

# **The Journal of American Science**

ISSN 1545-1003

Volume 6 - Number 11 (Cumulated No. 32), November 1 2010, ISSN 1545-1003

Marsland Press, Michigan, The United States

# The Journal of American Science

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## The Journal of American Science

ISSN 1545-1003

Volume 6, Issue 11, Cumulated No. 32, November 1, 2010

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**Abstract:** An experiment was carried out to investigate the use of long yam bean (*Sphenostylis sternocarpa*) as soil amendment for the growth and yield of white yam (*Dioscorea rotundata L*) between 1999 and 2003 at Akure in the rain forest zone of Nigeria. There were four treatment namely; NPK 15 – 15 – 15 fertilizer applied at 250kg/ha, poultry manure at 6 t/ha, long yam beans planted at two seeds per hole at a spacing of 1m x 0.5m between rows of yam plots (soil amendment) and a control (no fertilizer). The treatments were arranged in a randomized complete block design (RCB) and replicated five times. The soil analyses before planting and after harvesting were carried out. Each plot size is 4m x 4m (16m<sup>2</sup>). The growth parameters measured for the yam were vine length (cm), leaf population and stem girth (cm). At harvest, yam tuber weight (kg), tuber length (cm) tuber girth; root length and seed yield of long yam bean plants were determined. The leaf and soil N, P, K, Ca, Mg, pH and organic matter contents were also analysed at end of the experiment. The results showed that there were significant ( $p<0.05$ ) increases in the vine length, leaf population, stem girth, tuber weight, tuber length, tuber girth, soil and leaf N, P, K, Ca, Mg; pH and organic matter of white yam cultivated under the different fertilizer treatments compared to the control treatment. Long yam bean plants used as soil amendment increased the yam vine length, stem girth, leaf population, tuber weight, tuber length and tuber girth by 81%, 88.4%, 69.5%, 88.97%, 76% and 94% compared to the control. The same treatment (long yam bean plants) also increased the leaf population, tuber weight, tuber length and tuber girth of yam by 11%, 31%, 30% and 55% respectively compared to NPK fertilizer treatment. Long yam plants also increased the soil pH, O.M, K, Ca and Mg by 29%, 92%, 97%, 86%, 96%, 97% and 89% respectively compared to the control treatment. It increased soil pH, organic matter, K Ca and Mg by 31%, 87%, 1.42, 98% and 98.5% compared to NPK fertilizer. Long yam plants gave seed yield of 2.3 t/ha and produced yam tuber yield of 4900kg/ha amounting to \$6,050 compared to \$3453.00 and \$3,380.00 estimated on yam yields alone under poultry manure and NPK fertilizer treatments. Finally, the use of long yam bean plants as biological fertilizer source for yam production could substitute for 250kg/ha NPK fertilizer and 6t/ha poultry manure. [Journal of American Science. 2010;6(11):10-17]. (ISSN: 1545-1003).

**Keywords:** Long yam bean, soil amendment, white yam performance

#### Biochemical and Molecular Profiles of Gibberellic Acid Exposed Albino Rats

Hanan A.E.Soliman<sup>1</sup>; Mona M. Mantawy<sup>2</sup> and Hany M. Hassan<sup>3</sup>

<sup>1</sup>Chemistry Departement, Biochemistry Branch, Faculty of Science, Beni suef University, Egypt. <sup>2</sup>; Department of Medicinal chemistry , National Research Center, Dokki , Egypt and <sup>3</sup> Immunobiology and immunopharmacology unit , Animal Reproduction Research Inst., Giza , Egypt

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**Abstract:** The present study casts the light on the influence of the plant growth regulator, Gibberellic acid (GA3), on antioxidant defense systems [glutathione peroxidase, superoxide dismutase (SOD), and catalase (CAT)], lipid peroxidation level (malondialdehyde = MDA), AST, ALT , alkaline phosphatase, creatinine, total protein , albumin globulin , total lipids , total cholesterol, calcium and glucose . Moreover, histopathological examination of kidney and liver was done. On the molecular level, the DNA damage was determined. The rats were received 75 ppm of GA3 in drinking water ad libitum for 50 days. Gibberellic acid (GA3) treatments caused different effects on the estimated parameters compared to control. Gibberellic acid exposure induced significant elevations of plasma AST, ALT, alkaline phosphatase, creatinine and malondialdehyde. However, Gibberellic acid produced non significant alterations in plasma total protein, albumin globulin, total lipids, total cholesterol, calcium and glucose. On the other hand, exposure elucidate significant reductions of catalase, superoxide dismutase and glutathione peroxidase in comparison to control group. The histopathological findings revealed that Kidney sections of Gibberellic acid treated rats suffered from areas of interstitial fibrosis which appear as segmental and global glomerular sclerosis tubulointerstitial injury. On the similar ground, liver section of Gibberellic acid treated rats revealed that Gibberellic acid induced liver fibrosis; fatty metamorphosis and necrosis. The total genomic DNA electrophoretic pattern of lymphocytes deprived from Gibberellic acid treated rats revealed strong and obvious DNA damage as represented by a lot of fragments migrated from the wells. As a conclusion, Gibberellic acid (75 ppm) produce hepatonephrotoxicity, subsequently has oxidative stress role and DNA damage in albino rats 50 days post treatment. [Journal of American

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Science. 2010;6(11):18-23]. (ISSN: 1545-1003).

**Keywords:** plant growth regulator; Gibberellic acid (GA3); antioxidant defense systems; superoxide dismutase (SOD); catalase (CAT); lipid peroxidation

### An Efficient Algorithm for Transforming XML Schema into Relational Schema

<sup>1</sup> Abad Shah, <sup>2</sup>Amjad Farooq, <sup>3</sup>Syed Ahsan

<sup>1,3</sup> Al-Khawarizmi Institute of Computer Science, University of Engineering and Technology, Lahore

<sup>2</sup> Department of Computer Science, University of Engineering and Technology, Lahore

Amjadfarooquet@gmail.com

**ABSTRACT:** The Web and XML have influenced all walks of life especially those that involve business activities over the Internet. People like to do their business activities and transactions from their homes to save time and money. Many business and commercial companies such as insurance companies and banks maintain their records using relational database management systems. But the traditional relational database technology is unable to provide all these new facilities to the users. To enable the traditional relational database technology to cope with the new challenges of the Web and XML technologies, we need a transformation between the XML technology and the relational database technology as a middleware. To achieve this objective, we already proposed and reported an algorithm. In this paper, we extend our previous work and present automation details, testing, and performance report of our proposed algorithm. The result shows that the implementation of the algorithm is more efficient than the existing algorithms for the same purpose [Journal of American Science. 2010;6(11):24-37]. (ISSN: 1545-1003).

**Keywords:** XML, web, rational database, transforming algorithm

### Effect of injector types, irrigation and nitrogen levels on II-Garlic yield, water and nitrogen use efficiency

Tayel, M.Y., \*Shaaban, S.M., Ebtisam I. El-Dardiry and Sabreen Kh.

Water Relations and Field Irrigation Dept., National Research Centre, Dokki, Cairo, Egypt.

\*shaabansm@yahoo.com

**Abstract:** Field experiments were conducted during two consecutive growing seasons in split split plot design on a clay loam soil at Shalaquan, Qalubia Governorate, Egypt. Experiments investigated the effect of three injectors types by-bass pressurized mixing tank ( $J_1$ ), venturi ( $J_2$ ) and piston pump ( $J_3$ ); three rates of irrigation 50, 75; 100% of  $ET_c$  ( $I_1, I_2, I_3$ ); three nitrogen levels 60, 90; 120 kg fed<sup>-1</sup> ( $N_1, N_2, N_3$ ) on garlic yield, water use efficiency (WUE) and nitrogen use efficiency (NUE). The main results could be summarized as follows; the maximum and minimum garlic yields (6.34, 2.38 ton fed<sup>-1</sup>) were obtained with treatment  $J_3 I_2 N_3$  and  $J_1 I_1 N_1$ , respectively. Maximum value of WUE was 3.29 kg garlic m<sup>-3</sup> of irrigation water as recorded with the treatment  $J_3 I_1 N_3$ , while the minimum value was 1.30 kg garlic m<sup>-3</sup> of irrigation water as recorded with the treatment  $J_1 I_3 N_1$ . The maximum and minimum values of NUE in kg garlic kg<sup>-1</sup> N were 83.22 and 29.17 for  $J_2 I_2 N_1$  and  $J_1 I_1 N_3$ , respectively. A positive linear relationship was found between WUE and NUE. [Journal of American Science. 2010;6(11):38-46]. (ISSN: 1545-1003).

**Keywords:** Field experiments; clay loam soil; water use efficiency (WUE); nitrogen use efficiency (NUE)

### Software Cost Estimation through Entity Relationship Model

Arshid Ali <sup>1</sup>, Salman Qadri <sup>2</sup>, Syed Shah Muhammad <sup>2</sup>, Jalil Abbas <sup>3</sup>, Muhammad TariqPervaiz <sup>2</sup>,

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Sarfaraz Awan<sup>2</sup>

<sup>1</sup>. Department of Computer Science, GCU Faisalabad, Pakistan

<sup>2</sup>. Department of Computer Science, Virtual University of Pakistan, Lahore, Pakistan

<sup>3</sup>. Department of Computer Science, University of Central Punjab, Lahore, Pakistan

sayyed\_qadri@hotmail.com

**Abstract:** Software Cost Estimation is essential for efficient control and management of the whole software development process. Today, Constructive Cost Model (COCOMO 11) is very popular for estimating software cost. In Constructive Cost Model lines of code and function, points are used to calculate the software size. Actually, this work represents the implementation stages but in early stages in software development, it was not easy to estimate software cost. The entity relationship model (ER Model) is very useful in requirement analysis for data concentrated systems. This paper highlights the use of Entity Relationship Model for software cost estimation. Pathway Density is ushered in. By using the Pathway Density and other factors, many regression models are built for estimating the software cost. So in this paper, Entity Relationship Model is based on estimated cost of software. [Journal of American Science. 2010;6(11):47-51]. (ISSN: 1545-1003).

**Keywords:** ER Model, Cost Estimation, Entity

**Web-Ontology Design Quality Metrics**

<sup>1</sup>Amjad Farooq, <sup>2</sup>Syed Ahsan, <sup>2</sup>Abad Shah

<sup>2</sup> Al-khawarizmi Institute of Computer Science

<sup>1</sup>Department of Computer Science and Engineering

University of Engineering and Technology, Lahore

amjadfarooq@uet.edu.pk

Full Text

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**Abstract:** Semantic Web is an extension of current web in which the web resources are equipped with formal semantics about their interpretation for the machines. These web resources are integrated in the form of web information systems, and their formal semantics are normally represented in the form of web-ontologies. Using the database terminology, we can say that web-ontology of a semantic web system is schema of that system. Since web-ontology is an integral element of semantic web systems, therefore, design quality of a semantic web system can be measured by measuring the quality of its web-ontology. The key consideration is that after completing design of a web-ontology, it is appropriate time to assess its quality so that in case, the design is of low quality, it can be improved before its instantiation. This can save a considerable amount of cost and effort for developing high quality semantic web systems. Metrics are considered as suitable tools for evaluating quality. In this paper, we propose certain metrics for web-ontology quality evaluation. These metrics may contribute in developing a high quality semantic web system. [Journal of American Science. 2010;6(11):52-58]. (ISSN: 1545-1003).

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**Keywords:** Semantic web; Ontology metrics; quality measurement

**Determining Regression Models of Almond and its Kernel Mass Based on Geometric Properties  
(Shahrud 12 and Mama'e Varieties)**

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A.Mohamadi<sup>1</sup>, M.A.Ghazavi<sup>2</sup>, B.Hosseinzadeh<sup>2</sup>

<sup>1</sup>Payame-noor university, Farsan, Iran

Full Text

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<sup>2</sup> Department of mechanical farm machinery, University of Shahrekord, Shahrekord 115 Iran

bahram\_hs@yahoo.com

**Abstract:** Almond (*Prunus amygdalus*) belongs to Rosaceae family and sub-family of Pomoideae. Physical traits of agricultural products are main parameters in designing of grading, conveying, processing, and packing systems. In this study the physical traits such as dimensions, mass, volume, sphericity, geometric average of Mama'e and Shahrud 12 almonds and their kernels were measured and calculated. The average amounts of length, width, and thickness for both almond varieties were 37.41, 23.21, and 16.63 mm, respectively, and for almonds' kernel were 28.05, 13.4, and 7.82 mm, respectively. Results from modeling of almond and its kernel masses based on dimensions and volume showed that there exists a great correlation coefficient between the samples actual volumes and masses, but since determining actual volume of almond and its kernel is a time-taking task, it was suggested to use calculated volume and presuming that the cross-sectional area of the almond is oval. Also the mass model based on the thickness had the highest determination coefficient and lowest regression error which was the best option for industrial and economical applications. [Journal of American Science. 2010;6(11):59-64]. (ISSN: 1545-1003).

**Keywords:** Almonds, Physical Properties, Mass Modeling, Dimensional Models, Volumetric Models

Full Text

**Regional assessment of groundwater vulnerability in Tamtsag basin, Mongolia using drastic model**

Fanomezantsoa Hasiniaina<sup>1</sup>, Jianwei Zhou, Luo Guoyi

<sup>1</sup>School of Environmental Studies, China University of Geosciences (Wuhan)

Lumo Road 388, Wuhan City, 430074 Hubei Province, P.R. China.

hasiniainaf@hotmail.com

**ABSTRACT:** Groundwater is one of the most valuable natural resources and for that reason, its protection and management is vital for human evolution, socio-economic development and ecological diversity. Because of the known health and economic impacts associated with groundwater contamination, steps to assess groundwater vulnerability must be taken. This study aimed to assess groundwater pollution potentials of the north-eastern part of the deep confined aquifer of block XIX, Tamtsag basin, Mongolia. The normal DRASTIC model was applied to the study area with the help of GIS. DRASTIC parameters were calculated from geological data, soil and elevation contour maps, and groundwater level data of the study area. ArcInfo/GIS was used to demarcate vulnerable zones based on their vulnerability index. Finally, a sensitivity analysis of the parameters constituting the model was performed in order to evaluate the relative importance of the each DRASTIC model parameters. The aquifer vulnerability map revealed that only 2% of the study area is under moderate vulnerability to contamination, the remaining zone was determined to be in a low risk category. GIS greatly facilitated the implementation of the sensitivity analysis applied on the DRASTIC vulnerability index which otherwise could have been impractical. Appropriate methods for keeping groundwater resource sustainability in the study area have been suggested. [Journal of American Science. 2010;6(11):65-78]. (ISSN: 1545-1003).

**Keywords:** Groundwater vulnerability / DRASTIC / Tamtsag basin

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**Structure Of Whey Protein Consequence For Dairy Industry (Review)**

Khorshid,M.A.and Fatma,A.M.

10 Dairy Department, National Research Center, Dokki , Cairo , Egypt

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khorshid88@hotmail.com

**Abstract:** Milk proteins play a range of roles which make dairy products and products containing dairy

components are valuable. Theses include nutrition, physical functionality and breakdown under controlled condition to produce nutritional, functional or flavour full products. This article reviews the structure of whey protein consequence for dairy industry. [Journal of American Science. 2010;6(11):79-84]. (ISSN: 1545-1003).

**Keywords:** Milk proteins; dairy components; nutrition; review; dairy industry

**The Application Of Micro-Relief Meter For Soil Tillage Studies**

M.A.Ghazavi<sup>1</sup>, A.Yosufvand<sup>1</sup>, B.Hosseinzadeh<sup>1</sup>

<sup>1</sup> Department of mechanical farm machinery, University of Shahrekord, Shahrekord 115 Iran

Bahram\_hs@yahoo.com

**Abstract:** Measuring the physical properties of soil provides a good opportunity for careful study of processes such as evaporation from the soil surface, formation of water runoff, sediments and erosion. In this research, the change of some soil hydrological properties was studied in four kinds of primary soil cultivation activities by using a mechanical micro-relief meter. This study was conducted in a randomized complete block design. Data was collected in frames with 9025 cm<sup>2</sup> area and 400 data height were collected for every frame. Measured soil properties were: Root Mean Square (RMS) of height data, superficial Profile Length Ratio (RZ) of soil roughness, Infiltration Recession Factor (RECS), plough depth, the volume of displaced soil, comparison of the area change in relation to superficial evaporation of soil. The RMS of height data was higher ( $p<0.05$ ) with moldboard plow and modified disk plow than chisel and traditional ploughshares. The analysis of height data collected from plots showed that surface evaporation of soil moisture didn't differ by plowing with moldboard plow or traditional ploughshares, compared with two other ploughshares. This statistic was less than the recorded value of developed dish-like ploughshare ( $p<0.01$ ). Also displaced soil mass by these two ploughshares was much more than chisel and traditional ones ( $p<0.01$ ). The developed ploughshare prevents more evaporation. Therefore, plowing surface with this instrument provides high pot-hole store and penetration coefficient compared with other ploughshares. [Journal of American Science. 2010;6(11):85-89]. (ISSN: 1545-1003).

**Keywords:** RMS of height data, RZ, plough, height data, surface evaporation, pot-hole store

**Variations in some heavy metal concentrations in soil and *Manihot esculanta* tuber from the East and North eastern part of Nigeria**

S. T. Garba <sup>1\*</sup>, J. T., Barminas, <sup>2</sup> and A. H. Santuraki,<sup>1</sup>

<sup>1</sup>Department of Chemistry, University of Maiduguri, Borno state. P. M. B. 1069. Nigeria.

<sup>2</sup>Department of Chemistry, Federal University of Technology Yola (FUTY), P. M. B. 2076. Adamawa state. Nigeria. stelagarba@yahoo.com

**Abstract:** The levels of the heavy metals: Cr, Cd, Pb, Cu, Zn and Mn were determined in *Manihot esculanta* tubers and the soil used for its cultivation. This was done to asses the pollution level of the farmland and hence the safety status of *Manihot esculanta* tubers produced. Samples were collected from Konduga local government area of Borno state in the North East and from Umuahia local government area of Abia state in the East, all in Nigeria. These were treated and digested and the heavy metal concentrations were determined using Atomic Absorption Spectroscopy (AAS). The result obtained shows that, the samples from the North East had the highest levels of the elements Pb 30.14, Zn 88.65, Cd 3.15, Cu 16.00, Cr 6.74, and Mn 13.00 ppm in the soil sample while 5.47, 9.09, 5.05, 2.60, 3.37 ppm for the elements Pb, Zn, Cu, Cd, and Mn respectively were observed in *manihot esculanta* tubers sample. All these were found higher than what was observed in the soil and *manihot esculanta* samples collected from the East. Cr, was however, found below detection limits in *manihot esculanta* tuber samples from both the regions. [Journal of American Science. 2010;6(11):90-94]. (ISSN: 1545-1003).

**Keywords:** Health, Safety, Environment, Development, Borno State, Abia State, Nigeria

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**Exploitation of palms wine in the municipality of Ze (Benin): socio-economic and physical impacts**

Full Text

Ade Romaric Herman<sup>1\*</sup>, Bio Bigou Bani Leon<sup>2</sup>, Luo Zhaohui<sup>3</sup>

1. School of Environmental Studies, China University of Geosciences, Hubei province, 388 lomo Road, 430074 Wuhan, P.R China, , 008613797056028
2. University of Benin, (Abomey-Calavi), General Secretary of university Box: 526- Abomey-Calavi- Benin
3. China University of Geosciences, Department of Environmental Sciences, 388 Lomo Road, Wuhan City, Hubei province, 430074, P.R. China

**Abstract:** The exploitation of palm wine is one of activities which many people from southern Benin particularly the municipality of Ze have engaged. This study aims to analyze the socio-economic and environmental impacts of the exploitation of palm wine in the municipality. The methodology consisted of collecting demographic, agricultural, socio economic data, processing and analysis of data collected in real area based on components of the Leopold matrix. The exploitation of palm wine has changed the agricultural landscape of the municipality of Ze. The soil is becoming unproductive for food crops after a long fallow palm wine causing impoverished land due to overuse. The vegetation is endangered because of the rarity of certain plant species like Acacia sp (acacia), Antiaris africana, sapadi Blighia (bligia tasty), Chlorophora excelsa, Cola nitida, etc. But, in other aspect this activity is contributing to improve the living conditions of farmers and reduce the production of oil palm in this area. The production of alcohol provides employment to about 42% of the active population in the municipality of Ze, (municipality of Ze, 2007). At various levels of production that is from felling to alcohol distillation there are large numbers of people being employed. [Journal of American Science. 2010;6(11):95-102]. (ISSN: 1545-1003).

**Key words:** palm wine; sodabi; socio- economic and physical impact; municipality of Ze

**Sorption characteristics of copper in some calcareous soils of western Iran**

Full Text

A. R. Hosseinpur<sup>1</sup> and F. Dandanmozd<sup>2</sup><sup>1</sup>.Soil Sci. Dep. Shahrekord Univ. Shahrekord, Iran<sup>2</sup>.Soil Sci. Dep. Bu-Ali Sina Univ. Hamadan, Iran.*E-mail:* hosseinpur-a@agr.sku.ac.ir

**Abstract:** The environmental impact of metal additions to soil depends on its sorption ability. To evaluate the sorption of copper (Cu) on to some soils an experiment was conducted with ten calcareous soils of Hamadan province in the west of Iran. Half g soil samples were equilibrated at 25±1 and 45±1°C with 25 ml of 0.01 M CaCl<sub>2</sub> containing 0 to 30 mgL<sup>-1</sup> Cu as CuSO<sub>4</sub>. Suspensions were centrifuged, filtered and concentration of Cu in the clear extract solution was calculated. The thermodynamic parameters viz. K, G, H and S were determined by using sorption data and concentration of Cu in equilibrium solution at two different temperature. Thermodynamic parameters revealed that Cu sorption increased as the values of K and G increased with rise in temperature from 25 to 45 °C. The G° values at 25 and 45°C were negative and ranged from -18.39 to -24.10 and -21.167 to -26.267 kJ mol<sup>-1</sup> respectively. The values of enthalpy (H°) and entropy (S) were positive and ranged from 8.184 to 42.852 kJ mol<sup>-1</sup> and 102.457 to 206.184 J mol<sup>-1</sup> K<sup>-1</sup>. The results showed that Cu sorption is a spontaneous process and endothermic reaction. The results also showed that calcareous soils can sorb high amounts of Cu and that thermodynamic parameters are useful in describing Cu sorption. [Journal of American Science. 2010;6(11):103-108]. (ISSN: 1545-1003).

**Keywords:** Sorption isotherm; Calcareous soils; Thermodynamic parameter; Copper

**Association between Single nucleotide polymorphisms in Gallinacin genes and resistance to Marek's disease in White Leghorn chicken**

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Yacoub, H. A<sup>\*1</sup>, Galal, A<sup>2</sup>, El Fiky, S.A<sup>1</sup> and Fathi, M. M<sup>2</sup>,

<sup>1</sup>Cell Biology Department, National Research Center, Giza, Egypt

<sup>2</sup>Poultry Production Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt

\*Haitham\_yakoub@yahoo.com

**Abstract:** Gallinacins are antimicrobial peptides that play a significant role in innate immunity in chicken. The aim of this study was to determine the relationship between candidate genes of innate immunity and resistance to Marek's disease and to predict whether the amino acids substitutions lead to produce new phenotypes. We used in current study two inbred lines of White Leghorn chickens, line 6 which selected for resistant to Marek's disease and line 7 which selected to susceptible to Marek's disease from ADOL, ARS, USDA. We examined Gal-1 and Gal-2 in current study by sequenced a 1.38 kb in two directions from two inbred lines (6 and 7). A total of 6 SNPs were identified within the sequenced regions. This equates to an SNP rate of 4.34 SNPs/kb, nearly to the previously reported 5 SNPs/kb across the entire chicken genome. The current study showed that the gallinacin genes are polymorphic because there are many single nucleotide polymorphisms (SNPs) in both inbred lines of White Leghorn chickens and some of these SNPs are nonsynonymous and others are synonymous and some of them are located in intronic region and the rest are in exonic region. All identified SNPs were intronic; except for Gal-1 was exonic resulting in amino acids changes which have a non-synonymous SNP resulting in amino acids alterations of asparagine to serine, histidine to tyrosine and tyrosine to serine, respectively. From SIFT (Sorting Intolerant from tolerant) program which used to predict whether an amino acids substitutions can affect protein function resulting in phenotypic effect , that is may be made the inbred line 7 of White Leghorn chickens are susceptible to Marek's disease rather than line 6. We are concluded that a new chromosomal region with effects on the response to Marek's disease in chickens was characterized in this study. Within this region, the SNPs in the gallinacin candidate genes could potentially be used in a marker assisted selection program to enhance the response to Marek's disease. Analysis of the gallinacin genes in the protective pathways of disease resistance has also opened the possibilities for therapeutic strategies using endogenous antimicrobial peptides. [Journal of American Science. 2010;6(11):109-114]. (ISSN: 1545-1003).

**Keywords:** single nucleotide polymorphisms, Gallinacin, genes, Marek's disease, resistance

#### **Endometrial Cytology and Bacteriological Isolates From Buffaloes With Retained Fetal Membranes and Their Effects on the Reproductive Efficiency**

Amer H. A.<sup>1\*</sup>, AbouZeid N. Z.<sup>2</sup> and Barakat T. M.<sup>1</sup>

Department of Theriogenology<sup>1</sup> and Infectious Diseases<sup>2</sup>, Faculty of Veterinary Medicine, Zagazig University, Egypt  
samarmed84@yahoo.com

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**Abstract:** This study aimed to determine if the buffaloes with retained fetal membranes (RFM) and without systemic involvement had an effect on the subsequent reproductive efficiency. One hundred buffaloes with or without placental retention were allocated into 4 groups, including 25 buffaloes at day 15 post-calving had RFM (1<sup>st</sup> group), 25 buffaloes at 45 days post-calving had RFM (2<sup>nd</sup> group), 25 buffaloes without RFM at day 15 post-calving as control (3<sup>rd</sup> group ) and 25 buffaloes without RFM at day 45 post-calving as control (4<sup>th</sup> group). The intrauterine perfusion fluid (10ml) was collected and examined bacteriologically and cytologically to evaluate the intrauterine environment. The reproductive parameters were determined in both buffaloes with or without retained fetal membranes. The detection rate of bacterial spp. was significantly ( $P<0.05$ ) higher in buffaloes with RFM collected at day 15 after parturition than those in other groups. All 25 buffaloes with RFM at 15 days post-partum (100.0 %) showed positive results. From 22 of them (88.0%), more than one bacterial species was isolated. An *Archaeobacterium pyogenes* (A. pyogenes) was isolated from 56.0% of buffaloes with RFM after 15 days post-calving. On the other hand, 5 (20.0%) out of 25 buffaloes with RFM at 45 days post-partum showed positive results. Nine out of 25 (36.0%) buffaloes without RFM at 15 days post-partum showed positive results. Moreover, 4 out of 25 (16.0%) control buffaloes at 45 days post-partum showed positive results. The bacterial species most frequently

isolated was *Lactobacillus spp*. The number of buffaloes with 70% PMNs or 40% lymphocytes cells was higher (24/25, 96%) in the 1<sup>st</sup> group (RFM) at 15 days than those in 2<sup>nd</sup> group (RFM) at 45 days post-calving. The number of buffaloes with 70% PMNs or 40% lymphocytes cells was also significantly ( $P<0.01$ ) higher in control group (17/25, 65%) at 15 days than those in control group (6/25, 24%) at 45 days. There were no significant variations among the groups of the buffaloes with retained placenta and the control groups at 15 and 45 days post-calving in postpartum uterine involution, the number of days from parturition to initial insemination, the number of days to conception and the number of services per conception. The overall conception rate was 15(60%) and 16(64%) in the RFM group, meanwhile, it was 19(76%) and 20(80%) in the control groups. It could be concluded that, in most buffaloes, the retained fetal membranes without systemic involvement had no major effect on the postpartum reproductive performance. [Journal of American Science. 2010;6(11):115-121]. (ISSN: 1545-1003).

**Keywords:** Buffaloes, Bacteriologically, Cytologically, Insemination, Conception.

#### Toxoplasmosis in Naturally and Experimentally Infected Goats

AbouZeid N.Z.<sup>1</sup>, Amer H.A.<sup>2\*</sup>, T.M. Barakat<sup>2</sup>, Selim A.M.<sup>1</sup> and El-Balkemey F.A.<sup>1</sup>

Department of Infectious Diseases<sup>1</sup>, Theriogenology<sup>2</sup>, Faculty of Veterinary Medicine, Zagazig University, Egypt

samarmed84@yahoo.com\*

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**Abstract:** One hundred slaughtered goats (2-3 years old) were used for diagnosis of toxoplasmosis in naturally infected goats, and 12 healthy pregnant and nonpregnant goats were used to study the pattern of toxoplasmosis as experimental study. Prevalence of toxoplasmosis in 100 slaughtered goats revealed that 29 (29%) and 27 (27%) were seropositive by LAT and IHA tests, respectively. There were agreement between LAT and IHA 97.3% in seronegative and 93.1% in seropositive sera in goats. There were complete concordance between LAT and bioassay in cats and mice. While the agreement between IHA result and bioassay in cat and mice was 93.1% in goats. Clinical examination of experimentally infected goats revealed that all goats had slight rise of body temperature; depression, anorexia, cough, muscular hyperesthesia and diarrhea by day 5 and returned to normal by day 11. The age of fetus at the time of *T. gondii* infection is one of the known causes for the variability in clinical response. As infection of goats in early stage of pregnancy result in fetal reabsorption, while infection in mid pregnancy lead to abortion in one goat at 28 days post-infection and the other was aborted at 40 days post-infection. Moreover infections in late pregnancy resulted in delivery of viable kids. On the other hand controls goats were clinically normal and pregnant does were birth viable kids. LAT showed rapid response after 14 days post-infection, while IHA detected antibodies after 3 weeks post-infection. The antibody titers of both tests remained high until the end of experiment (48 weeks), while the titers were decreased around abortion or parturition and increased again after one week. Both LAT and IHA tests were insensitive in the pre-suckling kids from infected goats, whereas PCR gave positive results. In conclusion, PCR considered the most reliable tool for diagnosis of prenatal infection of toxoplasmosis, while LAT and IHA were considered unreliable tools for diagnosis of toxoplasmosis if they applied one week before or after kidding. [Journal of American Science. 2010;6(11):122-129]. (ISSN: 1545-1003).

**Keywords:** Prevalence, Toxoplasmosis, Goats, Abortion, Parturition.

#### Photocatalytic Degradation of Monoazo and Diazo Dyes in Wastewater on Nanometer-Sized TiO<sub>2</sub>

S.A. Abo-Farha

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Chemistry Department, Faculty of Science, Al-Azhar University (Girls), Naser City, Cairo, Egypt

samiaelhosieny@yahoo.com

**Abstract:** Advanced oxidation processes (AOPs) have proved very effective in treatment of the various hazardous organic pollutants in water. The photocatalytic degradation of two azo dyes, monoazo dye

Acid Orange 10(AO10) and diazo dye Acid Red114(AR114) present in wastewater were studied. Homogeneous photocatalytic degradation of the two azo dyes with UV/H<sub>2</sub>O<sub>2</sub> process was investigated. The rates of disappearance of the two azo dyes were monitored spectrophotometrically at the visible maximum absorption wavelengths. It was found that the rate of decolorization rises by increasing the initial dosage of H<sub>2</sub>O<sub>2</sub> up to a “critical” value at which it is maximum and beyond which it is inhibited. The rates of reactions follow pseudo-first-order kinetics. Also heterogeneous photocatalytic degradation of the two azo dyes with UV/TiO<sub>2</sub> (titanium dioxide) interface was investigated. The photocatalytic degradation rate depends on dye structure, dye concentration, TiO<sub>2</sub> concentration and pH of the medium. The mechanism of the photodegradation process under UV-visible light illumination involves an electron excitation into the conduction band of the TiO<sub>2</sub> semiconductor leading to the generation of very active oxygenated species that attack the dye molecules leading to photodegradation. Photocatalytic activity of TiO<sub>2</sub> was examined by focusing on its enhancement by electron scavengers in the photocatalytic decomposition of the two azo dyes. The electron scavengers employed was inorganic oxidant such as H<sub>2</sub>O<sub>2</sub>, adequate dose of H<sub>2</sub>O<sub>2</sub> led to a faster degradation of the two azo dyes in the TiO<sub>2</sub> photocatalytic system. The fast decolorization of monoazo dye (AO10) than diazo dye (AR114) is an indication that, the number of azo and sulphonate groups in the dye molecule may be a determining factor for increasing the degradation rates. [Journal of American Science. 2010;6(11):130-142]. (ISSN: 1545-1003).

**Keywords:** Azo dyes; UV/H<sub>2</sub>O<sub>2</sub> oxidation, Titanium dioxide; Photodegradation; Semiconductor.

**Phenotypic and genetic trends for milk production in Egyptian buffaloes**

Fooda, T. A.; Kawthar A. Mourad and Gebreel, I. A

Animal Production Research Institute-Buffalo Breeding Research Department- Dooki- Giza - Egypt

**Abstract:** A total of 3495 records collected from 904 buffalo cows progeny of 174 sires and 470 dams through period from 1990 to 2008 in all Stations belonging to Animal Production Research Institute, Ministry of Agriculture were used to estimate the phenotypic and genetic trends for total milk yield. LSM for total milk at different year of calving ranged between 1334 kg and 1692 kg, 1028 kg and 1561 kg, 1209 kg and 1633 kg, 1355 kg and 1415 kg and 1137 kg and 1355 kg for El-Nattafe El-Gidid (NG), El-Nattafe El-Kadim (NK), Mahalet Mousa (MM), Gemiza (G) and Sids (S) stations, respectively. Estimates of the positive breeding value (BV, %) at different year of calving ranged between 40 % and 52 %, 31 % and 52 %, 40 % and 56 %, 37 % and 55 % and 45 % and 59 % for NG, NK, MM, G and S stations, respectively. Annual phenotypic trend for milk production ranged between -11.7 kg and +36.7 kg for S and NK stations, respectively. While, the annual genetic trend ranged between -0.16 kg and +0.6 kg for G and NG stations, respectively. The results of the present study showed that there are increased of improvement of phenotypic and genetic trend in all MM farms from 1990 until now. [Journal of American Science. 2010;6(11):143-147]. (ISSN: 1545-1003).

**Keywords:** buffalo, phenotypic trend, genetic trend, breeding value and milk production

**Computer Aided Design, Synthesis and Biological Evaluation of Novel Acridine Derivatives as Topoisomerase I Inhibitors**

Gamil Mahmoud El Taliawi<sup>1</sup>, Enayat Ibrahim Ali<sup>1</sup>, Gehan Hegazy Hegazy<sup>\*1</sup>, Nasser S. M. Ismail<sup>2</sup> and Walaa Ramadan<sup>1</sup>

<sup>1</sup>Department of Pharmaceutical Chemistry, Faculty of Pharmacy Cairo University, <sup>2</sup> Pharmaceutical Chemistry Department, Faculty of Pharmacy Ain Shams University, Egypt.

<sup>\*</sup>gehan\_hegazy@yahoo.com

**Abstract:** A series of novel 9- anilinoacridines was designed and their molecular docking studies into the active site were examined as topoisomerase I inhibitor. Several compounds showed significant high simulation docking score. The designed compounds were synthesized and biologically evaluated against mammary carcinoma cell line (MCF-7), where compounds 8,11e,11f,13b,14b,14e and 14f showed significant inhibitory activity at a concentration 10 $\mu$ g/mL. It appears that the *in vitro* activity of

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compounds 8,11e,11f,13b,14b,14e and 14f were consistent with their molecular modeling results, and compound 14b showed the highest activity with IC<sub>50</sub> value of 7.8 µg. [Journal of American Science. 2010;6(11):148-158]. (ISSN: 1545-1003).

**Keywords:** Molecular docking, Acridine derivatives, Antitumor

**Effect of Probiotic (*Saccharomyces cerevisiae*) Adding to Diets on Intestinal Microflora and Performance of Hy-Line Layers Hens**

Saadia M. Hassanein<sup>1</sup> and Nagla K. Soliman<sup>2</sup>

<sup>1</sup>Microbiology Dept, Faculty of Science, <sup>2</sup>Poultry Production Dept., Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

**Abstract:** An experiment was conducted to evaluate the effect of adding various levels of a live yeast to laying hen diets on their laying and feeding performance, egg shell, egg components and some blood constituents, as well as the intestinal microflora make-up. This was studied to validate the mode of a live yeast action in improving laying hens performance. For this purpose 75 Hy line (W-36) white layers were selected from 70 to 79 week of age in individual cages and randomly distributed into five experimental groups of 15 layers each. The individual hen was represented as an experimental unit. The five experimental groups were fed on five graded levels of a live yeast as 0.0% (control), 0.4%, 0.8%, 1.2% and 1.6%. The main results indicated an increase in egg production percentage of layers fed with 0.4% and 0.8% a live yeast which recorded 83.4% and 80.6% respectively compared with 74% of control which was similar to the groups of layers fed 1.2% (74.9%) and 1.6% (74.6%). Average egg weight was not influenced by adding yeast into diets. Egg mass results were parallel to these of egg production where the values of 46.7, 51.0, 50.2, 48.3 and 46.1 g egg/hen/day were recorded for the group of birds fed with 0%, 0.4%, 0.8%, 1.2% and 1.6% a live yeast respectively. Egg albumen and egg yolk were affected significantly. There was a slight improvement in egg shell thickness and percentage. Feed intake values were approximately similar within the different treatments. Feed conversion ratios (g feed/g egg) of layers fed yeast levels of 0.4% (2.08) and 0.8% (2.07) were better than the control group (2.27). Blood total protein levels of birds fed 0.4% (3.82), 0.8% (3.65) and 1.2% (3.97) yeast were lower than the control (4.16), while the value of 1.6% yeast (4.16) was slightly higher than control. Blood albumen levels were parallel to those of blood protein while blood globulin values were not affected. Blood cholesterol levels of layers fed yeast-supplemented diets were lower than the control. Blood total lipids were not affected by treatments. Ileal content pH of layers fed 0.8% and 1.2% yeast levels was lower than the control. Microbiological examination of ileal content indicated an obvious reduction in bacterial total count. While Lactobacilli bacterial count was increased. There were reductions in bacterial strains of *Escherichia coli* (*E.coli*), *Klebsiella sp.*, *Staphylococcus sp.*, *Micrococcus sp.*, *Campylobacter sp.*, and *Clostridium perfringens* of layers fed various yeast levels. The results of this study suggest adding live yeast at 0.4% or 0.8% into laying hen diets can enhance the productive performance and nutrients utilization via the inhibitory effect of yeast against pathogenic bacteria. [Journal of American Science. 2010;6(11):159-169]. (ISSN: 1545-1003).

**Keywords:** yeast level, laying hen, egg production, ileal microflora, blood constituents.

**Properties of enterotoxigenic *S. aureus* Isolated from mastitic cattle and buffaloes in Egypt**

Jakeen Kamal Abdel Haleem El-Jakee<sup>1</sup>, Emad Rizkalla Zaki<sup>2</sup>, Randa Samy Farag<sup>2</sup>

1-Microbiology Department Faculty of Vet. Medicine Cairo University

2-Buffaloes Diseases Department, Animal Health Research Institute, Dokki, Giza.

jeljakee@yahoo.com

**Abstract:** Enterotoxigenic *S. aureus* in milk poses a potential health hazard to consumers. In this paper 106 *S. aureus* isolated from cow and buffalo milk samples were investigated for production of

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enterotoxins. RPLA results showed high incidence of type C enterotoxin followed by type A and type B with incidence of 34 (32.1%), 19 (17.9%) and 15 (14.2%) respectively. Toxigenic *S. aureus* isolates produced golden yellow, creamy and white colonies on agar in percent of 69.11%, 27.94% and 2.94% respectively. Regarding to hemolytic activity on sheep blood agar, 92.65% of toxigenic *S. aureus* isolated from bovine milk samples were hemolytic. A correlation exists between toxigenic isolates and coagulase and DNase production. On crystal violet agar medium, 23.53% of the *S. aureus* isolates yielded yellow colonies, 64.71% yielded violet colonies, while 11.76% yielded white colonies from the toxigenic *S. aureus* isolates. It is clear that most of bovine isolates yielded violet colonies on the medium. Out of 68 isolates of toxigenic *S. aureus* isolates 51 (75%) showed SpA by agglutination test positive. Results obtained showed 100% agreement between RPLA and PCR techniques. [Journal of American Science. 2010;6(11):170-178]. (ISSN: 1545-1003).

**Keywords:** *S. aureus*, mastitis, enterotoxins, RPLA, PC

#### Hydro-Thermal Safety Control of Karun-1 Dam under Unusual Reservoir Level Reduction

Mojtaba Labibzadeh<sup>1</sup>

<sup>1</sup>. Department of Civil Engineering, Faculty of Engineering, Shahid Chamran University, Ahvaz, Iran

Labibzadeh\_m@scu.ac.ir

**Abstract:** Karun-1dam safety was examined through carrying over a 3D finite elements analysis. The dam as well as its foundation and abutments have been modeled in a relatively exact manner. Furthermore, the vertical contraction joints were simulated in calculations. Hydrostatic, gravity and thermal effects have been taken into account as the load collections. 10m reduction of reservoir level from normal water level of the dam reservoir was applied in the modeling and the possibility of initiate and development of cracks in dam body was investigated by means of monitoring of principal stresses. The obtained results showed that mentioned possibility existed and the downstream face of the dam in vicinity of the abutments near to crest level probably experiences the tensile cracks. [Journal of American Science. 2010;6(11):179-184]. (ISSN: 1545-1003).

**Keywords:** Arch dam, Thermal, Contraction joints, Cracks, Dam safety

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#### Prevalence of HBV Genotypes in Egypt among Hepatitis Patients

Iman A.El Aziz Khaled<sup>\*1</sup>, Ola M. Mahmoud<sup>1</sup>, Abeya F.Saleh<sup>1</sup>, and Emad A. Baioumi<sup>2</sup>

<sup>1</sup>Haematology &Blood Bank, <sup>2</sup>Tropical Medicine, Theodor Bilharz Research Institute (TBRI).Cairo, Egypt; <sup>\*</sup>iman\_khaled@yahoo.com

**Abstract:** Phylogenetic analysis has led to the classification of hepatitis B virus into eight genotypes, designated A to H. The genotypes have differences in biological properties and show heterogeneity in their global distribution. These attributes of the genotypes may account not only for differences in the prevalence of hepatitis B virus mutants in various geographic regions, but also makes them responsible for differences in the clinical outcome and response to antiviral treatment in different population groups. Africa is one of the highly endemic regions of HBV with five genotypes (A-E) identified. Almost all patients in the Mediterranean area are infected with genotype D. However, there is little information of genotype distribution in Egypt. A total of 140 Egyptian patients with hepatitis B surface antigen (HBsAg) positive were enrolled in this study. Of the 140 patients, only100 patients were HBV DNA positive and only these were included in the study. They were classified in to 20 patients with acute hepatitis (AH), 75 patients with chronic active hepatitis (CAH) and 5 patients with hepatocellular carcinoma (HCC)]. HBV genotypes were determined using INNO-LiPA methodology which is based on the reversed hybridization principle. Results: This study showed that genotype D constituted 87% of the total infections (75% CAH, 7% AH & 5% HCC). The other 13% showed mixed infections of D/F. Conclusion: These findings show that the most prevalent genotype in Egypt is genotype D especially in CAH and HCC patients while the mixed type D/F is mostly encountered in AH. [Journal of American Science. 2010;6(11):185-190]. (ISSN: 1545-1003).

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	<b>Keywords:</b> Phylogenetic, Genotypes , Hepatocellular		
	<p><b>Cytogenetic Study of the Effect of <i>Schistosoma mansoni</i> Infection on Human Peripheral Blood Lymphocytes and the Role of -Carotene and Vitamin E in Modulating this Effect.</b></p> <p>Mervat S.El-Ansary<sup>1</sup>, Imam A.Khaled<sup>2</sup>, Abeya F. Saleh<sup>2</sup>, Ola M.Mahmoud<sup>2</sup>, Emad A. Baioumi<sup>3</sup>, Heba A.Bakr<sup>4</sup></p> <p><sup>1</sup>Immunology (Cairo University), <sup>2</sup>Haematology (TBRI), <sup>3</sup>Hepatology (TBRI), <sup>4</sup>Science (Ain Shams University), Cairo Egypt, iman_khaled@yahoo.com</p> <p><b>Abstract:</b> Aim: This study has been made to determine the potential genotoxicity of <i>Schistosoma mansoni</i> on lymphocytes of infected patients using different mutagenic end points. The protective role of antioxidants pro vitamin -carotene and vitamin E in minimizing these genotoxic effect was also studied. The study focused on the effect of schistosomiasis on the induction of sister chromatid exchange (SCEs) and other chromosomal aberrations. Patients and Methods: This work was conducted on 24 Schistosoma mansoni infected patients and 10 healthy adults as a control group. Lymphocytes from peripheral blood of patients and control group were used for culture and subsequent cytogenetic studies. Results: The results indicated that schistosomiasis was genotoxic in all examined tests. It induced a significant increase in the percentage of structural chromosomal aberrations and the frequency of SCEs. It also inhibited cell division and caused cell cycle delay. Lymphocyte cultures of <i>S. mansoni</i> patients treated with 10 µg/ml -carotene or 20 mg/ml vitamin E showed a significant decrease in the percentage of structural chromosomal aberrations and the frequency of SCEs. Conclusion: schistosomiasis has a genotoxic effect on peripheral blood lymphocytes. The use of the antioxidants -carotene and vitamin E can be considered a promising approach not only toward inhibiting the genetic damage of schistosomiasis but also as prophylactic agents against infection with <i>S. mansoni</i>. Furthermore, higher doses of antioxidant drugs, -carotene and vitamin E, should be tried as an adjuvants to conventional therapy in a trial to improve treatment of schistosomiasis. [Journal of American Science. 2010;6(11):191-202]. (ISSN: 1545-1003).</p> <p><b>Key words:</b> Schistosomiasis, -carotene, vitamin E, chromosomal aberration</p>	Full Text	25
	<p><b>BIOCHEMICAL PATTERN FOR HEPATITIS C AND ACUTE LYMPHOBLASTIC LEUKEMIA IN HUMAN SERA</b></p> <p>Abulyazid<sup>1</sup>, I., Mohga S. Abdallah<sup>2</sup>, Hayate I. Sharada<sup>3</sup> and Sama H. Okasha<sup>4</sup></p> <p><sup>1</sup>, Molecular Biology Department, Atomic Energy Authority</p> <p><sup>2, 3, 4</sup> Chemistry Department, Faculty of Science, Helwan university</p> <p><b>Abstract:</b> Present experimental work aimed to show role of the molecular biology in diagnosis of hepatitis C liver disease (HCV) and acute lymphoblastic leukemia (ALL) which occur as a result of the disturbances of protein and enzymes fractions at the molecular level. The study carried out using vertical slab gel electrophoresis for detection of the protein pattern, catalase and peroxidase. Protein fractionation of the control samples produced 13 bands with Rf ranged between 0.17 and 0.96 and (amount, 3.14 - 7.24). Comparing hepatitis C with control one out of these 13 bands are completely disappeared at Rf 0.86 (amount 9.34). Ten bands appeared to be common bands in all HCV samples except one sample only nine common bands were produced while the band number ten was disappeared. The data showed that 5 characteristic bands were produced. One from these five bands determined at Rf 0.7 in all HCV sera samples except the first sample. Comparing leukemia samples with control only two were considered as common bands. These bands completely appeared in all sera samples. On the other side one band was completely disappeared in all leukemia samples. The rest bands distributed between different leukemia samples. 15 bands produced as characteristic bands. Electrophoresis pattern for catalase mentioned that six bands were produced in control samples. When hepatitis C compared with the control showed that two out these six bands were completely disappeared and other all HCV four bands considered as common bands. The amount of catalase enzyme completely decreased in all bands. In leukemia five common bands were produced with the appearance of one characteristic band, from the</p>	Full Text	26

other side one band was disappeared. A documentation of peroxidase pattern data showed that tow common bands were appeared with Rf 0.1 and 0.33, the amount of these two bands were decreased when the amount of HCV compared with control in the same rows. In leukemia there is only one common band was produced with appearance of a three characteristic bands. [Journal of American Science. 2010;6(11):203-216]. (ISSN: 1545-1003).

**Keywords:** HCV, Acute lymphoblastic leukemia, Protein electrophoresis, catalase, peroxidase

**Serum resistin levels and haemostatic changes in experimentally induced diabetic and high fat fed rats**

Mohammad I. Hoseen, Mai M .Hassan, Dalia I. Abd-Alaleem and Eman M. Faragallah.

Department of physiology, Faculty of medicine, Zagazig University.

**Abstract:** Adipose tissue is considered as an active endocrine gland that affects many aspects of body homeostasis. Adipose tissue derived molecules “adipokines” regulate energy homeostasis, dietary behavior, as well as insulin sensitivity and immunity; it refers to leptin, adiponectin, resistin, apelin, visfatin and omentin. Resistin is a cysteine-rich adipokine that is released by adipocytes and macrophages and has been involved in the development of insulin resistance in rodents. Moreover a strong link between diabetes, hypercoagulability and thrombogenesis, had been recognized for decades. Aim: In a trial to identify any possible relationship between resistin levels and some haemostatic changes in streptozotocin-induced diabetic and high fat diet-fed rats (HFD); the present work had been carried out. Design: A total number of 40 adult male albino rats were divided into 2 main groups: Group I (n= 24): To study the effect of streptozotocin-induced type 1 diabetes and was further divided into 3 equal subgroups (n= 8 in each) and survived for 30 days: Ia: (control group), Ib: (experimental diabetic non-treated group (by a single i.p. injection of streptozotocin (65mg/Kg B.W), Ic (experimental diabetic group treated with insulin). Group II (n= 16) : To study the effect of high fat diet and was further divided into 2 equal subgroups (n= 8 in each) and survived for 7 weeks: IIa: (control group), IIb (high fat diet fed (58% fat). In all groups, serum levels of glucose, insulin, resistin, total cholesterol(TC), triglycerides (TG), HDL, LDL, BT, WBCT, PT, aPTT, plasma fibrinogen level, plasma D-dimmers level and platelet count were measured. Results: The results of this study showed a significant decrease in serum resistin levels ( $p<0.001$ ) in streptozotocin-induced diabetic group in comparison with its control group and insulin-treated group. Moreover, no significant correlation could be detected between resistin levels and any of measured parameters in these groups except the significant positive correlation with body weight at the end of experimental period. In addition, our study revealed a significant increase in serum resistin levels ( $p<0.001$ ) in HFD-fed group in comparison with its controls, which was correlated positively and significantly with body weight, serum glucose levels, insulin levels and HOMA-IR index ( $p<0.001$ ), atherogenic lipid profile and markers of hyper-coagulability (except for platelet count). Conclusion: No role for resistin in metabolic and haemostatic changes in type 1 diabetic rats was detected. Although, hyperresistinemia may represent a link between metabolic signals, atherogenesis, and hypercoagulability in type 2 diabetic rats. However, further studies are needed to clarify this relationship in human cardiovascular diseases. [Journal of American Science. 2010;6(11):217-227]. (ISSN: 1545-1003).

**Keywords:** Resistin, Streptozotocin, high fat, diabetes, heamostasis

**Analytic Investigation and Numeric Prediction for Biodynamic Response of the Seated Human Body**

Mostafa A. M. Abdeen<sup>a</sup>, W. Abbas<sup>b</sup>

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<sup>a</sup> Dept. of Eng. Mathematics and Physics, Faculty of Eng. Cairo University, Egypt

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<sup>b</sup>Eng. Physics and Mathematics Dept., Faculty of Eng. (Mataria), Helwan University, Egypt

msotafa\_a\_m\_abdeen@hotmail.com; wael\_abass@hotmail.com

Full Text

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1. **Abstract:** The biodynamic response behaviors of seated human body subject to whole-body vibration have been widely investigated. The biodynamic response characteristics of seated human subjects have been extensively reported in terms of apparent mass and driving-point mechanical impedance while seat-to-head vibration transmissibility has been widely used to characterize response behavior of the seated subjects exposed to vibration. These functions (apparent mass, driving-point mechanical impedance) describe “to-the-body” force–motion relationship at the human–seat interface, while the transmissibility function describes “through-the-body” vibration transmission properties. The current study proposed a 4-DOF analytic biomechanical model of the human body in a sitting posture without backrest in vertical vibration direction to investigate the biodynamic responses of different masses and stiffness. Following the analytical approach, numerical technique developed in the present paper to facilitate and rapid the analysis. The numerical analysis used here applies one of the artificial intelligence technique to simulate and predict the response behaviors of seated human body for different masses and stiffness without the need to go through the analytic solution every time. The Artificial Neural Network (ANN) technique is introduced in the current study to predict the response behaviors for different masses and stiffness rather than those used in the analytic solution. The results of the numerical study showed that the ANN method with less effort was very efficiently capable of simulating and predicting the response behaviors of seated human body subject to whole-body vibration. [Journal of American Science. 2010;6(11):228-239]. (ISSN: 1545-1003).

**Keywords:** Biodynamic response, Analytic seated human body model, Numerical simulation model, Artificial

Neural Network.

**Design Synthesis of New Peptide Derivatives and Evaluated DNA Binding Activity, Anticancer and Antimicrobial Activity.**

A. A. EL-HENAWY

Chemistry Department, Faculty of Science, Al-Azhar University, Nasr City, Cairo-Egypt.

**Abstract:** Recently, sulfonamides have been reported to show significant antitumor activity in vitro and/or in vivo. There are a variety of mechanisms for the anticancer activity. The present work reports the synthesis some novel peptide sulfadiazine derivatives, this may play a role in their anticancer activity. All the newly synthesized compounds were evaluated for DNA binding activity and antimicrobial activity, some synthesized compounds showed high DNA binding activity and antimicrobial activity. Some selected compounds were evaluated for anticancer activity against breast cancer cell line (MCF7) in vitro. All selected compounds showed interesting cytotoxic activities compared to a reference drug. [Journal of American Science. 2010;6(11):240-249]. (ISSN: 1545-1003).

**Keywords:** Peptide; Anticancer; Antimicrobial; DNA Binding

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Full Text

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31	<p><b>Design Synthesis of New Peptide Derivatives and Evaluated DNA Binding Activity, Anticancer and Antimicrobial Activity.</b></p> <p>A. A. EL-HENAWY</p> <p>Chemistry Department, Faculty of Science, Al-Azhar University, Nasr City, Cairo-Egypt.</p> <p><b>Abstract:</b> Recently, sulfonamides have been reported to show significant antitumor activity in vitro and/or in vivo. There are a variety of mechanisms for the anticancer activity. The present work reports the synthesis some novel peptide sulfadiazine derivatives, this may play a role in their anticancer activity. All the newly synthesized compounds were evaluated for DNA binding activity and antimicrobial activity, some synthesized compounds showed high DNA binding activity and antimicrobial activity. Some selected compounds were evaluated for anticancer activity against breast cancer cell line (MCF7) in vitro. All selected compounds showed interesting cytotoxic activities compared to a reference drug. [Journal of American Science. 2010;6(11):240-249]. (ISSN: 1545-1003).</p> <p><b>Keywords:</b> Peptide; Anticancer; Antimicrobial; DNA Binding</p>	Full Text	31
32	<p><b>Margin Assessment and Fracture Resistance of Adhesively Luted Ceramic Crowns</b></p> <p>Jylan F. ElGuindy<sup>1</sup>, Dina H. Mostafa <sup>*2</sup> and Mona A. El Agroudi<sup>1</sup></p> <p><sup>1</sup>Fixed Prosthodontics Department and <sup>2</sup>Biomaterials Department, Faculty of Oral and Dental Medicine, Cairo University, Cairo, Egypt.</p> <p><sup>*</sup>dinamostafa@hotmail.com</p> <p><b>Abstract:</b> Objectives: The aim of this study was to investigate the effect of different adhesive systems on the vertical marginal gap distance and the fracture resistance of lithium disilicate based crowns.</p> <p>Methods: Forty premolars were prepared to receive forty e-max crowns. The crowns were divided into 4 groups (N=10 each) according to the adhesive luting systems. Group (U): using RelyX Unicem resin cement (self-adhesive system). Group (V): Variolink II (total-etch system). Group (GU) and group (GV): application of G-bond (self-etch) on dentin preceded previously used adhesive systems. A stereomicroscope was used to record the vertical marginal gap distance before and after cementation. The crowns were subjected to cyclic loading and fracture resistance test. Data were statistically analyzed using One-way Analysis of Variance (ANOVA) SPSS 15.0. A scanning electron microscope was used to qualitatively examine the dentin/resin interface. Results: Groups (GU) (<math>67.6 \pm 5.8 \mu\text{m}</math>) and (GV) (<math>68 \pm 6.4 \mu\text{m}</math>) recorded the significantly lowest vertical marginal gap, followed by group (V) (<math>82 \pm 6.8 \mu\text{m}</math>). Group (U) showed the highest marginal inaccuracy (<math>114 \pm 6.4 \mu\text{m}</math>). Group (GU) recorded the significantly highest fracture resistance (<math>2840.5 \pm 3.8 \text{ N}</math>), followed by group (GV) (<math>2411.3 \pm 3.3 \text{ N}</math>) and group (V) (<math>2365.8 \pm 3.6 \text{ N}</math>). Group (U) showed the lowest results (<math>2270.9 \pm 3.4 \text{ N}</math>). Conclusions: Ceramic restorations luted with total-etch system offer better vertical marginal gap distance and fracture resistance than restorations luted with self-adhesive system. Treatment of the dentin surface prior to the application of the bonding system is efficient. [Journal of American Science. 2010;6(11):264-273]. (ISSN: 1545-1003).</p> <p><b>Keywords:</b> Adhesives, marginal gap, fracture resistance, all-ceramics</p>	Full Text	32
33	<p><b>Do Desensitizers Affect the Retention of Questionable Preparations?</b></p> <p>Jylan F. ElGuindy<sup>1</sup>, Dina H. Mostafa <sup>*2</sup> and Mona A. El Agroudi<sup>1</sup></p> <p><sup>1</sup>Fixed Prosthodontics Department and <sup>2</sup>Biomaterials Department, Faculty of Oral and Dental Medicine,</p>	Full Text	33

**Abstract:** Objectives: This study aimed to investigate the effect of different desensitizers on the retention of short and over-converged preparations. Methods: Eighty molars were prepared with 3mm occluso-cervical height and 24 degrees convergence angle. Nickel-chromium copings were cast with a loop at the occlusal surface for tensile loading after cementation. The copings were assigned to two groups (N=40 each) according to the cement used. Group 1: resin cement (Duolink). Group 2: glass-ionomer (Ketac-Cem). Each group was assigned to four subgroups (N=10 each) according to desensitizers used prior each cement. Subgroup I: control (untreated), subgroup II: Gluma Comfort + Desensitizer, subgroup III: Oxalate (Bisblock) and subgroup IV: Fluoride varnish (Flor-Opal). The retention was determined by uniaxial tensile mode of force. Two-way Analysis of Variance (ANOVA) SPSS 16.0 was used to assess cements, desensitizers and their interactions on copings retention. Results: Resin group: Oxalate ( $212.10 \pm 7.41$ N) showed the significant highest mean of retention, followed by Gluma ( $201.52 \pm 6.93$ N), then control ( $177.52 \pm 6.14$ N). Fluoride ( $153.80 \pm 6.03$ N) recorded the lowest mean. Glass-ionomer group: control ( $135.54 \pm 4.58$ N) and Oxalate ( $132.62 \pm 4.84$ N) recorded the significant highest mean, followed by Gluma ( $126.84 \pm 4.75$ N). Fluoride ( $101.96 \pm 6.34$ N) recorded the lowest mean values. Conclusions: With questionable preparations, fluoride desensitizer drastically affected the retention of both cements. Oxalate and Gluma enhanced the retention with resin cement. Oxalate desensitizer can be efficiently used with glass-ionomer. [Journal of American Science. 2010;6(11):274-283]. (ISSN: 1545-1003).

**Keywords:** retention; Nickel-chromium coping; occlusal surface; Fluoride varnish

**Accuracy of working casts and dies produced by fast-setting polyvinyl siloxane impressions**

Mona El-Agroudi MD DDS<sup>1</sup> and Eman Essam, MD DDS<sup>2</sup>

Assistant professor of Fixed Prosthodontics, Faculty of Oral and Dental Medicine, Cairo University. , Egypt.

<sup>2</sup> Lecturer of Fixed Prosthodontics, Faculty of Oral and Dental Medicine, Al Azhar University. , Egypt.

**Abstract:** Purpose: This study aimed to evaluate the effect of spacer on the accuracy of working casts and dies produced from fast-setting polyvinyl siloxane impressions.

**Materials and Methods:** Twenty Impressions of the mandibular arch of a modified Dentoform master model incorporating a stainless steel circular crown preparation were made, using a fast-set Polyvinyl siloxane (Affinis perfect impressions Coltene/Whaledent AG) using 2/step impression technique with and without a spacer. Gypsum working casts and dies were produced from the poured impressions. Measurements of the master model and working casts were carried out including anteroposterior (AP) and cross-arch (CA) dimensions. The stainless steel circular crown preparation incorporated within the master model was also measured in buccolingual (BL), mesiodistal (MD), and occlusogingival (OG) dimensions and compared to measurements from recovered gypsum dies. Linear measurements were made using a measuring stereomicroscope. **Results:** Double impression technique without spacer showed statistically significant higher mean percent relative change than double impression technique with spacer. With each technique, the means percent relative change in die measurements showed statistically significant higher mean values than cast measurements. There was no statistically significant difference between means percent relative change in the BL and MD dimensions which showed the statistically significant highest mean values. The means percent relative change in the OG dimension showed the statistically significant lowest mean value. **Conclusion:** Accuracy of fast-setting polyvinyl siloxane impression material was favorably affected with the use of spacer, as the space resulted from contraction of the putty material was not enough to produce accurate detail reproduction by the light material. The working dies; from the fast- setting polyvinyl siloxane

impression material without spacer demonstrated an increase in (mesio-distal and bucco-lingual) dimensions, while for cast dimensions, there was no difference between the two techniques. [Journal of American Science. 2010;6(11):284-292]. (ISSN: 1545-1003).

**Keywords:** dies; fast-setting; polyvinyl siloxane

**Kinetics and Thermodynamics of Oil Extraction from Jatropha Curcas in Aqueous Acidic Hexane Solutions**

Full Text

Sh. K. Amin, S. Hawash, G. El Diwani\*, and S. El Rafei

Chemical Engineering and Pilot Plant Department, National Research Center, Cairo, Egypt.

\*geldiwani@yahoo.com

**Abstract:** Jatropha oil curcas (JOC) extraction was performed in aqueous HCl, H<sub>2</sub>SO<sub>4</sub>, and H<sub>3</sub>PO<sub>4</sub> solutions with n-hexane (C<sub>6</sub>H<sub>14</sub>) at 30, 40, 50, and 60 °C using 10 gm of Jatropha seeds over 1 hours with 10 minutes sampling intervals. The optimum acid concentration was 15 % by weight for each acid, and the highest oil yield was obtained in the extraction procedure with n-hexane containing HCl. The extraction process was observed with regard to the percent oil yield versus time, and the reaction order was found to be first-order kinetics by the differential method. The activation energy for the oil extraction kinetics of Jatropha seeds with 15 % HCl was E<sub>a</sub> = 26.6763 kJ/mol, and the activation thermodynamic parameters at 60 °C were H = 23.908 kJ/mol, S = -239.927 J/mol.K, and G = 103.803 kJ/mol. The enthalpy value was H = 0.1586 kJ/mol, and the other thermodynamic parameters at 60 °C were calculated to be S = 15.275 J/mol.K, and G = -4.928 kJ/mol. [Journal of American Science. 2010;6(11):293-300]. (ISSN: 1545-1003).

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**Key words:** kinetics, thermodynamics, oil extraction, Jatropha curcas

**Methodology for Selective Adsorption of Lithium Ions onto Polymeric Aluminium (III) Hydroxide**

Full Text

S. Hawash, E. Abd El Kader and G. El Diwani\*

Chemical Engineering and Pilot Plant Department, National Research Center, Cairo, Egypt.

\*geldiwani@yahoo.com

**Abstract:** The recovery of lithium as lithium aluminate from Egyptian bitterns was investigated. Studies were performed on synthetic Li<sup>+</sup> solution and on three high – salinity end brines which contain Li<sup>+</sup> of concentrations varying between 5.5- 19.5 ppm. Pretreatment with a mixture of Na<sub>2</sub>SO<sub>4</sub>- Na<sub>2</sub>CO<sub>3</sub> is achieved to precipitate BaSO<sub>4</sub>, SrCO<sub>3</sub>, CaCO<sub>3</sub> and possibly MgCO<sub>3</sub>. A co-precipitation method was employed using aluminum salt as (AlCl<sub>3</sub>.6H<sub>2</sub>O). Lithium ion is adsorbed onto aluminum hydroxide, which is freshly produced by adding AlCl<sub>3</sub>.6H<sub>2</sub>O and Na OH to the brines at Al<sup>3+</sup> / Li<sup>+</sup> molar ratio 5-7. Results obtained indicate that high Li<sup>+</sup> adsorption was performed at pH = 6-7 for Alexandria-Arish and Emissal salines, even for small concentration of aluminum salt added. Also, Lithium ions uptake decreased with increasing adsorption temperature from 10°C to 30°C but over 30°C increase in temperature does not affect lithium uptake on Al(OH)<sub>3</sub>, which proved that the process is physical adsorption. Equilibrium isotherms have been determined for the adsorption of Li<sup>+</sup> onto Al(OH)<sub>3</sub> at 30°C and pH= values (5 to 9), the maximum adsorption capacity of Al(OH)<sub>3</sub> at 30°C and pH = 9 is 123 mg/gm. The results indicated that applied isotherms were shown to be "favorable" and were fitted with Langmuir and Freundlich isotherms. Li<sup>+</sup> desorption from Al(OH)<sub>3</sub> was investigated using hydrofluoric acid (HF) or sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) with different concentrations, and results obtained showed that HF is more efficient than H<sub>2</sub>SO<sub>4</sub> concerning Li<sup>+</sup> desorption. From the obtained results, Li ion can be recovered successfully from bittern and saline solutions. [Journal of American Science. 2010;6(11):301-

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	309]. (ISSN: 1545-1003).		
	<p><b>Keywords:</b> lithium; lithium aluminate; hydrofluoric acid (HF); sulphuric acid (<math>H_2SO_4</math>); saline solution</p> <p>The Effects of Dietary Egyptian Propolis and Bee Pollen Supplementation against Toxicity if Sodium Fluoride in Rats</p> <p>Fatma A. Khalil and Nora M. El-Sheikh</p> <p>Biochemistry and Nutrition Department, Women's College, Ain Shams University, Cairo, Egypt.</p> <p><b>Abstract:</b> Propolis and bee pollen are substances produced by honey bees its components are strong antioxidant and free radical scavengers. The present study aimed to study the protective effects of propolis and bee pollen supplementation against toxicity of sodium fluoride in rats. After the end of experimental period, the rats sacrificed and biochemical analysis were carried out. The results showed that the administration of fluoride (F) alone causes significant increase of malondialdehyde (MDA) level and significant decrease of antioxidant system as erythrocyte superoxide dismutase (SOD) activity and reduced glutathione (GSH) levels in blood and brain. Also F causes significant increase alkaline phosphatase (ALP) activity, urea, creatinine, sodium and potassium levels. And significant decrease total protein, calcium, magnesium and phosphorus levels as compared to control group (<math>P &lt; 0.05</math>). Whereas administration of propolis or bee pollen with F led to significant decrease in MDA level and significant increase in SOD activity, GSH levels in blood and brain. And significant decrease ALP activity, urea, creatinine, sodium and potassium levels in serum. The propolis or bee pollen enhanced total protein, calcium, magnesium and phosphorus levels in serum as compared to F group alone.</p> <p>In conclusion; supplementation of natural antioxidant (propolis or bee pollen) during Fluoride administration, facilitate reduction of the toxic effects and enhanced the antioxidant system, the levels of minerals in serum. [Journal of American Science. 2010;6(11):310-316]. (ISSN: 1545-1003).</p> <p><b>Keywords:</b> Propolis, bee pollen, sodium fluoride, rats, antioxidant system minerals.</p>	Full Text	37
38	<p><b>Diet Selection, Feed Intake Capacity and Performance of Growing Female Camels: Effects of Type of Roughage and Level of Concentrates Offered</b></p> <p>M.F.A. Farid, A.M. Abdel-Wahed, Safinaz M. Shawket* and N.I. Hassan</p> <p>Animal Nutrition Department, Desert Research Centre, Al-Matareya, Cairo, Egypt.</p> <p>dr.safinazshawket@hotmail.com*</p> <p><b>Abstract:</b> The feeding trials were intended to investigate diet selection, feed intake capacity (FIC) and animal performance when concentrates (corn grains and commercial concentrates mixture) and roughages (Atriplex, clover hay or rice straw) were fed ad lib free-choice in a cafeteria feeding system, and also the effect of restricting concentrates offered. The roughages were selected to represent different grazing conditions prevailing in arid rangelands. Eighteen growing she-camels were randomly allotted to three groups. Each group was assigned one of the three roughages offered ad lib for the duration of the whole experiment. Results indicated that type of roughage and concentrate levels, and their interaction, affected (<math>P &lt; 0.05</math>) FIC and diet selection, and consequently live weight gain. Average total and roughage DMI were 78.9 and 16.1, 83.9 and 22.5, 96.4 and 33.4 g DM/day/Kg<sup>0.75</sup> for straw, hay and atriplex groups, respectively. Irrespective of the roughage fed, growing camels consumed three-times as much corn grains as that from the cottonseed meal. Limiting concentrates offered to 75% or 50% of ad lib intake, decreased FIC, while the proportion of roughages in DMI increased significantly, total OMI and total protein decreased and crude fibres intake increased, more so in the straw fed camels. The Atriplex fed camels recorded the higher ADG, followed by the hay fed ones and the straw fed mates grew the least, 516, 429 and 240 g/d, respectively. Restricting the level of concentrates offered decreased significantly (<math>P &lt; 0.05</math>) the ADG (691, 305 and 189 g/d in camels fed 100, 75 and 50% of ad lib concentrate intake. These results tend</p>	Full Text	38

to indicate that growing camels having free choice to select their diets from both concentrates and roughages were capable of regulating their voluntary food intake predominantly through physiological mechanisms to satisfy energy requirements. This was true for the atriplex and hay groups but not for the straw group or when concentrates offered was limited. [Journal of American Science. 2010;6(11):317-326]. (ISSN: 1545-1003).

**Key words:** camels, diet selection, feed intake capacity, weight gain

### **Application of Proposed Distribution Network Planning Rules on Fast Developing Countries**

Salem M. Elkhodary<sup>1</sup>, and M. Khafagy<sup>2</sup>

<sup>1</sup> Faculty of Engineering, Ain Shams University,, Cairo, Egypt,<sup>2</sup> Saudi Electricity Company, KSA.

**Abstract:** With the ever increasing need to electric energy and the fast development of loads in many countries especially in the fast developing ones such as the GULF countries, the load growth as well as the forecasted loads, are highly increased depending on new and arising factors and conditions. In turn, Electricity Companies build rapidly generating plants, transmission and distribution networks to meet the rapid load demand. Usually, power system expansion follows the load growth which may exist at random locations. This adds to the absence of prior proper planning, especially medium and long term planning, resulting in network configurations that do not match with optimum siting and sizing planning rules. Operation of such networks faces several problems that may sacrifice the power quality. Thus, proper planning of new networks, expansion or rehabilitation of existing ones should be based on most accurate and proper planning rules. This calls for the investigation of a new exact cost function for optimum sizing and siting of network substations, and hence the H.V. feeds (incoming) and the M.V. distribution (outgoing) feeders. Therefore, this paper presents a newly proposed methodology that takes into consideration the capital costs of all electrical components, losses in these components, operation and maintenance costs. The inflation rate can be also taken into consideration. This methodology gives important results, which conclude that the optimum distance between substations and hence the optimum number of substations, greatly depends on different factors that were not taken into consideration before, for example : the kWh price, cost of the HV incoming feeders (66-110 kV feeders) besides the cost of the MV outgoing feeders (6.6-22 kV feeders), cost of the distribution substations (MV/LV), cost of losses in transformers, cost of losses in all feeders, incoming and outgoing, Operation and maintenance costs ....etc. [Journal of American Science. 2010;6(11):327-]. (ISSN: 1545-1003).

**Keywords:** Cost function. Objective function. , Distribution network planning. Optimum siting and sizing of substation

### **New Proposed Method of Damping Temporary Overvoltages on Power System Interconnections**

Salem M. Elkhodary<sup>1</sup>, and Ali S. Abd El-Munem<sup>2</sup>

<sup>1</sup> Faculty of Engineering, Ain Shams University , <sup>2</sup>Egyptian Electricity Holding Company, Cairo, Egypt

**Abstract:** The interconnection between countries links different networks. These interconnections may be exposed to several disturbances. These disturbances (such as transient and temporary overvoltages phenomena, faults ...etc.) threaten the interconnection security and reliability. This paper presents actual field measurements of transient and temporary overvoltages appearing on the Egyptian – Libyan system interconnection as an example. These overvoltages were recorded for different cases of operation. These cases were modeled and simulated using the most recent version of Alternative Transient Program (ATP) computer package to compare the results of the computational method with the actual field measurements. The comparison between the ATP output results and the actual field measurements were found less than  $\pm 4\%$ . Within the research activities of the Egyptian Electricity Holding Company (EEHC) temporary overvoltage phenomena on the Egyptian – Libyan interconnection network were detected. EEHC carried field measurements of the temporary overvoltage by using a special transient mobile test laboratory. This detected temporary overvoltage was due to the generated reactive power along the line on switching, in spite of this leading reactive

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power was compensated by connecting number of reactors at different nodes. The economical aspect has been taken into consideration to reduce the number of reactors to the network, which showed the best effect on damping the temporary overvoltage. This paper, thus, presents a proposed technique to damp the temporary overvoltage and keep the system voltage within the permissible limits by estimating the optimum number and location of reactors that must be connected to the network. [Journal of American Science. 2010;6(11):336-342]. (ISSN: 1545-1003).

**Keywords:** interconnection; networks; disturbances; Alternative Transient Program (ATP); Egyptian Electricity Holding Company (EEHC)

**Evaluation of *Corynebacterium variabilis* Sh42 as a degrader for different poly aromatic compounds**

Yasser M. Moustafa<sup>1</sup>, Nour Sh. El-Gendy<sup>1</sup>, Salem A. Habib<sup>2</sup>, Sherif Ali<sup>1\*</sup>

<sup>1</sup>Egyptian Petroleum Research Institute, Cairo, P.O. 11727, Egypt.

<sup>2</sup>Mansoura University, Faculty of Science, Damietta, Egypt.

**Abstract:** *Corynebacterium variabilis* sp. Sh42 is used to investigate the biodegradation potentials and metabolic pathways of different poly aromatic compounds (PACs) in batch flasks. Effects of PACs size, molecular weight, alkylation and their presence individually or in mixture on biodegradation potentials of Sh42 were studied; Naphthalene (Nap) as a model compound for di-aromatic ring; Anthracene (Ant) and Phenanthrene (Phe) as model compounds for tri-aromatic ring; while Pyrene (Pyr) as a model compound for four-aromatic ring compounds were used as representatives for different PAHs. Dibenzothiophene (DBT), 4-methyldibenzothiophene (4-MDBT) and 4,6-dimethyldibenzothiophene (4,6-DMDBT) were taken as representative models for PASHs compounds. While, 2-hydroxybiphenyl (2-HBP) and 2, 2'-bihydroxybiphenyl (2, 2'-BHP) were taken as models for phenolic compounds. The experimental results show that biodegradation rate decrease with increase ring size, alkylation's group within homologous series and Sh42 has the highest capability to biodegradation of toxic phenolic compounds either in single (BD% 90%) or mixed substrates cultures (BD% 48%). To ensure detoxification and mineralization of these toxic PACs; metabolic pathways of representative model compounds (Pyr, DBT and 2,2'-BHP) were elucidated by GC/MS analysis which confirmed that, Sh42 completely metabolized all representative compounds to CO<sub>2</sub> and H<sub>2</sub>O. [Journal of American Science. 2010;6(11):343-356]. (ISSN: 1545-1003).

**Keywords:** Polynuclear aromatic compounds, Biodegradation, Metabolic pathways.

**In vitro assessment of gastrointestinal viability of potentially probiotic Lactobacilli**

Kawther,.EL-Shafei, N.F.Tawfik, Nadia, M.A.Dabiza, O.M.Sharaf, and B.A.Effat

Dairy Science Department, National Research Center Dokki, Cairo, Egypt.

**Abstract:** The objectives of this study were to assess the potential of four probiotic lactobacillus strains, *Lactobacillus bulgaricus*, *Lactobacillus johnsonii* B-2178, *Lactobacillus gasseri* B-14168 and *Lactobacillus salivarius* B-1950 in human upper gastrointestinal tract in vitro and evaluate the effect of milk proteins addition on viability of these strains in simulated gastric juices and in yoghurt during storage for 15 days at 4°C. The viability of lactobacilli strains in simulated gastric transit conditions (pH 2.0, pH 3.0 and pH 4.0) gastric juices with or without milk proteins singly or in combination with starch was tested. All the treatments were determined with three replicates. The simulated gastric transit tolerance of *L. johnsonii*, *L. gasseri* and *L. salivarius* strains was pH-dependent and correspondingly showed lower viability at pH 2.0 after 180 min compared with pH 3.0 and pH 4.0. The addition of milk proteins singly or in combination with starch enhanced the survival of probiotic lactobacilli strains in simulated gastric juices different tested pH values. Results showed that addition of milk proteins in combination with starch improved the viability of *L.johnsonii* B-2178, *L. gasseri* B-14168 and *L. salivarius* B-1950 in yoghurt during storage. Sensory evaluation showed that yoghurt

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fortified with milk proteins plus starch recorded the highest score for and overall acceptability than the other treatments. However, yoghurt manufactured with *L. johnsonii* and *L. gasseri* and fortified with sodium caseinate plus starch showed the highest organoleptic score. It is suggested that the yoghurt of acceptable quality and high total probiotic bacterial count during storage can be made from milk supplemented with 0.5% (w/v) starch plus 0.5% (w/v) sodium caseinate. [Journal of American Science. 2010;6(11):357-367. (ISSN: 1545-1003).

**Keywords:** Probiotics, Gastric tolerance, *L. johnsonii*, *L. gasseri*, *L. salivarius*

### Extraction of oil from canola seeds with supercritical carbon dioxide: Experimental and Modeling

Soroush Zarinabadi<sup>1\*</sup>, Riyaz Kharrat<sup>2</sup>, Ali Vaziri Yazdi<sup>3</sup>

1, 3-Islamic Azad University- Science & Research Branch – Tehran, Iran

2-Petroleum University of Technology - Tehran, Iran

[avy123@behta.com](mailto:avy123@behta.com) , 3- 2-kharrat@put.ac.ir , 1-zarinabadi@yahoo.com

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**ABSTRACT:** In this work extraction oil from canola (*Brassica Napus*) seed with supercritical CO<sub>2</sub> extraction at pressure of 1500 to 2750 Psi , temperature of 308 to 333 k, and particles size 0.08 to 0.2 mm in flow rate 5 Lit/hr was investigated in a bench scale apparatus, The extraction was modeled by the sovova extended lack's model. The fluid phase mass transfer coefficient (k<sub>f</sub>), solid phase mass transfer coefficient (k<sub>s</sub>), and hardly accessible solute (x<sub>p</sub>) were a just able parameter of Models. The broken and intact cells model fit the experimental data, quite well, showing the applicability of the model to the supercritical extraction system studied here. [Journal of American Science. 2010;6(11):368-373. (ISSN: 1545-1003).

**Keywords:** supercritical fluid extraction, canola oil, mathematical modeling, sovova model

### Biochemical Significance of Proinflammatory Cytokines in Psoriasis vulgaris among Egyptian Patients

[Full Text](#)

Halla M. Ragab\*, Nabila Abd El Maksoud\* and Mohamed M. Farid Roaiah\*\*

\*Department of Biochemistry, Genetic Engineering and Biotechnology Division, National Research Centre, Dokki, Cairo, Egypt. \*\* Dermatology & Andrology and S. T. D.S, Kasr El Aini Hospital, Cairo university. [hmrugab@yahoo.com](mailto:hmrugab@yahoo.com)

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**Abstract: Background:** Psoriasis has been characterized by hyperproliferation accompanied by acanthosis and aberrant differentiation of keratinocytes. Several growth factors and cytokines, are assumed to be important. Recent studies indicate that various cytokines including tumor necrosis factor - ( TNF - ), IL - 2R and IL - 6 play an essential role in the induction and maintenance of psoriatic lesion. **Objectives:** To analyse relevant inflammatory mediators in the serum of patients with active psoriasis ( Psoriasis vulgaris ) of mild-to-moderate and severe psoriasis compared to healthy controls. **Patients / Methods:** Forty psoriasis patients were recruited from the dermatology outpatient clinic of Cairo University Hospital. Patients body mass index ( BMI ), waist circumference and psoriasis area and severity index. ( PASI ) were recorded. Fasting serum samples were obtained on enrolment. All the patients did not receive any treatment (locally or systemically), for at least four weeks before enrolment. Age, sex and ( BMI ) matched with forty healthy controls were also recruited. Serum TNF - , IL - 2R and IL - 6 levels were estimated using an Enzyme-Linked Immunosorbant Assay ( ELISA ) technique. The patients group were subdivided to two groups according to the diseases severity, PASI , into, mild-to-moderate psoriasis group and severe psoriasis group. **Results:** Serum TNF - , IL - 2R and IL - 6 were all statistically significant elevated in the patients group compared to healthy controls ( p < 0.05 ). Also they were all statistically significant increased in severe psoriasis compared to mild-to-moderate psoriasis ( p <0.05 ). **Conclusions:** These data support the view that serum TNF - , IL - 2R and IL - 6 are involved in the pathogenesis of

psoriasis, possibly by induction and maintenance of psoriatic lesion. We recommend a use of an array of these cytokines as a useful follow-up marker for monitoring of psoriatic patients and optimizing therapeutic strategies. Also we suggest the study of antisense therapy using the antibody of these cytokines in psoriatic patients. [Journal of American Science 2010;6(11):374-380]. (ISSN: 1545-1003).

**Keywords:** Psoriasis vulgaris, Cytokines, TNF - , IL - 2R and IL - 6

**Antihepatotoxic Effect of *Eruca Sativa* Extracts on Alcohol Induced Liver Injury in Rats**

Jihan Hussein<sup>1</sup>, Azza Salah<sup>2</sup>, Fatma Oraby<sup>1</sup>, Amany Nour El-Deen<sup>2</sup> and Zakarya El-Khayat<sup>1</sup>

<sup>1</sup> Medical Biochemistry Department, National Research Center,Doki,Giza, 12311, Egypt

<sup>2</sup> Biochemistry Department, Faculty of Science, Ain Shams University, 12311, Egypt

[jihan\\_husein@yahoo.com](mailto:jihan_husein@yahoo.com)

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**Abstract:** Food derived antioxidants have a strong potential for long term use as chemopreventive agents in disease states involving oxidative stress, such as hepatitis and alcoholic liver diseases. This study aimed to investigate the effect of different extracts of *Eruca Sativa* in ethanol induced liver injury in rats. Eighty eight male albino rats were divided into 3 main groups included control, prophylactic and treated groups using different extracts of *Eruca sativa*. Serum liver functions tests, lipid profile and oxidants/antioxidants profile were estimated. The results showed that *Eruca Sativa* extracts improved liver functions, Lipid profile and antioxidants parameters. We concluded that, *Eruca sativa* extracts may exert their prophylactic and treatment role against oxidative stress produced by ethanol by increasing/maintaining the levels of antioxidant molecules and antioxidant enzymes. [Journal of American Science 2010;6(11):381-389]. (ISSN: 1545-1003).

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**Key words:** *Eruca sativa*, Ethanol, Liver, Ethanolic extract, Antioxidants, Oxidative stress

[Full text](#)

**In vivo and in vitro studies on *Thevetia* Species Growing in Egypt**

**I: Isolation, Identification, and Quantification of cardiac glycosides in *in vivo* and *in vitro* cultures of immature seeds.**

Taha H. S.<sup>1\*</sup>, Farag H.S.<sup>2</sup>, Shams A. K.<sup>2</sup>, Abdel-Azim S.N.<sup>2</sup>, Hanna G. A.<sup>3</sup> Ewais E. E.<sup>4</sup> and Seif El-Nasr M. M.<sup>2</sup>

<sup>1</sup> Plant Biotechnology Department, National Research Centre, Dokki, Giza, Egypt.

<sup>2</sup>Phytochemistry Department, National Research Centre, Dokki, Giza, Egypt.

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<sup>3</sup>Chemistry of Natural Compounds Department, National Research Centre, Dokki, Giza, Egypt.

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<sup>4</sup> Botany and Microbiology Department, Faculty of Science, Al-Azhar University ,Cairo, Egypt

[Corresponding author hussein.taha2@yahoo.com](mailto:Corresponding author hussein.taha2@yahoo.com)

**ABSTRACT:** *In vivo* and *in vitro* extracted cardiac glycosides of immature seeds (IS) cultures of *Thevetia nerifolia* Jussieu. and *T. thevetioides* Kunth. were chemically identified. Calli were grown on modified Murashige & Skoog (MS) medium supplemented with 1mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) +3mg/l kinetin (Kin). The content of cardiac glycosides in IS cultures of *T. nerifolia* and *T. thevetioides* were monitored by HPLC. Two major compounds were detected and isolated from IS extracts i.e. digitoxigenin and thevetin B. The different structures of the *in vivo* and *in vitro* isolated compounds were verified by means of MS and NMR spectral analysis, as well as those compounds were identified and determined using HPLC technique. [Journal of American Science 2010;6(11):390-395].

[Full text](#)

(ISSN: 1545-1003).

**Key words:** Cardiac glycosides, callus, *Thevetia spp.*, HPLC, MS medium, immature seed cultures

**Comparative Analysis Of Resource Use Efficiency In Rice Production Systems In Abia State Of Nigeria**

[Full text](#)

Nwaru, J. C. and O. R. Iheke

Department of Agricultural Economics

Michael Okpara University of Agriculture, Umudike

PMB 7267 Umuahia, Abia State, Nigeria

E- mail: [nwaruj@yahoo.com](mailto:nwaruj@yahoo.com)

**ABSTRACT:** Arresting the observed low productivity and continued decline in the output of rice especially in the face of rising population and the concomitant escalating increases demand has been a lingering socioeconomic problem. Continued increase in rice production through a number of options including expansion into high potential areas especially the inland valleys has been proposed. This study was designed to examine resource use efficiency in rice production systems in Abia State of Nigeria. Primary data collected from a sample of 142 farmers consisting of 46 inland valley, 41 upland and 55 swamp rice farmers were analysed by the ordinary least squares multiple regression analysis and analysis of variance (ANOVA). Results indicate that the upland rice farmers are technically more efficient than the swamp and inland rice farmers and that there is no difference in technical efficiency between the swamp and inland rice farmers. None of the farmer groups achieved absolute allocative efficiency. The upland rice farmers achieved least allocative efficiency ( $W_{ij}$  is farther from unity), underutilized all farm resources ( $W_{ij} > 1$ ) while both the inland valley and the swamp rice farmers under utilized farmland, other inputs and capital and over utilised ( $W_{ij} < 1$ ) family labour and hired labour. There was no significant difference in the mean output of rice from the production systems; upland, inland valley and swamp while each operated in region one on the production surface indicating that overall, resource levels could be increased to achieve higher levels of productivity in each system. Economic policies and programmes that could encourage the reallocation and if possible the redistribution of farm production inputs for increased farm productivity and efficiency were recommended. [Journal of American Science 2010;6(11):396-408]. (ISSN: 1545-1003).

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**Key words:** Resource use efficiency, rice production systems, Nigeria

**Knowledge Discovery In Al-Hadith Using Text Classification Algorithm**

[Full text](#)

Khitam Jbara

Jordan University, King Abdullah II School for Information Technology. [ktjlc2000@yahoo.com](mailto:ktjlc2000@yahoo.com)

**Abstract:** Machine Learning and Data Mining are applied to language datasets in order to discover patterns for English and other European languages, but Arabic language belongs to the Semitic family of languages, which differs from European languages in syntax, semantic and morphology. One of the difficulties in Arabic language is that it has a complex morphological structure and orthographic variations. This study is conducted to examine knowledge discovery from AL-Hadith through classification algorithm in order to classify AL-Hadith to one of predefined classes (books), where AL-Hadith is the saying of Prophet Mohammed (Peace and blessings of Allah be upon him (PBUH)) and the second religious source for all Muslims, so because of its importance for Muslims all over the world knowledge discovery from AL-Hadith will make AL-Hadith more understandable for both muslims and nonmuslims. [Journal of American Science 2010;6(11):409-419]. (ISSN: 1545-1003).

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**Keywords:** AL-Hadith, Classification, Stem, feature, Class, Expansion, Training set

	Organic amendment effect on soil properties and yield of potato ( <i>Solanum tuberosum</i> ) under irrigated condition: a case study from Kombolcha, Eastern Harergie, Ethiopia	<a href="#">Full text</a>
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Eyasu Mekonnen<sup>1</sup>, Fassil Kebede<sup>2,3,\*</sup> and Nurhussien Taha<sup>2</sup>

<sup>1</sup>Kombolcha Agricultural TVET College, Kombolcha, Eastern Harrargie; <sup>2</sup> Department of Land Resource Management and Environmental Protection, Mekelle University, Ethiopia

\* Corresponding Author: Address: E-mail- [fyimamu@gmail.com](mailto:fyimamu@gmail.com)

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**Abstract:** Field experiment was conducted in 2005/06 cropping season in Kombolcha to understand the comparative effect of organic and inorganic sources of soil ameliorant for managing surface soil crust under basin and furrow irrigation practices to boost potato production. A factorial experiment was conducted on plots of 12 m<sup>2</sup> (4 m x 3 m) and arranged in RCBD with three replicates, which combine irrigation methods and soil amendments. The treatments were the control (no amendment), FYM, *chat* residue (decayed leaves of *Chata edulis*) and sediment (sub surface inorganic material locally known as ‘decay dimma’). Results have, therefore, revealed that FYM and *chat* made compost significantly ( $p \leq 0.05$ ) improved moisture content, bulk density, porosity and infiltration rate over the sediment amended plot and the control. However, yield harvested from plots, which were amended with *chat*-made compost was significantly ( $p \leq 0.05$ ) lower than FYM under furrow irrigation practice. [Journal of American Science 2010;6(11):420-425]. (ISSN: 1545-1003).

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**Keywords:** Chata edulis, decay dimma, organic amendment, potatoes, soil properties, irrigation

[Full text](#)

### The Effect of Women's Socio-demographic Variables on their Empowerment

Hidayat Allah Nikkhah

Department of Social and Development Sciences, Faculty of Human Ecology, University Putra Malaysia, [hnik2003@yahoo.com](mailto:hnik2003@yahoo.com)

Ma'ruf Redzuan (Corresponding author)

Department of Social and Development Sciences, Faculty of Human Ecology, University Putra Malaysia. [marof@putra.upm.edu.my](mailto:marof@putra.upm.edu.my)

Asnarulkhadi Abu-Samah

Department of Social and Development Sciences, Faculty of Human Ecology, University Putra Malaysia. [asnarl@putra.upm.edu.my](mailto:asnarl@putra.upm.edu.my)

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**Abstract:** Social scientists and development agencies have long been interested in the conditions that empower women. Since the empowerment could give women freedom of choice, equal access to domestic and community resources, opportunities and powers, thus, empowerment of women and the improvement of their status, particularly in respect of education, health and economic opportunities (occupation), are highly important ends in themselves. However, there are many factors which stop women benefiting from such development and contribute to women’s powerlessness such as inequality in economic opportunity, lack of knowledge, skills, and lack of access to education. This study elucidates the relationship between socio-demographic variable of women and their empowerment in Shiraz, Iran. Indeed, this study discovered the effect of respondents’ background i.e., age, education, occupation, family income and marital status on their empowerment. Data was collected from 195 women who participated in empowerment process which organized by NGOs in the whole city of Shiraz. The result of study showed that among the socio-demographic variables, only education and marital status had effect on empowerment. It can be concluded that access to education and knowledge play an important role increasing women empowerment. The result of study also showed that the divorced women have high level of empowerment compare to married and widow women. This is might due to that married women couldn’t get their husband permission to participate in empowerment

process. [Journal of American Science 2010;6(11):426-434]. (ISSN: 1545-1003).

**Key words:** empowerment, socio-demographic variables, women, Iran

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**Nutritive Aspects of *Oxalis corniculata* L. Used by Tribals of Central India During Scarcity of Food.<sup>2</sup>**

3.

Ashok k. Jain<sup>1</sup>, Preeti Tiwari Barua<sup>2</sup> and Mudasir Bashir<sup>3</sup>

2. <sup>1</sup>Professor, School of Studies in Botany, Jiwaji University Gwalior -474011, Madhya Pradesh, India; E-mail: [asokjain2003@yahoo.co.in](mailto:asokjain2003@yahoo.co.in)

3. <sup>2</sup>Assistant Professor, Department of Biotechnology, IPS Academy Indore-452012, Madhya Pradesh, India; E-mail: [preetibarua26@gmail.com](mailto:preetibarua26@gmail.com);

4. <sup>3</sup>Research Scholar, Plant Tissue Culture Laboratory, School of Studies in Botany, Jiwaji University Gwalior-474011, Madhya Pradesh, India; E-mail: [mudasirbot@gmail.com](mailto:mudasirbot@gmail.com)

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**Abstract:** Reports on ethnobotanical surveys reveal that a good number of plant species are being used by various tribal communities as emergency food. The present work deals with some parameters regarding nutritive value of leaves of *Oxalis corniculata*. L. used as alternative vegetable during emergency by some tribes of central India. The leaves have been found to be rich in moisture ( $82.42\pm 0.5\%$ ), total carbohydrate ( $24.67\pm 0.4\%$ ), crude protein ( $22.28\pm 0.5\%$ ), crude lipid ( $23.7\pm 0.5\%$ ), sodium ( $1.12\pm 0.02\%$ ), potassium ( $2.17\pm 0.31\%$ ), calcium ( $2.5\pm 0.08\%$ ), nitrogen ( $3.56\pm 0.70\%$ ) and magnesium ( $0.25\pm 0.03\%$ ). [Journal of American Science 2010;6(11):435-437]. (ISSN: 1545-1003).

**Key words:** Nutritive status; *Oxalis cornicula*; Tribes; Scarcity of food

**Effects of sports participation on psychological stress in female students in region 3 of Kermanshah**

Ali Feyzkhademi<sup>1</sup>, Saadat Hajipoor<sup>1</sup>, Shahram Azimi<sup>2</sup>, Mehrdad Jalalian<sup>3, 4, 5</sup>

<sup>1</sup>Faculty Member of Izeh Branch, Islamic Azad University, Izeh, Iran

<sup>2</sup>Lecturer, Sama Branch (Kermanshah), Islamic Azad University, Kermanshah, Iran

<sup>3</sup>Research Center of Iranian Blood Transfusion Organization, Khorasan-e Razavi Blood Center, Mashhad, Iran

<sup>4</sup>Department of Community Health, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor D.E., Malaysia

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<sup>5</sup>Editorial Office, Electronic Physician Journal, Mashhad, Iran

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[khademisport@gmail.com](mailto:khademisport@gmail.com)

**Abstract:** The aim of this study was to investigate the effects of sports participation on the psychological stress levels of female students 15-18 years old. Psychological stress is defined as a collection of nonspecific reactions against organisms in reflections and exposure to any factor that should be faced. Stress control includes several factors, and, in particular, sports participation is thought to be effective. This quasi-experimental research was performed using pre-test plan-test, after-test, and control groups. Research subjects for the control group were 30 people chosen randomly. The subjects of stress were tested by a 40-item stress questionnaire and then tested in step independent variable "Sports participation" included volleyball education and skills training for three months and three weekly sessions of 75-90 minutes. This was carried out to investigate its effect on the dependent variable "stress." We did not observe any statistically meaningful difference between the mean scores of stress-control group and experiment group scores at pre-test in  $p<0.05$ ; however, statistically meaningful differences were observed between the mean scores of stress control group and experiment group scores on the post test stage ( $p<0.05$ ) and between the mean scores of stress in the control group pre-test and

post-test in ( $p<0.05$ ). In addition, A statistically meaningful difference was observed statistically meaningful differences were observed between the mean scores of stress in the experiment group pre-test and post-test ( $p<0.05$ ). [Journal of American Science 2010;x(x):xx-xx]. (ISSN: 1545-1003). [Journal of American Science 2010;6(11):438-441]. (ISSN: 1545-1003).

**Keywords:** Sports Participation; Psychological stress; Students

**Plotting An Improved Orbital Elements Through Speckle Interferometry For Two Binary Systems, 00122+5337=Bu 1026ab And WDS 04136+0743 =A1938.**

<sup>1</sup>S. PATTNAIK, S. K. KAMILA<sup>2</sup>, G. S. ROY<sup>3</sup>, B. B. Acharya<sup>4</sup>

<sup>1</sup>Pathani Samanta Planetarium, Bhubaneswar, Orissa (INDIA),

<sup>2</sup>Department of Physics, ITER, Bhubaneswar, Orissa (INDIA),

<sup>3</sup>Department of Physics, Govt. (Auto) college, Bhawanipatana, Orissa (INDIA)

<sup>4</sup>Christ college, Cuttack, Orissa (INDIA)

[subhendu\\_patnaik@yahoo.com](mailto:subhendu_patnaik@yahoo.com)

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**ABSTRACT:** The effect of atmospheric turbulence on the diffraction-limited imaging of celestial bodies is one of the major problems in observational astronomy. The speckle interferometric technique was introduced in the 1970s to solve this problem. The technique is used to decode the diffraction-limited spatial Fourier spectrum and image features of the celestial objects, using a series of short-exposure (< 20 ms) images. Since most common binary orbit periods vary from 10 to 30 years, a large number of these binary systems, studied using the speckle data, completed one or two revolutions. In this study, an algorithm developed by the Indian Institute of Astrophysics (IIA) and an algorithm developed by Hartkopf's group at Georgia State University were used to plot the orbits of two binary systems, 00122+5337=Bu 1026AB and WDS 04136+0743 = A1938. The orbital parameters of these binary systems have been calculated using speckle data and other interferometric data. The former algorithm is based on standard least square technique with iterative improvement of the orbital parameters. Unlike the latter algorithm, the former algorithm does not require any previous knowledge of the period and the eccentricity of the binary systems. The results of this comparative study have shown that both algorithms generate almost the same orbital parameters. However, the algorithm developed by the IIA requires fewer steps to calculate the orbital parameters of these binary systems. [Journal of American Science 2010;6(11):442-448]. (ISSN: 1545-1003).

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**Key words:** close binaries: visual stars: Speckle interferometric technique

**Hepatoprotective and Therapeutic Activity of *Origanum syriacum* Aqueous Extract in Paracetmol Induced cell Damage in Albino Mice**

Abeer Y. Ibrahim<sup>1</sup>, Nermene M. Shaffie<sup>2</sup> and Hemaia M. Motawa<sup>3</sup>

<sup>1</sup>Medicinal and Aromatic Plants Dept., Pharmaceutical and Drug Industries Division, National Research Centre.

<sup>2</sup> Pathology Department, Medical researches Division, National Research Centre, Egypt.

<sup>3</sup>,Pharmacognosy Department, Pharmaceutical and Drug Industries Division, National Research Centre, 12622, Cairo, Egypt.

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[abeeryousry@yahoo.com](mailto:abeeryousry@yahoo.com)

**Abstract:** Ethnomedically genus *Origanum* L. is one of the most commonly used herb in many countries as a stimulant, analgesic, antitussive, expectorant, sedative, anti-inflammatory and antihelminthic agent. The hepatoprotective and therapeutic effects of *Origanum syriacum* aqueous methanolic extract on paracetamol induced liver cell damage in mice with respect to antioxidant status was investigated. Mice were treated with extract and sylimarin in recommended dose after or before paracetamol administration (400mg/ kg/ day). Lipid peroxides concentration was considerably

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decreased due to the elevation of reduced glutathione concentration(GSH) and enhancing of glutathione reductase(GR), glutathione transferase(GST), glutathione peroxidase (GPx)and superoxide dismutase(SOD) activities as compared to paracetamol or sylimarin treated mice. Liver function parameters are still in the normal levels in extract treated mice as compared to control. Using extract as a treating agent after subjecting mice to paracetamol gave better results, the liver tissue showing a nearly normal liver tissue except for a little cellular infiltrate around main blood vessels while sylimarin showing a noticeable dilatation of blood vessels that are surrounded by fibrosis and cellular infiltration. Liver tissue from mouse received *Origanum* extract and then paracetamol showing mild dilatation of blood sinusoids and cellular infiltration around main blood vessels while sylimarin treated mice showed marked dilatation of blood sinusoids, vacuolar degeneration in many of the hepatocytes and focal necrotic areas among the hepatocytes. In conclusion, *Origanum syriacum* extract has potent therapeutic activity than hepatoprotective activity and it is more effective than sylimarin in two cases. The plant extract was screened for its phytochemical constitutions.

[Abeer Y. Ibrahim<sup>1</sup>, Nermene M. Shaffie<sup>2</sup> and Hemaia M. Motawa. Hepatoprotective and Therapeutic Activity of *Origanum syriacum* Aqueous Extract in Paracetmol Induced cell Damage in Albino Mice. Journal of American Science 2010;6(11):449-458]. (ISSN: 1545-1003).

**Key words:** Hepatoprotective, Antioxidant, Oregano, Paracetamol, Therapeutic

**Water quality status of Golden Key Lake in Clement Town, Dehradun, Uttarakhand**

Avnish Chauhan\*, Mayank Pawar\* and Showkat Ahmad Lone

\* Department of Applied Sciences, College of Engineering, Teerthanker Mahaveer University,  
Moradabad-244001

Department of Environmental Science, Uttaranchal College of Science and Technology, Dehradun-  
248001

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**Abstract:** An attempt has been made to understand to provide information on the physico-chemical characteristics of Golden Key Lake which is being used for aquaculture, were studied between Nov 2008 to Feb 2009. All the parameters has been correlated with each other and each parameters has shown correlation matrix with different parameters at selected sites.

[Avnish Chauhan, Mayank Pawar and Showkat Ahmad Lone. Water quality status of Golden Key Lake in Clement Town, Dehradun, Uttarakhand. Journal of American Science 2010;6(11):459-464]. (ISSN: 1545-1003).

**Keywords:** TDS, TSS, pH, DO, COD, Ca, Mg, K, Golden Key Lake

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**Influence of freeze-shocked mesophilic lactic starter bacteria and adjunct lactobacilli on the rate of ripening Gouda cheese and flavor development**

El-Sayed El-Tanboly, Mahmoud El-Hofi, Y. B. Youssef,\*Wahed El-Desoki, and \*\*Reda A. Jalil

Dairy Science Department, National Research Center, Dokki, Cairo, Egypt.

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\*Dairy Science Department, Al-Azhar Univ., Agriculture Faculty, Assuet Branch, \*\*Chamber of Food Industries, 1195 Cornish El-Nil, Beaulac, Cairo, Egypt.

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[tanboly1951@yahoo.com](mailto:tanboly1951@yahoo.com)

**Abstract:** The objective of the present study was to determine the effects of *Lactobacillus acidophilus* on the sensory attributes, ripening time, and composition of Gouda cheese and to investigate the survival of *L. acidophilus* during ripening. Five types of Gouda cheeses, control cheese (Tc), made with with mesophilic lactic starter bacteria, Ta1, Ta2 , Tb1 and Tb2 cheeses made using modified mesophilic

lactic starter bacteria by freeze-shocked at -10°C/-20°C for 24 , 96 hrs and probiotic Lactobacillus, as adjunct culture. Cheese samples were assessed for microbiological and compositional properties, proteolysis, and sensory evaluation at different ripening stages. The composition and the pH value were almost identical between control and experimental vats within a single trial cheese. Characterization of proteolysis of gel electrophoresis of cheese samples at various stages of ripening showed that the extent of casein degradation varied between samples in all cheeses,  $\alpha_1$ -Casein was more extensively degraded than  $\beta$ -casein. However, levels of soluble nitrogen (SN/TN) increased with ripening period for all cheeses, only moderate enhancement of proteolysis as in amino acid -N in all trials. The formation of non protein nitrogen (NPN/TN) was slightly increased compared to control at the end of ripening. Organoleptic evaluation showed that probiotic cheese had higher sensory evaluation than control cheese, without probiotic strain. The population of Lactobacillus survived to numbers  $> 10^7$  cfu/g, which is necessary for positive effects on health. These results showed that the contribution of modified mesophilic lactic starter bacteria by freeze-shocked and probiotic strain as adjunct culture can be successfully used in production of Gouda cheese.

[El-Sayed El-Tanboly, Mahmoud El-Hofi, Y. B. Youssef, Wahed El-Desoki, and Reda A. Jalil. Influence of freeze-shocked mesophilic lactic starter bacteria and adjunct lactobacilli on the rate of ripening Gouda cheese and flavor development. Journal of American Science 2010;6(11):465-471]. (ISSN: 1545-1003).

**Keywords:** Physically freeze-shock mesophilic starter, probiotic bacteria, proteolysis Gouda cheese

#### **Gap Analysis for Protected Areas of Andhra Pradesh, India for conserving biodiversity**

C. Sudhakar Reddy

Forestry and Ecology Division, National Remote Sensing Centre, Indian Space Research Organisation, Balanagar, Hyderabad -500 625, India. [drsudhakarreddy@gmail.com](mailto:drsudhakarreddy@gmail.com)

**Abstract:** A gap analysis was carried out to assess the Protected Area (PA) network system in Andhra Pradesh, India. The decisive factors of vegetation type distribution, elevation and endemism was used to determine the representativeness of PA system. In Andhra Pradesh, vegetation cover occupies 23.03% of geographical area and distributed in Coastal Plains, Deccan Plateau and Eastern Ghats. There are 27 PAs for conservation in Andhra Pradesh. The total area protected for biodiversity is about 12,555 km<sup>2</sup> or 4.56% of geographical area of Andhra Pradesh. Of the three physiographic regions, Eastern Ghats represents very high area under PAs which was estimated as 7811.38 km<sup>2</sup> followed by Deccan plateau of 3526.89 km<sup>2</sup>. Three main forest types (semi evergreen forests, thorn forests and dry evergreen forests) missing in the existing PA network were identified. Moist deciduous forests of Eastern Ghats of northern Andhra Pradesh were under-represented in PAs. The land area in an elevation range of 900m-1527m was not included in PA network. Of the 103 species of endemics, 64 species were not included in PA system. Many PAs are experiencing threat from invasive species, forest fires, grazing pressure etc. There is a need to consider for possible ways for effective conservation and to extend the present PA network system in India.

[Journal of American Science 2010;6(11):472-484]. (ISSN: 1545-1003).

**Keywords:** gap area; vegetation; protected area; semi evergreen forests; Andhra Pradesh; India

#### **Knowledge Discovery in Al-Hadith Using Text Classification Algorithm**

Khitam Jbara

Department of Computer Science, King Abdullah II School for Information Technology, The University Of Jordan, P.O. Box 710481 Amman 11171 Jordan. [ktjlc2000@yahoo.com](mailto:ktjlc2000@yahoo.com)

**Abstract:** Machine Learning and Data Mining are applied to language datasets in order to discover patterns for English and other European languages, Arabic language belongs to the Semitic family of

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languages, which differs from European languages in syntax, semantic and morphology. One of the difficulties in Arabic language is that it has a complex morphological structure and orthographic variations. This study is conducted to examine knowledge discovery from AL-Hadith through classification algorithm in order to classify AL-Hadith to one of predefined classes (books), where AL-Hadith is the saying of Prophet Mohammed (Peace and blessings of Allah be upon him (PBUH)) and the second religious source for all Muslims, and because of its importance for Muslims all over the world knowledge discovery from AL-Hadith will make AL-Hadith more understandable for both Muslims and nonmuslims.

[Khitam Jbara. Knowledge Discovery in Al-Hadith Using Text Classification Algorithm. Journal of American Science 2010;6(11):485-494]. (ISSN: 1545-1003).

**Keywords:** AL-Hadith, classification, stem, feature, class, expansion, training set

**Efficacy of some Biocontrol Agents on Reproduction and Development of *Meloidogyne incognita* Infecting Tomato**

Moussa Lobna\* and Hanaa Zawam\*\*

\*Soils, Water & Environment Research Institute, Agricultural Research Centre (ARC), Giza, Egypt,

\*\* Plant Pathology Research Institute, Agricultural Research Centre (ARC), Giza, Egypt  
[mlobnamy@yahoo.com](mailto:mlobnamy@yahoo.com), [hn\\_zawam@yahoo.com](mailto:hn_zawam@yahoo.com)

**Abstract:** Three rhizobacteria and two yeasts isolates were used as biocontrol agents against *Meloidogyne incognita* in laboratory and greenhouse. The used biocontrol agents were identified as *Bacillus amyloliquefaciens*, *Brevibacterium otitidis*, *Sanguibacter inulinus*, *Candida incommunis* and *Wicherhamiella domercqiae*. They inhibited the egg-masses hatching *in vitro* and exhibited strong nematicidal activity by killing the second stage juveniles of *Meloidogyne incognita* to various degrees in greenhouse. The most effective treatment was the complete culture of the four biocontrol agents (propagules and filterate) suppressed galls and egg-masses formation by 100% *Br. otitidis* reduced galls and egg-masses by 43.7 and 52.19 %, respectively compared with the untreated control. The microorganisms used in greenhouse test reduced nematode populations in the rhizosphere and promoted the growth of tomato plants over the control treatment.

[Moussa Lobna and Hanaa Zawam. Efficacy of some Biocontrol Agents on Reproduction and Development of *Meloidogyne incognita* Infecting Tomato. Journal of American Science 2010;6(11):495-509]. (ISSN: 1545-1003).

**Key words:** Biocontrol; *Meloidogyne incognita*; tomato; Rhizobacteria; yeast

**Phytochemical and Elemental Analysis of *Acalyphe wilkesiana* Leaf**

\*Madziga, H. A.<sup>1</sup>, Sanni S.<sup>2</sup> and Sandabe U. K.<sup>1</sup>

<sup>1</sup>Department of Veterinary Physiology, Pharmacology and Biochemistry, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria. <sup>2</sup>Department of Veterinary Pharmacology, University of Abuja, Nigeria.

[hannamadziga@yahoo.com](mailto:hannamadziga@yahoo.com)

**ABSTRACT:** Phytochemical and Elemental determination of *Acalyphe wilkesiana* was conducted. The result of the Phytochemical analysis of the aqueous leaf extract of *A. wilkesiana* revealed a high presence of carbohydrates, Tannins and Flavonoid, a moderate presence of Phlobatannins, Saponins. Alkaloids and Cardiac glycosides and minute quantity of Terpenes and Steroids. Anthraquinone derivatives was not present. The Elemental analysis showed presence of chloride, sodium, potassium, calcium, iron, magnesium, zinc copper and manganese in moderate quantity while cadmium and lead were not detected. It is therefore concluded that the aqueous leaf extract of *A. wilkesiana* contains

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Pharmacologically useful active principles elements. Thus the aqueous leaf extract of the plant could play a vital roles in health and disease.

[Madziga, H. A.<sup>1</sup>, Sanni S.<sup>2</sup> and Sandabe U. K. Phytochemical and Elemental Analysis of *Acalypha wilkesiana* Leaf. Journal of American Science 2010;6(11):510-514]. (ISSN: 1545-1003).

**Key words:** *Acalypha wilkesiana*, aqueous leaf extract , Phytochemical analysis, Elemental analysis

**Nutritional studies on some different sources of iodine on productive performance, ruminal fermentation and blood constituents of Buffalo. 1 – Effect of two different iodine levels on productive and reproductive performance of buffalo cows.**

Kh. I. I. Zeedan<sup>1</sup>, O. M. El-Malky<sup>2</sup>, Kh. M. M. Mousa<sup>1</sup> , A. A. El.Giziry<sup>1</sup> and K. E.I. Etman<sup>1</sup>

1. Department of Animal Nutrition Research.
2. Department of Buffalo Research.

Animal Production Research Institute, Agricultural Research Centre, Dokki, Giza, Egypt.

[khzeedan@yahoo.com](mailto:khzeedan@yahoo.com)

**Abstract:** This study was conducted to evaluate the effect of feeding buffalo cows on ration supplemented with two levels from iodine (I) during late pregnancy (three months before parturition) and postpartum period (six months after parturition) on nutrients digestibility, some blood constituent, birth weight of their offspring, Concentrations of immunoglobulin in colostrums, milk (yield and composition) and reproductive parameters. Eighteen buffalo cows (2-4 lactations) in late pregnancy period were selected to carry out the experimental work. The animals were divided into three similar groups (6 female buffaloes in each). Concentrate feed mixture (CFM), berseem hay (BH) and rice straw (RS) were given to animals as a control ration ( $I_0$ ) without supplementation, while the other groups  $I_1$  and  $I_2$  received the control ration with iodine at levels of 0.3 and 0.5 mg I per kg DM intake /h/d, respectively. Results indicated that supplementation ration of buffalo cows with different levels of I had improved the digestibility of all nutrients, TDN, DCP at pre and post partum, feed efficiency, increased milk yield, 7% fat correct milk yield and its composition. Birth and weaning weight of calves in treated groups were higher than that control group. Immunoglobulin concentration in colostrums indicated higher values with animals feed supplemented rations than those fed the control. Moreover, addition of I improved RBC, WBC, Hb, PCV, plasma total protein, globulin, glucose, T<sub>3</sub> and T<sub>4</sub>. Supplemented rations of buffalo cows with 0. 5 mg I/h/d tend to significantly ( $P < 0.05$ ) higher in actual milk yield, 7% FCM yield, fat %, protein %, lactose %, SNF % and TS %, while supplemented with 0. 3 mg I/h/d appeared to the same higher trend with no significantly differences. Moreover, better feed efficiency was observed with animals fed supplemented rations. The periods required for fetal membrane expulsion was significantly reduced in  $I_2$  group when compared to  $I_1$  or control groups. Moreover, only control group showed a case of abortion and still birth, while treated dams delivered 100% healthy calves. Buffaloes of group  $I_2$  had the least ( $P < 0.05$ ) calving interval due to the shorter intervals for uterine involution, onset of the 1<sup>st</sup> postpartum heat and days open. Iodine supplementation showed significant differences among groups in studied parameters such as NSPC and CI. Mean period elapsed from calving until placenta drop significantly decreased  $I_2$  than the control group. Generally, it concluded that I supplementation for ration of buffalo cows improved immunity, nutrients digestibility, calves birth weight and increased milk (yield and composition) and showed better feed efficiency as well as higher some traits of reproductive performance.

[Kh. I. I. Zeedan, O. M. El-Malky, Kh. M. M. Mousa, A. A. El.Giziry and K. E.I. Etman. Nutritional studies on some different sources of iodine on productive performance, ruminal fermentation and blood constituents of Buffalo. 1 – Effect of two different iodine levels on productive and reproductive performance of buffalo cows. Journal of American Science 2010;6(11):515-530]. (ISSN: 1545-1003).

**Keywords:** buffalo cows, iodine, performance, reproductive parameters, blood components, milk yield, digestibility, immunity

[Full text](#)

**Integrated theoretical model to enhance neonatal screening for sickle hemoglobinopathies in the wake of predictive, preventive, personalized and participatory medicine**

[Full text](#)

E. William Ebomoyi, Ph.D., Professor

Department of Health Studies, College of Health Sciences, Chicago State University, Chicago Illinois and he serves as a Consultant in International Health for the American Public Health Association, 9501 South King Drive, Douglas Hall 127, Chicago, Illinois 60628-1598, USA

[eebomoyi@csu.edu](mailto:eebomoyi@csu.edu), 773-995-2527

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**ABSTRACT:** This study utilized the integrated theoretical model (ITM) to assess strategies to ameliorate screening for sickle hemoglobinopathies in the age of genomic medicine. Also discussed, is the relevance of predictive, preventive, personalized and participatory interventions. Comparison was made between universal and targeted screening. The international guidelines for neonatal screening were reiterated. The self-efficacy and empowerment of mothers is crucial in ensuring that they effectively participate in the treatment and follow-up of their new-born babies. We emphasized the compliance with the ethical, legal and social implications of newborn screening for genetic diseases.

[E. William Ebomoyi. Integrated theoretical model to enhance neonatal screening for sickle hemoglobinopathies in the wake of predictive, preventive, personalized and participatory medicine. Journal of American Science 2010;6(11):531-537]. (ISSN: 1545-1003).

**Keywords:** integrated theoretical model (ITM); ameliorate; sickle hemoglobinopathies; genomic medicine; neonatal screening; ethical; legal; social; genetic diseases

**Mixed Infection of Bovine Viral Diarrhea Virus, Mycoplasma Species and Mannheimia Haemolytica in Calves Showed Chronic Pneumonia with Reference to the Histopathological Findings of the Affected Lungs**

[Full text](#)

Hanaa, A. Ghoneim\*, Naglaa, I. Hassan, Hanaa, A. Elhalawany and A.M.Nabih

Animal Reproduction Research institute (ARRI) Giza, Egypt

[\\*hanaeg2002@yahoo.com](mailto:*hanaeg2002@yahoo.com)

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**Abstract:** A total of 100 nasal swabs as well as blood samples were collected from 75 diseased calves suffered from respiratory manifestations and 25 apparently healthy calves of ages ranges from 2-12 month old from three herds. Also 80 clinically pneumonic lung specimens of slaughtered calves were collected from El-warak and El- moneeb abattoir. All were examined to establish the extent of involvement of Bovine Viral Diarrhea (BVDV), *Mycoplasma* species (*M. spp.*) and *Mannheimia haemolytica* (*M. haemolytica*) in cases of chronic calf pneumonia. On virological studies, AGPT and commercial ELISA kits were rapid and accurate tests for detection of BVDV antigen. BVDV was isolated on MDBK cell line from Buffy coat, nasal swabs collected from diseased calves and lung specimens. The isolated virus was identified by IFAT using reference antisera. Also 100 serum samples collected from diseased and apparently healthy calves were tested by VNT for the detection of neutralizing antibodies against BVDV. Moreover, on bacteriological investigation. *M. haemolytica* were recovered from lung specimens of slaughtered calves as well as nasal swabs of diseased ones and apparently healthy ones. The isolated strains were biotyped as biotype A (56 isolates, 80 %) and biotype T (14 isolates , 20 %). The resistance of the isolates to most antimicrobial agents was high to ceftiofur, nalidixic acid, gentamicin, oxytetracycline, and cephalexin. While they were highly sensitive to norfloxacin, ampicillin and erythromycin. Although, *Mycoplasma* species recovery rate from the examined nasal swabs of pneumonic calves was (46.67%) relatively higher than that recovered from apparently healthy calves (32.00%), the isolation rate from the examined lung tissues reached to (25.0%). The most prevalent isolated species was *M.bovis* followed by *M.dispar*, then glucose positive, arginine negative species. Considering the mixed infection, results showed that, simultaneous isolation of the three pathogens from nasopharyngeal swabs of the examined pneumonic calves was relatively

high (12.00%), followed by simultaneous isolation of BVDV & *Mycoplasma sp* as well as *M.haemolytica* & *Mycoplasma sp.* (9.33%). On the other hand, there was simultaneous isolation of both BVDV and *M.haemolytica* from nasopharyngeal swabs of (8.00%) out of the examined pneumonic calves. Examination of 80 clinically pneumonic lung tissues of slaughtered calves that were collected from abattoirs revealed that, a high percentage (17.50%) of examined lung tissues colonized both *Mycoplasma sp.* and *M.haemolytica* together. On the other hand, simultaneous isolation of the three pathogens was detected in (3.75%). However, simultaneous isolation of both BVDV and *Mycoplasma sp.* as well as BVDV and *M.haemolytica* was recorded in (2.50%) of examined lung tissues. Regarding histological studies of lung tissue specimens, there were five types of pneumonia distinguished according to types of necrosis, and cellular infiltrations in relation to microbial isolation, Caseonecrotic bronchopneumonia, 3.75%, Fibrino-necrotizing bronchopneumonia 12.5%, Acute and chronic fibrinosuppurative bronchopneumonia 13.75%. In conclusion *M. bovis* showed two necrotic patterns, where an original focus of coagulative necrosis evolves with time into caseous necrosis ended by fibrosis.

[Hanaa, A. Ghoneim, Naglaa, I. Hassan, Hanaa, A. Elhalawany and A.M.Nabih. Mixed Infection of Bovine Viral Diarrhea Virus, Mycoplasma Species and Mannheimia Haemolytica in Calves Showed Chronic Pneumonia with Reference to the Histopathological Findings of the Affected Lungs. Journal of American Science 2010;6(11):538-555]. (ISSN: 1545-1003).

**Keywords:** Infection; Bovine Viral Diarrhea Virus; Mycoplasma Species; Mannheimia Haemolytica; Calves; Chronic Pneumonia; Lungs

#### **Comparative Study between Different Denture Adhesives in Improving Phonation in Complete Denture Wearers**

Essam Adel Aziz<sup>1</sup>, Azza Adel Aziz<sup>2</sup> Dina Essam Eldeen Ibrahim<sup>1</sup> and Ali Eldeen Mohammed Ahmed<sup>1</sup>

<sup>1</sup>Prosthodontic Department, Faculty of Oral and Dental Medicine, Cairo University, Cairo Egypt

<sup>2</sup>Phoniatric Unit, ENT Department, Cairo University, Cairo Egypt.

[dr\\_mona\\_zaki@yahoo.co.uk](mailto:dr_mona_zaki@yahoo.co.uk)

**Abstract:** Objectives: the aim of this study was to evaluate the efficiency of denture adhesives in improving phonation in complete denture wearers and to compare the efficacy of three different types of denture adhesives. Methodology: Fifteen completely edentulous patients with flat mandibular ridge shared in this study, complete denture was constructed for each patient according to the conventional method. Phonetic analysis was performed in the Phoniatric Unit via both Perceptual and Acoustic techniques to compare the efficacy of three chemically different denture adhesives (Super corega, Fittydent and Fixodent) on Arabic phonemic production. Results: a marked improvement in patients' articulation after application of the denture adhesives was reported, perceptually and acoustically, where the Fixodent denture adhesive gave the highest values. Conclusion: Whenever possible, denture adhesives should be used to improve retention and articulation. The polymethylvinyl ether malate-based adhesives (Fixodent) are strongly recommended as a highly reliable type of denture adhesives.

[Essam Adel Aziz, Azza Adel Aziz Dina Essam Eldeen Ibrahim and Ali Eldeen Mohammed Ahmed. Comparative Study between Different Denture Adhesives in Improving Phonation in Complete Denture Wearers. Journal of American Science 2010;6(11):556-561]. (ISSN: 1545-1003).

**Keywords:** Different Denture; Adhesives; Improving Phonation; Complete Denture Wearer

#### **Assessment of Working Memory in Normal Children and Children Who Stutter**

Hazem Aboul Oyoun<sup>1</sup>; Hossam El Dessouky<sup>2</sup>; Sahar Shohdi \*<sup>2</sup> and Aisha Fawzy<sup>2</sup>

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<sup>1</sup>Otorhinolaryngology, ENT Department, Faculty of Medicine, Cairo University. Cairo, Egypt

<sup>2</sup>Phoniatrics, Phoniatic Unit, ENT Department, Faculty of medicine, Cairo University. Cairo, Egypt

\* sshohdi@hotmail.com

**Abstract:** The aim of this study is to assess working memory (WM) abilities in normal children and Children Who Stutter (CWS) then to compare the results in order to detect if WM deficits have a role in the development of stuttering. 30 normal children and 30 children who stutter were subjected to WM recall abilities tests and nonword repetition tasks. The WM recall tests included recall of word sets different in length and rhyming, digit span, letter sequences and picture-number test. The nonword repetition test was used to assess phonological encoding through measuring number of phonological errors produced on repeating the task, and to measure the reaction time. The children who stutter (CWS) had performed poorly on some working memory tests compared to the control group. Conclusion: Children who stutter may have diminished ability to recall nonwords and some of working memory abilities and that further investigation into this possibility may shed light on the emergence and characteristics of childhood stuttering.

[Hazem Aboul Oyoun; Hossam El Dessouky; Sahar Shohdi and Aisha Fawzy. Assessment of Working Memory in Normal Children and Children Who Stutter. Journal of American Science 2010;6(11):562-569]. (ISSN: 1545-1003).

**Key words:** working memory; children; stutter nonword repetition; phonological encoding, phonological errors, reaction time

#### CHEMICAL STUDIES ON 3,6-DICHLOROPYRIDAZINE

**Mohamed H. Sherif, Gamal A. Ahmed, Adel A. Elbahnasawy and Eman O. Helal**

Department of Chemistry, Faculty of Science, Zagazig University, Egypt. [meahsherif@hotmail.com](mailto:meahsherif@hotmail.com)

**ABSTRACT:** Reaction of 3,6-dichloropyridazine (**1**) with acid hydrazides, p-toluene sulfonylhydrazine, anthranilic acid derivatives and ammonium hydroxide afforded the compounds (**2a,b**), (**3**), (**4a,b**) and (**5**) respectively. Compound (**5**) reacted with aromatic aldehydes yielded the Schiff's bases (**6**) and (**7**). Compound (**6**) reacted with anthranilic acid derivatives and gave (**8**). Also, compound (**1**) easily reacted with 2-chlorobenzylamine, sodium azide and thiosemicarbazide afforded the compounds (**9**), (**10**) and (**11**) respectively.

[Mohamed H. Sherif, Gamal A. Ahmed, Adel A. Elbahnasawy and Eman O. Helal. CHEMICAL STUDIES ON 3,6-DICHLOROPYRIDAZINE. Journal of American Science 2010;6(11):570-574]. (ISSN: 1545-1003).

**Keywords:** 3,6-dichloropyridazine; acid hydrazides; p-toluene sulfonylhydrazine; anthranilic acid derivative; ammonium hydroxide

#### Citizens' Attitude toward's Local Government and Citizen's Participation in Local Government

Seyed Hamid Mohammadi

Department of Social and Development Sciences, Faculty of Human Ecology, Putra University, Malaysia. Tel: 60-17-2118806; Email: [hmd\\_mohamadi@yahoo.com](mailto:hmd_mohamadi@yahoo.com)

Sharifah Norazizan

Department of Social and Development Sciences, Faculty of Human Ecology, Putra University, Malaysia. Email: [sharifah@putra.upm.edu.my](mailto:sharifah@putra.upm.edu.my)

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Nobaya Ahmad

Department of Social and Development Sciences, Faculty of Human Ecology, Putra University, Malaysia. Email: [nobaya@putra.upm.edu.my](mailto:nobaya@putra.upm.edu.my)

**Abstract:** The purpose of this paper is to describe the citizen's attitude toward local government and its relationship with level of participation. Participation in local government issues, requires a favorable attitude towards local government, councilors and councils' performance in terms of efficiency. The paper is based on the study of citizens' attitude towards local government, which was carried out in Torbat Hedarieh city, Iran. The analysis of data uses Pearson correlation to determine the relationship between variables involved. The findings revealed that two level of ladder participation (Tokenism and Citizen-power) have positive and significant relationship with attitude, while Non-participation level of ladder participation has negatively significant relationship with attitude. The findings of the study imply that those respondents who have positive attitude toward local government, councilors and council performance, would have the higher tendency to be actively involved in higher levels of participation, whereas who have negative attitude toward local government, would put less effort in higher levels of participation.

[Seyed Hamid Mohammadi. Citizens' Attitude toward's Local Government and Citizen's Participation in Local Government. Journal of American Science 2010;6(11):575-583]. (ISSN: 1545-1003).

**Keywords:** Citizen attitude, Citizen participation, Local government, social exchange

**Algal Abundances and Growth Performances of Nile Tilapia (*Oreochromis niloticus*) as Affected by Different Fertilizer Sources**

M.A. Elnady\*, H.A. Hassanien, M.A. Salem and H. Marian Samir

Department of Animal Production, Faculty of Agriculture, Cairo University, Giza , Egypt.  
[melnadyahmed@yahoo.com](mailto:melnadyahmed@yahoo.com)

**Abstract:** The experiment was designed to study the effect of different fertilizer sources (chemical fertilizer, organic fertilizer or combined chemical +organic fertilization) on plankton abundances , growth performances of Nile tilapia juveniles and water quality parameters in concrete tanks compared to feeding fish at satiation .The average secchi disk readings were shallower in the chemical and combined fertilizer treatments compared to those of the ration and organic fertilizer treatments as a result of increased algal density and abundances. Ammonia and orthophosphate concentrations in the chemical and combined fertilizer treatments were higher with an increase in algal growth, abundance. Within fertilizer treatments, the daily weight gains of Nile tilapia reared in the chemical and combined fertilizer treatments (0.43 and 0.5 g/fish/day, respectively) were significantly higher than those reared in the organic fertilizer treatment (0.32g/ fish/ day). This indicated that the use of chemical fertilizer in a fertilization program is superior in increasing fish growth compared to that of the organic fertilizer .It can be concluded that Nile tilapia juveniles can obtain major nutritional requirements for growth( 48% of its total feed requirements) from feeding only on algae during this stage of growth. Results of the current experiment recommended that organic fertilizer should not be used as sole source in fertilizer programs and should be combined with chemical fertilizer in order to produce good algal growth necessary for the nourishment of farmed fish.

[M.A. Elnady, H.A. Hassanien, M.A. Salem and H. Marian Samir. Algal Abundances and Growth Performances of Nile Tilapia (*Oreochromis niloticus*) as Affected by Different Fertilizer Sources. Journal of American Science 2010;6(11):584-593]. (ISSN: 1545-1003).

**Key words:** Fertilizers, manure, algae, plankton, Nile tilapia

**Age as Moderated Influence on the Link of Spiritual and Emotional Intelligence with Mental Health in High School Students**

[Full text](#)

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Jafar Shabani\*, Siti Aishah Hassan, Aminah Ahmad, Maznah Baba

Faculty of Educational Studies, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

[jshabani@yahoo.com](mailto:jshabani@yahoo.com)

**Abstract:** This study examined whether, spiritual intelligence (SI) and emotional intelligence (EI) can be considered as predictor for mental health. The present investigation was also to test the moderating effects of age on the relationship of SI and EI with mental health among high school students. The participants in the study were 247 High school students (124 male and 123 female) in the age range of 14-17 years old, at the Gorgan City, north of Iran. Three valid and reliable instruments were used to assess SI, EI and mental health. Descriptive statistics, multiple and moderated regression analysis were used to analyses the data. The result demonstrated that mental health can be influences by SI and EI. In addition, the moderated effect of age on the relationship of SI and EI with mental health was not found.

[Jafar Shabani\*, Siti Aishah Hassan, Aminah Ahmad, Maznah Baba. Age as Moderated Influence on the Link of Spiritual and Emotional Intelligence with Mental Health in High School Students. Journal of American Science 2010;6(11):394-400]. (ISSN: 1545-1003).

**Keywords:** Psychology, education, high school students, spiritual and emotional intelligence, mental health

**Morphometrical, Histopathological, and Cytogenetical ameliorating Effects of Green tea Extract Nicotine Toxicity of the Testis of Rats**

**<sup>1</sup>\*Azza M. Gawish, <sup>2</sup>Aliaa M. Issa, <sup>3</sup>Aziza M. A., and Sherin Ramadan**

<sup>1</sup>Department of Zoology, Faculty of Science, Cairo University, Cairo, Egypt

<sup>2</sup> Cell biology Department, National Research Centre, Dokki, Cairo, Egypt

<sup>3</sup> National Organization for Drug control and Research Dokki – Giza, Cairo, Egypt

[azzagawish@ymail.com](mailto:azzagawish@ymail.com)

[Full text](#)

**Abstract:** Nicotine is a major toxic component of cigarette smoke and it is a major risk factor in the development of functional disorder of several organ systems. The natural diet contains a variety of compounds that exhibit protective effects towards different toxicities of nicotine as green tea. Four groups of male Swiss albino mice were divided: untreated control group; Nicotine-treated group (2.5 mg/kg/day); Green tea-treated group (40 mg/kg./day); and Nicotine and green tea treated group interperitoneal administration for successive 28 days. Results showed that disorganization of the seminiferous tubules associated with reduction of spermatogenic cells, leading to widening of lumen of tubules upon nicotine toxicity. Many of seminiferous tubules exhibited degenerative phases of spermatocytes and spermatides as well as missing of sperms and hypo-spermatogenesis. The recorded data in nicotine intoxicated group showed significant and gradual decrease of number of leydig cells throughout all intervals of experiment. In the last, cytogenetically examination demonstrated significant increased in the number of nucleated polychromatic erythrocytes (MnPCE) and decreased in number of polychromatic erythrocytes (PCE) in bone marrow of nicotine-treated animals using micronucleus assay. Green tea treatment reduced number of nucleated polychromatic erythrocytes (MnPCE) and restored number of polychromatic erythrocytes (PCE) to nearly normal. In conclusions, intake of green tea might suppress the toxicity and mutagenic activity of nicotine.

[Azza M. Gawish, Aliaa M. Issa, Aziza M. A., and Sherin Ramadan. Morphometrical, Histopathological, and Cytogenetical ameliorating Effects of Green tea Extract on Nicotine Toxicity of the Testis of Rats. Journal of American Science 2010;6(11):401-411]. (ISSN: 1545-1003).

**Keywords:** Smoking - Nicotine – Fertility – Antioxidants – Green tea

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**Tourism as an Economic Development Tool**

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### Mohammad Taleghani

Assistant professor, Islamic Azad University - Rasht Branch, IRAN. [Taleghani@iaurasht.ac.it](mailto:Taleghani@iaurasht.ac.it)

**ABSTRACT** - Probably, the greatest single deterrent to tourism development is the lack of appreciation and enthusiasm for tourism by civic and business leaders. When tourism is not understood and its benefits are unclear, planning and implementation of measures to improve the industry are often lacking. Notably, global tourism has become the largest industry in the world, with nearly 500 million consumers of tourism services per year spending hundreds of billions of dollars. The industry provides employment to over 100 million people worldwide. Thus, in view of tourism's increasing role in economic activity, the factors affecting its performance should be analyzed. An understanding of these factors is crucial to determine the ways in which national and international financial institutions, NGOs and other entities can play the most value-adding role. This paper provides a brief profile of key factors and trends in tourism and their economic effects at the global, national and regional levels.

[Mohammad Taleghani. Tourism as an Economic Development Tool. Journal of American Science 2010;6(11):412-416]. (ISSN: 1545-1003).

**Keywords:** Tourism, Economic Development, Supply and Demand, GDP

### Impact of Metformin on Immunity and Male Fertility in Rabbits with Alloxan- Induced Diabetes

**Naglaa, Z.H. Eleiwa<sup>\*</sup>; Hesham, A.M.; Hosny Abdel Fadil and Abdel Motal, S.M.**

Dept. of Pharmacology, Faculty of Veterinary Medicine, Zagazig University, Egypt.  
<sup>\*</sup>[eleiwa02@yahoo.com](mailto:eleiwa02@yahoo.com)

[Full text](#)

**Abstract:** A study was designed to explore the possible side effects of metformin on immunity and fertility of male rabbits with alloxan- induced diabetes. Sixteen adult male rabbits were used in this study, they were classified into four equal groups as follows: the first group received neither alloxan nor metformin and remained as control group. Rabbits in the 2<sup>nd</sup> group were orally treated with metformin at a dose of 120 mg/kg b.wt once a day for 3 months .Rabbits in the 3<sup>rd</sup> group were administered alloxan, I/V, at a single dose of 100 mg/kg b.wt.Rabbits in the 4<sup>th</sup> group were administered alloxan ( 100 mg/kg b.wt, single I/Vdose ) then treated orally with metformin (120 mg/kg b.wt.) once daily for 3 months. Rabbits in all groups were subcutaneously injected with 2 ml polyvalent rabbit pasteurellosis vaccine after two months from the beginning of experiment for studying the immunological profile of the drug. Treatment of diabetic and non-diabetic rabbits with metformin evoked a significant decrease ( $P < 0.05$ ) in nitric oxide production on the 1<sup>st</sup> and the 2<sup>nd</sup> day post vaccination .In response to treatment with metformin, rabbits demonstrated a significant decrease ( $P < 0.05$ ) in serum lysozyme activity on the 1<sup>st</sup>, 2<sup>nd</sup> , 3<sup>rd</sup> day and in the 1<sup>st</sup> week post vaccination while diabetic rabbits treated with metformin showed a significant decrease ( $P < 0.05$ ) in serum lysozyme activity on the 3<sup>rd</sup> day and on the 1st , 3rd and 4th week post vaccination . In addition, treatment with metformin of diabetic and non-diabetic rabbits resulted in a significant decrease( $P < 0.05$ ) in testicular weight , sperm cell count, sperm motility and serum testosterone with a significant increase in sperm abnormalities and dead sperm %. Summing up our observations, the present study calls into question the justification for the use of metformin in a frame of a therapeutic strategy for diabetes due to its resulting negative impact on immunity and male fertility.

[Naglaa, Z.H. Eleiwa; Hesham, A.M.; Hosny Abdel Fadil and Abdel Motal, S.M. Impact of Metformin on Immunity and Male Fertility in Rabbits with Alloxan- Induced Diabetes. Journal of American Science 2010;6(11):417-426]. (ISSN: 1545-1003).

**Key words:** Metformin – alloxan – diabetes - rabbits

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### Amniotic Membrane Extract for Acute Ocular Chemical Burns

**Hosam Sheha, Hisham Hashem, Lingyi Liang, Mohamed Ramzy, , Ahmed ZaKi**

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**Abstract:** Background: Ocular chemical burns induce devastating and permanent damage to the ocular surface. Rapid intervention is required for maximal visual rehabilitation. Amniotic membrane transplantation (AMT) may save the ocular surface, however it introduces a potentially unnecessary surgical trauma in such compromised eyes. Amniotic membrane extracts (AME) could be a practical substitute of AMT in acute chemical burn. Aim: To evaluate the efficacy of topical AME in the management of acute ocular chemical burn. Methods: Non-comparative interventional case series. Six eyes of 4 consecutive patients with mild to moderate acute chemical burn, exhibiting persistent epithelial defect, inflammation and haze despite extensive conventional therapy were recruited. Topical AME was prepared and added to the conventional treatment within 2 days of the injury. Pain relief, inflammation, haze, and corneal epithelial healing were monitored. Results: Pain was significantly relieved, and inflammation was markedly reduced in all cases. The corneal epithelial defects rapidly healed while visual acuity improved within 11 (range 4-23) days. During an average follow-up period of 6 months (range, 3-8 months), all eyes retained stable surface with improved corneal clarity without neovascularization or symblepharon. Conclusions: Topical application of AME could be an effective adjunct in the treatment of mild to moderate cases of acute chemical burns. It allows non-traumatic and economic early intervention to promote epithelialization, reduce pain, haze and inflammation in acute phase, and prevent cicatricial complications in chronic phase. This result justifies additional large series controlled studies in the future.

[Hosam Sheha, Hisham Hashem, Lingyi Liang, Mohamed Ramzy, Ahmed ZaKi. Amniotic Membrane Extract for Acute Ocular Chemical Burns. Journal of American Science 2010;6(11):427-433]. (ISSN: 1545-1003).

**Key words:** Acute chemical burn, amniotic membrane extract, corneal epithelial defect

**Phylogenetic Diversity and Relationships of Some Tomato Varieties by Electrophoretic Protein and RAPD analysis**

**Elham A. A. Abd El-Hady<sup>\*</sup>, Atef A. A. Haiba , Nagwa R. Abd El-Hamid, and Aida A. Rizkalla**

Department of Genetics and Cytology, National Research Center, Dokki, Giza, Egypt.  
<sup>\*</sup>[elhamabdelhady@hotmail.com](mailto:elhamabdelhady@hotmail.com)

**Abstract:** Biochemical and molecular characterization of eight tomato varieties were carried out based on seed storage proteins electrophoresis and RAPD markers. The electrophoretic pattern of water soluble protein produced 4 monomorphic bands, 6 polymorphic band and 3 unique bands .The pattern of non soluble protein produced 9 bands, one band is unique and considered a positive specific band of tomaten cartago variety and the others are polymorphic bands. RAPD results revealed a high level of polymorphism among the studied genotypes. All of the seven random primers screened gave reproducible polymorphic DNA bands. A total number of 81 amplified DNA bands were generated across the studied genotypes with average of 11.57 bands /primer. 37 bands out of the total number were polymorphic and 19 were unique. Combination of the all data derived from the SDS-protein markers of both water soluble and non soluble proteins produced a dendrogram almost similar to that obtained by the RAPD analysis. It could be concluded that, both of SDS-Protein and RAPD markers are equally important for genetic analysis and indicate a considerable amount of genetic diversity between the different studied varieties of *Lycopersicon esculentum* L.

[Elham A. A. Abd El-Hady<sup>\*</sup>, Atef A. A. Haiba , Nagwa R. Abd El-Hamid, and Aida A. Rizkalla. Phylogenetic Diversity and Relationships of Some Tomato Varieties by Electrophoretic Protein and RAPD analysis. Journal of American Science 2010;6(11):434-441]. (ISSN: 1545-1003).

**Keywords:** Tomato, Genetic diversity, SDS-protein, RAPD-PCR

**Protective effect of three different fluoride treatments on dental erosion in primary and permanent teeth**

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**Sherine B Y Badr<sup>1</sup>, Mohamed A Ibrahim<sup>2</sup>**

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<sup>1</sup> Pediatric dentistry Dept., Cairo University

<sup>2</sup> Restorative Dentistry Dept., Misr University for Science and Technology

[Shbadr5@hotmail.com](mailto:Shbadr5@hotmail.com)

**Abstract:** **Objective:** To assess the effect of acidulated phosphate fluoride gel (APF), sodium fluoride varnish (NaF) and casein phosphopeptide-amorphous calcium phosphate fluoride paste (CPP-ACPF) on the dental erosion produced by coca cola in primary and permanent teeth. **Design:** Sixty extracted human primary molars ( $n = 30$ ) and young permanent premolars ( $n = 30$ ) were used in this study. The coronal portion of each tooth was removed and transversely sectioned from the mesial to distal surface using a diamond coated saw blade. The crown sections were embedded in acrylic resin blocks leaving the enamel surfaces exposed. The enamel surfaces were ground and polished. Test specimens were randomly assigned to one of three groups each of 10 according to the protective agent used: APF gel (1.23% F), NaF varnish (2.26% F), and CPP-ACPF paste (0.2% F). Half of the exposed enamel surface was protected with adhesive tape during the treatment of the remaining surface according to their group. Fluoride gel and CPP-ACPF paste were applied for 4 minutes and fluoride varnish for 24 hours. Six daily demineralisation–remineralization cycles of 5 minutes of immersion in a cola drink (pH 2.3) and 30 minutes in artificial saliva were conducted for 14 days. All specimens were stored in artificial saliva between and after cycles. Surface Vickers Micro-hardness readings were recorded at baseline and 14 days later for both halves. Percentage surface microhardness reduction (%SMHR) was then calculated. Data were analyzed using ANOVA and Duncan's post-hoc test ( $p < 0.05$ ). **Results:** For primary enamel, the mean ( $\pm SD$ ) % surface microhardness reduction (SMHR) for treated and untreated half was, respectively: gel ( $18.6 \pm 3.3$  and  $29.6 \pm 6.7$ ), varnish ( $29.3 \pm 9.6$  and  $33.9 \pm 12.2$ ), and CPP-ACPF paste ( $29.8 \pm 15.4$  and  $34.8 \pm 12.2$ ). For permanent enamel, such values were: gel ( $28.7 \pm 16.3$  and  $35 \pm 16$ ), varnish ( $42.9 \pm 2$  and  $49.6 \pm 1.6$ ) and CPP-ACPF paste ( $23.6 \pm 8.8$  and  $36.7 \pm 16.3$ ). Analysis of Variance (ANOVA) was used to compare between means of the three groups at  $P < 0.05$ . **Conclusions:** All of the tested fluoride treatments were able to reduce erosive enamel loss in both primary and permanent groups but both CPP-ACPF paste and APF gel showed significantly higher protective anti-erosive effect in permanent teeth. In primary teeth only APF gel showed significantly higher anti-erosive effect. Primary and permanent enamel substrates reacted differently to different fluoridated compounds.

[Sherine B Y Badr<sup>1</sup>, Mohamed A Ibrahim. Protective effect of three different fluoride treatments on dental erosion in primary and permanent teeth. Journal of American Science 2010;6(11):442-451]. (ISSN: 1545-1003).

**Keywords:** dental erosion, fluoride, CPP-ACPF paste, microhardness, Primary, permanent

#### Diatoms of Tropical Eutrophic Lagoon

[Full text](#)

<sup>1</sup>Paul. Chuks. Onuoha, <sup>2</sup>Dike Ikeagwu Nwankwo and <sup>3</sup>Vyverman, Wim

<sup>1</sup>Department of Fisheries and Marine Biology, Federal College of Fisheries and Marine Technology, Bar-beach Victoria Island, Lagos Nigeria.

<sup>2</sup>Department of Marine Sciences University of Lagos, Akoko, Lagos, Nigeria

<sup>3</sup>Protistology and Aquatic Ecology Research Laboratory, University of Ghent, Belgium

[hydro\\_vision@yahoo.com](mailto:hydro_vision@yahoo.com)

**Abstract:** The diatoms of Ologe lagoon for the first time were studied at monthly intervals for two years (February 2002-January 2004). A total of forty-eight species belonging to eighteen genera was found in diatoms, with pinnate forms being more diverse and less abundance than the centric forms. *Aulacoseira granulata*, *A. granulata* var. *angustissima*, *A. granulata* var. *angustissima f.spiralis*, *A. granulata* var. *angustissima f. curvata*, *A. granulata* var. *muzzaensis*, *A. islandica* and *Cyclotella meneghiniana* were

the more abundant and frequently occurring centric species throughout the study period. More frequently occurring pennate diatoms include: *Synedra ulna*, *Nitzschia closterium*, *Pinnularia major*, *Navicula oblonga*, *Cymbella minuta*, *Nitzschia palea*, *Suirella elegans* and *Gomphonema parvulum*. Rarely occurring diatoms at this station included *Biddulphia laevis*, *Melosira varians*, *Nitzschia accicularis*, *Pinnularia laevis*, *Cocconeis placentula* and *Eunotia gracilis*. In this study, six new diatoms species were recorded for Lagos lagoon complex. Community structure analysis shows a highly diverse environment.

[Paul Chuks Onuoha, Dike Ikeagwu Nwankwo and Vyverman, Wim. Diatoms of Tropical Eutrophic Lagoon. Journal of American Science 2010;6(11):452-456]. (ISSN: 1545-1003).

**Keywords:** diatom; Olage lagoon; genera; Lagos lagoon complex; diverse environment

#### Can Dermatoglyphics be used as an Anatomical Marker in Egyptian Rheumatoid Patients?

Hanan M. Elsaadany<sup>1</sup>, Elham Kassem<sup>1</sup>, Mervat El-Sergany<sup>\*1</sup> and Abdel -Razek A. Sheta<sup>2</sup>,

<sup>1</sup>Rheumatology & Rehabilitation, <sup>2</sup>Anatomy Departments, Faculty of Medicine, Tanta University, Tanta, Egypt. [\\*elahm77@hotmail.com](mailto:*elahm77@hotmail.com)

**Abstract:** Background/aim: Rheumatoid arthritis (RA) is supposed to be influenced by genetic and environmental factors and so also dermatoglyphics. Therefore, the present study was undertaken to find out a possible correlation of some quantitative and qualitative dermatoglyphic variables with rheumatoid arthritis (RA) and its radiological grading. Materials and methods: This study was conducted on 60 clinically confirmed RA patients and an equal number of controls. Different qualitative dermatoglyphic patterns (ulnar& radial loops, whorls and arches) and quantitative dermatoglyphic measures (total finger ridge count, pattern intensity and a-b ridge count) in addition to palmar creases were studied on rheumatoid arthritis patients and controls. Comparison between patients and controls in both sexes was done and recorded. Also, correlation between significant dermatoglyphic changes in RA patients and radiological changes were studied. Results: Loops were the most common type of the qualitative dermatoglyphic patterns of the fingers, followed by whorls then arches. In both male and female patients, there was significant marked decrease in ulnar loops and significant increase in arches. Total ridge count and pattern intensity of patients were decreased in both hands of both sexes; however, this decrease was significant in the left hand of males and right hand of females. Moreover, the a-b ridge count was significantly decreased in both hands of female and left hand of male patients. Regarding the unusual palmer flexion creases, there was significant increase only in the Sydney line in female right hands. Significant inverse correlation was noted between total ridge count of the fingers and the radiological erosion in both males and females. Conclusion: The findings of the present work demonstrate the association between some dermatoglyphic patterns and RA suggesting that dermatoglyphics can represent an anatomical, non-invasive, inexpensive tool for screening high-risk population, and thus facilitate early detection and management. Also the relationship between total ridge count and the aggressive type of RA indicate that this dermatoglyphic variable might play a significant role not only for screening but also for studying the behavior of the disease.

[Hanan M. Elsaadany, Elham Kassem, Mervat El-Sergany and Abdel-Razek A. Sheta. Can Dermatoglyphics be used as an Anatomical Marker in Egyptian Rheumatoid Patients. Journal of American Science 2010;6(11):457-466]. (ISSN: 1545-1003).

**Keywords:** dermatoglyphics, fingerprints, rheumatoid arthritis

#### High Efficiency Production of Mosquitocidal Toxin by a novel *Bacillus sphaericus* isolate from Egyptian Soils on Local Agroindustrial Byproducts

**M.S.Foda,<sup>1\*</sup> Fawkia M. El-Beih,<sup>2</sup> Maysa E. Moharam.<sup>1</sup> Nora N.A.El-Gamal<sup>1</sup>**

<sup>1\*</sup> Microbial Chemistry Department, National Research Center, Dokki, Giza, Egypt.

[Full text](#)

[Full text](#)

<sup>2</sup> Faculty of Science, Ain Shams University, Cairo, Egypt. \*[foda302002@yahoo.com](mailto:foda302002@yahoo.com)

**Abstract:** Eighty six cultures were isolated from soil of different Egyptian Governorates including Quina, El-Menofeya, El-Gharbia, El-Sharkia, El-Behera and Kafr EL-Sheikh Governorates. Investigations on the mosquitocidal Egyptian isolates have revealed that isolate No.1 have the ability to form more toxin than the international reference strain *Bacillus sphaericus* 2362(Bs 2362).The selected isolate No.1 exhibited a lower LC<sub>50</sub> and LC<sub>90</sub>values than the International strain *B.s* 2362 upon bioassay against second instars' larvae of *Culex pipiens*. The Egyptian isolate No.1was identified morphologically and biochemically as *Bacillus sphaericus*. Physiological factors affecting growth and toxin formation in *B. sphaericus* No 1 in comparison to *B.s* 2362 were carried out. THE organism grown on modified Nutrient broth medium yielded the highest larval toxicity against the second instars' of *Culex pipiens* for both *Bacillus sphaericus* isolate No 1 and the international strain *Bacillus sphaericus*. The Optimum air : medium ratio were 9:1 and 4:1 of the flask volume for 4 and 3 days incubation periods using 2%and 3% sizes of inocula for *B. sphaericus* 2362 and the Egyptian isolate *B. sphaericus*No.1, respectively. Sodium acetate was the suitable carbon source for the isolate *B. sphaericus* No.1, while *B.s* 2362 was capable to utilize both sodium acetate and sodium succinate as carbon sources.The Egyptian isolate *B. sphaericus* No.1exhibited the highest mosquitocidal activity upon growth on kidney beans seeds and sesame meal as nutrient substrates at 3% final concentration, while *B.s* 2362 exhibited the highest mosquitocidal activity by utilizing soy beans, lentils and sesame meal as complete media for growth and mosquitocidal toxin production.

[M.S. Foda, Fawkia M. El-Beih, Maysa E. Moharam, Nora N.A.El-Gamal. High Efficiency Production of Mosquitocidal Toxin by a novel *Bacillus sphaericus* isolate from Egyptian Soils on Local Agroindustrial Byproducts. Journal of American Science 2010;6(11):467-475]. (ISSN: 1545-1003).

**Key words:** *Bacillus sphaericus*, isolation, characterization, mosquitocidal toxin, physiology, agroindustrial byproducts

#### Novel Validated Chromatographic Method for Determination of Some Anti-hypertensive Drugs

M. Farouk<sup>1</sup>, O. Abd ELAziz<sup>1</sup>, A. Hemdan<sup>\*2</sup>, M. Shehata<sup>2</sup>

[Full text](#)

<sup>1</sup> Analytical Pharmaceutical Chemistry Department, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt. <sup>2</sup> Pharmaceutical Chemistry Department. Faculty of Pharmacy, Ahram Canadian University, 6<sup>th</sup> October, Egypt. \*[hemmdan@yahoo.com](mailto:hemmdan@yahoo.com)

**Abstract:** Accurate, precise and reproducible isocratic RP-HPLC method was developed and subsequent validated for the analysis of Torasemide (I), Irbesartan (II) and Olmesartan medoxomil (III) at ambient temperature, using Atlantis 4.6 mm x 250 mm RP-C18 Column, with a flow rate of 1.5 ml.min<sup>-1</sup>, and UV. detector at 288 nm and 260 nm for (I) and (II and III), respectively. By adopting the mentioned chromatographic technique, (I) and (III) were determined in the presence of their acidic and alkaline-degradates separately as stability-indicating methods utilizing phosphate buffer pH = 3:acetonitrile (60:40, v/v), phosphate buffer pH = 3.2:acetonitrile (60:40, v/v) as a mobile phase, respectively, while (II) was determined in presence of Hydrochlorothiazide (HCTZ), using phosphate buffer pH = 4:acetonitrile (70 :30, v/v). All the proposed methods were validated according to the International Conference on Harmonization (ICH) guidelines and successfully applied to determine the mentioned studied drugs in pure form, in laboratory prepared mixtures and in pharmaceutical preparations. The obtained results were statistically compared to the reference methods of analysis [for I and "II and III", respectively] and no significant differences were found.

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[M. Farouk, O. Abd ELAziz, A. Hemdan, M. Shehata. Novel Validated Chromatographic Method for Determination of Some Anti-hypertensive Drugs. Journal of American Science 2010;6(11):476-486]. (ISSN: 1545-1003).

**Keywords:** Torasemide, Irbesartan, Olmesartan medoxomil, High Performance Liquid Chromatography, Stability Indicating method

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Diversity of *Staphylococcus aureus* Isolated from Human and Bovine Estimated by PCR - Gene Ana

**<sup>1</sup>J.El-Jakee, <sup>2</sup>Ata S. Nagwa , <sup>1</sup>Gad El-Said, W.A., <sup>2</sup>Bakry,M.A., <sup>2</sup>Samy, A.A., <sup>2</sup>Khairy E.A., <sup>2</sup>Elgabry , E.A.**

<sup>1</sup> Department of Microbiology Faculty of Veterinary Medicine

<sup>2</sup> Department of Microbiology & Immunology National Research Center, Cairo Egypt

**Abstract:** The present investigation studied the diversity of 19 *S. aureus* isolates (9 from bovine and 10 from human sources) in comparison with the standard Cowan I strain by conventional methods and by PCR technology. The latter uses primers targeted to species-specific parts of genes encoding coagulase (*coa*), enterotoxin A (*sea*) and B (*seb*), *mec A* gene encoding methicillin resistant *S. aureus* (MRSA) and *Staphylococcus* protein A (*spa*) gene. *S. aureus* isolates (19) as well as the Cowan 1 strain were tested for antimicrobial sensitivity with 15 antibiotics by disk diffusion method and classified as susceptible, intermediate and resistant. 57.9% of isolates had a relatively high molecular weight plasmid. The *mec A* gene among the chosen MRSA *S. aureus* isolates recovered from human and bovine sources was discussed. Polymorphisms of *coa* and *spa* genes were detected among *S. aureus* isolates. The examined isolates had coagulase gene ranging from 423 bp to 658 bp and the Cowan -1 strain had amplified fragment at 642 bp. All examined *S. aureus* isolates gave an amplified *spa* gene product at approximately from 396-464 bp. The prevalence of enterotoxin genes *sea* and *seb* were determined and the diversity among the chosen isolates was recorded.

[J.El-Jakee, Ata S. Nagwa, Gad El-Said, W.A., Bakry,M.A., Samy, A.A., Khairy E.A., Elgabry, E.A. Diversity of *Staphylococcus aureus* Isolated from Human and Bovine Estimated by PCR - Gene Analysis. Journal of American Science 2010;6(11):487-498]. (ISSN: 1545-1003).

**Keywords:** *S. aureus*, antibiogram sensitivity, MRSA, Enterotoxins, coagulase gene, *spa* gene

[Full text](#)

**Implementation of a rapid procedure for distinguishing enterotoxigenic Clostridium perfringens**

**<sup>1</sup>J. El-Jakee, <sup>2</sup>Ata S. Nagwa, <sup>2</sup>Bakry,M.A., <sup>2</sup>Sohier, M. Syame, <sup>2</sup>Samy A.A., <sup>2</sup>Khairy E.A.**

1Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt

2Department of Microbiology and Immunology National Research Center, Cairo, Egypt

**Abstract:** The objective of the present study is to develop an easy method for detection of toxigenic *C. perfringens* isolates .4 *C. perfringens* isolates (type A, B, C & D) were collected from chickens and reconfirmed on the basis of conventional tests and multiplex PCR. Antisera were prepared from *C. perfringens* types A, B, C & D separately in different groups of rabbits. The titres of the prepared hyperimmune sera were estimated by ELISA & staphylococci protein A (SpA) agglutination test. An attempt was carried out to detect *C. perfringens* toxins in infected fecal samples. The fecal samples were infected by 20 &40 µg /ml *C. perfringens* toxins (A, B, C & D) and examined by double sandwich ELISA & SpA agglutination methods. In addition the sensitivity of PCR for detection of *C. perfringens* types were compared with conventional culture technique among fecal samples contaminated with *C. perfringens* types and the results were discussed.

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[J. El-Jakee, Ata S. Nagwa, Bakry, M.A., Sohier, M. Syame, Samy A.A., Khairy E.A. Implementation of a rapid procedure for distinguishing enterotoxigenic Clostridium perfringens. Journal of American Science 2010;6(11):499-508]. (ISSN: 1545-1003).

**Keywords:** *C. perfringens*, ELISA, SpA agglutination, PCR

[Full text](#)

**Comparative Study of Software Engineering Processes in Egyptian Cmmi Companies**

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Alaa El-Din Hamouda and Mohammad Abdrabo Elwahsh

Computers & Systems Engineering Dept., Al-Azhar University Cairo, Egypt.

[Alaa\\_ham@giga.net](mailto:Alaa_ham@giga.net), [eng.md.elwahsh@gmail.com](mailto:eng.md.elwahsh@gmail.com); [www.elwahsh.com](http://www.elwahsh.com)

**Abstract:** The Egyptian government has paid special attention to the software industry as Egypt to provide it with a competitive advantage that makes this emerging industry promising. Thus, the State has supported the Egyptian companies to make use of the Capability Maturity Model Integration (CMMI). Since 2009, more than thirty companies obtained the CMMI at different levels. However, these companies suffer from lack of a mechanism to exchange experience and information among themselves although they could be similar in the culture of their engineers and perhaps in the nature and size of their software projects. So, we provide in this research a survey to gauge the quality of methods, tools and processes used in these Egyptian companies winning the CMMI. Then we analyzed the results to reach the recommendations aimed at enriching the software industry in Egypt.

[Alaa El-Din Hamouda and Mohammad Abdрабو Elwahsh. Comparative Study of Software Engineering Processes in Egyptian Cmmi Companies. Journal of American Science 2010;6(11):509-514]. (ISSN:1545-1003).

**Keywords:** CMMI in Egypt, software engineering processes, survey

**Prevalence of *S. aureus* and *S. epidermidis* among patients with indwelling catheters and their antibiogram using some commonly used antibiotics.**

[Full text](#)

\*De, N. and Godlove, M.

Department of Microbiology, Federal University of Technology, Yola, Adamawa State, Nigeria

**e-mail:** nanditamicrobio@yahoo.com; \*corresponding author

**Abstract:** The objectives of this study were to find the prevalence of *S. aureus* and *S. epidermidis* in urine samples of patients placed on catheter in Federal Medical Centre, Yola (FMCY) and State Specialist hospital, Yola (SSHY) and the efficacy of some commonly used antibiotics against the isolates of *S. aureus* and *S. epidermidis*. A total of one hundred and five samples (60 from SSHY and 45 from FMCY) were collected and inoculated into Cystine lactose electrolyte deficient (CLED) agar for isolation of Staphylococcal species. A total of seventy six presumptive Staphylococcal isolates were obtained on CLED agar and these isolates were identified using gram staining, morphological characteristics and standard biochemical tests. Serological studies revealed that out of 76 isolates, 56 were *S. epidermidis* (coagulase negative) and 20 were *S. aureus* (coagulase positive). Fifty one point eight percent (51.8%) of the isolates of *S. epidermidis* were sensitive to ceftazidime followed by ciprofloxacin (46.4 %) whereas 45% of the isolates of *S. aureus* were sensitive to ceftriaxone followed by cefotaxime and ciprofloxacin (40%).

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Key words: coagulase, CLED agar, catheters, *S. aureus*, *S. epidermidis*

[De, N. and Godlove, M. Prevalence of *S. aureus* and *S. epidermidis* among patients with indwelling catheters and their antibiogram using some commonly used antibiotics. Journal of American Science 2010;6(11):515-520]. (ISSN: 1545-1003).

**Keywords:** ciprofloxacin; coagulase; CLED agar, catheters, *S. aureus*, *S. epidermidis*

[Full text](#)

**Biochemical and Molecular Genetic Studies on Rice Tolerance to Salinity**

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El-Mouhamady, A. A.; I. S. El-Demandash and K. A. Aboud\*

\*Genetics Department , National Research Centre , Cairo, Egypt

**Abstract:** The present investigation was carried under green house conditions during 2009 and 2010

seasons included two conditions (normal irrigation and salinity) using model of half diallel analysis by five cultivars of rice “Gz1368-s-5-4, Hybrid1, Sakha102, Giza 181 an IE1444”. Five parents and ten crosses were grown under two conditions and the results showed that: (1) The most desirable mean value and positive and highly significant for heterosis, general and specific combining ability effects for all genotypes under normal and salinity conditions were observed from the genotypes.; Gz1368-s-5-4, hybrid1, IET1444, Gz1368-s-5-4 x hybrid 1, Gz1368-s-5-4 x IET1444, Hybrid 1 x IET1444, Sakha 102 x IET1444 and Giza 181 x IET 1444 for all traits studied. (2) From the foreign discussion, it could be concluded that the crosses Gz1368-s-5-4 x Hybrid 1, G21368-s-5-4 x IET1444, Hybrid 1 x IET1444 and Giza 181 x IET1444 were contained of 1, 5, 1 and 56 and 5 using PM15 primer, 6, 3, 6 and 6 bands using AY334988 and 6, 2, 4 and 5 bands using HL-17 primer, which indicated that these bands were found to be index and marker for salinity tolerance in rice by increasing K<sup>+</sup> content and decreasing of Na<sup>+</sup> content.

[El-Mouhamady, A. A.; I. S. El-Demardash and K. A. Aboud. Biochemical and Molecular Genetic Studies on Rice Tolerance to Salinity. Journal of American Science 2010;6(11):521-535]. (ISSN: 1545-1003).

**Keywords:** heterosis,, half diallel analysis, salinity tolerance, RAPD

**Effect of Adding Urea or Ammonium Sulphate on some Herbicides Efficiency in Controlling Weeds in Onion Plants**

[Full text](#)

<sup>1</sup>El-Metwally, I. M.; <sup>\*1</sup>Kowthar G. El-Rokiek; <sup>1</sup>Salah A. Ahmed; <sup>1</sup>Ebrahim R. El-Desoki and  
<sup>2</sup>Emad E. H. Abd-Elsamad

<sup>1</sup>Botany Dept., National Research Centre, Dokki, Cairo,Egypt.

<sup>2</sup>Vegetable Crops Research Dept., National Research Centre, Dokki, Cairo, Egypt.  
[ahmed\\_ezat2000@yahoo.com](mailto:ahmed_ezat2000@yahoo.com)

**Abstract:** Two field experiments were conducted during two successive seasons of 2008/2009 and 2009/2010 at the Agricultural Experiments Station of the National Research Centre at Nobariya, Behaira Governorate, Egypt, to study the effect of adding urea or ammonium sulphate at 2% to herbicide solution on weed control efficiency in onion fields. Weed control treatments were as follows: Metosulam at 20 ml/fed or Clodinafop-propargyl at 70g/fed with or without addition of urea or ammonium sulphate (AMS) at 2% of herbicide solution in comparison to Metosulam at 40 ml/fed, Clodinafop- propargyl at 140g/ fed, Metosulam at 20 ml + Clodinafop- propargyl at 70 g / fed, two hand hoeing and unweeded check. All weed control treatments significantly depressed weed growth when compared to the unweeded one. Two hand hoeing showed the best control of broadleaved weeds in both seasons, followed by that of Metosulam at 40 ml, Metosulam + urea and Metosulam + AMS treatments, respectively. Clodinafop – propargyl at 140 g, Clodinafop – propargyl at 70 g, Clodinafop – propargyl + urea, Clodinafop – propargyl + AMS and Metosulam + Clodinafop – propargyl were very effective in controlling most grass weeds. Meanwhile, hand hoeing , Metosulam + Clodinafop – propargyl, Metosulam at 40 ml and Clodinafop – propargyl at 140 g /fed were the most effective in controlling onion weeds. All herbicidal treatments as well as hand hoeing markedly increased onion yield in both seasons. Maximum values of bulb length, diameter, weight and bulb yield (t/fed) were recorded from Metosulam + Clodinafop – propargyl, Metosulam at 20 ml and hand hoeing twice.

[El-Metwally, I. M.; Kowthar G. El-Rokiek; Salah A. Ahmed; Ebrahim R. El-Desoki and Emad E. H. Abd-Elsamad. Effect of Adding Urea or Ammonium Sulphate on some Herbicides Efficiency in Controlling Weeds in Onion Plants. Journal of American Science 2010;6(11):536-543]. (ISSN: 1545-1003).

**Keywords:** Onion, Urea, Ammonium sulphate, Metosulam, Clodinafop-propargyl, weeds

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**Prevalence of gastrointestinal parasites infections in sheep in the Zoo garden and Sinai district and**

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**study the efficacy of anthelmintic drugs in the treatment of these parasites.**

Abouzeid. N.Z.<sup>1</sup>; Selim. A. M.<sup>1</sup> and El-Hady K. M.<sup>2</sup>

1. Department of Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, Egypt.

2. Veterinay Clinic Faculty of Veterinary Medicine, Zagazig University, Egypt.

[dr\\_nasser\\_zeidan@yahoo.com](mailto:dr_nasser_zeidan@yahoo.com)

**Abstract:** A survey of the prevalence of gastro-intestinal tract (GIT) parasites in 240 sheep was conducted in different area in the zoo garden (110) and in Sinai district (130) during the period of March 2009 to February 2010. The overall prevalence of infections with nematodes; fasciola and coccidiosis in sheep in Sinai and zoo garden were 66/240 (27.5%); 24/240 (10.0%) and 16/240 (6.7%) respectively. Of the 240 examined sheep, 12.5%; 0.0% and 8.6 % young lambs (1-6 month), 37.7%; 6.9 % and 9.2 % immature sheep (>6-12 months) and 17.1 %; 21.4 % and 1.4 % adult sheep (>one yr) were infested with nematodes, fasciola and coccidia respectively. Most of the animals examined during the present survey had low to moderate infestation. Serum biochemical parameters revealed that serum calcium, inorganic phosphorus, magnesium, copper and iron levels were significantly decreased in all parasitic infested animals. All treated sheep showed significant improvement & disappearance of most clinical signs and significant decrease of egg per gram (EPG) with complete disappearance of eggs in 5<sup>th</sup> day; 4<sup>th</sup> day and 6<sup>th</sup> day post treatment with albendazole (valbazine); doramectin (dectomax) and trichlabendazole (fasinex) respectively. There were gradual increases in the levels of biochemical parameters in 3 groups after one and two weeks post treatment and their levels reached nearly similar to standard levels after 3 week post treatment. Study surveys suggest, appropriate parasitic control approach be explored and tried in order to alleviate the problem of worm burden.

[Abouzeid. N.Z.; Selim. A. M. and El-Hady K. M. Prevalence of gastrointestinal parasites infections in sheep in the Zoo garden and Sinai district and study the efficacy of anthelmintic drugs in the treatment of these parasites. Journal of American Science 2010;6(11):544-551]. (ISSN: 1545-1003).

**Key words:** gastro intestinal parasites, sheep, Zoo garden, Sinai, anthelmintics & biochemical parameters

#### Diet quality in Egyptian Obese Children and Adolescents

[Full text](#)

**Nayera El-morsi Hassan<sup>\*1</sup>, Safaa,T, Zaki<sup>2</sup>, Azza,Gabr<sup>2</sup> and Hala El gindi<sup>2</sup>**

Biological Anthropology Department<sup>1</sup>, Child Health Department<sup>2</sup> National Research Center, Cairo, Egypt

[\\*safaazaki@hotmail.com](mailto:*safaazaki@hotmail.com)

**Abstract:** The epidemic increase in the prevalence of obesity is now seen in most countries. Dietary composition, the relative proportions of calories coming from fats, carbohydrates, protein and intake of fiber has been suspected of playing a role in obesity. So, the aim of the present study was to analyze the diet quality and also to determine if an association exists between obesity and the relative percentage of fats, carbohydrates, protein and fiber in the diets of children and adolescent. A cross-sectional survey, comprised 5760 children (2638 boys and 3122 girls) was recruited from 6 public schools. Each child underwent a complete physical examination, including anthropometric measures. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared. Only one thousand and one hundred children of the total sample (19.1%), (417 boys and 683 girls, aged 13.43±2.65 years), with the complaint of obesity, were included. Repeated Twenty-four hour recall method, record food intake for three scattered days (3 recalls), includes one day as a holiday was done to assess the nutritional status of obese children. Nutrient intake were calculated using the computer program World Food Dietary Assessment System<sup>(1)</sup> compared with National Research Council USA1989<sup>(2)</sup>, while vitamins and minerals were compared with USDA, 2005<sup>(3)</sup>. This study highlights the importance of

nutritional data that it is not what you eat but rather how much the total number of calories consumed which contributes to obesity. Success in obesity prevention is most likely to be achieved when preventive measures are initiated early and sustained throughout childhood and adolescence. More researches must be done for more evaluation, also, to achieve physical activity and life style for obese children and adolescence.

[Nayera El-morsi Hassan, Safaa,T, Zaki, Azza,Gabr and Hala El gindi. Diet quality in Egyptian Obese Children and Adolescents. Journal of American Science 2010;6(11):552-558]. (ISSN: 1545-1003).

**Keywords:** Diet quality, obese, children, adolescents

**Improvement Growth, and Yield of Wheat Plants Grown Under Salinity Stress by Using Silicon**  
Abd El-Monem M. Sharaf

[Full text](#)

Botany and Microbiology Dept. Faculty of Science, Al-Azhar Univ. Cairo, Egypt,  
[sharaf5858@yahoo.com](mailto:sharaf5858@yahoo.com)

**Abstract:** The present study aims to improvement of wheat production under saline conditions using silicon (Si) treatment. Salinity caused significant reduction in the growth parameters, photosynthetic pigments and yield components of wheat plants. The magnitude of reduction increased by increasing salinity level. Significant increases were observed in activities of superoxide dismutase (SOD), peroxidase (POX), catalase (CAT) and glutathione reductase (GR) in shoots of salt stressed plants. Silicon treatment in absence and presence of NaCl had great changes on most of the assayed parameters. The adverse effects of salinity as regards the growth characters, photosynthetic pigments and yield components were significantly mitigated by Si supplement. Application of Si caused great variations in the activities of antioxidant enzymes. Under normal (Non-saline) condition, addition of Si, especially at 1 mM, markedly increased the activity of both SOD and CAT, however activity of both POX and GR was significantly decreased. Addition of Si markedly reduced the increases in the activities of SOD, POX, CAT and GR were observed in salt stressed plants. Great variations were also observed as regards the contents of endogenous phytohormones in response to Si, NaCl and their interactions. Generally, it could be concluded that Si have (to more extent) a beneficial regulatory role in plants grown under salt stress conditions.

[Abd El-Monem M. Sharaf. Improvement Growth and Yield of Wheat Plants Grown Under Salinity Stress by Using Silicon. Journal of American Science 2010;6(11):559-566]. (ISSN: 1545-1003).

**Key words:** *Triticum aestivum*; NaCl; Silicon; Photosynthetic pigments; Antioxidant enzymes

**Re-introduction of Elephant's Infant into Wild Group: First Attempt and Case Study from North-West India**

[Full text](#)

**Ritesh Joshi**

Doon Institute of Engineering and Technology, Shyampur, Rishikesh, Dehradun, 249 204, Uttarakhand, India. E-mail: [ritesh\\_joshi2325@yahoo.com](mailto:ritesh_joshi2325@yahoo.com)

**Abstract:** Elephant's infant is considered extremely difficult to re-introduce into the wild. On 21<sup>st</sup> of November 2009, an eight day old elephant's infant was found strayed from its group at Shyampur forest of the Haridwar forest division. For the first time in the history of Uttarakhand, attempts had been made by forest officials to re-introduce the orphaned baby elephant into the wild. It is noteworthy that during the introduction attempts, group of seven elephants had taken the baby within group, but left her behind after a while. They had responded from all directions to the cries of the baby elephant but the attempts were in vain. Radha – the domesticated elephant at Chilla forest of the Rajaji National Park nurtured the infant for 10 days before infant's death and Radha's behaviour always illuminated something new about elephant's life. It was the first attempt to re-introduce the infant to wild in north-west India in which some lessons came forward and could be helpful in management of elephants and in documentation of conservation-oriented action plan. Additionally, studies on the behaviour of wild elephants are highly

required and recommended so that we can ensure the future survival of this endangered species.

[Ritesh Joshi. Re-introduction of Elephant's Infant into Wild Group: First Attempt and Case Study from North-West India. Journal of American Science 2010;6(11):567-570]. (ISSN: 1545-1003).

**Keywords:** Asian elephants, infant, re-introduction, conservation, north-west India

An Approach To Partially Import The Ontologies On Semantic Web Based Upon User Choice

Tayybah Kiren<sup>1</sup>, Muhammad Shoaib<sup>1</sup>, Muhammad Tariq Pervez<sup>2</sup>, Sonia Majid<sup>3</sup>, Qazi Mudassar Illyas<sup>4</sup>

<sup>1</sup>Department of CS & E, University of Engineering & Technology, Lahore Pakistan

<sup>2</sup>Department of CS, Virtual University of Pakistan, Shadman Campus, Shadman Market, Lahore, Pakistan

<sup>3</sup>Lahore College for Women University, Lahore Pakistan

<sup>4</sup>COMSATS Institute of Information Technology, Abbottabad, Pakistan

[tariq\\_cp@hotmail.com](mailto:tariq_cp@hotmail.com)

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**Abstract:** With the increase in applications using ontologies to represent semantic information, the issue of partially reusing the ontologies is getting more focus of researchers. Ontology construction from scratch is protracted and labor intensive job. Therefore, it is good to fabricate the ontologies by reusing the existing ontologies. Existing techniques for partially importing the ontology do not consider the user choice while selecting the most relevant ontologies for reusing. Most of the approaches have restriction on the size of ontology that is to be modularized. An approach for partially importing the ontologies has been presented in this paper. The proposed technique selects important keywords from a document by calculating term frequency, IR measure and precision along with class match measure to rank the most relevant ontologies. An algorithm to extract ontology fragments has been presented. This algorithm is independent of the size of ontology being reused.

[Tayybah Kiren, Muhammad Shoaib, Muhammad Tariq Pervez, Sonia Majid, Qazi Mudassar Illyas. An Approach To Partially Import The Ontologies On Semantic Web Based Upon User Choice. Journal of American Science 2010;6(11):571-581]. (ISSN: 1545-1003).

**Keywords:** semantic web; ontology; partial import; knowledge management; user choice; term frequency

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### The Use of Lemongrass Extracts as Antimicrobial and Food Additive Potential in Yoghurt

**Shaaban, M. Abd-El Fattah<sup>\*1</sup>; Abo sree, Yahia Hassan<sup>1</sup>; Hala M. Bayoum<sup>2</sup> and Hesham A. Eissa<sup>3</sup>**

Food Toxins and contaminants Department<sup>1</sup>, Dairy Department<sup>2</sup>, Food Technology Department<sup>3</sup>, National Research Centre, Cairo, Egypt. [shaabanmostafa@yahoo.com](mailto:shaabanmostafa@yahoo.com)

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**Abstract:** The following study was conducted to investigate the antifungal and food additive potential of medicinal plants. herbal decoction and essential oil (EO) extracts of *Cymbopogon flexuosus* (lemongrass) leaves and stems were tested for their inhibitory action against spoilage organisms and mycotoxins formation in two separated experiments. In the first experiment, yeast- extract sucrose medium (YES) was used as a basal medium to examine the mold growth and mycotoxin production by three pathogenic fungi: *Aspergillus flavus* (*A. flavus*), *Aspergillus parasiticus* (*A. parasiticus*) and *Aspergillus ochraceus* (*A. ochraceus*). The YES medium was supplemented with four different concentrations of Lemongrass oil, inoculated with 1-mL of a spore suspension containing  $10^5$ - $10^6$  conidia of each test mold and then incubated at 28° C for 14 days. After incubation period, cultures were analyzed for mycelial dry weight and mycotoxin accumulation. In the second experiment, yoghurt

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medium was used as a basal medium and the same system of study was applied in two different degrees of temperature (5°C and 28°C) for 4 weeks. Evaluation of the Lemongrass oil activity in yoghurt samples focused on the microbial stability of yoghurt, sensory evaluation as well as mold growth and mycotoxin formation. In the 1<sup>st</sup> experiment, the level of 0.1% of the EO extract was effective in inhibition both mold growth and mycotoxin production for all tested molds, and 0.3 % extract completely prevented the growth and toxin production. whereas, 1% of the decoction extract was effective. So, the EO extract was the suitable agent in the second experiment. It is of interest to note that while reduction in mold growth due to increasing extract concentrations was observed, the most striking effect was the reduction of mycotoxin production. The obtained data from the second experiment showed that the EO extract (0.1% concentration) inhibited viable yeasts and preserved yoghurt for over 28 days at 5°C. Also, the inhibitory action of the EO extract against yeasts was concentration dependent. The maximum inhibitory effect of was found when the extract level increased above 0.1%. Incubation temperature had an important role in growth and mycotoxin production in yoghurt medium. During cold storage for 28 days at 5°C, the different concentrations of the EO extract added to yoghurt samples displayed different titratable acidity and total bacterial cells and pH than the control yoghurt ( $p < 0.05$ ). Overall sensory acceptability of yoghurt supplemented with the EO extract was higher than that of the control yoghurt prepared without the EO extract. The results indicate that the addition of the appropriate the EO concentration (0.1%, w/v) improved the physicochemical properties as well as sensory characteristics of yoghurt, could be used for decontamination of dairy products such as yoghurt from mycotoxicogenic fungi and mycotoxins formation, beside its beneficial properties as a functional food.

[Shaaban, M. Abd-El Fattah; Abo sree, Yahia Hassan; Hala M. Bayoum and Hesham A. Eissa. The Use of Lemongrass Extracts as Antimicrobial and Food Additive Potential in Yoghurt. Journal of American Science 2010;6(11):582-594]. (ISSN: 1545-1003).

**Key words:** Yoghurt, lemongrass, molds, yeasts, mycotoxins, aflatoxins, ochratoxin A, food additives

#### The Assessment Of Mycotic Settlement Of Freshwater Fishes In Egypt

[Full text](#)

Refaai, M.K.<sup>1</sup>, Laila, A. Mohamed<sup>2</sup>, Amany, M. Kenawy<sup>2</sup>, and Shima, El-S.M.A.\*<sup>2</sup>

<sup>1</sup> Microbiology Dept., Faculty of Vet.Medicine, Cairo University, Giza, Egypt.

<sup>2</sup> Hydrobiology Dept., National Research Center. Dokki, Giza, Egypt.

\* [shimaakhalifa2003@yahoo.com](mailto:shimaakhalifa2003@yahoo.com)

**Abstract:** This study was carried out on 360 freshwater fishes (240 *Oreochromis* species and 120 *Clarias gariepinus*). They were collected from different governorates and during different seasons. Naturally infected fishes showed clinical abnormalities such as skin darkening, exophthalmia, corneal opacity, abdominal distention, ulceration of the skin and cotton wool like growths on various parts of the body. Fishes were then subjected to post mortem examination which revealed many abnormalities. Mycological examination revealed the isolation of 2081 fungal isolates from 150 diseased and 210 apparently healthy fish samples (1658 mould and 423 yeast isolates), of which 1334 were isolated from *Oreochromis* species and 747 isolates from *Clarias gariepinus*. Isolated moulds belonged to the following genera: Saprolegnia (4.2%), Aspergillus (43.0%), Fusarium (14.1%), Mucor (14), Penicillium (17.2), Rhizopus (4.8%), Scopulariopsis (1.2%), Paeciliomyces (1%) and Curvularia (0.4%). Yeasts isolated also from both fish species had the following incidence: *Candida albicans* (35.9 %), other *Candida* species (19.1%), *Rhodotorula* species (31.4%) and *Torulopsis* species (13.5%). Experimental infection with the most predominant fungi (Aspergillus flavus, Fusarium species and *Candida albicans*) was conducted to evaluate the pathogenicity of these isolates. Clinical pictures of experimentally infected fish were similar to those of natural infection. Inoculated fungi were re-isolated from different organs. Results were confirmed with histopathological examination, which revealed the presence of fungal hyphae and spores in different organs.

[Refaai, M.K., Laila, A. Mohamed, Amany, M. Kenawy, and Shima, El-S.M.A. The Assessment Of Mycotic Settlement Of Freshwater Fishes In Egypt. Journal of American Science 2010;6(11):595-602].

**Keywords:** Mycotic infection, Oreochromis species, Clarias gariepinus, Moulds, Yeasts, Aspergillus, Fusarium, Candida, Penicillium

### Bacterial Infections Affecting Marine Fishes In Egypt

**M. Moustafa<sup>1</sup>, Laila. A. Mohamed<sup>2</sup>, M.A. Mahmoud<sup>3</sup>, W.S. Soliman<sup>2</sup>, A.E. Eissa<sup>1</sup> and M.Y. El-gendy<sup>\*2</sup>**

[Full text](#)

<sup>1</sup> Department of Fish Diseases and Management, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt; <sup>2</sup> Department of Hydrobiology, National Research Center, Dokki, Egypt; <sup>3</sup> Department of Pathology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

<sup>\*</sup>[mamdochyouosif@yahoo.com](mailto:mamdochyouosif@yahoo.com)

**Abstract:** Marine fishes are suffering from continuous depletion due to bacterial pathogens infections triggered by devastating environmental changes at their native aquatic environment. Qarun Lake and Suez Gulf are among the most vulnerable areas. 600 fish samples of Six different fish species; *Epinephelus tukula*, *Siganus rivulatus*, and *Dedalechilus labiosus* native to Suez-gulf at Suez governorate; *Mugil capito*, *Solea vulgaris* and *Tilapia zilli* native to Qarun Lake at El-Fayoum governorate were examined throughout the different year seasons. Gram positive and negative fish pathogenic bacteria were isolated from a total of 245 fish sample. Among those samples, the following bacteria were retrieved in the following percentages respectively, 17.55% (*Vibrio anguillarum*), 16.73% (*Vibrio alginolyticus*), 15.51% (*Pasteurella piscicida*), 15.91% (*Pseudomonas fluorescens*), 13.46% (*Streptococcus faecalis*), 11.02% (*Aeromans hydrophila*), 6.12% (*Aeromans sobria*) and 3.67% were infected with *Staph. aureus*. The *Siganus rivulatus* was the highest infected fish species with a prevalence of 8.33%, while *Mugil capito* was the lowest infected species (5.67%). The highest total prevalence of bacterial infection was recorded in summer season (40.81%) while the lowest was recorded in winter (15.91%). The aforementioned bacterial isolates were successfully re-isolated from experimentally infected fish. The retrieved isolates were confirmed by semi-automated (API 20 E) and conventional biochemical tests.

[M. Moustafa, Laila. A. Mohamed, M.A. Mahmoud, W.S. Soliman, A.E. Eissa and M.Y. El-gendy. Bacterial Infections Affecting Marine Fishes In Egypt. Journal of American Science 2010;6(11):603-612]. (ISSN: 1545-1003).

**Keywords:** Marine fishes, Bacterial diseases, Diagnosis, seasonal variation

### Effect of Antioxidant on Lead-Induced Oxidative Damage and Reproductive Dysfunction in Male Rabbits

[Full text](#)

<sup>\*</sup>**El-Tohamy, M.M., and El-Nattat,W.S.**

Department of Animal Reproduction and A.I., National Research Centre, Cairo, Egypt.

<sup>\*</sup>[eltohamymagda@yahoo.com](mailto:eltohamymagda@yahoo.com)

**Abstract:** The objective of this study was to characterize the Lead toxicity syndrome, to assess biomarkers that may be most useful for detecting toxicant-induced reproductive dysfunction, and to determine whether supplemental vitamin C would tend to alleviate the lead toxicity in rabbits. To test the hypothesis that the level of lead exposure is associated with an adverse effect on semen quality, in terms of sperm concentration, morphology, motility to assess antioxidant as important markers of disease using total antioxidant status. Adverse effects of lead on the testes may be mediated by oxidative damage and subsequent lipid peroxidation. The effect of lead acetate administration on testicular, hepatic and renal functions and the biomarker of effect for them were investigated in the present study with a trial of treatment by vitamin C. 35 male rabbits were divided into five groups. One control group and four groups received orally low and high doses of lead acetate (10.8 and 15 mg/kg b.wt.,

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respectively). One low and one high received, in addition, 1 g vitamin C / L in drinking water. SOD,  $\gamma$ -GT, AST, ALT, cholinesterase, acid phosphatase, and LDH activities were measured in both serum and semen. Also semen characteristics were measured. Results concerning all the enzymes were promising. SOD, LDH, ALT and acid phosphatase activities in serum and semen were obviously affected by lead. Vitamin C was a good antioxidant that recuperates from the normal enzymatic status in both serum and semen. In conclusion, lead levels led to testicular hypo function, which is supported by the results of semen picture. The hazardous effect of lead led to disturbance in the activities of enzymes under investigation such as SOD,  $\gamma$ -GT, LDH, AST, ALT, Cholinesterase, Acid phosphatase. Vitamin C proved its antioxidant effect on recuperating from the normal status of enzymes in semen and serum. LDH and prostatic acid phosphatase are shown to be biomarkers of testicular dysfunction, while LDH, ALT may be used as biomarkers for hepatic and renal dysfunction. This study established the principle that lead toxicity can be prevented and makes it worthwhile to establish an acceptable treatment or preventive regimen in the light of the present results.

[El-Tohamy, M.M., and El-Nattat,W.S. Effect of Antioxidant on Lead-Induced Oxidative Damage and Reproductive Dysfunction in Male Rabbits. Journal of American Science 2010;6(11):613-622]. (ISSN: 1545-1003).

**Keywords:** Effect; Antioxidant; Lead; Oxidative Damage; Reproductive Dysfunction; Male; Rabbit

#### **Tara Gum Carbamate: A New Thickening System for Cotton Printing Using Vat Dyes**

A. Hebeish<sup>1</sup>, A.A. Ragheb<sup>1</sup>, S.H.Nassar<sup>1</sup>, E.Allam<sup>2</sup> and J.I. Abd El -Thalouth<sup>2</sup>

<sup>1</sup>Textile Research Division, National Research Center Dokki , Cairo Egypt

<sup>2</sup> Faculty of Applied Arts, Helwan University, Cairo, Egypt

**Abstract:** Green technology-based textile thickeners, namely, glactomannan was isolated from tara seeds and harnessed to vat printing of cotton fabrics before and after being carbamated. Carbamation was effected through reaction with urea at 160 °C for 15 and 90 min. to produce tara carbamate derivatives having 1% N and 3.12 % N respectively. These derivatives are soluble in water at room temperature and characterized by non-Newtonian pseudoplastic behaviour. However, for a given rate of shear, tara carbamate derivative having 1% N exhibits lower apparent viscosity than the derivative with 3.12 % N. On the other hand the apparent viscosity of pastes prepared from these two derivatives increases by storing for 24 or 48 hours before commence measuring. Prints could successfully be achieved using either of the two new tara carbamate derivatives in single use or in admixture with conventional thickener viz. Lameprint A6. Colour strength (K/S) values of prints prepared using the new tara carbamate -based thickeners are higher than those obtained with the conventional thickener, meanwhile the overall fastness properties of all prints are equal, irrespective of the thickener used.

[A. Hebeish, A.A. Ragheb, S.H.Nassar, E.Allam and J.I. Abd El -Thalouth. Tara Gum Carbamate: A New Thickening System for Cotton Printing Using Vat Dyes. Journal of American Science 2010;6(11):623-631]. (ISSN: 1545-1003).

**Keywords:** Tara Gum Carbamate; System; Print; Vat Dye

#### **Use of GIS and Remote Sensing for Environmental Sensitivity Assessment of North Coastal Part, Egypt.**

**Ahmed A. Afifi \*<sup>1</sup>; Gad, A<sup>2</sup>. and Refat, A.<sup>1</sup>**

<sup>1</sup> Soils and water use dept., National Research Centre, Dokki, Giza, Egypt.

<sup>2</sup> National Authority for Remote Sensing and Space Sciences, Egypt.

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**Abstract:** Desertification is considered as an important problem facing arid and semi-arid regions, as Egypt. These processes are resulted either from human activities or adverse natural conditions. However, the combination of both is often applicable. The aim of this study is the identification of areas sensitive to desertification in the north coast of Egypt. Based on the MEDALUS approach and the characteristics of the study area regional model developed using GIS. Three main indicators of desertification, including: soil, vegetation and climate were considered. The several sub-indicators affecting the quality of each main indicator were identified. Based on the MEDALUS approach, each sub-indicator was quantified according to its quality and given a weighting of between 1 and 2. Arc-GIS 9.2 was used to analyze and prepare the layers of quality maps using the geometric mean to integrate the individual sub-indicator. ETM and SRTM satellite images, geologic and soil maps were used as main sources for calculating the Environmental Sensitivity Areas Index (ESAI) for desertification. The results show that the soil of the north coast is characterized mainly by high sensitive areas for desertification (44.01 % of the total area), distributed mostly in the north western coast and the northern part of Sinai, where the soil quality, climatic quality and vegetation quality are low, while, 9.37 % of the total area exhibit are sensitive. The areas of moderate sensitive to desertification revealed in the studied area, representing an area of 3834.577 Km<sup>2</sup> (11.04 %) of the total area. The low sensitivity areas for desertification exhibit the whole area of the Nile Delta, as they represent 27.17 % of the total area (i.e. 9434.928 Km<sup>2</sup>). The low sensitivity for desertification is due to the good vegetation cover and soil quality. It can be concluded that implementing the maps of sensitivity to desertification is rather useful in the arid and semi arid areas as they give a more likely quantitative trend for frequency of sensitive areas. The integration of different factors contributing to desertification sensitivity may lead to plan a successful combating. The usage of space data and GIS proved to be suitable tools to rely on estimation and to fulfill the needed large computational requirements. They are also useful in visualizing the sensitivity situation of different desertification parameters.

[Ahmed A. Afifi; Gad, A. and Refat, A. Use of GIS and Remote Sensing for Environmental Sensitivity Assessment of North Coastal Part, Egypt. Journal of American Science 2010;6(11):632-646]. (ISSN: 1545-1003).

**Keywords:** Remote sensing, GIS, Environment, Desertification, Egypt

**Efficiency of Natural Minerals in Presence of Different Nitrogen Forms and Potassium Dissolving Bacteria on Peanut and Sesame Yields**

[Full text](#)

**Gehan H. Youssef, Wafaa M. A. Seddkik and Mona A. Osman**

Soil, Water and Environ. Research Institute, agricultural Research Center (ARC), Giza, Egypt

**Abstract:** A field experiment was carried out for two summer seasons at Ismailia Agric. Res. Station to study the effect of some natural minerals combined with potassium dissolving bacteria inoculation in the presence of different nitrogen forms on chemical properties of soil, nutritional status and yield of peanut-sesame. Each experiment was designed in a split-split design with three replications. Three forms of nitrogen fertilizer were included along with two natural minerals, in a presence of potassium dissolving bacteria inoculation, as well as one mineral fertilizer as source potassium fertilizer. Furthermore, data show high significant increases in available N due to the application of ammonium nitrate in combination with feldspar, and calcium nitrate in combination with potassium sulfate in a presence of inoculation for peanut and sesame, respectively. However, application of calcium nitrate combined with potassium sulfate, and ammonium nitrate in combination with feldspar, in a presence of inoculation, led to significant increases in K available in soil for peanut and sesame, respectively. Oppositely, the pH values, different to those of EC, decreased either for inoculation or non-inoculation as compared to control. In spite of that, the values of EC and pH of soil were higher with application of either bentonite or bentonite + feldspar in a presence of all nitrogen fertilizer forms. Generally, the highest EC values in soil, after the two studied seasons were encountered with calcium nitrate fertilizer as well as bentonite mineral. Moreover, applying feldspar mineral and ammonium nitrate treatments had recorded the highest values of yield components as well as nutrient (N and K) uptake by either peanut or

sesame crops, particularly in the presence of inoculation as compared to those given by other treatments.

[Gehan H. Youssef, Wafaa M. A. Seddk and Mona A. Osman. Efficiency of Natural Minerals in Presence of Different Nitrogen Forms and Potassium Dissolving Bacteria on Peanut and Sesame Yields. Journal of American Science 2010;6(11):647-660]. (ISSN: 1545-1003).

**Keywords:** Efficiency; Natural Mineral; Nitrogen; Potassium; Bacteria

**Clinical Prospective of Repeat Breeding Syndrome in Buffaloes**

**Ahmed W.M.\* , El-khadrawy H.H., Emtenan M. Hanafi , Amal H. Ali , Shalaby S.A.**

Department of Animal Reproduction and Artificial Insemination, National Research Centre  
Dokki,Cairo, Egypt. \*[wahidmma@hotmail.com](mailto:wahidmma@hotmail.com)

[Full text](#)

**Abstract:** Local meat production in Egypt is in continuous decrease and can not meet the local market requirement. So this study was designed to throw light on true repeat breeding syndrome (RBS) as one of the reproductive disorders that hinders the buffalo meat and milk production. A field survey was carried out on 1358 female buffaloes which were subjected to clinical and gynecological examination, and blood samples were collected for carrying out some relevant analyses. Treatment trials were practiced using different ways to control the condition and the economic impact of this syndrome has been studied. Results revealed that the incidence of clinical repeat breeding (RB) in the examined buffalo cows was 4.34 %. Typical repeat breeders represented 7.25 % of total reproductive disorders in female buffaloes. Serum progesterone level was  $1.44 \pm 0.39$  and  $3.66 \pm 0.84$  in RB and normal buffaloes (NB), respectively. Oxidant/antioxidant markers in RB buffalo-cows showed increased malondialdehyde (MDA) and nitric oxide (NO) and decreased catalase (CAT), superoxide dismutase (SOD), ascorbic acid (ASCA), reduced glutathione (R-GSH) and total antioxidant capacity (TAC). Serum zinc, copper, iron and selenium values were lower in repeat breeder cows compared to normal animals. Repeat breeder buffalo-cows responded to the treatments with mineral mixture, GnRH and Lugol's solution with recovery rates; 63.64, 61.54 and 60.00%, respectively. The study concluded that special care should be paid for food additives to control this syndrome.

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[Ahmed W.M., El-khadrawy H.H., Emtenan M. Hanafi , Amal H. Ali , Shalaby S.A. Clinical Prospective of Repeat Breeding Syndrome in Buffaloes. Journal of American Science 2010;6(11):661-666]. (ISSN: 1545-1003).

**Key words:** Repeat breeding buffaloes - progesterone - oxidant/antioxidants and trace elements

**Biologically evaluation of pan bread supplemented with vital gluten, lupin, fenugreek and turmeric flour blends**

**Aly, M. H\*; El Nikeety, M. M\*; Saleh, M. A. M\*\*. and Abd El-Hak, N. A. M.\* \*\***

\*Cairo University, Faculty of Agric., Food Science & Technology Dept.

\*\* Food Technology Research Institute, Special Food & Nutrition Dept.

99 \*\*\*Food Technology Research Institute, Experimental Kitchen Unit.

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**ABSTRACT:** The current study was carried out to utilize each of whole meal wheat flour (control), some legumes (lupin and fenugreek), turmeric and vital gluten flour in blends for preparation of pan bread more nutrients and healthy in order to enhance the dietary fiber and amino acids contents. The biological parameters of rats (non and induced diabetic) fed on such pan bread was also estimated. A significant higher amount of soluble, insoluble and total dietary fiber contents was found in the turmeric, fenugreek and lupin, pan bread compared to that found in control once( whole wheat flour). Normal rats (nondiabetic and fed on basal diet) exhibited an insignificant decrement in blood glucose. However, in the diabetic rats a significantly lowered blood glucose trend was found. The tested pan bread samples were more slightly effective in lowering liver and kidney function in the diabetic rats in a relation to

diabetic rats, when compared with the positive control. Finally, it is recommended to utilize whole meal flour to prepare healthy diets to deal with diabetic status and control of some biological parameters.

[Aly, M. H; El Nikeety, M. M; Saleh, M. A. M. and Abd El-Hak, N. A. M. Biologically evaluation of pan bread supplemented with vital gluten, lupin, fenugreek and turmeric flour blends. Journal of American Science 2010;6(11):667-79]. (ISSN: 1545-1003).

**Key words:** Whole meal wheat, Vital gluten, Fenugreek seeds, Legumes, Turmeric, Diabetes

### Scleral Fixation Intraocular lenses

<sup>1</sup>Ayman Shouman, <sup>1</sup>Mohamed Marzouk, <sup>1</sup>Hesham Ali and <sup>1</sup>Ehab Zakzook

<sup>1</sup>Ophthalmology Department, Research Institute of Ophthalmology, Giza

[shoumanaes@yahoo.co.uk](mailto:shoumanaes@yahoo.co.uk)

**Abstract:** Background: The 1ry indication for scleral fixation of intraocular lenses (IOL) is dislocation as a principal complication of cataract surgery. Inadequate capsular support is the most common cause of IOL dislocation. Other indications include traumatic phakic lens dislocation (cataractous or clear), surgically aphakic eyes or anterior chamber IOL with complications (persistent hyphema, uveitis). Methods: 20 eyes of 20 patients were done, surgery was done only when the IOL was dislocated peripheral to the visual axis and was causing symptoms of visual loss sufficient to interfere with the patient's activities of daily living, or patients who were left aphakic for a 2ry implantation procedure. A modification of the technique was done which made the procedure faster and preserved the surrounding conjunctiva. Results: Best corrected visual acuity (BCVA) preoperatively ranged from 1/60 -6/60 and postoperatively between 6/60 – 6/6. Statistical analysis of the logarithm of the minimum angle of resolution (LogMAR) between the preoperative and postoperative visual acuity revealed significant improvement ( $p < 0.05$ ). Intraoperative complications included one case of accidental iris injury, two cases of mild vitreous hemorrhage, two cases of moderate vitreous hemorrhage. Early postoperative complications included pupillary block. Midterm post operative complications occurred in one case with the occurrence of cystoid macular edema. Conclusion: Scleral fixation of IOL is a safe procedure with minimal complications, but needs surgical skills to be managed optimally.

[Ayman Shouman, Mohamed Marzouk, Hesham Ali and Ehab Zakzook. **Scleral Fixation Intraocular lenses**. Journal of American Science 2010;6(11):680-687]. (ISSN: 1545-1003).

**Key words:** Scleral fixation, Intraocular lenses, Aphakia, IOL dislocation

### Technological Properties of some Egyptian New Wheat Varieties

**Ahmed M. S. Hussein, Mohie M. Kamil and Gamal H. Ragab**

National Research Centre, Food Technology Department, Dokki, Cairo, Egypt

email: [ResearchTeamMMK@yahoo.com](mailto:ResearchTeamMMK@yahoo.com)

**Abstract:** Whole meal and flour 72% of Gemmeiza 7, Gize 168, Sohage 3 and Sakha 93 wheat varieties were evaluated to produce pan bread, pasta and biscuits. Pan bread of whole meal wheat varieties had higher contents of moisture, protein, fat, ash and fiber than wheat flour 72% of the same varieties. Pan bread of Sakha 93 characterized with its higher baking quality (weight, volume and specific volume) than pan breads of other varieties. Crust color of pan bread slightly affected with whole-meal wheat varieties, where its color score maximized in Sakha 93 (7.7) and Sohage 3 (6.7). This result agreed with the obtained color parameter of Hunter, where lightness (L) maximized to 55.95 and 49.79 in pan bread crust of Sohage 3 and Sakha 93, respectively. Pasta characterized with its higher protein (13.12%), fat (2.59%) and crude fiber (2.82%) in case of using whole meal of Sohage 3, Giza 168 and Gemmeiza 7 varieties, respectively. Pasta cooking quality ranked first in case of using Sohage 3 whole meal, where its weight increase, volume increase and cooking loss reached to 265%, 305.3% and 8.3%, respectively.

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Pasta color parameter showed that, wheat flour 72% and whole meal of Sakha 93 characterized with its higher lightness (L). Sensory evaluation showed that, pasta of wheat flour 72% accepted slightly in appearance and color if compared with whole meal pasta of the same variety. In addition, there were no significant difference between pasta of wheat flour 72% and whole meal in flavor, tenderness and stickiness. Biscuit of whole meal characterized with its higher content of protein, fat, ash and crude fiber than wheat flour 72%. Whole meal biscuit of Sohage 3 characterized with its higher protein (12.13%), fat (31.0%) and ash (2.51%) contents; and lowest carbohydrate content (52.18%). Biscuit of Sakha 93 variety (whole meal or flour 72%) was higher in baking quality. Hunter color parameter and sensory evaluation showed that, biscuit of whole meal varieties was slightly darker than biscuit of wheat flour 72% varieties. In addition, biscuits flavor, taste, texture, appearance and overall acceptability of wheat flour 72% not affected significantly in case of using whole meal flour of the same variety.

[Ahmed M. S. Hussein, Mohie M. Kamil and Gamal H. Ragab. Technological Properties of some Egyptian New Wheat Varieties. Journal of American Science 2010;6(11):688-699]. (ISSN: 1545-1003).

**Keywords:** Egyptian wheat varieties – technological properties - Pan bread – Pasta – Biscuit - whole meal – wheat flour 72%

#### **Circulating Endothelial Cells And Cardiovascular Risk In Systemic Lupus Erythematosus**

**Elham Kassem<sup>1</sup>, Mervat El-Sergany<sup>2</sup>, Hanan El-Saadany<sup>3</sup> Abeer Shahba<sup>4</sup> and Wesam Salah<sup>5</sup>**

Departments of Rheumatology & Rehabilitation <sup>1,2,3</sup>, Internal Medicine<sup>4</sup> and Clinical Pathology<sup>5</sup>

Faculty of Medicine, Tanta University, Egypt. [Elahm77@hotmail.com](mailto:Elahm77@hotmail.com)

[Full text](#)

**ABSTRACT:** Premature atherosclerosis seen in systemic lupus erythematosus (SLE) patients is not explained by traditional risk factors. Circulating endothelial cells (CECs) have been shown to be a surrogate marker of endothelial dysfunction. The aim of this study was to assess the number of CECs in SLE patients and to determine any potential correlation between CEC count and endothelial function (FMD%), disease activity, organ involvement and therapy used. Also, to investigate VCAM-1 and ICAM-1 levels as markers of vascular inflammation and injury. This study was performed on 30 premenopausal female SLE patients and 20 age and sex matched healthy controls (HC). Patients were subjected to full history taking, complete clinical examination and assessment of disease activity using (SLAM) score. For both patients and controls, endothelial function (FMD%), laboratory estimation of CEC count, and serum level of VCAM-1 and ICAM-1 were performed. CEC count was significantly elevated in SLE patients comparing to HC ( $P<0.001$ ). CEC count was positively correlated with SLAM score, while negatively correlated with FMD%. Serum levels of VCAM-1 and ICAM-1 were significantly increased in SLE patients than controls. Moreover, VCAM-1 correlated significantly with disease activity and CEC count while ICAM-1 did not correlate with any of them. There was significant correlation between CEC count and skin vasculitis, renal involvement and anti-malarial medications. In conclusion, increased number of CEC may be a biomarker of disease activity and disseminated vasculopathy occurring in the course of SLE and may represent one of the first specific cellular markers to provide a direct link with the pathophysiology of cardiovascular disease (CVD). VCAM-1 is considered a marker of activation of endothelial cells. Taking together, this may predict patients at increased risk of CVD complications, lupus nephritis or vasculitic skin affection.

[Elham Kassem, Mervat El-Sergany, Hanan El-Saadany Abeer Shahba and Wesam Salah. Circulating Endothelial Cells And Cardiovascular Risk In Systemic Lupus Erythematosus. Journal of American Science 2010;6(11):700-707]. (ISSN: 1545-1003).

**Key words:** Circulating endothelial cells (CEC), SLE, adhesion molecules

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#### **Degradation Of Polycyclic Aromatic Hydrocarbons As Affected By Some Lactic Acid Bacteria**

**Abou-Arab, A.A.K\*; Abou-Bakr Salim<sup>\*</sup>; Maher,R.A. ; El-Hendawy, H.H. \*\* and Awad, A.A. \*\***

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\*Food Toxicology and Contaminants, National Research Center.

\*\*Botany and Microbiology Department, Faculty of Science, Helwan University.

[Aak\\_abouarab2007@yahoo.com](mailto:Aak_abouarab2007@yahoo.com)

**ABSTRACT:** Polycyclic aromatic hydrocarbons (PAHs) are a group of chemicals composed of two or more fused aromatic rings that are formed from the incomplete combustion or high-temperature pyrolysis of coal, oil, gas, wood, fossil fuel, garbage or other organic substances, such as tobacco, charbroiled meat and exhaust from automobile and trucks. They enter the environment and release to air, soil, water and food. Some PAHs have shown to have toxicological, carcinogenic and mutagenic effects on animals and humans. Biodegradation of PAHs in the presence of the three types of lactic acid bacteria (*Bifidobacterium bifidum*, *Streptococcus thermophilus* and *Lactobacillus bulgaricus*) were studied during the different incubation periods (2, 4, 6, 8, 10, 12, 24, 48 and 72 h) at 37°C. The reduction of PAHs concentration proved that these compounds were affected by the previous lactic acid bacteria. At the end of incubation period (72 h), the reduction percent were 46.6, 87.7 and 91.5% with *Bifidobacterium bifidum*, *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, respectively. These results could be explained as the bacterial cell is a high proteinous material and so may adsorbs PAHs which could interfere with cellular metabolism. Also, the variation of pH values during the incubation periods may control in the adsorbed PAHs on the cells. The biodegradation of PAHs by yoghurt starter during yoghurt manufacture were studied. Slightly reduction was observed during the incubation periods (1, 2 and 3 h). The reduction percent was 3.46 at the final product. It could be revealing that the persistence of PAHs depend on a number of factors such as the type of microorganism, the interaction between microorganisms, the microbial concentration, the composition of the medium, and the microbial growth conditions of temperature and pH. The foregoing information reveal that extra care must be taken when comparing the results since in-vitro studies are not always relevant to real situation in food products.

[Abou-Arab, A.A.K; Abou-Bakr Salim; Maher, R.A.; El-Hendawy, H.H. and Awad, A.A. Degradation Of Polycyclic Aromatic Hydrocarbons As Affected By Some Lactic Acid Bacteria. Journal of American Science 2010;6(11):708-715]. (ISSN: 1545-1003).

**Key words:** PAHs, Lactic acid bacteria, Degradation, MRS, Milk, Yoghurt

#### **Expression of The Antiapoptotic Gene Survivin in Acute Leukemias**

Hoda Sadek,<sup>1</sup> Shadia Ragab,<sup>2</sup> Hanan Rasmy,<sup>2\*</sup> Nancy M. El Guindy,<sup>1</sup> Wafaa Ezzat,<sup>3</sup> Mona Hamed<sup>2</sup>

<sup>1</sup> Clinical and Chemical Pathology Department, Faculty of Medicine, Cairo University, Egypt

<sup>2</sup> Clinical and Chemical Pathology Department, National Research Center, Egypt

<sup>3</sup> Internal Medicine Department, National Research Center, Egypt

[Full text](#)

**Abstract:** *Objectives:* To assess the level of expression of the antiapoptotic signal "Survivin" in Egyptian patients with acute leukemias and to delineate any significant correlation between the level of Survivin with the clinical and haematological findings in those patients. *Patients and Methods:* Survivin expression was quantitatively determined by a real-time PCR technique in 30 acute leukemia patients; ALL and AML in two age groups; pediatric group (<18 years) and adult group (≥18 years) and in age and sex matched control healthy subjects. *Results:* Statistically significant higher expression was noted in both ALL and AML groups when compared to the control group (p-value = 0.0001). A statistically significant negative correlation was detected between Survivin expression and RBCs count, HB level and Platelet count with p-values = 0.01, 0.01 and 0.0001 respectively. Positive correlations were found with T.L.C, peripheral blood blasts, bone marrow malignant cells, LDH, ALP and uric acid levels with p-values = 0.0001, 0.0001, 0.03, 0.0001, 0.006 and 0.001 respectively. During the post-induction phase,

Survivin expression showed a statistically insignificant difference between patients achieving complete remission and those showing unfavorable response with a p-value = 0.7. After follow up, the expression change between patients achieving complete remission and those showing unfavorable response was statistically insignificant with a p-value = 0.6. *In summary*, The previous data emphasized important correlations between Survivin expression and established risk factors in acute leukemia patients. Thus Survivin could be used as a marker for assessment of bone marrow infiltration that in future could be used to refine treatment stratification.

[Hoda Sadek, Shadia Ragab, Hanaa Rasmy, Nancy M. El Guindy, Wafaa Ezzat, Mona Hamed. Expression of The Antiapoptotic Gene Survivin in Acute Leukemias. Journal of American Science 2010;6(11):716-725]. (ISSN: 1545-1003).

Key words: Survivin - Antiapoptosis function - Hematological malignancies

**Biochemical and molecular characterization of three colored types of roselle (*Hibiscus sabdariffa* L.)**

[Full text](#)

**Refaei M. Hussein<sup>a</sup>, Yasser E. Shahein<sup>b</sup>, Amr E. El Hakim<sup>b</sup> and Hanem M. Awad<sup>c,\*</sup>**

<sup>a</sup>Genetics and Cytology Department; <sup>b</sup>Molecular Biology Department and <sup>c</sup>Department of Tanning Materials and Leather Technology, National Research Centre, El-Behouth St., Dokki; P. Box; 12622; Cairo; Egypt. [hanem\\_awad@yahoo.com](mailto:hanem_awad@yahoo.com)

**ABSTRACT:** Roselle (*Hibiscus sabdariffa* L.) has a considerable industrial, pharmaceutical and economic values in Egypt and many other countries around the world, mainly for its pleasant sepals. There are many colored types of Roselle depends on sepals color. The biochemical and molecular characterization of three roselle types, green (G), light red (LR) and dark red (DR), were studied. RAPD-PCR patterns for their genomic-DNA were significantly different. The total protein electrophoretic profile of their seeds was similar except for some inter-individual variation in band density. Their total protein contents were 46.0, 66.5 and 68.1 mg/g seed, respectively. In addition to the water-soluble antioxidant capacity, the total polyphenolic-content and the antioxidant activity of 12 roselle extracts, three colored types in 2 solvent systems (aqueous, A and 30% ethanolic, E) and 2 extraction temperatures (hot, H and cold, C), were determined by Folin-Ciocalteu reagent and 1,1-diphenyl-2-picrylhydrazyl (DPPH) methods, respectively. The ability of these roselle extracts to inhibit the formation of nitrous acid-induced tyrosine nitration decreases in the order of LREC > DREC > LREH > GEC > DREH > GEH > GAC > DRAH > LRAC > GAH > LRAH > DRAC.

[Refaei M. Hussein, Yasser E. Shahein, Amr E. El Hakim and Hanem M. Awad. Biochemical and molecular characterization of three colored types of roselle (*Hibiscus sabdariffa* L.). Journal of American Science 2010;6(11):726-733]. (ISSN: 1545-1003).

**KEYWORDS:** Antioxidant activity; DPPH; HPLC; molecular characterization; polyphenol content; reactive nitrogen species (RNS); sepals, Roselle (*Hibiscus sabdariffa* L.)

**Management of obstructive sleep apnea using oral appliance with magnetic versus increase vertical dimension**

[Full text](#)

Mohamed. A. Saad-Eldeen<sup>1</sup>, Shawky M. Elmorsy<sup>2</sup>, Shaza. M. Hammad<sup>3</sup>

<sup>1</sup> Ass. Prof., Prosthodontic Department, Faculty of Dentistry, Mansoura University

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<sup>2</sup> Ass. Prof, ENT Department, Faculty of Medicine, Mansoura University

<sup>3</sup>Lecturer, Orthodontic Department, Faculty of Dentistry, Mansoura University.

[mohamed.elkhodary@hotmail.com](mailto:mohamed.elkhodary@hotmail.com)

**Abstract:** Statement of Problem: Oral devices may be helpful in the management of obstructive sleep apnea by improving upper airway potency. Purpose: Management of obstructive sleep apnea using oral appliance with magnetic versus oral appliance with increased vertical dimension. Material and Methods: 12 patients with mild to moderate obstructive sleep apnea were evaluated in this study before and after wearing devices for six months. The patients randomly divided into two equal groups(A and B ). Group A used oral appliances with magnetic for six month Patients in group B wear oral appliances with increased vertical dimension, Evaluation was done by Polysomnograph, clinical findings and cephalometric x-rays. Results: The results of this study revealed that improvement of clinical finding, symptoms and apnea index for patients wearing two types of oral appliance. Conclusions: It can be concluded that oral appliance, with magnetic and increase vertical dimension, make improvement for OSA patients oral appliances with magnets are more effective in management of mild and moderate obstructive sleep apnea in comparison to appliances with increased vertical dimension.

[Mohamed. A. Saad-Eldeen, Shawky M. Elmorsy, Shaza. M. Hammad. Management of obstructive sleep apnea using oral appliance with magnetic versus increase vertical dimension. Journal of American Science 2010;6(11):734-741]. (ISSN: 1545-1003).

**Keywords:** Management; obstructive; sleep; apnea; oral appliance; magnetic; dimension

#### **Biochemical Changes in Glutathione Redox System and Glucose Regulation in Late Pregnant Ossimi Ewes**

**Ali Hafez El-Far<sup>1</sup>, Mohamed K. Mahfouz<sup>2</sup>, Hussein A. Abdel maksoud<sup>2</sup>**

<sup>1</sup> Department of Biochemistry, Faculty of Veterinary medicine, Alexandria University, Damanhour Branch (Al-Bostan), Egypt.

<sup>2</sup>Department of Biochemistry, Faculty of Veterinary medicine, Moshtohor, Banha University, Egypt.

[aboufares90@yahoo.com](mailto:aboufares90@yahoo.com)

**Abstract** Pregnancy is the more prevalent stress in under feeding small ruminant with multiple bearing. Fifty Ossimi ewes of two years old and their body weight ranging between 35 and 50 kg were allotted into three groups; Group I: contains ten non pregnant non lactating ewes were used as control group. Group II: contains twenty single pregnant ewes\* and Group III: contains twenty twin pregnant ewes used as experimental animals. Our study focused on the comparison between single and twin bearing ossimi ewes in the last three weeks of pregnancy and the day of parturition by measurement of reduced glutathione (GSH) level and the activities glutathione peroxidase (GSH-Px); glutathione reductase (GR-ase); glutathione-S-transferase (GST) and total superoxide dismutase (t-SOD) in erythrocytic haemolysate. In addition, glucose, non esterified fatty acid (NEFA), Beta hydroxyl butyric acid (BHBA), cortisol, insulin and protein electrophoretic patterns were measured in serum. Our results concluded that, In erythrocytic haemolysate the mean values of GSH-Px and GST in group II and III during the period of 2<sup>nd</sup> and one week before parturition and at the day of parturition were high significantly increased. While, GSH and t-SOD were high significantly decreased ( $P<0.01$ ) and GR-ase activities were significantly decreased. While serum insulin level decreased while serum NEFA, BHBA and cortisol were increased in single and twin but in twin the values is more significant. The data showed that twin bearing ewes are more susceptible to pregnancy toxemia than single bearing that may be decrease the productivity and performance of those animals.

[Ali Hafez El-Far, Mohamed K. Mahfouz, Hussein A. Abdel maksoud. Biochemical Changes in Glutathione Redox System and Glucose Regulation in Late Pregnant Ossimi Ewes. Journal of American Science 2010;6(11):742-748]. (ISSN: 1545-1003).

**Keywords** pregnancy, glutathione, single bearing, twin bearing, ewes

#### **Study the Efficiency of Investment and its Determinants in the Agricultural Sector**

Khairy Hamed Eleshmawy, Enaam Abd elFattah Mohamed, Laila Mustafa ELShrif, Haitham Bauomy

[Full text](#)

**Abstract:** The promotion of increased rates of the investment growth is the main priority of the economic development, where there can be no development without adequate levels of investment. The problem has been narrowed to study in lower volume of investment goes to agriculture in spite of the importance of this sector to increase the rate of economic growth, the study aims to identify the relative importance of the investment total agricultural and agricultural domestic and foreign farm, as well as identify the most important factors affecting each. In addition to, identify the efficiency of agricultural investment. The results indicated that, overall agricultural investment and agricultural domestic and foreign farm represented about 9.38%, 7.98%, 1.4% of the total investments, and investments amounted to local agriculture, and foreign to 84.88%, 15.12% of the total agricultural investment. Estimating the efficiency indicators of agricultural investments shows that, there is efficiency in agricultural investment despite lower Kimpalastmarat directed to the agriculture sector during the study period. The results showed that, the agriculture sector capital intensive, in addition to increasing the coverage of agricultural savings to agricultural investment as much as about 46% in 2008. While the share of one acre of agricultural investment from 283.65 pounds in 1999 to about 194 pounds in 2008. The results showed that, the most important factors affecting the local agricultural investments are in value-added farm income and saving agricultural and domestic liquidity and interest rate on farm loans. While the GDP and the budget deficit and non-agricultural investments, the most important factors affecting foreign investments in agriculture. Therefore, the study recommends the need to increase investments directed to the agricultural sector given the importance of this sector and its contribution to economic growth. You need to follow monetary policies that reduce the interest rate on agricultural loans to encourage investment in agricultural projects, in addition to the need to reduce taxes on agricultural projects as a means to stimulate the agricultural investor.

[Khairy Hamed Eleshmawy, Enaam Abd elFattah Mohamed, Laila Mustafa ELShrif, Haitham Bauomy Hassan. Study the Efficiency of Investment and its Determinants in the Agricultural Sector. Journal of American Science 2010;6(11):749-755]. (ISSN: 1545-1003).

**Keywords:** Agricultural Investments, Domestic Agricultural Investments, Foreign Agr. Investments, Gross Investments

#### Measurement of Family Economic Status

<sup>1</sup>Mehdi Yadollahi & <sup>2</sup>Laily Hj Paim

<sup>1</sup> Faculty of Human Ecolog, Putra University, Malaysia &

Dept. of Management, University of Payam e Noor, Sirjan

E-mail: [mfma155@yahoo.com](mailto:mfma155@yahoo.com)

[Full text](#)

<sup>2</sup> Dept. of Resources Management & Consumer Studies, Putra University, Malaysia

**Abstract:** The concept of family economic has become important around the world. It has been realized that communities based family economic can play a fundamental role in poverty alleviation. Measuring of family economic status is an important step in developing family economic strategies to achieve poverty reduction. This paper used qualitative approaches to illustrated family economic status. The purpose of this study is to explore the concept and indictors of family economic. The literature derived from my study in family economic management.

[Mehdi Yadollahi & Laily Hj Paim. Measurement of Family Economic Status. Journal of American Science 2010;6(11):756-760]. (ISSN: 1545-1003).

**Key Words:** Family Economic, Income, Physical Assets, Expenditure

## **High Efficiency Production of Mosquitocidal Toxin by a novel*Bacillus sphaericus*isolate from Egyptian Soils on Local Agroindustrial Byproducts**

[Full text](#)

M.S.Foda.,<sup>1</sup>\*Fawkia M. El-Beih.,<sup>2</sup>Maysa E. Moharam.,<sup>3</sup>Nora N.A.El-Gamal<sup>4</sup>

Microbial Chemistry Department, Genetic Engineering & Biotechnology Division, National Research Center, Dokki, Giza, Egypt.<sup>1,3,4</sup>Faculty of Science, Ain Shams University, Cairo, Egypt.<sup>2</sup>

[foda302002@yahoo.com](mailto:foda302002@yahoo.com)

**Abstract:** Eighty six cultures were isolated from soil of different Egyptian Governorates including Quina, El-Menofeya, El-Gharbia, El-Sharkia, El-Behera and Kafr EL-Sheikh Governorates. Investigations of the mosquitocidal Egyptian isolates have revealed that isolate No.1 have the ability to form more toxin than the international reference strain *Bs*2362. The selected isolate No.1 exhibited a lower LC<sub>50</sub> and LC<sub>90</sub>values than the International strain *B. sphaericus* 2362 upon bioassay against secondinstars' larvae of *Culex pipiens*. The Egyptian isolate No.1 was identified morphologically and biochemically as *Bacillus sphaericus*. Physiological factors affecting growth and toxin formation in *B. sphaericus*No 1 in comparison to *B.s* 2362 were carried out. THE organism grown on modified Nutrient broth medium yielded the highest larval toxicity against the second instars' of *Culex pipiens*for both *Bacillus sphaericus*isolate No 1 and the international strain *Bacillus sphaericus*. The Optimum air : medium ratio were 9:1 and 4:1 of the flask volume for 4 and 3 days incubation periods using 2%and 3% sizes of inocula for *B. sphaericus* 2362 and the Egyptian isolate *B. sphaericus*No.1, respectively. Sodium acetate was the suitable carbon source for the isolate *B. sphaericus* No.1, while *B. sphaericus* 2362 was capable to utilize both sodium acetate and sodium succinate as carbon sources. The Egyptian isolate *B. sphaericus* No.1exhibited the highest mosquitocidal activity upon growth on kidney beans seeds and sesame meal as nutrient substrates at 3% final concentration, while *B. sphaericus*2362 exhibited the highest mosquitocidal activity by utilizing soy beans, lentils and sesame meal as complete media for growth and mosquitocidal toxin production.

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[M.S.Foda., Fawkia M. El-Beih., Maysa E. Moharam., Nora N.A.El-Gamal. High Efficiency Production of Mosquitocidal Toxin by a novel*Bacillus sphaericus*isolate from Egyptian Soils on Local Agroindustrial Byproducts. Journal of American Science 2010;6(11):761-769]. (ISSN: 1545-1003)

**Key words:** *Bacillus sphaericus*, isolation, characterization, mosquitocidal toxin, physiology, agroindustrial byproducts

[Full text](#)

## **Global Analysis of an Epidemic Model with General Incidence Rate**

**M. M. A. El-Sheikh<sup>(1),(2)</sup> and S. A. A. El-Marouf<sup>(1),(2)</sup>**

(1) Department of Mathematics, Faculty of Science, Taibah University, Kingdom of Saudi Arabia

(2) Permanent Adress: Department of Mathematics, Faculty of Science,

Menoufia University, Shebin El-Koom, Egypt.

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**Abstract:** A general four-dimensional SIQR epidemic model is considered. Threshold, equilibria and their stability are established. The dynamics of the system is discussed in the case of this general form of incidence rate. The global stability of both free-deisease and endemic equilibria are deduced. Hopf bifurcation , boundedness, dissipativity and persistence are studied.

[M. M. A. El-Sheikh and S. A. A. El-Marouf. Global Analysis of an Epidemic Model with General Incidence Rate. Journal of American Science 2010;6(11):770-783]. (ISSN: 1545-1003).

**Keywords:** Epidemic model; Nonlinear incidence rate; quarantine; uniformaly persistence; global

	stability; Hopf bifurcation		
112	<p><b>The Effect of Combining Herbal Therapy with Conventional Chemotherapy on the Incidence of Chemotherapy Side Effects in 2nd Stage Breast Cancer Patients</b></p> <p>Nagla Hamdi Kamal Khalil, Sanaa Alaa El- Din, Maha Adel Salem</p> <p>Medical-Surgical Nursing Department, Faculty of Nursing,</p> <p>Ahmed Adel Seif El-Din, Pharmacognosy Department, Faculty of pharmacy</p> <p>Waleed Osman Arafat, Oncology department,Faculty of Medicine, Alexandria University</p> <p style="text-align: center;"><a href="mailto:Mahaadel52@yahoo.com">Mahaadel52@yahoo.com</a></p> <p><b>Abstract:</b> The purpose of this study is to identify the effect of the combination of herbal mixture and conventional chemotherapy on minimizing the incidence of chemotherapeutic side effects for 2<sup>nd</sup> stage breast cancer. Forty adult female patients aging 20 to 65 years old diagnosed with breast cancer stage (II), receiving chemotherapy for at least one month and will continue to receive it for 3 months- were selected randomly and divided equally into study and control groups. They were free from any associated co-morbid diseases as diabetes, renal, cardiac. The patients were interviewed in the oncology outpatient clinic. Study group patients were instructed about the importance of taking herbal capsules regularly with chemotherapeutic cycles, on a scheduled dose of 1 capsule three times per day for 3months. Complete assessment for both groups as baseline data to assess the chemotherapeutic side effects, laboratory investigations and the nutritional status of the patients were done, and then after 45days and after 3 months. The results revealed that (45%) of cancer breast women were in age group 49-65 years. The greater proportion of the sample (62.5%) breast fed and lactated for three times and more through their life. The least affected systems with chemotherapeutic side effects and the most affected systems when combined herbal to conventional therapy were: liver functions and endocrine studies, renal functions, reproductive system, urinary system, and weight changes. While psychological status, nervous system, and skin, hair, and nails were the most affected systems with the side effects of chemotherapy, and they were the least affected systems when combined herbal to conventional chemotherapy. Also it was found that there was significant difference between the study and control groups in relation to second and third assessments related to all body systems. It is recommended that herbal education should be introduced in nursing and medical curriculum. Further researches related to these herbal components to measure its efficacy on minimizing the side effects of chemotherapy for breast cancer and/ or other types of cancer. Further researches are also needed on larger number of sample. Clinical studies should be done to identify the effect of these herbs on different cancer therapies, different chemotherapeutic protocols, specifically pre or post mastectomy.</p> <p>[Nagla Hamdi Kamal Khalil, Sanaa Alaa El- Din, Maha Adel Salem. The Effect of Combining Herbal Therapy with Conventional Chemotherapy on the Incidence of Chemotherapy Side Effects in 2nd Stage Breast Cancer Patients. Journal of American Science 2010;6(11):784-801]. (ISSN: 1545-1003).</p> <p><b>Keywords:</b> Herbal Therapy; Conventional Chemotherapy; Chemotherapy; Side Effect; Breast Cancer</p>	<a href="#">Full text</a>	112
113	<p style="text-align: center;"><b>Women's Awareness of Danger Signs of Obstetrics Complications</b></p> <p style="text-align: center;"><b>Wafaa A. Rashad * , Rasha M. Essa **</b></p> <p>* Assistant Professor of Obstetric and Gynecologic Nursing, Faculty of Nursing, University of Alexandria, Alexandria, Egypt</p> <p>** Lecturer of Obstetric and Gynecologic Nursing, Faculty of Nursing, University of Alexandria, Damnhour Branch, Alexandria, Egypt</p>	<a href="#">Full text</a>	113

[wafaa.rashad@alex-nursing.edu.eg](mailto:wafaa.rashad@alex-nursing.edu.eg); [wafaara@yahoo.com](mailto:wafaara@yahoo.com); [rashaessa111@yahoo.com](mailto:rashaessa111@yahoo.com)

**Abstract:** An exploratory descriptive study was conducted at two Maternal and Child Health Centers (MCH) selected randomly in Albeheira Governorate to assess women's awareness of danger signs of obstetric complications. The study subjects consisted of 200 pregnant women attending the previously mentioned setting for tetanus toxoid immunization during pregnancy was enrolled in the study. (100 from each) A structured interview schedule was developed by the researcher after reviewing of the relevant literature and used to collect the necessary data. It comprised the following parts: Part I: Socio-demographic data such as age, level of education, occupation and number of family members...etc Part II: Obstetric characteristics such as gravidity, parity, abortions, antenatal follow up and presence of any complications. etc. Part III: questions related to knowledge about signs of obstetric complications, complaining of any obstetric complication, what to do if the woman has any of these signs. The study revealed that slightly more than one quarter of the study subjects (26.5 %) were unaware of obstetric danger signs compared to almost the same proportion (26.0 %) that had good awareness about such signs, while 47.5 % of the study subjects exhibited fair awareness. Lack of awareness about obstetric danger signs was related younger age, low level of education, gravidity and parity, previous experiences with any obstetric complications and lack of antenatal care. This study reflects the need for strategic plane to increase the awareness to shape health seeking behavior of the public related to signs of obstetric complications.

[**Wafaa A. Rashad**, Rasha M. Essa, Women's Awareness of Danger Signs of Obstetrics Complications. Journal of American Science 2010;6(11):802-]. (ISSN: 1545-1003).

**Keywords:** obstetric danger signs, awareness, signs of obstetric complications

#### The Use of lemongrass extracts as Antimicrobial and food additive potential in yoghurt

[Full text](#)

Abd-El fattah<sup>1</sup>; Abo sree, Yahia Hassan<sup>1</sup>; Hala M. Bayoum<sup>2</sup> and Hesham A. Eissa<sup>3</sup>

Food Toxins and contaminants Department<sup>1</sup>, Dairy Department<sup>2</sup>, Food Technology Department<sup>3</sup>, National Research Centre, Cairo, Egypt.

[shaabanmostafa@yahoo.com](mailto:shaabanmostafa@yahoo.com)

**ABSTRACT:** The following study was conducted to investigate the antifungal and food additive potential of medicinal plants. herbal decoction and essential oil (EO) extracts of *Cymbopogon flexuosus* (lemon grass) leaves and stems were tested for their inhibitory action against spoilage organisms and mycotoxins formation in two separated experiments. In the first experiment, yeast- extract sucrose medium (YES) was used as basal medium to examine the mold growth and mycotoxin production by three pathogenic fungi: *Aspergillus flavus* (*A. flavus*), *Aspergillus parasiticus* (*A. parasiticus*) and *Aspergillus ochraceus* (*A. ochraceus*). The medium was supplemented with four different concentrations of Lemongrass oil, inoculated with 1-mL spore suspension containing  $10^5$ - $10^6$  conidia of each test mold and then incubated at 28°C for 14 days. After incubation period, cultures were analyzed for mycelial dry weight and mycotoxin accumulation. In the second experiment, yoghurt medium was used as a basal medium and the same system of study was applied in different degrees of temperature (5°C and 28°C) for 4 weeks. Evaluation of the Lemongrass oil activity on yoghurt samples focused on the microbial stability of yoghurt, sensory evaluation as well as mold growth and mycotoxin formation. In the 1<sup>st</sup> experiment, the level of 0.1% of the EO extract was effective in inhibiting both mold growth and mycotoxin production for all tested molds, and 0.3 % extract completely prevented mold growth and toxin production, whereas, 1% of the decoction extract was effective. So, the EO extract was a suitable agent in the second experiment. It is of interest to note that while reduction in mold growth decreased increasing extract concentrations was observed, the most striking effect was the reduction of mycotoxin production. The obtained data from the second experiment showed that the EO extract (0.1% concentration) inhibited viable yeasts and preserved yoghurt for over 28 days at 5°C. Also, the inhibitory action of the extract against yeasts was concentration dependent. The maximum inhibitory effect of was found when the extract level increased above 0.1%. Incubation temperature had an important role in growth and mycotoxin production in yoghurt medium. During cold storage for 28 days at 5°C, the different concentrations of the extract added to the yoghurt samples displayed different titratable acidity and total bacterial cells and pH than the control yoghurt ( $p < 0.05$ ). Overall sensory acceptability of yoghurt supplemented with the EO extract

higher than that of the control yoghurt prepared without the EO extract. Total sensory evaluation of experimental yoghurt used as a control was up to 4.3 scores lower compared to yoghurt samples treated with the EO extract. The results indicate that the addition of the appropriate EO concentrations (0.1%, v/v) improved the physicochemical properties as well as sensory characteristics of yoghurt, could be used for decontamination of dairy products such as yoghurt from mycotoxicogenic fungi and mycotoxins formed beside its beneficial properties as a functional food.

[Abd-El fattah; Abo sree, Yahia Hassan; Hala M. Bayoum and Hesham A. Eissa. The Use of lemon grass extracts as Antimicrobial and food additive potential in yoghurt. Journal of American Science 2010;6(11):810-822]. (ISSN: 1545-1003).

**Key words:** Yoghurt, lemongrass, molds, yeasts, mycotoxins, aflatoxins, ochratoxin A, food additives.

#### The Assessment Of Mycotic Settlement Of Freshwater Fishes In Egypt

Refai, M.K.<sup>1</sup>, Laila, A. Mohamed<sup>2</sup>, Amany, M. Kenawy<sup>2</sup>, and Shimaa, El-S.M.A<sup>\*2</sup>.

<sup>1</sup> Microbiology Dept., Faculty of Vet.Medicine, Cairo University, Giza, Egypt.

<sup>2</sup> Hydrobiology Dept., National Research Center. Dokki, Giza, Egypt.

<sup>\*</sup>[shimaakhalifa2003@yahoo.com](mailto:shimaakhalifa2003@yahoo.com)

**Abstract:** This study was carried out on 360 freshwater fishes (240 *Oreochromis* species and 120 *Clarias gariepinus*). They were collected from different governorates and during different seasons. Naturally infected fishes showed clinical abnormalities such as skin darkening, exophthalmia, corneal opacity, abdominal distention, ulceration of the skin and cotton wool like growths on various parts of the body. Fishes were then subjected to post mortem examination which revealed many abnormalities. Mycological examination revealed the isolation of 2081 fungal isolates from 150 diseased and 210 apparently healthy fish samples (1658 mould and 423 yeast isolates), of which 1334 were isolated from *Oreochromis* species and 747 isolates from *Clarias gariepinus*. Isolated moulds belonged to the following genera: Saprolegnia (4.2%), Aspergillus (43.0%), Fusarium (14.1%), Mucor (14), Penicillium (17.2), Rhizopus (4.8%), Scopulariopsis (1.2%), Paeciliomyces (1%) and Curvularia (0.4%). Yeasts isolated also from both fish species had the following incidence: Candida albicans (35.9 %), other Candida species (19.1%), Rhodotorula species (31.4%) and Torulopsis species (13.5%). Experimental infection with the most predominant fungi (Aspergillus flavus, Fusarium species and Candida albicans) was conducted to evaluate the pathogenicity of these isolates. Clinical pictures of experimentally infected fish were similar to those of natural infection. Inoculated fungi were re-isolated from different organs. Results were confirmed with histopathological examination, which revealed the presence of fungal hyphae and spores in different organs.

[Refai, M.K., Laila, A. Mohamed, Amany, M. Kenawy, Shimaa, El-S.M.A. The Assessment Of Mycotic Settlement Of Freshwater Fishes In Egypt. Journal of American Science 2010;6(11):823-831]. (ISSN: 1545-1003).

**Keywords:** Mycotic infection, *Oreochromis* species, *Clarias gariepinus*, Moulds, Yeasts, Aspergillus, Fusarium, Candida, Penicillium.

#### Impact of Balanced Caloric Diet and Physical activity on Body Composition and Fat Distribution of Obese Egyptian Adolescent Girls

\*Nayera El-morsi Hassan, \*\*Safaa T. Zaki ,\*Sahar El-masry, \*\*Manal A. Mohsen, \*\*Eman Elashmawy

\*Biological Anthropology, \*\* Child Health Depts., National Research Centre, Cairo, Egypt

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[masrysa@yahoo.com](mailto:masrysa@yahoo.com)

**Abstract:** **Objective:** The aim of this study was to evaluate the effects of 6 months of balanced caloric moderately deficit diet program combined with individualized moderate Physical exercise on the body weight, body composition and fat distribution of adolescent girls. **Subjects & methods:** It was a longitudinal survey comprised 1244 adolescent girls, aged from 14 to 18 years. Their body weight and height were measured, and the BMI was calculated. Of the total sample, only one hundred and eleven girls (8.9%), with mean age was  $15.82 \pm 0.75$  years, were suffering from obesity based on their body mass index; which is greater than the 95<sup>th</sup> percentile for age and gender based Egyptian Growth Reference Charts. These obese girls were undergoing nutritional intervention (specific dietary program, nutritional education and exercise) for 6 months. At the start of this program, the obese girls were assessed for their anthropometric measures: the body weight, body height (or stature), body mass index (BMI), waist and hip circumferences, waist/hip ratio, skin folds thickness at 5 sites and, according to BIA, their body composition. This assessment was repeated after 6 months. Only thirty eight girls completely finished the program till the end. **Results:** The current study showed that after following the dietary program and physical activity, there were highly significant reduction in waist circumference, the skin fold thickness at the 5 sites (triceps, biceps, sub scapular, suprailiac and abdominal), peripheral and central adiposity, and fat mass, and significant reduction in body weight, hip circumference and fat%. The change in BMI was not significant. On the other hand, there was a highly significant increase of the total body water and Basal metabolic rate after following the dietary program and physical activity. **Conclusion:** The nutritional intervention program was succeeded in 38 obese adolescent girls. These girls show highly significant reduction in body composition and body fat distribution. This revealed that the combined program of diet restriction and exercise is necessary.

[Nayera El-morsi Hassan, Safaa T. Zaki, Sahar El-masry, Manal A. Mohsen, Eman Elashmawy. Impact of Balanced Caloric Diet and Physical activity on Body Composition and Fat Distribution of Obese Egyptian Adolescent Girls. Journal of American Science 2010;6(11):832-842]. (ISSN: 1545-1003).

**Keywords:** Egyptian adolescents, obese girls, diet restriction, exercise training, body composition, anthropometry

**Morphological and Molecular Evidences Among Four Heteroforms of *Avicennia marina* (Forssk) Vierh.**

**Wafaa M. Said and Nahla O. M. Ehsan\***

Botany Department, Women's College for Arts, Science, and Education, Ain Shams University.

[dr.nahla.osman@gmail.com](mailto:dr.nahla.osman@gmail.com)\*

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**Abstract:** Morphological characteristics and random amplified polymorphic DNA (RAPD) marker were used to assess inter-specific relationships among four heteroforms of gray mangrove (*Avicennia marina* (Forssk) Vierh.) grown in Al-Sharm Al-Bahari site, 33Km south Al-Qussier, Red Sea Coast, Egypt. The four heteroforms viz. I, II, III and IV were detected in two distinct habitats (marine and desert). The morphological and molecular data indicated high variation between form I&III and II&IV. On the other hand, low variation between form I&II and III&IV. Dendrogram based on morphological, anatomical and genetic data supported the segregation of the four heteroforms of *Avicennia marina* into two groups; one includes form I & III and the second include form II & IV. The study concluded that the four heteroforms can be classified as two subspecies, *A. marina* subsp. *eucalyptifolia* (form I) and the *A. marina* subsp. *marina* (form II). In addition, forms III and IV considered as phenotypes from I and II, respectively.

[Wafaa M. Said and Nahla O. M. Ehsan. Morphological and Molecular Evidences Among Four Heteroforms of *Avicennia marina* (Forssk) Vierh. Journal of American Science 2010;6(11):843-856]. (ISSN: 1545-1003).

**Keywords:** Mangrove; *Avicennia marina*; Morphology, RAPD; Red Sea

**A High Temperature Sensitive Micro-Planes Damage Model for Plane Concrete**

Mojtaba Labibzadeh<sup>1</sup>, Ali Edalatbehbahani<sup>1</sup>, Hamid Reza Ghafouri<sup>1</sup>

<sup>1</sup>Department of Civil Engineering, Faculty of Engineering, Shahid Chamran University, Ahvaz, Iran  
[Labibzadeh\\_m@scu.ac.ir](mailto:Labibzadeh_m@scu.ac.ir)

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**Abstract:** A computational model simulating the behavior of the concrete subjected to the high temperature environment has been presented here by means of micro-planes framework. The constitutive equations using damage formulations developed earlier by (Labibzadeh and Sadrnejad, 2006) have been adapted here to account for the effects of elevated temperatures. These damage formulations have been founded upon five fundamental damage functions which are directly related to the loading history of each micro-plane. The characteristic features of the proposed model have been verified through making comparison with published experimental results for uniaxial compression and tension tests in the literatures. This suggested model could be easily implemented into a 3D finite elements code to detect damages of concrete structures subjected to fire events.

[Mojtaba Labibzadeh, Ali Edalatbehbahani, Hamid Reza Ghafouri. A High Temperature Sensitive Micro-Planes Damage Model for Plane Concrete. Journal of American Science 2010;6(11):857-864]. (ISSN: 1545-1003).

**Keywords:** Constitutive equations, Micro-planes, Damage, High temperatures, Plane concrete

**Toxic Impact of Titanium Dioxide ( $TiO_2$ ) In Male Albino Rats with Special Reference to its Effect on Reproductive System**

**Nabela, I., EL- Sharkawy\*, Salah, M. Hamza and Ehsan, H., Abou-Zeid**

Dept. of Forensic Medicine & Toxicology. Fac. of Vet. Med. Zagazig University, Zagazig, Egypt.

\* [nabelaimam@hotmail.com](mailto:nabelaimam@hotmail.com)

[Full text](#)

**Abstract:** The present study was directed to explore the toxic effects of orally administered  $TiO_2$  in mature male albino rats . Eighteen mature male albino rats were classified into three equal groups. The first group was used as control and fed on  $TiO_2$  free ration (C), the second and the third groups (T1) and (T2) were fed on ration containing 1% and 2%  $TiO_2$  respectively for 65days . The body weight of male albino rats fed 1% and 2%  $TiO_2$  showed a significant decrease along the experimental period. Animals were scarified after termination of the experimental period. The sera were separated for estimation of nitric oxide and testosterone levels. Liver samples were preserved for antioxidants enzyme activities determination. Liver, testes and seminal vesicle samples were preserved in formalin for histopathological study. The results indicated that  $TiO_2$  resulted in a significant decrease in body weight gain, sperm motility %, sperm cell concentration, sperm viability and serum testosterone level. While, a significant increase in sperm abnormalities, serum nitric oxide (NO), hepatic superoxide dismutase (SOD), glutathione reductase (GR) enzyme activities and malondialdehyde (MDA) concentration were recorded. Histopathological findings revealed reduction in the number and size of the epithelial lining of the tubuloalveolar gland and hyperplastic glandular epithelium of seminal vesicle. Testes showed mild spermatogenesis besides congested testicular blood vessels. Liver showing vacuolar, hydropic degeneration and cell death of some hepatic cells and steatosis .The present study concluded that,  $TiO_2$  elicited a marked ruinous effect on male fertility and biochemical parameters as well as histopathological picture.

[Nabela, I., EL- Sharkawy, Salah, M. Hamza and Ehsan, H., Abou-Zeid. Toxic Impact of Titanium Dioxide ( $TiO_2$ ) In Male Albino Rats with Special Reference to its Effect on Reproductive System. Journal of American Science 2010;6(11):865-872]. (ISSN: 1545-1003).

**Keywords:** Toxic; Titanium Dioxide ( $TiO_2$ ); Rat; Reproductive System

**Protective Effect of *Lepidium sativum L.* Seeds Powder and Extract on Hypercholesterolemic Rats**

**Wafeqa Abdulah Al Hamedan**

Department of Nutrition and Food Science, Home Economic, Collage, Princess Nora Bent abdul – rahman -University, Riyadh, Saud Arabia

[Full text](#)

**Abstract:** The present study was designed to investigate the effects of *Lepidium sativum L* (LS) on the nutritional status and some biochemical analyses of serum and liver in hypercholesterolemic rats. Forty-two adult albino male rats Sprague Dawley strain were classified into six groups. One was fed on standard diet and kept as control (-ve) group. The other five hypercholesterolemic rat groups were control (+ve), drug, LS extract, 5 % or 10 % LS powder rat groups. In comparison to control (- ve) group, the control (+ve) group showed a significant higher value of weight gain , feed efficiency ratio (FER), serum cholesterol, triglycerides , LDL-c ,VLDL-c, cholesterol/ HDL-c and LDL-c/ HDL-c, serum (AST& ALT) ,creatinine ,urea, liver cholesterol and total lipids but significant decrease in HDL-c,

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globulin and liver triglycerides .Also, LS extract and 5% LS powder rat groups showed a significant increase in weight gain, cholesterol/ HDL-c and LDL-c/ HDL-c, serum (AST& ALT) however, drug and 10% LS powder rat groups showed a significant increase in cholesterol/ HDL-c. On the other hand, The drug, LS extract , 5% and 10% LS powder rat groups showed a significant increase in serum cholesterol ,triglycerides VLDL-c , LDL-c ,serum creatinine and urea level when compared to control (- ve) group. In comparing with control (+ ve) group, The drug, LS extract , 5% and 10% LS powder rat groups showed a significant lower value of weight gain , feed efficiency ratio, serum cholesterol ,triglycerides VLDL-c , LDL-c level, cholesterol/ HDL-c , LDL-c/ HDL-c , serum (AST& ALT) ,serum creatinine, urea, liver cholesterol and total lipids with a significant increase in both serum globulin and liver triglycerides.

[Wafeqa Abdulah Al Hamedan. Protective Effect of *Lepidium sativum L.* Seeds Powder and Extract on Hypercholesterolemic Rats. Journal of American Science 2010;6(11):873-879]. (ISSN: 1545-1003).

Key wards : *Lepidium sativum* – aqua extract- cholesterol and rats

**Investigation of MLS<sub>B</sub> and tetracycline resistance in coagulase-negative staphylococci isolated from the skin of Egyptian acne patients and controls**

**El-Mahdy, T.S. <sup>\*1,2</sup>; Abdalla, S. <sup>3</sup>; El-Domany, R. <sup>2</sup> and Snelling, A.M.<sup>1</sup>**

<sup>1</sup>Dept. of Biomedical Sciences, University of Bradford, UK <sup>2</sup>Faculty of Pharmacy, University of Helwan, Egypt

<sup>3</sup>Faculty of Pharmacy, University of Suez-Canal, Egypt; \*[Sata186@hotmail.com](mailto:Sata186@hotmail.com)

[Full text](#)

**Abstract:** A total of 335 antibiotic-resistant coagulase-negative staphylococci (CNS) were isolated from face of 53 Egyptian acne patients, 13 dermatology staff and 36 controls. Prevalence of tetracycline resistant CNS was the most common with a rate of 87.3% of total population sampled. Acne patients treated with antibiotics were found to have significant higher risk of carrying erythromycin and clindamycin resistant CNS than patients not under treatment. Staff group was the most common cohort to carry multi-resistant CNS strains with a prevalence of 81.2%. Four erythromycin-resistance genes were screened for 43 CNS strains from patients. The most widely distributed determinants were *msr(A)* alone (48.8%), followed by *erm(C)* alone (39.6% strains) while both determinants together were accounted in 11.6% of the isolates. In addition, 48 non-duplicate tetracycline resistant CNS strains from patients were screened for the presence of four tetracycline resistance genes. Forty-seven of the isolates (97.9%) had *tet(K)* gene. *Tet(L)* gene was only found in four isolates (8.3%), from which three isolates were found to carry also *tet(K)* gene. This study revealed that the high carriage rate of *msr(A)* in our isolates suggests the effective therapy with clindamycin for most of erythromycin resistant CNS infections. In addition, the mechanism of tetracycline resistance in our isolates is mainly by active efflux and we might expect the success of treatment with minocycline in most of tetracycline resistant CNS from Egypt.

[El-Mahdy, T.S.; Abdalla, S.; El-Domany, R. and Snelling, A.M. Investigation of MLS<sub>B</sub> and tetracycline resistance in coagulase-negative staphylococci isolated from the skin of Egyptian acne patients and controls. Journal of American Science 2010;6(11):880-888]. (ISSN: 1545-1003).

**Key words:** acne, coagulase-negative staphylococci, MLS<sub>B</sub>, resistance, tetracycline. **Running title:** Antibiotic resistance of Egyptian CNS.

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**Evaluation of Rural Development in Guilan Province, Iran**

Hamidreza Alipour<sup>1</sup> and Mohammad Sadegh Allahyari<sup>2</sup>

<sup>1,2</sup> Department of Agricultural Management, College of Agriculture, Islamic Azad University, Rasht Branch, Iran; [Allahyari@iaurasht.ac.ir](mailto>Allahyari@iaurasht.ac.ir)

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**Abstract:** The main purpose of this study was to measure the development level of Guilan rural districts based on Morris Inequality Index. The study employed a descriptive survey design. The statistical population of this study was all Guilan rural districts consisting of 109 rural districts in 2006. In order to investigate and to determine the key indexes of development or backwardness in each region, some variables in five groups (agricultural, health, infrastructure and social) had been used. For data analysis and assessment of development level, Morris Inequality Index was used. Findings revealed that out of the total Guilan rural districts in developmental situation, six rural districts were underdeveloped and more percent of villages were in less developed

	<p>situation.</p> <p>[Hamidreza Alipour and Mohammad Sadegh Allahyari. Evaluation of Rural Development in Guilan Province, Iran. Journal of American Science 2010;6(11):889-893]. (ISSN: 1545-1003).</p> <p><b>Keywords:</b> Development, Rural, coefficient of variation, Morris Inequality Index</p>		
123	<p><b>Diagnosis and Epidemiological Studies of Bovine Trypanosomiasis in Kaliobia Governorate</b></p> <p><b>Mervat E.I. Radwan<sup>*1</sup> and Reham El Madawy<sup>2</sup></b>            Department of Infectious Diseases, Vet. Hospital<sup>1</sup>, Department of Parasitology<sup>2</sup>, Benha-University, Benha, Egypt; <a href="mailto:dr_mervat19@yahoo.com">dr_mervat19@yahoo.com</a></p> <p><b>Abstract:</b> This investigation was performed on 131 animals (cattle and buffaloes) from farms located in different places in kaliobia aged from 1.5-5 years the samples were collected from clinically infected animals that suffer from "surra" disease and animals apparently healthy in contact with infected animals (subacute or chronic) Infected animals .This investigation reported that 51animals showed the Clinical signs of illness as pyrexia, parasitaemia,progressive emaciation, generalized edema and recurrent episodes of fever occur during course of disease .The microscopic examination of blood film revealed (<i>Trypanosoma evansi</i>) in 5 out of 80 apparently healthy animals (7.8%) while PCR examination found that 35 out of 75animals positive (46.7) so PCR is the most suitable diagnosis for early diagnosis and consequently controlling programs and consider the confirmatory test.</p> <p>[Mervat E.I. Radwan and Reham El Madawy. Diagnosis and Epidemiological Studies of Bovine Trypanosomiasis in Kaliobia Governorate. Journal of American Science 2010;6(11):894-898]. (ISSN: 1545-1003).</p> <p><b>Keywords:</b> Diagnosis; Epidemiological; Bovine Trypanosomiasis; Kaliobia Governorate</p>	<a href="#">Full text</a>	123
124	<p><b>Noise Prediction for Outdoor Cooling Systems; Case Study</b></p> <p><b>Ahmed A. Medhat A.Fahim<sup>1</sup> and Hoda S. Seddeq<sup>**</sup></b>  <sup>1</sup>HVAC Consultant &amp; Fire Engineering Auditor in Electro-Mechanical Research Institute at Housing and Building National Research Center, HBRC, Cairo, Egypt, P.O.Box 1770 Giza,  <sup>2</sup> Acoustic laboratories in Building Physics research Institute at Housing and Building National Research Center, HBRC, Cairo, Egypt, P.O.Box 1770 Giza  <a href="mailto:luukki@live.com">luukki@live.com</a> <a href="mailto:hodasoliman@yahoo.com">hodasoliman@yahoo.com</a></p> <p><b>Abstract:</b> Outdoor noise analyses are commonly required to estimate the sound levels at the property line of adjacent buildings. Outdoor cooling units, such as cooling towers, air-cooled chillers and rooftop units, all create noises at different levels that can disturb neighbors or occupants inside the building itself. Creating a comfortable acoustic environment in most of Heating, Ventilating and Air-Conditioning, HVAC, applications falls on the mechanical engineering disciplines because most background noise sources are generated by the mechanical apparatuses and cooling devices. This paper investigates the prediction of sound pressure levels emitted from outdoor HVAC systems. The sound level of outdoor units in various applications is dependent upon several significant factors. These factors include equipment location, directivity of the source, barrier shielding, sound path, and attenuation due to distance, atmospheric sound absorption and ground attenuation. A developed simplified model called "Outdoor Modeling Acoustic Code, OMAC" has been utilized taking into consideration the influences of previously mentioned parameters. This OMAC code has been used to analyze and predict the noise level emitted from roof-top air-cooled chillers located on office building as a case study. Predicted noise regimes were compared with the collected field measurements for the validation and verification purposes. Detailed analyses and comparisons between predicted and measured noise spectrums were carried out based on the local and international standards. These comparisons show a good agreement among predicted noise criterions, measured data and dedicated standard thresholds. It was concluded that it is mandatory to utilize such prediction "modeling" tool during the early stages of HVAC design process to allow the authority having jurisdictions to predict the impact outdoor noises within the new development urban.</p> <p>[Ahmed A. Medhat A.Fahim and Hoda S. Seddeq. Noise Prediction for Outdoor Cooling Systems; Case Study. Journal of American Science 2010;6(11):898-905]. (ISSN: 1545-1003).</p>	<a href="#">Full text</a>	124

	<p>Key wards: outdoor sound propagation, HVAC, sound power, directivity, barrier, atmospheric sound absorption, ground attenuation</p>	
125	<p><b>Seed Exomorphic Characters of some Taxa from Saudi Arabia</b></p> <p><b>Soliman, M.S.A.* Al-Tarras. A. and Al-Awady, M.</b> Biotech. &amp; Genet. Eng. Res. Unit, Taif University, Taif, KSA  <sup>*</sup><a href="mailto:prof.msoliman@yahoo.com">prof.msoliman@yahoo.com</a></p> <p><b>Abstract:</b> Seed exomorphic characters of seven species collected from Taif province, Saudi Arabia, were investigated by the aid of Scanning Electron microscopy (SEM). The seed exomorphic characters that are diagnostic at the generic and specific level are seed shape, dimensions, epidermal cells, seed surface sculpture and aspects of anticlinal and periclinal walls. The seed coat of the studied taxa exhibit a wide range of morphological characters. Seed shapes varied from globoid, elliptic, oblong and kidney shaped. They showed either smooth or papilate surface. SEM investigation at higher magnifications revealed different types of seed surface pattern viz, is tuberculate, reticulate, scalariform and tunicostate. Seeds of <i>Cloeme droserifolia</i> and <i>Fagonia schweinfurthia</i> showed a deposition of wax on their surface. The present study is a modest contribution to previous studies on the flora of Saudi Arabia.  [<b>Soliman, M.S.A. Al-Tarras. A. and Al-Awady, M.</b> Seed Exomorphic Characters of some Taxa from Saudi Arabia. Journal of American Science 2010;6(11):906-910]. (ISSN: 1545-1003).</p> <p><b>Key words:</b> SEM, seed coat, exomorphic characters, <i>Cloeme droserifoli</i>, <i>Fagonia schweinfurthia</i>, flora, Saudi Arabia</p>	<a href="#">Full text</a> 125
126	<p><b>Biomarkers Characteristics of Crude Oils from some Oilfields in the Gulf of Suez, Egypt.</b></p> <p><b>M. I. Roushdy, M. M. El Nady, Y. M. Mostafa, N.Sh. El Gendy and *H. R. Ali</b> Egyptian Petroleum Research Institute, Nasr City, Cairo, Egypt.  <sup>*</sup><a href="mailto:hugochem@yahoo.com">hugochem@yahoo.com</a></p> <p><b>Abstract:</b> Seven representative crude oil samples from the Gulf of Suez were chosen for this study. The studied crude oils are Ras Badran, Belayim marine, Belayim Land, Rahmi, West Bakr, Esh El Mellaha and Geisum. The oils were fractionated by medium pressure liquid chromatography into saturated hydrocarbons, aromatic hydrocarbons and polar compounds. The saturated hydrocarbons were determined by gas chromatography and gas chromatography/mass spectrometry (GC/MS). Ratios of certain biomarkers, (Pristane/phytane, isoprenoids/n-alkanes, CPI, Homohopane, Diasteranes, Gammacerane index, C<sub>29</sub> 20S/20S+20R, C<sub>29</sub>/C<sub>30</sub> hopanes and Ts/Tm) referred to as source correlation indices, are sensitive to the geological source of oil. The results of evaluation suggest that two types of oils could be recognized as marine oils. These oils are characterized by high level of maturation and sourced mainly from source rocks rich in marine organic matters with few inputs from terrestrial origin.  [<b>M. I. Roushdy, M. M. El Nady, Y. M. Mostafa, N.Sh. El Gendy and *H. R. Ali.</b> Biomarkers Characteristics of Crude Oils from some Oilfields in the Gulf of Suez, Egypt. Journal of American Science 2010;6(11):911-925]. (ISSN: 1545-1003).</p> <p><b>Key words:</b> Egypt, Gulf of Suez, Homohopanes, Diasteranes, Gammacerane index, C<sub>29</sub> 20S/20S+20R, C<sub>29</sub>/C<sub>30</sub> hopanes and Ts/Tm, Crude oils</p>	<a href="#">Full text</a> 126
127	<p><b>Drug resistance and recent therapeutic measures in controlling of fascioliasis</b></p> <p><b>A. Z. Mahmoud<sup>1</sup>; Mokhtar M. Taha<sup>1</sup>; Salah M. H. Afifi<sup>1</sup>; Khaled M. A. Hassanein<sup>1</sup> and Amal Mohamed Abdo<sup>*2</sup></b> Department of Pathology &amp; Clinical Pathology, Faculty of Veterinary Medicine, * Parasitology department, Faculty of Medicine, Assiut University, Assiut, Egypt.  <sup>*</sup><a href="mailto:amalalmatary@yahoo.com">amalalmatary@yahoo.com</a></p> <p><b>Abstract:</b> Fascioliasis is a widely distributed disease affecting herbivorous animals. As a result of drug resistance a mixture of two antifasciola drugs (<b>Triclobendazole and Superivomec</b>) was used in trial to overcome this drug resistance. Twenty eight newly weaned white Boskat rabbit</p>	<a href="#">Full text</a> 127

aging 1.5 month were divided into 7 groups, six of them were experimentally infected with metacercaria of *Fasciola gigantica* and one kept as -ve control group. Faecal egg count during the clinical course of the disease, counting the worm and its morphological studies and lesion score after postmortem examination were the parameters used to evaluate the effect of different drug mixtures. It had been concluded that the mixture of triclabendazole and superivomec was the mixture of choice.

[A. Z. Mahmoud; Mokhtar M. Taha; Salah M. H. Afifi; Khaled M. A. Hassanein and Amal Mohamed Abdo. Drug resistance and recent therapeutic measures in controlling of fascioliasis. Journal of American Science 2010;6(11):926-933]. (ISSN: 1545-1003).

**Key words:** Fascioliasis, Metacercaria, Triclabendazole, Rabbit and Superivomec

**The Effect of Green, Roasted and Decaffeinated Coffee on Serum Glucose, Insulin and Serum Lipid Profile in Diabetic Experimental Animals**

**Eman A.Sadeek<sup>\*1</sup>, Hala, A. Abd El;-Rahman<sup>2</sup> and Waffa, Sh. Ali<sup>3</sup>**

<sup>1</sup>Department of Biochemistry & Nutrition -Women's College –Ain –Shams University. <sup>2</sup> Food Tech. Res. Ins. Agric. Res. Center. <sup>3</sup>College of Home Economics, Helwan University. Cairo, Egypt

<sup>\*</sup>dr.emansadeek@yahoo.com

[Full text](#)

**Abstract:** Aim of the work: Assessing the Effect of green, roasted and decaffeinated coffee on serum glucose, insulin and serum lipid profile in diabetic rat models. Methods: Design of the Study: Thirty female wistar rats weighing  $124.5 \pm 5.41$ g (mean  $\pm$ S.D) were divided into 5 groups. The first group served as a control and consumed a standard diet according to (AIN – 93). The other 4 groups were injected intraperitoneally with 105 mg / kg body weight of alloxan . One group was kept without further treatment and served as a positive diabetic control. Groups 3, 4, 5 consumed 5% green, roasted and decaffeinated coffee in drinking water, respectively. The feeding trial continued for four weeks. At the end of the experiments, the animals were sacrificed, blood samples were collected, and the liver, kidney ,spleen and heart were separated, washed ,dried and weighed. Laboratory investigations Consisted of serum glucose, insulin, calcium, phosphorus and complete lipid profile was determined to test the magnitude of antioxidant potential green, roasted and decaffeinated coffee. Results: The present study show a significant difference ( $p < 0.05$ ) in body weight gain and food intake between all treatment groups , with non significant difference in water intake , relative weight of organs including liver , kidney , spleen and heart . the study also shows significant elevation ( $p < 0.05$ ) in serum glucose and insulin in diabetic control group as compared to normal control group. This indicates uncontrolled hyperglycemia in alloxan diabetic rats. While consumption of green, roasted and decaffeinated coffee resulted in a decrease in serum glucose and insulin ( $p < 0.05$ ) .There is a significant decrease ( $p < 0.05$ ) in serum calcium and serum phosphorus in groups 3,4 and 5 fed green, roasted and decaffeinated coffee respectively indicating an association between coffee consumption and bone health. our results also shows that alloxan injection produced a significant increase( $p < 0.05$ ) in serum total- cholesterol(TC); triacylglycerol(TAG); LDL-C ; VLDL-C and in LDL\ HDL ratio and TC \ HDL ratio however a significant decrease ( $p < 0.05$ ) in serum HDL-C is observed ; In diabetic rats compared to normal control .green, roasted and decaffeinated coffee resulted in a significant decrease ( $p < 0.05$ ) in triacylglycerol (TAG); LDL-C ; VLDL-C and in LDL\ HDL ratio and TC \ HDL ratio .on the other hand a significant increase ( $p < 0.05$ ) in serum HDL-C is observed in green, roasted and decaffeinated coffee groups compared to diabetic rats compared to normal control with the highest value for green coffee .Non significant effect on serum total- cholesterol(TC) reported in this study .

Conclusion: The observed improvement in glucose, insulin profile, triacylglycerol and HDL-C confirm the potent biological action of green, roasted and decaffeinated coffee and suggest that chlorogenic acid (a component in coffee) might have an antagonistic effect on glucose transport. Suggesting a novel function of coffee on lowering the risk factors of diabetes and delaying the progress of diabetes complications as well.

[Eman A.Sadeek, Hala, A. Abd El;-Rahman and Waffa, Sh. Ali. The Effect of Green, Roasted and Decaffeinated Coffee on Serum Glucose, Insulin and Serum Lipid Profile in Diabetic Experimental Animals. Journal of American Science 2010;6(11):934-943]. (ISSN: 1545-1003).

**Key words:** Green, roasted, decaffeinated coffee, glucose, insulin and lipid profile

**The Impact of Obesity and Weight Reduction Program with Xenical Drug Treatment on Health Status of Obese Adolescent Girls in Saudi Arabia**

[Full text](#)

**Hend Hassan Ali Ganbi**

Nutrition and Food Science Dept., Faculty of Education for Home Economics and Art Education , King Abd – Elaziz University, Jeddah , Saudi Arabia  
[dr.gamal\\_rawayshed@yahoo.com](mailto:dr.gamal_rawayshed@yahoo.com)

**Abstract:** The present investigation aimed to assess the health problems and diseases associated with obesity in Saudi Arabian adolescent girls, and to evaluate the impact of obesity and weight reduction program with xenical (Orlistat) drug treatment on health status of tested obese adolescent patients. This study was performed on a group of 160 obese adolescent girls, aged 15 – 20 years, attending Physical Rehabilitation and Obesity Treatment Centers at Jeddah, Saudi Arabia. The obese patients group, under investigation, was selected from the adolescent girls who want in the treatment of obesity after obtaining their consent to participate in this study. The present results revealed that most tested obese adolescent girls suffered with a lot of health problems and diseases. The obesity was associated with deterioration of health status for obese subjects. Also, the weight reduction program with xenical (Orlistat) drug treatment caused a significant loss ( 0.01) in body weight of obese adolescent girls by 7.42 and 11.35 % after 30 and 60 days respectively. In addition that tested weight reduction program with orlistat treatment exhibited a significant enhancing impact ( 0.01) on all tested health status parameters of obese patients ; especially when obese patients engaged with tested weight – reduction program based upon being on a nutritionally balanced , reduced – calorie dietary regimen and practicing the physical activities regularly at least 6 hours a day , as showing by its enhancing effect on liver functions, serum lipid profile , liver and renal functions and by its lowering effect on blood glucose , insulin and LDL - cholesterol levels ; within the reference reported range of all health status items for health individual adolescent girls. Therefore, it is recommended that the obese adolescent girls and women should be orally treated with xenical drug capsules with their obligation by being on a dietary regimen ; a nutritionally balanced , reduced – calorie diet (800 – 1200 calorie) , and practicing the physical activities regularly at least 6 hours a day.

[Hend Hassan Ali Ganbi. The Impact of Obesity and Weight Reduction Program with Xenical Drug Treatment on Health Status of Obese Adolescent Girls in Saudi Arabia. Journal of American Science 2010;6(11):944-958]. (ISSN: 1545-1003).

**Key words :** Obesity; Weight reduction program; Obese adolescent girls; Liver functions; lipid profile; Renal functions; Health status; Xenical drug; Physical activity; Dietary regimen

**Molecular Characterization of Egyptian Isolates of *Lactobacillus* and *Bifidobacterium***

**Hashem S.<sup>1</sup>; H. H. Sabit<sup>2</sup>; M. Amin<sup>3</sup>; W. Tawakkol<sup>4</sup>; and A. F. Shamseldin<sup>4</sup>**

<sup>1</sup>Microbiology Dept., College of Medicine, Assiut University, Assiut, Egypt

<sup>2</sup>Microbial Genetics Dept., College of Biotechnology, Misr University for Science and Technology, Cairo, Egypt

<sup>3</sup>Microbiology Dept., College of Pharmacy, Cairo University, Cairo, Egypt

<sup>4</sup>Microbiology Dept., College of Pharmacy, Misr University for Science and Technology, Cairo, Egypt

**Abstract:** Strains of *Lactobacillus* and *Bifidobacterium* were isolated from processed milk collected in Cairo, Egypt. *Lactobacilli* was isolated on Acetate media (SL) of Rogosa and Mitchell-Weisman. While *Bifidobacterium* was isolated on DSM medium (Difco Sporulation Medium). The isolates were characterized microscopically, morphologically and by some biochemical tests. DNA was extracted from the specified isolates using (Qiagen, Germany. Cat #51306) and species-specific primers for *Lactobacillus* and *Bifidobacterium* were designed to amplify the 16S rDNA gene as a conserved region in the bacterial DNA. Elution of the target band from the gel was performed efficiently and the 16S rDNA region was subjected to sequencing using Sequencer ABI PRISM 3730XL Analyzer. The sequencing data obtained suggested that the two studied isolates were (at the genus level) designated as *Lactobacillus* and uncultured *Bifidobacterium*. When the sequencing data was aligned on <http://www.ncbi.nlm.nih.gov>, it shows 88% homology and expected value of 7e-164 to

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*Lactobacillus kiranofaceins* but dendogram tree shows more homology to *Lactobacillus plantarum* family. While the other sample showed 91% homology and expected value of 3e-113 with Uncultured *Bifidobacterium* Clone R333 16S rRNA gene.

[Hashem S.; H. H. Sabit; M. Amin; W. Tawakkol; and A. F. Shamseldin. Molecular Characterization of Egyptian Isolates of Lactobacillus and Bifidobacterium. Journal of American Science 2010;6(11):959-964]. (ISSN: 1545-1003).

**Keywords:** Molecular Characterization of Egyptian Isolates of Lactobacillus and Bifidobacterium

Maturation and Histological characteristics of ovaries in Mudskipper, *Periophthalmus papilio* from Lagos lagoon, Nigeria

[Full text](#)

LAWSON, Emmanuel Olugbenga

Department Of Fisheries, Faculty of Science, Lagos State University, Ojo.

P.O. Box 001, LASU Post Office Box, Lagos, Nigeria.

[ollulawson@yahoo.com](mailto:ollulawson@yahoo.com).

**Abstract:** Maturation and histological characteristics of female gonads in mudskipper, *Periophthalmus papilio* from Lagos lagoon, Nigeria were investigated between July 2004 and July 2006. This species is found in abundance in the mud flats of the mangrove swamps of Lagos lagoon where it forms part of its fisheries. Its importance lies on its availability as food for man and as baits for artisanal and offshore fisheries. Diurnal collections were made with non return valve traps. Biometric data were recorded and sexes separated. Ovaries were carefully removed from 1390 individual specimens that were with no abnormalities or pathological changes. The histological structure of the ovaries was based on a temporal scale after intensive sampling. The ovaries were observed macroscopically and processed by standard histological technique. ICES, BITS and IBTS scales and Bucholtz manuals were employed in the classifications of its maturity and gonadal stages. Seven stages of maturity which included: immature (stage I), immature and developing (stage II), ripening (stage III), ripe (stage IV), ripe running (Stage V), spent (stage VI) and recovering-spent (stage VII) were observed among the specimens. These constituted 1.15, 47.99, 15.32, 9.86, 19.50, 4.68 and 1.51% of the specimens examined in the study respectively. The pre-spawning phase was represented by I, II and III; the spawning by IV and V; and post-spawning by VI and VII. Histological development of the species indicated six (6) developmental stages of oocytes development viz: oogonium, primary oocyte, primary, secondary, and tertiary vitellogenic and hyaline oocytes. Specimens were found with oocytes which had developed over the migratory nucleus stage, indicating maturation can still proceed in the fish on the mudflats before migrating to spawning nests in the burrows. Stages V and VI ovaries contained all stages of oocyte. The GSI of the species increased at initial phase and then became stable at the later period. The species was a multiple and synchronous spawner, spawning in February, March, and October. The mean GSI varied from  $1.03 \pm 0.09\%$  in May to  $8.40 \pm 1.67\%$  in February 2006. Less than  $8.40 \pm 1.67\%$  of the body biomass was converted by the species to development of ovaries. The minimum size of spawning females was 110 mm TL. Therefore, this study provides the necessary information on maturation and histological development of oocytes as an appropriate strategy for optimum utilization and conservation of this commercially valued fish species in Lagos lagoon, Nigeria.

[LAWSON, Emmanuel Olugbenga. Maturation and Histological characteristics of ovaries in Mudskipper, *Periophthalmus papilio* from Lagos lagoon, Nigeria. Journal of American Science 2010;6(11):965-976]. (ISSN: 1545-1003).

**Key words:** Chromatin, zona radiata interna, externa, maturation, nucleolar, vitellogenic

Residual Available Copper and Boron in Soil as Affected by Zinc sulfate and Boric acid in a Zinc and Boron Deficient Soil

[Full text](#)

Farshid Aref

Department of Soil Science, Firouzabad Branch, Islamic Azad University, Iran

Tel: +989173383896, [farshidaref@yahoo.com](mailto:farshidaref@yahoo.com)

**Abstract:** Micronutrients such as copper (Cu) and boron (B) are needed in small amounts, and

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there are also likely to be residual effects for some years after their application. A field experiment with maize plant grown on Zn and B deficient soil was conducted to evaluate the effect of Zn and B interaction on the residual available Cu and B content in the soil during 2009 at Fars Province, Iran. Treatments including five levels of Zn (0, 8, 16 and 24 kg ha<sup>-1</sup> and Zn foliar spray) and four levels of B (0, 3, and 6 kg ha<sup>-1</sup> and B foliar spray) in a completely randomized block design were set up. The findings showed that in all treatments, the residual available Cu and B in the soil increased compared to its initial levels (before culture). The main effect of Zn and B on the residual Cu was insignificant relative to the no Zn and B level. No treatments, showed a significant difference on the residual Cu in the soil as compared with the control and also the effect of Zn-B interaction on the residual Cu was insignificant. In most treatments, the residual B in the soil decreased compared to its initial level levels (before culture). The Zn-B interaction was significant on the residual available B content in the soil. The presence of Zn prevented from increase of the available B remaining in the soil by B use relative to the soil B content before culture. Application of a high amount of Zn in the soil decreased residual available B in the soil relative to the no Zn level.

[Farshid Aref. Residual Available Copper and Boron in Soil as Affected by Zinc sulfate and Boric acid in a Zinc and Boron Deficient Soil. Journal of American Science 2010;6(11):977-984]. (ISSN: 1545-1003).

**Keywords:** Interaction, Zinc, Boron, Copper, Residual available nutrients

Preparation of spherical silica nanoparticles: Stober silica

[Full text](#)

**Ismail A.M. Ibrahim\*, A.A.F. Zikry, Mohamed A. Sharaf**  
**Chemistry Department, Faculty of science, Helwan University, 11795 Egypt**  
*\* [ismailscience@gmail.com](mailto:ismailscience@gmail.com)*

**Abstract:** The diameter of silica nanoparticles is mainly affected by the relative contribution from nucleation and growth. Once the total number of nuclei is fixed, the resultant particle size is then determined via the growth process by the total quantity of TEOS. In this work, we will demonstrate the effect of TEOS and NH<sub>3</sub> concentrations on particle size of silica nanoparticles. Experimental results indicate that the size of silica colloids decreases with increasing with TEOS and ammonia concentrations where both the rate of hydrolysis and condensation become Faster and influence the solubility of intermediate [Si (OC<sub>2</sub>H<sub>5</sub>)<sub>4-x</sub>(OH)<sub>x</sub>] and hence the supersaturation for the nucleation process. With higher catalyst concentration, the number of nuclei is increased and therefore smaller silica colloids are obtained. Also surface modification of the silica nanoparticles by hexamethyldisilazane was studied to prevent the particles aggregation and to give good dispersion of silica nanoparticles in hydrophobic mediums.

[Ismail A.M. Ibrahim, Amina Zikry, Mohamed A. Sharaf, Chemistry Department, Faculty of science, Helwan University, Egypt. Journal of American Science 2010;6(11):985:989]. (ISSN: 1545-1003).

**Keywords:** Stober silica; nucleation; hydrolysis; condensation; nanoparticles; surface modification

**Early postpartum dietary practices among a group of Saudi women**

[Full text](#)

**Samar k. Hafez<sup>1&2</sup> and Sahar M. Yakout<sup>1&3</sup>**  
<sup>1</sup>Maternity and Gynecologic Nursing Dep., Alexandria University Alexandria, Egypt.  
<sup>2</sup>Nursing Dep. Taif University, Sudia Arabian  
<sup>3</sup>Nursing Dep. King Sa'ud University, Saudi Arabian  
*\*[sakamal2000@yahoo.com](mailto:sakamal2000@yahoo.com)*

**Abstract:** This work aimedto study the early postpartum dietary practices among a group of Saudi women.A retrospective study was carried out on a convenient sample of 300 women during their post-partum period who attended seven primary health centers in Riyadh and Taif, KSA. The subjects were interviewed individually throughout a period of four months fromSeptember 2009 to January 2010.An interview questionnaire and a dietary scale of King and Jakobson were used for data collection. The results showed that73.3% of the study subjects had incomplete knowledge about post-partum nutrition and anequal proportion of them (28.3%) had

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either excellent or borderline dietary practices during their early post-partum period and about one-fifth of them (19.3%) had dangerous dietary practices. The study concluded that Saudi women's post-partum dietary practices were significantly associated with their general characteristics such as age, education, employment and number of family member as well as with their obstetrical characteristics including their gravidity and parity.

[Samar k. Hafez and Sahar M. Yakout. Early postpartum dietary practices among a group of Saudi women. Journal of American Science 2010;6(11):990-998]. (ISSN: 1545-1003).

**Keywords:** postpartum; dietary; Saudi; women

#### **Data Mining Methodology in Perspective of Manufacturing Databases**

Muhammad Shahbaz<sup>1,2</sup>, Syed Athar Masood<sup>2</sup>, Muhammad Shaheen<sup>3</sup>, Ayaz Khan<sup>4</sup>

<sup>1,3</sup> Department of Computer Science & Engineering, UET Lahore, Pakistan

<sup>2</sup> Department of Engineering Management, College of E&ME, Rawalpindi, Pakistan

<sup>4</sup> Forensic Expert/ Project Coordinator, National Response Center for Cyber Crimes (NR3C), FIA, Islamabad

<sup>1</sup> [m.shahbaz@uet.edu.pk](mailto:m.shahbaz@uet.edu.pk), <sup>2</sup> [atharmasood2000@hotmail.com](mailto:atharmasood2000@hotmail.com), <sup>3</sup> [m.shaheen@uet.edu.pk](mailto:m.shaheen@uet.edu.pk), <sup>4</sup> [chaudhary.ayaz@gmail.com](mailto:chaudhary.ayaz@gmail.com)

[Full text](#)

**Abstract:** In recent years data mining has become a very popular technique for extracting information from the database in different areas due to its flexibility of working on any kind of databases and also due to the surprising results. This paper is an attempt to introduce application of data mining techniques in the manufacturing industry to which least importance has been given. A taste of implementable areas in manufacturing enterprises is discussed with a proposed architecture, which can be applied to an individual enterprise as well as to an extended enterprise to get benefit of data mining technique and to share the discovered knowledge among enterprises. The paper proposes conceptual methods for better use of different data mining techniques in product manufacturing life cycle. These techniques include statistical techniques, neural networks, decision trees and genetic algorithms. An integrated and unified data mining platform is anticipated then to improve overall manufacturing process.

[Muhammad Shahbaz, Syed Athar Masood, Muhammad Shaheen, Ayaz Khan. Data Mining Methodology in Perspective of Manufacturing Databases. Journal of American Science 2010;6(11):999-1012]. (ISSN: 1545-1003).

**Keywords:** Data Mining, Manufacturing, Industrial application, Data Mining methodologies, Data Warehousing

#### **Available Zn Distribution, Response and Uptake of Rice (*Oriza sativa*) to Applied Zn Along a Toposequence of Lake Gerio Fadama Soils at Yola, North-eastern Nigeria.**

H. E. Shehu and G. Y. Jamala

Department of Crop Science, Adamawa State University, Mubi, P. M. B 25 Mubi, 650001, Adamawa State, Nigeria. [harushe2003@gmail.com](mailto:harushe2003@gmail.com)

[Full text](#)

**Abstract:** A screen house pot experiment was conducted at FAO/TCP farm of the Adamawa State University, Mubi north-eastern Nigeria, to study the response of rice to Zn fertilizer application and the distribution of Zn along toposequence of the Lake Gerio Fadama soils of North-eastern Nigeria which was used for the study. The experiment consisted of four Zn rates of 0, 5, 7.5 and 10 kg Zn ha<sup>-1</sup> as ZnSO<sub>4</sub>.7H<sub>2</sub>O. The effect of treatment on Zn concentration and dry matter yields response were determined. The 0.1N HCl and DTPA extractable Zn ranged from 8.5 to 9.5 mg kg<sup>-1</sup> soil, 2.2 to 2.7 mg kg<sup>-1</sup> soil with mean values of 9.08 and 2.35 mg kg<sup>-1</sup> soil respectively. Available Zn soil status is therefore assessed as medium. Dry matter yields and Zn uptake were optimum at 5 kg ha<sup>-1</sup> with corresponding values of 2.04 and 11.86 mg kg<sup>-1</sup> respectively.

[H. E. Shehu and G. Y. Jamala. Available Zn Distribution, Response and Uptake of Rice (*Oriza sativa*) to Applied Zn Along a Toposequence of Lake Gerio Fadama Soils at Yola, North-eastern Nigeria. Journal of American Science 2010;6(11):1013-1016]. (ISSN: 1545-1003).

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(<http://www.americanscience.org>)

**Key words:** Zinc uptake; Toposequence; Fadama soils; Rice

**Approximate Optimal Control for a Class of Nonlinear Volterra Integral Equations**

Akbar H. Borzabadi, Akram Abbasi and Omid S. Fard

Department of Applied Mathematics, Damghan University, Damghan, Iran

[borzabadi@dubs.ac.ir](mailto:borzabadi@dubs.ac.ir)

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**Abstract:** In this study an iterative approach to extract approximate solutions of optimal control problems which are governed by a class of nonlinear Volterra integral equations is presented. The structure of approach is based on the parametrization of the control and state functions. Considering some conditions on the problem, the convergence of the given approach is studied. Numerical examples illustrate the efficiency of the given approach.

[Akbar H. Borzabadi, Akram Abbasi and Omid S. Fard. Approximate Optimal Control for a Class of Nonlinear Volterra Integral Equations. Journal of American Science 2010;6(11):1017-1021]. (ISSN: 1545-1003). (<http://www.americanscience.org>).

**Keywords:** Optimal control; Volterra integral equations; iterative schemes; approximation

[Full text](#)

[Full text](#)

**Virulence Factors, Plasmid Profiling and Curing analysis of Multi-drug Resistant *Staphylococcus aureus* and Coagulase negative *Staphylococcus* spp. isolated from Patients with Acute Otitis Media.**

<sup>a</sup> Akinjogunla O. J. and <sup>b</sup> Enabulele, I. O.

<sup>a</sup> Department of Microbiology, Faculty of Science, University of Uyo, P.M.B 1017, Uyo, Akwa Ibom State, Nigeria.

<sup>b</sup> Department of Microbiology, Faculty of Life Sciences, University of Benin, P.M.B.1154. Benin City, Edo State, Nigeria. [papajyde2000@yahoo.com](mailto:papajyde2000@yahoo.com)

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**ABSTRACT:** Microbiological and molecular techniques were used to determine the virulence factors, plasmid profile and antibiotic susceptibility spectrum of *Staphylococcus aureus* and CON-*Staphylococcus* spp isolated from patients with acute otitis media attending University of Uyo Teaching Hospital, General Hospital, Ikot-Ekpene and Nekede Paediatric Hospital between January, 2009 and January, 2010. 42 (30.9%) *Staphylococcus aureus* and 21 (15.4%) CON *Staphylococcus* spp were isolated from the aural swab samples collected. *Staphylococcus aureus* produced 16 (38.1%), 22 (52.4%) and 4 (9.5%) of alpha, beta and gamma haemolysis, respectively, while CON-*Staphylococcus* spp. produced more of beta haemolysis than both alpha and gamma. The results showed that 14 (33.3%) *Staphylococcus aureus* and 9 (42.9%) of CON-*Staphylococcus* spp are beta-lactamase producer. The antibiotics susceptibility testing showed that 29 (69.0%), 26 (61.9%), 27 (64.3%), 28 (66.7%) and 29 (69.0%) of *Staphylococcus aureus* were sensitive to penicillin, ceftriaxidime, cefoxitin, ciprofloxacin and levofloxacin, respectively. 12 (28.6%) of *Staphylococcus aureus* were resistant to streptomycin and imipenem, while about 45.2% -50.0% were resistant to cephalothin and amoxicillin. CON-*Staphylococcus* spp also showed varied percentages of sensitivity ranging between 42.9% for streptomycin and 71.4% for moxifloxacin. The result also showed that 19.2 % of *Staphylococcus aureus* and 9.6% of CON-*Staphylococcus* spp. were resistant to more than eight antibiotics with (MAR) index ranging from 0.25 to 1.00 and 0.25 to 0.75 for *Staphylococcus aureus* and CON-*Staphylococcus* spp. respectively. The results obtained in this study are statistically significant (p < 0.05). Most of the *Staphylococcus aureus* and CON-*Staphylococcus* spp were cured of their plasmids showing that they are plasmid borne. Large molecular weight plasmids ranging from 23.13 kbp to 50.0 kbp were harboured by both *Staphylococcus aureus* and CON-*Staphylococcus* spp obtained from acute otitis media. However,

continuous surveillance to monitor antimicrobial resistance and guide the antibacterial therapy is overwhelmingly necessary.

[Akinjogunla Olajide Joseph, Enabulele Idahosa Onaiwu. Journal of American Science 2010;6(11):1022-1033]. (ISSN: 1545-1003). (<http://www.americanscience.org>)

### **Barriers of Agricultural Development in Iran: A Case study of Fars Province**

[Full text](#)

Farshid Aref

Department of Soil Science, Firouzabad Branch, Islamic Azad University, Iran

[farshidaref@yahoo.com](mailto:farshidaref@yahoo.com), Tel: +989173383896

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**Abstract:** This article attempts to illustrate the barriers of agricultural development in Fars, Iran. Agriculture is certainly a major contributor to rural development in many countries. It is one of the most important economic sectors in Iran. But, there are a significant number of barriers to effectively using agriculture industry as a tool for rural development. The findings through focus group discussion indicated that there are some organizational barriers in agricultural development in some villages in Fars. The finding can assist the local agriculture organizations for remove this problem in face of agriculture for rural development.

[Farshid Aref. Barriers of Agricultural Development in Iran: A Case study of Fars Province. Journal of American Science 2010;6(11):1034-1037]. (ISSN: 1545-1003). (<http://www.americanscience.org>).

**Keywords:** agriculture development, rural area, barriers

### **Immunostimulatory and Protective Properties of *Lactobacillus brevis* Used as a Biocontrol Agent *in Vivo***

[Full text](#)

**Agarry, O. O.**

Department of Biological Sciences, Microbiology Unit, University of Abuja , P. M. B. 117,Abuja, Nigeria

E-mail: [oluagarry@yahoo.com](mailto:oluagarry@yahoo.com)

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**Abstract:** The immunostimulatory and protective properties of *Lactobacillus brevis* isolated from cassava starch were studied in vitro and in vivo. Antagonism was measured by the zone of inhibition between the bacterium streak/ring and fungus plug. Subsequent increases in inhibition were observed and complemented by a small but progressive decrease in the distance between the bacterium and the fungus. *L. brevis* significantly (>74%) inhibited the growth rate of *Fusarium moniliforme* after 168 h. Biochemical indices of albino rat plasma showed that the bacterium had liver improvement functions. Plasma aspartate aminotransferase (AST) activity of the rats dosed with *L. brevis* alone was lower (8.33 IU/L) than the control. A mild elevation of AST and alanine aminotransferase (ALT) activities was observed in rats administered with *L. brevis* and *F. moniliforme* implying that the bacterium possesses antimycotic properties capable of reducing the severity of pathogen attack on the host. However, there was a significant ( $P<0.05$ ) decrease in the plasma globulin and protein levels. There was a reduction in the count of *F. moniliforme* in rats dosed with both organisms during feeding trials. The weight gain by rats in the treatment group compared favourably with the control. Further pathological investigation confirmed a pale and friable liver while the small intestine was inflamed. The administration of *L. brevis* had an immunostimulatory effect. *Lactobacillus brevis* has not only potent in vitro antifungal activity against *F. moniliforme* but also in vivo control efficacy against *Fusarium* infection. Further evaluation of its effectiveness for disease control and applications should be done

[Agarry, O. O. Immunostimulatory and Protective Properties of *Lactobacillus brevis* Used as a

Biocontrol Agent *in Vivo*. Journal of American Science 2010;6(11):1038-1045]. (ISSN: 1545-1003). (<http://www.americanscience.org>).

**Keywords:** Immunostimulatory properties, Fusarium moniliforme, Lactobacillus brevis

### Accelerating Vector Quantization Based Speaker Identification

Muhammad Afzal<sup>1</sup>, Shaiq A. Haq<sup>2</sup>

<sup>1</sup>Department of Computer Science and Engineering,  
University of Engineering and Technology, Lahore-54890, Pakistan  
<sup>2</sup>Dean Faculty of Engineering, Wah Engineering College,  
University of Wah, Wah Cantt., Pakistan

E-mails: [shmafzal@yahoo.com](mailto:shmafzal@yahoo.com), [shaiq\\_haq@yahoo.com](mailto:shaiq_haq@yahoo.com)

[Full text](#)

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**Abstract:** Matching of feature vectors extracted from speech sample of an unknown speaker, with models of registered speakers is the most time consuming component of real-time speaker identification systems. Time controlling parameters are size and count of extracted test feature vectors as well as size, complexity and count of models of registered speakers. We studied vector quantization (VQ) for accelerating the bottlenecking component of speaker identification which is less investigated than Gaussian mixture model (GMM). Already reported acceleration techniques in VQ approach reduce test feature vector count by pre-quantization and reduce candidate registered speakers by pruning unlikely ones, thereby, introducing risk of accuracy degradation. The speedup technique used in this paper partially prunes VQ codebook mean vectors using partial distortion elimination (PDE). Acceleration factor of up to 3.29 on 630 registered speakers of TIMIT 8kHz speech data and 4 on 91 registered speakers of CSLU speech data is achieved respectively.

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[Muhammad Afzal, Shaiq A. Haq. Accelerating Vector Quantization Based Speaker Identification, Journal of American Science 2010;6(11):1046-1050]. (ISSN: 1545-1003).

<http://www.americanscience.org>.

**Keywords:** Speaker identification, vector quantization, partial distortion elimination, speaker pruning

[Full text](#)

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### Curcumin/Irbesartan Combination Improves Insulin Sensitivity and Ameliorates Serum Pro-inflammatory Cytokines levels in Diabetes Rat model

Mohamed Khaled Mohamed. Mahfouz

Department of Biochemistry, Faculty of Vet Medicine, Benha University, [Banha](#), [Al Qalyubiyah](#), Egypt

[drm\\_mahfouz@hotmail.com](mailto:drm_mahfouz@hotmail.com)

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**Abstract:** To evaluate the impact of chronic administration of an angiotensin II receptor antagonist (Irbesartan) and/or curcumin on fasting blood glucose level (FBG), insulin sensitivity, and proinflammatory cytokines in experimentally-induced diabetes in albino rats. Materials and Methods: The study included 80 (20 as control group) male albino rats; diabetes mellitus (DM) was induced using intraperitoneal injection of a single dose of 50 mg/kg of streptozotocin (STZ) after animals were maintained on high-fat diet for 2-weeks (30 rats) for induction of non-insulin dependent DM (NIDDM) or without dieting regimen (30 rats) for induction of IDDM. One-week later, rats received oral irbesartan (2.5 mg/kg/day), oral curcumin (200 mg/kg) or both lines for 6 weeks. Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) and rapid insulin

sensitivity test (RIST) were used for clinical assessment. Two fasting venous blood samples were obtained prior to initiation and at 6-wks after treatment for estimation of FBG and ELISA estimation of fasting plasma insulin (FPI), serum interleukin (IL)-1 and -6 and tumor necrosis factor- (TNF-). Results: Both lines of treatment induced significant reduction of FBG and FPI levels compared to pre-treatment levels with significant reduction of FBG on using curcumin compared to irbesartan, but combination therapy significantly lowered FPI levels compared to either drug alone. Post-treatment serum levels of studied cytokines in all groups were significantly lower compared to pre-treatment levels, but curcumin alone significantly reduced serum levels of IL-6 and TNF- compared to irbesartan alone. Post-treatment HOMA-IR and RIST indices were significantly improved compared to pre-treatment levels. Conclusion: Chronic administration of irbesartan/curcumin combination showed anti-diabetic effect manifested as decreased FBG and FPI levels and ameliorated the increased serum levels of pro-inflammatory cytokines. The use of such combination could be recommended for clinical trials so as to document its use for control of both types diabetes.

[Mohamed Khaled Mohamed. Mahfouz. **Curcumin/Irbesartan Combination Improves Insulin Sensitivity and Ameliorates Serum Pro-inflammatory Cytokines levels in Diabetes Rat model.** Journal of American Science 2010;6(11):1051-1059]. (ISSN: 1545-1003). (<http://www.americanscience.org>).

**Keywords:** Curcumin, Irbesartan, Proinflammatory cytokines

Evaluation of some Inflammatory Cytokines in Children with Type1 Diabetes Mellitus

Azza A.A<sup>\*1</sup>, Mohga S. A<sup>2</sup>, Wafaa GH. SH<sup>2</sup>, Karam AM<sup>3</sup>, Enas R.A<sup>1</sup>, Tarek AS. H<sup>4</sup>, Salwa M E<sup>5</sup>

[Full text](#)

<sup>1</sup> Child Health Department, National Research Centre, Cairo, Egypt

<sup>2</sup> Biochemistry Department, Faculty of Science Helwan University, Helwan, Egypt

<sup>3</sup>Medical Biochemistry Department, National Research Centre, Cairo, Egypt

<sup>4</sup> Biology Department, Animal Reproduction Research Institute, Agriculture Research centre, Cairo, Egypt

<sup>5</sup> Biochemistry Department, Faculty. of Science, Ain shams University, Cairo, Egypt

[\\*drazzaaa@yahoo.com](mailto:*drazzaaa@yahoo.com)

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**Abstract:** Objectives: Recent evidence favors primary role of cellular autoimmunity and its humoral mediators in pathogenesis and following Type I diabetes mellitus (IDDM) The present study is carried out to investigate serum concentration of TNF- $\alpha$ , IL-6 and sIL-2 R in children with IDDM. Potential role of glycemic control, body mass index and disease duration were evaluated. Design and Methods: Thirty five children with IDDM and 30 age and sex matched non diabetic healthy subjects were recruited for this study from the out patients Clinic of diabetes of National Institute of Diabetes and Endocrinology. Results: Circulating level of TNF- $\alpha$  IL-6 and sIL-2R were elevated in children with type I DM ( $39.91 \pm 17.46$  pg/ml,  $14.89 \pm 10.69$  pg/ml and  $779.0 \pm 467.06$  pg/ml respectively). Compared with nondiabetic controls ( $5.67 \pm 1.88$  pg/ml,  $6.23 \pm 2.78$  pg/ml and  $254.33 \pm 173.6$  pg/ml respectively). These differences were statistically highly significant ( $<0.0001$ ). Glycemic control, Insulin dose and disease duration were not significant predictors of cytokine concentration in children with IDDM. A significant negative correlation was obtained between TNF - $\alpha$  with age, weight, BMI and sIL-2R in diabetic patients. However there was a significant positive correlation between IL-6 with weight and BMI in those children. Conclusion: Circulating levels of inflammatory cytokines were elevated in patients with IDDM suggesting activation of the inflammatory immune response system. Their levels were not affected by glucose level , insulin dose or duration of the disease.

[Azza A.A, Mohga S. A, Wafaa GH. SH, Karam AM, Enas R.A, Tarek AS. H, Salwa M E.

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Evaluation of some Inflammatory Cytokines in Children with Type1 Diabetes Mellitus. Journal of American Science 2010;6(11):1060-1067]. (ISSN: 1545-1003). (<http://www.americanscience.org>).

**Keywords:** Inflammatory; Cytokines; children; Diabetes Mellitus

**Postoperative Pain Control in Patients after Lower Third Molar Extraction**

\*Hanaa El Shenawy ; \*\*Neveen Helmy Aboelsoud; \*\*\*Ahmed Abbass Zaki; ;\*\*\*\*Mohamed El Zawahry ; \*Amr Shaibeta

\*Oral Surgery and Medicine , National Research Centre

\*\*Complementary Medicine , National Research Centre

\*\*\*\*\* Fixed and Removable Prothodontics Departments –National Research Centre –

\*\*\* Oral Surgery Departments -National Institute of Laser Enhanced Sciences- Cairo-Egypt.

Corresponding author: Name: Prof. Dr. Neveen Helmy Aboelsoud.

Prof. of Complementary Medicine/ Complementary Medicine Department

National Research Centre – 33 El Bohouth Street – Dokki- Cairo- Egypt-12311

Phone: +202 0124359509; E-mail: neveenster@gmail.com

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**Abstract:** The most valuable treatment objective in dental practice is to afford the patient a pain-free treatment. The **aim of this study** was to compare the use of low-power laser irradiation and the non-steroidal anti-inflammatory drug diclofenac sodium, as dental analgesic postoperative tools.

**Materials and Methods:** Ninety patients undergoing non- surgical extraction of lower third molar with local anaesthesia (2% lidocaine with epinephrine 1:80.000) were enrolled in this study. Sixty received a preoperative single dose of 100 mg diclofenac sodium; thirty patients of them had postoperative low power laser irradiation in addition. They were compared to a third group with only regular postoperative recommendations (30 patients). **Results** showed that low-power laser irradiation significantly reduced postoperative pain intensity than in patients pre-medicated with diclofenac alone, or depend only on regular recommendations (controls).**In conclusion:** We suggested that the use of low-power laser irradiation enables the best postoperative analgesic effect and the most comfortable postoperative course after non surgical extraction of lower third molar than non-steroidal anti-inflammatory drugs or regular postoperative treatment.

[Hanaa El Shenawy; Neveen Helmy Aboelsoud; Ahmed Abbass Zaki; Mohamed El Zawahry; Amr Shaibeta. **Postoperative Pain Control in Patients after Lower Third Molar Extraction.** Journal of American Science 2010;6(11):1068-1072]. (ISSN: 1545-1003). (<http://www.americanscience.org>).

**Key words:** Post operative pain- laser therapy- Diclofenac sodium - VAS

**Organizational, Operational and Interactional Processes of People's Participation in Community Activities in Malaysia**

<sup>1</sup>Asnarulkhadi A. S & <sup>2</sup>Fariborz Aref

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1. Dept. of Social and Development Science, Faculty of Human Ecology, Putra University, Malaysia
2. School of Management and Economics, Science and Research Branch, Islamic Azad University, Tehran, Iran; [fariborz.aref@gmail.com](mailto:fariborz.aref@gmail.com)

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**Abstract:** This study focuses particularly on how people living as one community organize themselves to fulfill their needs and expectations through various groups, as revealed and directed by respondents in the research process. Therefore, the analysis and interpretation of the data is based on the people's expressed experiences of participating in such processes by treating those experiences as one entity, regardless of the type of groups they represented.

[Asnarulkhadi A. S & Fariborz Aref. **Organizational, Operational and Interactional Processes of People's Participation in Community Activities in Malaysia.** Journal of American Science 2010; 6(11):1073-1077]. (ISSN: 1545-1003). (<http://www.americanscience.org>).

**Keywords:** participation, community activities, planning

**Influence of Organic Matter and Different Rates of Sulphur and Nitrogen on Dry Matter and Mineral Composition of Wheat Plant in New Reclaimed Sandy Soil**

**El-Fatah., M.S. and Khaled, S.M.**

Plant Nutrition Dept., National Research Centre. Dokki, Cairo, Egypt

[Full text](#)

**Abstract:** A pot experiment was carried out in greenhouse on reclamation sandy soil from (Abu-Rwash) region north of Egypt to evaluation effect of organic matter at rate 2% of soil weight and different rates of elemental sulphur at a rate, i.e. (100 and 200) ppm ( $S_1$  and  $S_2$ ) respectively and nitrogen, i.e. (50, 100 and 150) ppm ( $N_1$ ,  $N_2$  and  $N_3$ ) respectively at from ammonium sulphate ( $NH_4$ )  $SO_4$ . Dihydrogen potassium phosphate  $H_2KPO_4$  was added as at a rate 200 ppm as sources to phosphorus and potassium. All treatments were added before the culture of a week at one dose. The growth stages were divided to three stages (planting, elongation and maturity) each stage for two months about. The determination was performed each stage to soil and plant (whole plant). The results can be summarized as follows: (1) Soil pH decreasing at significantly especially at rates, i.e. 200 ppm S and 150 ppm N treatment in each of planting and elongation stages then began a gradual return to initial in maturity stage. (2) Electric conductivity (E.C) is rising at significantly especially with  $S_2 - N_3$  treatment then starting the gradual return to initial in maturity stage. (3) Thiosulphate  $S_2O_3$  was found in soil as a result sulphur oxidation as it affects inhibition on the nitrification process. (4) Available nitrogen ( $NH_4^+$  and  $NO_3^-$ ) continued a long experiment period. (5) Dry weight was more significantly with  $S_2-N_3$  treatment in comparison to other treatments. (6) Mineral contents were more significantly with  $S_2-N_3$  treatment along of time experiment except potassium and zinc elements as decreasing in maturity stage.

[**El-Fatah., M.S. and Khaled, S.M. Influence of Organic Matter and Different Rates of Sulphur and Nitrogen on Dry Matter and Mineral Composition of Wheat Plant in New Reclaimed Sandy Soil.** Journal of American Science 2010;6(11):1078-1084]. (ISSN: 1545-1003). (<http://www.americanscience.org>).

**Key Words:** organic matter-Sulphur-Nitrogen-wheat plant reclamation sandy soil-mineral composition-dry matter

**Presumed Chronological, Developmental and Clinical Classification of Human Dentitions**

**Said Mahmoud Hani**

Oral Biology Department, Faculty of Dental Medicine, Al-Azher University, Cairo, Egypt

[hani.said@yahoo.com](mailto:hani.said@yahoo.com)

[Full text](#)

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**Abstract:** Diphyodont, including man, have traditionally two dentitions the deciduous and

permanent. The significances of the presence of two dentition may lie behind the fact that once the teeth have been developed, they are unable to grow, by the common sense of the word. Since the individual organs and tissues grow by time and the jaws also do, therefore, other generation of dentition is needed to match the new situation, that is, the permanent dentition. However, this typing of dentition into two sets is oversimplified and nonindcative for the condition in which the teeth are variably represented. It also, does not exhibit the different and definite cases by which the teeth are expressed. Taking these drawbacks into consideration, a presumed classification has been presented indicating the developmental, clinical and chronological situations of the different sets of dentitions. The presumed classification may be valuable not only for pedagogic purposes but also for the developmental and clinical studies.

[. Journal of American Science 2010;6(11):1085-1090]. (ISSN: 1545-1003). (<http://www.americanscience.org>).

**Keywords:** Classification, Dentitions, Human

**Prognostic and Predictive Significance of Haemostatic and Angiogenic Parameters in Cancer Bladder Patients**

**Madkour B. S.; Bekheet I.W.\* , El Baz A.G.\* , Ghobashy S.; El-Ganzory H. and, Essawy F.M.**

Haematology and Urology Department, Theodor Bilharz Research Institute, Imbaba, Giza, Egypt.

\*[iman.william@yahoo.com](mailto:iman.william@yahoo.com)

**Abstract:** Recent studies demonstrated a key role of angiogenesis, thrombosis and fibrinolysis in tumour invasion and metastasis. We aimed to clarify the potential link between angiogenic factor {vascular endothelial growth factor (VEGF)}, prothrombotic factor {von Willebrand factor (vWF)} and fibrinolytic markers {tissue plasminogen activator (t-PA), plasminogen activator inhibitor-1 (PAI-1) and D-dimer} with disease progression and metastatic dissemination in bladder cancer patients. The study enrolled forty five bladder cancer patients classified into three groups: 20 patients with locally invasive tumours, 15 patients with regional lymph nodes involvement and 10 patients with distant metastasis. In addition to 15 subjects served as a control group. Enzyme linked immunoassay method was used for measurement of VEGF, vWF, t-PA, PAI-1 and D-dimer. Enhanced angiogenesis was evident by high level of VEGF with subsequent high release of endothelial vWF. Also activation of fibrinolytic system was pronounced by elevated t-PA, PAI-1 and D-dimer. In addition, highest values of these factors were associated with relatively advanced tumour stage, as they showed a significant direct correlation with the stage of bladder cancer. Regression analysis proved that VEGF, vWF, t-PA and D-imer are independent determinant for the stage of bladder cancer. Conclusion: These results suggest that VEGF, t-PA, PAI-1 and D-dimer are potential prognostic markers in bladder cancer patients. These findings may have future implications for the treatment of patients with metastastatic disease.

[**Madkour B. S.; Bekheet I.W.\* , El Baz A.G.\* , Ghobashy S.; El-Ganzory H. and, Essawy F.M. Prognostic and Predictive Significance of Haemostatic and Angiogenic Parameters in Cancer Bladder Patients.** Journal of American Science 2010;6(11):1091-1097]. (ISSN: 1545-1003). (<http://www.americanscience.org>).

**Key words:** cancer bladder; vWF; VEGF; t-PA; PAI-1; D-dimer.

**Oil content and yield of *Foeniculum vulgare* Mill. cv. Soroksary seeds as affected by different plant cultivation densities**

Jalal Khorshidi, Seyed Fazel Mirahmadi, Mohammad Fakhr Tabatabaei

Department of Horticulture Science, Faculty of Agricultural Science and Engineering, University of Tehran, Karaj, Iran. [Mirahmadif@ut.ac.ir](mailto:Mirahmadif@ut.ac.ir)

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**Abstract:** In this study, the effect of different plant cultivation densities on the oil content and yield of *Foeniculum vulgare* Mill. Cv. Soroksary seeds was studied at the Faculty of Agricultural Sciences and Engineering, Karaj, Iran (Latitude 35° 47' N and Longitude 50° 59' E) in 2008. Five plant spaces studied were 10, 15, 20, 25, and 30 cm and the distance between rows in all treatments was 40 cm using a complete randomized block design with three replicates. According to results, the effect of plant density on oil content and yield was significant ( $P<0.01$ ). The highest oil content (3.33%) and yield per hectare (116.73 liter) was obtained with the lowest plant density.

[Jalal Khorshidi, Seyed Fazel Mirahmadi, Mohammad Fakhr Tabatabaei. **Oil content and yield of *Foeniculum vulgare* Mill. cv. Soroksary seeds as affected by different plant cultivation densities.** Journal of American Science 2010;6(11):1098-1100]. (ISSN: 1545-1003). (<http://www.americanscience.org>).

**Keywords:** *Foeniculum vulgare* Mill. cv. Soroksary; Plant density; Oil content; Oil yield

**Cyanobacteria of a Tropical Lagoon, Nigeria.**

Adesalu, Taofikat Abosede<sup>1</sup>, Nwankwo, Dike Ikegwu.<sup>2</sup>

<sup>1</sup>Department of Botany and Microbiology, University of Lagos, Nigeria.

<sup>2</sup>Department of Marine sciences, University of Lagos, Nigeria.

[boseadesalu@yahoo.com](mailto:boseadesalu@yahoo.com).

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**Abstract:** Investigations for the first time into the blue green algae of Lekki lagoon were carried out for 24 months (June 2003- May 2005) at monthly intervals using standard plankton net of mesh size 55µm. One hundred and seventy nine species belonging to thirty genera were observed. The filamentous blue green algae *Oscillatoria* formed the most abundant genus making up twenty three species followed by *Phormidium* eighteen species. *Anabaena* and *Chroococcus* recorded thirteen species each while the genera, *Gleocapsa*, *Merismopedia* and *Microcystis* recorded ten, eight and twelve species respectively. Only one genus each of *Cyanoarcina*, *Calothrix* and *Scytonema* were encountered. Bloom forming species identified were *Microcystis aeruginosa*, *M. flos-aquae*, *M. wesenbergii* and *Anabaena flos-aquae*. In this study, thirty-nine new species were recorded for Lagos lagoon complex in which Lekki lagoon is one of it while *Cyanoarcina hueberliorum* is new record for Nigeria.

[Adesalu, Taofikat Abosede, Nwankwo, Dike Ikegwu. **Cyanobacteria of a Tropical Lagoon, Nigeria.** Journal of American Science 2010;6(11):1101-1107]. (ISSN: 1545-1003). (<http://www.americanscience.org>).

**Key words:** Cyanophytes, tropical, bloom, Lagos lagoon complex

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Emails: [editor@americanscience.org](mailto:editor@americanscience.org); [americansciencej@gmail.com](mailto:americansciencej@gmail.com)

# Indirect Boundary Element Method for Calculation of Compressible Flow past a Symmetric Aerofoil with Linear Element Approach Using Doublet Distribution Alone

Muhammad Mushtaq\* &amp; Nawazish Ali Shah

Department of Mathematics, University of Engineering &amp; Technology Lahore – 54890, Pakistan

Corresponding Author, e-mail: [mushtaqmalik2004@yahoo.co.uk](mailto:mushtaqmalik2004@yahoo.co.uk)

**Abstract:** In this paper, an indirect boundary element method is applied to calculate the compressible flow past a symmetric aerofoil. The velocity distribution for the flow over the surface of the symmetric aerofoil has been calculated with linear boundary element approach using doublet distribution alone. To check the accuracy of the method, the computed flow velocity is compared with the exact velocity. The comparison of these results has been given in the tables and graphs. It is found that the computed results are in good agreement with the analytical results. [Journal of American Science. 2010;6(11):1-9]. (ISSN: 1545-1003).

**Keywords:** Indirect boundary element method, Compressible flow, Velocity distribution, Symmetric aerofoil, linear element.

## 1. Introduction

In the past, many numerical techniques such as finite difference method, finite element method, and boundary element method etc. came into being making possible to solve various practical fluid flow problems. Boundary element method has received much attention from the researchers due to its various advantages over the other domain methods. One of the advantages is that with boundary elements one has to discretize only the surface of the body, whereas with domain methods it is essential to discretize the entire region of the flow field. Moreover, this method is well-suited to problems with an infinite domain. The boundary element method can be classified into two categories i.e. direct and indirect. The direct method takes the form of a statement which provides the values of the unknown variables at any field point in terms of the complete set of all the boundary data. On the other hand, the indirect method utilizes a distribution of singularities over the boundary of the body and computes this distribution as the solution of integral equation. The equation of indirect method can be derived from that of direct method. (Lamb, 1932; Milne-Thomson, 1968, Kellogg, 1929 and Brebbia and Walker, 1980). The indirect method has been used in the past for flow field calculations around arbitrary bodies (Hess and Smith, 1967; Muhammad, 2008&2009, Luminita, 2008, Mushtaq, 2008, 2009 & 2010). Most of the work on fluid flow calculations

using boundary element methods has been done in the field of incompressible flow. Very few attempts have been made on flow field calculations using boundary element methods in the field of compressible flow. In this paper, the indirect boundary element method has been used for the solution of compressible flows around a symmetric aerofoil with linear element approach using doublet distribution alone.

## 2. Mathematical Formulation

We know that equation of motion for two-dimensional, steady, irrotational, and isentropic flow is

$$(1 - Ma^2) \frac{\partial^2 \Phi}{\partial X^2} + \frac{\partial^2 \Phi}{\partial Y^2} = 0 \quad (1)$$

where  $Ma$  is the Mach number and  $\Phi$  is the total velocity potential of the flow. Here  $X$  and  $Y$  are the space coordinates.

Using the dimensionless variables,  $x = X$ ,

$$y = \beta Y, \text{ where } \beta = \sqrt{1 - M a^2},$$

equation (1) becomes

$$\frac{\partial^2 \Phi}{\partial x^2} + \frac{\partial^2 \Phi}{\partial y^2} = 0$$

$$\text{or } \nabla^2 \Phi = 0 \quad (2)$$

which is Laplace's equation.

### 3. Symmetric Aerofoil

The Joukowski transformation

$$z = \zeta + \frac{a^2}{\zeta} \quad (3)$$

transforms the circle shown in figure (1) in the  $\zeta$ -plane on to symmetric aerofoil in the  $z$ -plane.

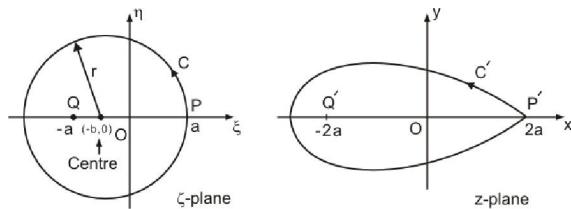


Figure 1

### 4. Flow Past a Symmetric Aerofoil

Consider the flow past a symmetrical aerofoil and let the onset flow be the uniform stream with velocity  $U$  in the positive direction of the  $x$ -axis as shown in figure (2).

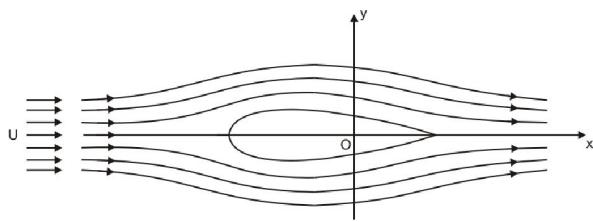


Figure 2: Flow past a symmetric aerofoil.

#### Exact Velocity

The magnitude of the exact velocity distribution over the boundary of a symmetric aerofoil is given by Chow[3] as

$$V = U \left| \frac{1 - \left( \frac{r}{z-b} \right)^2}{1 - \left( \frac{a}{z} \right)^2} \right|$$

where  $r$  = radius of the circular cylinder,  
 $a$  = Joukowski transformation constant  
and  $b = a - r$  =  $x$ -coordinates of the centre of the circular cylinder

In Cartesian coordinates, we have

$$\begin{aligned} V &= U \\ &\sqrt{\left[ \left\{ (x-b)^2 + y^2 \right\}^2 - r^2 \left\{ (x-b)^2 - y^2 \right\} \right]^2 + 4r^4y^2(x-b)^2} \\ &\quad \left[ (x-b)^2 + y^2 \right]^2 \\ &\times \sqrt{\left[ (x^2 + y^2)^2 - a^2(x^2 - y^2) \right]^2 + 4a^4x^2y^2} \\ &\quad \left[ (x^2 + y^2)^2 - 2a^2(x^2 - y^2) + a^4 \right] \end{aligned}$$

#### Boundary Conditions

Now the condition to be satisfied on the boundary of a symmetric aerofoil is

$$\nabla \cdot \hat{n} = 0 \quad (4)$$

where  $\hat{n}$  is the unit normal vector to the boundary of the aerofoil .

Since the motion is irrotational

$$\nabla = -\nabla \Phi$$

where  $\Phi$  is the total velocity potential . Thus equation (4) becomes

$$(-\nabla \Phi) \cdot \hat{n} = 0$$

$$\text{or } \frac{\partial \Phi}{\partial n} = 0 \quad (5)$$

Now the total velocity potential  $\Phi$  is the sum of the perturbation velocity potential  $\phi_{s.a}$  where the subscript  $s.a$  stands for symmetric aerofoil and the velocity potential of the uniform stream  $\phi_{u.s}$  .

$$\text{i.e. } \Phi = \phi_{u.s} + \phi_{s.a} \quad (6)$$

$$\text{or } \frac{\partial \Phi}{\partial n} = \frac{\partial \phi_{u.s}}{\partial n} + \frac{\partial \phi_{s.a}}{\partial n} \quad (7)$$

From equations (5) and (7) , we get

$$\frac{\partial \phi_{s.a}}{\partial n} + \frac{\partial \phi_{u.s}}{\partial n} = 0$$

$$\text{or } \frac{\partial \phi_{s.a}}{\partial n} = -\frac{\partial \phi_{u.s}}{\partial n} \quad (8)$$

But the velocity potential of the uniform stream , given in Milne – Thomson [ 6 ], Shah [ 7 ], is

$$\phi_{u.s} = -Ux \quad (9)$$

$$\begin{aligned} &= -U \frac{\partial x}{\partial n} \\ &= -U (\hat{n} \cdot \hat{i}) \quad (10) \end{aligned}$$

Thus from equations (8) and (10), we get

$$\frac{\partial u_{s.a}}{\partial n} = U (\hat{n} \cdot \hat{i}) \quad (11)$$

Now from the figure (3)

$$\hat{A} = (x_2 - x_1) \hat{i} + (y_2 - y_1) \hat{j}$$

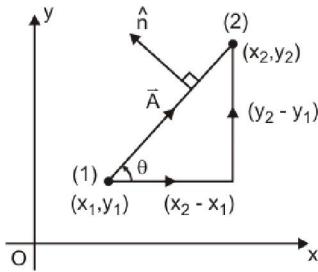


Figure 3

Therefore the unit vector in the direction of the vector  $\underline{A}$  is given by

$$\underline{A} = \frac{(x_2 - x_1) \hat{i} + (y_2 - y_1) \hat{j}}{\sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}}$$

The outward unit normal vector  $\hat{n}$  to the vector  $\underline{A}$  is given by

$$\hat{n} = \frac{-(y_2 - y_1) \hat{i} + (x_2 - x_1) \hat{j}}{\sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}}$$

$$\text{Thus } \hat{n} \cdot \hat{i} = \frac{(y_1 - y_2)}{\sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}} \quad (12)$$

From equations (11) and (12), we get

$$\frac{\partial \phi_{s.a}}{\partial n} = U \frac{(y_1 - y_2)}{\sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}} \quad (13)$$

Equation (13) is the boundary condition which must be satisfied over the boundary of a symmetric aerofoil.

#### Equation of Indirect Boundary Element Method

The equation of indirect boundary element method for two-dimensional flow in the case of doublet distribution alone [Muhammad,2008 & Mushtaq, 2009 & 2010] is :

$$-c_i \Phi_i + \frac{1}{2\pi} \int_{\Gamma-i} \Phi \frac{\partial}{\partial n} \left( \log \frac{1}{r} \right) d\Gamma + \phi_\infty \\ = -(\phi_{u.s})_i \quad (14)$$

where  $c_i = 0$  when 'i' is within  $R'$   
 $= 1$  when 'i' is within  $R$   
 $= \frac{1}{2}$  when 'i' is on  $S$  and  $S$  is smooth

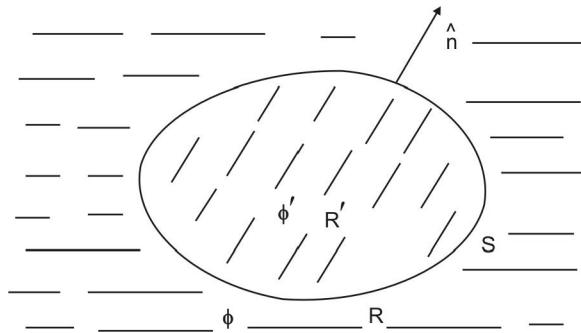


Figure 4

#### Matrix Formulation

Let the boundary of the region be discretized into  $m$  linear elements, then equation (14) can be written as

$$-c_i \Phi_i + \sum_{j=1}^m \left[ \frac{1}{2\pi} \int_{\Gamma_{j-i}} \Phi \frac{\partial}{\partial n} \left( \log \frac{1}{r} \right) d\Gamma \right] + \phi_\infty = -(\phi_{u.s})_i \quad (15)$$

where  $\Gamma_{j-i}$  is the length of the element 'j' excluding the point 'i'.

For the linear boundary element approach, the number of nodes will be more than the number of elements. Suppose that  $m$  is the number of nodes in this case. Since  $\Phi$  varies linearly over the element, its value at any point can be defined in terms of the nodal values and the two shape functions  $N_1$ ,  $N_2$ , that is

$$\Phi = N_1 \Phi_1 + N_2 \Phi_2 = [N_1 \ N_2] \begin{Bmatrix} \Phi_1 \\ \Phi_2 \end{Bmatrix} \quad (16)$$

where  $N_1 = \frac{1}{2}(1-\delta)$ ,

and  $N_2 = \frac{1}{2}(1+\delta)$ ,  $-1 \leq \delta \leq 1$

The integrals along the element 'j' i.e.

$$\frac{1}{2\pi} \int_{\Gamma_{j-i}} \Phi \frac{\partial}{\partial n} \left( \log \frac{1}{r} \right) d\Gamma$$

can be written as

$$\frac{1}{2\pi} \int_{\Gamma_{j-i}} \Phi \frac{\partial}{\partial n} \left( \log \frac{1}{r} \right) d\Gamma$$

$$= \frac{1}{2\pi} \int_{\Gamma_j-i} [N_1 N_2] \frac{\partial}{\partial n} \left( \log \frac{1}{r} \right) d\Gamma \begin{Bmatrix} \Phi_1 \\ \Phi_2 \end{Bmatrix}$$

$$= \begin{bmatrix} h_{ij}^1 & h_{ij}^2 \end{bmatrix} \begin{Bmatrix} \Phi_1 \\ \Phi_2 \end{Bmatrix} \quad (17)$$

where  $h_{ij}^1 = \frac{1}{2\pi} \int_{\Gamma_j-i} \Phi_1 \frac{\partial}{\partial n} \left( \log \frac{1}{r} \right) d\Gamma$ ,

$$h_{ij}^2 = \frac{1}{2\pi} \int_{\Gamma_j-i} \Phi_2 \frac{\partial}{\partial n} \left( \log \frac{1}{r} \right) d\Gamma$$

$$\text{or } h_{ij}^k = \frac{1}{2\pi} \int_{\Gamma_j-i} k \frac{\partial}{\partial n} \left( \log \frac{1}{r} \right) d\Gamma \quad (18)$$

$$k = 1, 2$$

The  $h_{ij}^k$  are influence coefficients during the interaction between the point 'i' under consideration and a particular node  $k$  on an element 'j'.

To write the equation (15) corresponding to the node 'i' the contributions from all elements associated with the node 'i' are to be added into one term, defining the nodal coefficients. This will give the following equation

$$-c_i \Phi_i + \left[ \hat{H}_{i1} \hat{H}_{i2} \dots \hat{H}_{im} \right] \begin{Bmatrix} \Phi_1 \\ \Phi_2 \\ \vdots \\ \Phi_m \end{Bmatrix} + \phi_\infty = -(\phi_{u.s.})_i \quad (19)$$

where  $\hat{H}_{ij}$  term is the sum of the contributions from all the adjoining elements of the node 'i'. Hence equation (19) represents the assembled equation for node 'i' and can be written as

$$-c_i \Phi_i + \sum_{j=1}^m \hat{H}_{ij} \Phi_j + \phi_\infty = -(\phi_{u.s.})_i \quad (20)$$

$$\text{or } \sum_{j=1}^m H_{ij} + \phi_\infty = -(\phi_{u.s.})_i \quad (21)$$

where  $H_{ij} = \begin{cases} \hat{H}_{ij} & \text{when } i \neq j \\ \hat{H}_{ij} - c_i & \text{when } i = j \end{cases}$

When all nodes are taken into consideration, equation (21) is  $M \times (M+1)$  system of equations. Which can put in the matrix form in case of linear element as

$$[H] \{U\} = \{R\} \quad (22)$$

where as usual  $[H]$  is a matrix of influence coefficients,  $\{U\}$  is a vector of unknown total potentials  $\Phi_i$  and  $\{R\}$  on the R.H.S. is a known vector whose elements are the negative of the values of the velocity potential of the uniform stream at the nodes on the region of the body. Note that  $\{U\}$  in equation (22) has  $(M+1)$  unknowns  $\Phi_1, \Phi_2, \dots, \Phi_m, \phi_\infty$ . To solve precisely this system of equations, the value of  $\Phi$  at some position must be specified. For convenience  $\phi_\infty$  is chosen as zero. Thus  $M \times (M+1)$  system reduces to an  $M \times M$  system of equations which can be solved as before but now the diagonal coefficients of  $[H]$  will be found by

$$H_{ii} = - \sum_{\substack{j=1 \\ j \neq i}} H_{ij} - 1 \quad (23)$$

### Process of Discretization

Now for the discretization of the boundary of the symmetric aerofoil, the coordinates of the extreme points of the boundary elements can be generated within computer programme using Fortran language as follows:

Divide the boundary of the circular cylinder into  $m$  elements in the clockwise direction by using the formula.

$$\theta_k = [(m+2)-2k] \frac{\pi}{m}, \quad k = 1, 2, \dots, m \quad (24)$$

Then the extreme points of these  $m$  elements of circular cylinder are found by

$$\xi_k = -b + r \cos \theta_k$$

$$\eta_k = r \sin \theta_k$$

Now by using Joukowski transformation in equation (3), the extreme points of the symmetric aerofoil are

$$x_k = \xi_k \left( 1 + \frac{a^2}{\xi_k^2 + \eta_k^2} \right)$$

$$y_k = \eta_k \left( 1 - \frac{a^2}{\xi_k^2 + \eta_k^2} \right)$$

where  $k = 1, 2, \dots, m$ .

The coordinates of the middle node of each boundary element are given by

$$x_m = \frac{x_k + x_{k+1}}{2}$$

$$y_m = \frac{y_k + y_{k+1}}{2}$$

$$k, m = 1, 2, \dots, n \quad (25)$$

and therefore the boundary condition (13) in this case takes the form

**Table 1: The comparison of the computed velocity with exact velocity over the boundary of a symmetric aerofoil using 8 linear boundary elements.**

ELEMENT	X	Y	$R = \sqrt{X^2 + Y^2}$	VELOCITY	EXACT VELOCITY
1	-1.94	.39	1.98	.72096E+00	.83769E+00
2	-1.39	.94	1.68	.17375E+01	.20086E+01
3	-.62	.93	1.12	.17174E+01	.20216E+01
4	-.01	.38	.38	.75534E+00	.70748E+00
5	-.01	-.38	.38	.75534E+00	.70748E+00
6	-.62	-.93	1.12	.17174E+01	.20216E+01
7	-1.39	-.94	1.68	.17375E+01	.20086E+01
8	-1.94	-.39	1.98	.72096E+00	.83768E+00

**Table 2: The comparison of the computed velocity with exact velocity over the boundary of a symmetric aerofoil using 16 linear boundary elements.**

ELEMENT	X	Y	$R = \sqrt{X^2 + Y^2}$	VELOCITY	EXACT VELOCITY
1	-2.06	.21	2.07	.38546E+00	.39565E+00
2	-1.90	.60	1.99	.10974E+01	.11264E+01
3	-1.60	.89	1.84	.16415E+01	.16923E+01
4	-1.22	1.05	1.61	.19341E+01	.20055E+01
5	-.79	1.05	1.32	.19293E+01	.20115E+01
6	-.40	.89	.98	.16249E+01	.16985E+01
7	-.10	.58	.59	.10539E+01	.10938E+01
8	.11	.20	.23	.42683E+00	.30764E+00
9	.11	-.20	.23	.42683E+00	.30764E+00
10	-.10	-.58	.59	.10539E+01	.10938E+01
11	-.40	-.89	.98	.16249E+01	.16985E+01
12	-.79	-1.05	1.32	.19293E+01	.20115E+01
13	-1.22	-1.05	1.61	.19341E+01	.20055E+01
14	-1.60	-.89	1.84	.16415E+01	.16923E+01
15	-1.90	-.60	1.99	.10974E+01	.11264E+01
16	-2.06	-.21	2.07	.38546E+00	.39565E+00

$$\frac{\partial \phi_{s.a}}{\partial n} = U \frac{(y_1)_m - (y_2)_m}{\sqrt{[(x_2)_m - (x_1)_m]^2 + [(y_2)_m - (y_1)_m]^2}} \quad (26)$$

The following tables show the comparison of computed and analytical velocity distribution over the boundary of a symmetric aerofoil for 8, 16, 32, and 64 linear boundary elements for  $r = 1.1$ ,  $a = 0.1$  and  $Ma = 0.7$ .

**Table 1: The comparison of the computed velocity with exact velocity over the boundary of a symmetric aerofoil using 8 linear boundary elements.**

ELEMENT	X	Y	$R = \sqrt{X^2 + Y^2}$	VELOCITY	EXACT VELOCITY
1	-1.94	.39	1.98	.72096E+00	.83769E+00
2	-1.39	.94	1.68	.17375E+01	.20086E+01
3	-.62	.93	1.12	.17174E+01	.20216E+01
4	-.01	.38	.38	.75534E+00	.70748E+00
5	-.01	-.38	.38	.75534E+00	.70748E+00
6	-.62	-.93	1.12	.17174E+01	.20216E+01
7	-1.39	-.94	1.68	.17375E+01	.20086E+01
8	-1.94	-.39	1.98	.72096E+00	.83768E+00

**Table 2: The comparison of the computed velocity with exact velocity over the boundary of a symmetric aerofoil using 16 linear boundary elements.**

ELEMENT	X	Y	$R = \sqrt{X^2 + Y^2}$	VELOCITY	EXACT VELOCITY
1	-2.06	.21	2.07	.38546E+00	.39565E+00
2	-1.90	.60	1.99	.10974E+01	.11264E+01
3	-1.60	.89	1.84	.16415E+01	.16923E+01
4	-1.22	1.05	1.61	.19341E+01	.20055E+01
5	-.79	1.05	1.32	.19293E+01	.20115E+01
6	-.40	.89	.98	.16249E+01	.16985E+01
7	-.10	.58	.59	.10539E+01	.10938E+01
8	.11	.20	.23	.42683E+00	.30764E+00
9	.11	-.20	.23	.42683E+00	.30764E+00
10	-.10	-.58	.59	.10539E+01	.10938E+01
11	-.40	-.89	.98	.16249E+01	.16985E+01
12	-.79	-1.05	1.32	.19293E+01	.20115E+01
13	-1.22	-1.05	1.61	.19341E+01	.20055E+01
14	-1.60	-.89	1.84	.16415E+01	.16923E+01
15	-1.90	-.60	1.99	.10974E+01	.11264E+01
16	-2.06	-.21	2.07	.38546E+00	.39565E+00

**Table 3: The comparison of the computed velocity with exact velocity over the boundary of a symmetric aerofoil using 32 linear boundary elements.**

ELEMENT	X	Y	$R = \sqrt{X^2 + Y^2}$	VELOCITY	EXACT VELOCITY
1	-2.09	.11	2.10	.19581E+00	.19530E+00
2	-2.05	.32	2.08	.57987E+00	.57871E+00
3	-1.97	.51	2.04	.94154E+00	.94085E+00
4	-1.85	.69	1.98	.12669E+01	.12682E+01
5	-1.70	.84	1.90	.15433E+01	.15485E+01
6	-1.52	.96	1.80	.17601E+01	.17706E+01
7	-1.32	1.04	1.68	.19088E+01	.19256E+01
8	-1.11	1.08	1.55	.19836E+01	.20067E+01
9	-.90	1.08	1.41	.19814E+01	.20097E+01
10	-.69	1.04	1.25	.19022E+01	.19331E+01
11	-.49	.96	1.07	.17484E+01	.17783E+01
12	-.31	.84	.89	.15255E+01	.15493E+01
13	-.16	.68	.70	.12408E+01	.12519E+01
14	-.04	.50	.50	.90285E+00	.89212E+00
15	.06	.29	.29	.52553E+00	.47258E+00
16	.15	.09	.17	.23435E+00	.17793E+00
17	.15	-.09	.17	.23435E+00	.17793E+00
18	.06	-.29	.29	.52553E+00	.47258E+00
19	-.04	-.50	.50	.90286E+00	.89212E+00
20	-.16	-.68	.70	.12408E+01	.12519E+01
21	-.31	-.84	.89	.15255E+01	.15493E+01
22	-.49	-.96	1.07	.17484E+01	.17783E+01
23	-.69	-.104	1.25	.19022E+01	.19331E+01
24	-.90	-.108	1.41	.19814E+01	.20097E+01
25	-1.11	-.108	1.55	.19836E+01	.20067E+01
26	-1.32	-.104	1.68	.19088E+01	.19256E+01
27	-1.52	-.96	1.80	.17601E+01	.17706E+01
28	-1.70	-.84	1.90	.15433E+01	.15485E+01
29	-1.85	-.69	1.98	.12669E+01	.12682E+01
30	-1.97	-.51	2.04	.94154E+00	.94085E+00
31	-2.05	-.32	2.08	.57985E+00	.57871E+00
32	-2.09	-.11	2.10	.19581E+00	.19529E+00

**Table 4: The comparison of the computed velocity with exact velocity over the boundary of a symmetric aerofoil using 64 linear boundary elements.**

ELEMENT	X	Y	$R = \sqrt{X^2 + Y^2}$	VELOCITY	EXACT VELOCITY
1	-2.10	.05	2.10	.98261E-01	.97607E-01
2	-2.09	.16	2.10	.29388E+00	.29139E+00
3	-2.07	.27	2.09	.48665E+00	.48261E+00
4	-2.04	.37	2.07	.67469E+00	.66939E+00
5	-2.00	.47	2.05	.85622E+00	.85001E+00
6	-1.95	.56	2.03	.10295E+01	.10228E+01
7	-1.89	.65	2.00	.11927E+01	.11861E+01
8	-1.82	.74	1.96	.13445E+01	.13383E+01
9	-1.74	.81	1.92	.14832E+01	.14781E+01
10	-1.66	.88	1.88	.16076E+01	.16040E+01
11	-1.57	.94	1.83	.17164E+01	.17148E+01
12	-1.47	.99	1.78	.18086E+01	.18093E+01
13	-1.37	1.03	1.72	.18832E+01	.18867E+01

14	-1.27	1.06	1.66	.19396E+01	.19459E+01
15	-1.17	1.08	1.59	.19772E+01	.19864E+01
16	-1.06	1.09	1.52	.19955E+01	.20075E+01
17	-.95	1.09	1.45	.19945E+01	.20090E+01
18	-.84	1.08	1.37	.19740E+01	.19906E+01
19	-.74	1.06	1.29	.19342E+01	.19524E+01
20	-.63	1.03	1.21	.18756E+01	.18946E+01
21	-.53	.99	1.12	.17985E+01	.18174E+01
22	-.44	.93	1.03	.17036E+01	.17213E+01
23	-.35	.87	.94	.15919E+01	.16072E+01
24	-.27	.80	.85	.14642E+01	.14756E+01
25	-.19	.73	.75	.13216E+01	.13275E+01
26	-.12	.64	.65	.11653E+01	.11637E+01
27	-.06	.55	.55	.99650E+00	.98490E+00
28	-.00	.45	.45	.81656E+00	.79156E+00
29	.04	.34	.35	.62733E+00	.58415E+00
30	.08	.23	.24	.43330E+00	.36688E+00
31	.12	.11	.16	.25449E+00	.18028E+00
32	.17	.03	.17	.13772E+00	.17941E+00
33	.17	-.03	.17	.13772E+00	.17941E+00
34	.12	-.11	.16	.25448E+00	.18028E+00
35	.08	-.23	.24	.43330E+00	.36688E+00
36	.04	-.34	.35	.62733E+00	.58415E+00
37	-.00	-.45	.45	.81657E+00	.79156E+00
38	-.06	-.55	.55	.99650E+00	.98490E+00
39	-.12	-.64	.65	.11653E+01	.11637E+01
40	-.19	-.73	.75	.13216E+01	.13275E+01
41	-.27	-.80	.85	.14642E+01	.14756E+01
42	-.35	-.87	.94	.15919E+01	.16072E+01
43	-.44	-.93	1.03	.17036E+01	.17213E+01
44	-.53	-.99	1.12	.17985E+01	.18174E+01
45	-.63	-1.03	1.21	.18756E+01	.18946E+01
46	-.74	-1.06	1.29	.19342E+01	.19524E+01
47	-.84	-1.08	1.37	.19740E+01	.19906E+01
48	-.95	-1.09	1.45	.19945E+01	.20090E+01
49	-1.06	-1.09	1.52	.19955E+01	.20075E+01
50	-1.17	-1.08	1.59	.19772E+01	.19864E+01
51	-1.27	-1.06	1.66	.19396E+01	.19459E+01
52	-1.37	-1.03	1.72	.18832E+01	.18867E+01
53	-1.47	-.99	1.78	.18086E+01	.18093E+01
54	-1.57	-.94	1.83	.17164E+01	.17148E+01
55	-1.66	-.88	1.88	.16076E+01	.16040E+01
56	-1.74	-.81	1.92	.14833E+01	.14781E+01
57	-1.82	-.74	1.96	.13445E+01	.13383E+01
58	-1.89	-.65	2.00	.11928E+01	.11861E+01
59	-1.95	-.56	2.03	.10295E+01	.10228E+01
60	-2.00	-.47	2.05	.85624E+00	.85001E+00
61	-2.04	-.37	2.07	.67471E+00	.66939E+00
62	-2.07	-.27	2.09	.48660E+00	.48261E+00
63	-2.09	-.16	2.10	.29389E+00	.29139E+00
64	-2.10	-.05	2.10	.98278E-01	.97606E-01

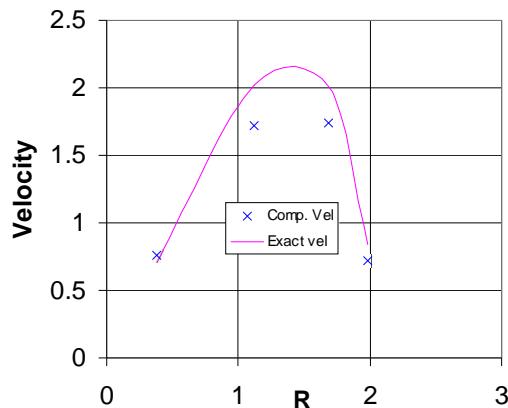


Figure 5: Comparison of computed and analytical velocity distributions over the boundary of a symmetric aerofoil using 16 boundary elements with linear element approach.

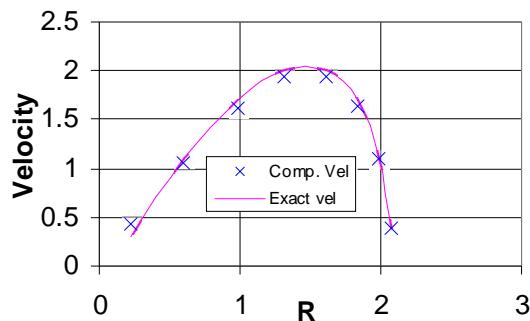


Figure 6: Comparison of computed and analytical velocity distributions over the boundary of a symmetric aerofoil using 16 boundary elements with linear element approach.

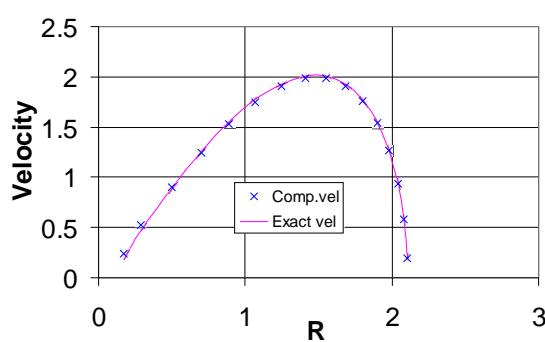


Figure 7: Comparison of computed and analytical velocity distributions over the boundary of a symmetric aerofoil using 32 boundary elements with linear element approach.

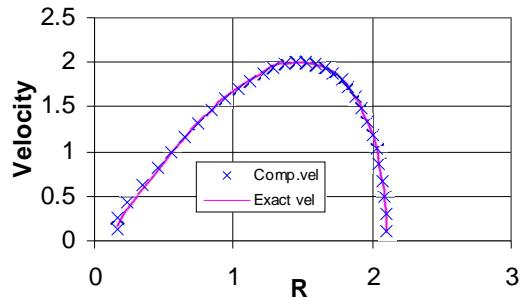


Figure 8: Comparison of computed and analytical velocity distributions over the boundary of a symmetric aerofoil using 64 boundary elements with linear element approach.

## 5. Conclusion

An indirect boundary element method has been applied for the calculation of compressible flow past a symmetric aerofoil with linear element approach using doublet distribution alone. The calculated flow velocities obtained using this method are compared with the analytical solutions for flow over the boundary of a symmetric aerofoil. It is found from the tables and figures that the computed results obtained by this method are excellent in agreement with the analytical ones for the body under consideration and the accuracy of the result increases due to increase of number of boundary elements.

## 6. Acknowledgement

We are thankful to the University of Engineering & Technology, Lahore – Pakistan for the financial support.

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2010/6/19

# Use of Long Yam Bean (*Sphenostylis stenocarpa*) as Soil Amendment for the Growth, Leaf Chemical Composition and Yield of White Yam (*Dioscorea rotundata* L)

Emmanuel Ibikunoluwa Moyin-Jesu<sup>1</sup> and Francis Omotayo Adekayode<sup>2</sup>

<sup>1</sup>Agronomy Department, Federal College of Agriculture Akure, Nigeria

[moyinjesu2004@yahoo.com](mailto:moyinjesu2004@yahoo.com)

<sup>2</sup>Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Nigeria.

[adekay98@yahoo.com](mailto:adekay98@yahoo.com)

**Abstract:** An experiment was carried out to investigate the use of long yam bean (*Sphenostylis stenocarpa*) as soil amendment for the growth and yield of white yam (*Dioscorea rotundata* L) between 1999 and 2003 at Akure in the rain forest zone of Nigeria. There were four treatment namely; NPK 15 – 15 – 15 fertilizer applied at 250kg/ha, poultry manure at 6 t/ha, long yam beans planted at two seeds per hole at a spacing of 1m x 0.5m between rows of yam plots (soil amendment) and a control (no fertilizer). The treatments were arranged in a randomized complete block design (RCB) and replicated five times. The soil analyses before planting and after harvesting were carried out. Each plot size is 4m x 4m (16m<sup>2</sup>). The growth parameters measured for the yam were vine length (cm), leaf population and stem girth (cm). At harvest, yam tuber weight (kg), tuber length (cm) tuber girth; root length and seed yield of long yam bean plants were determined. The leaf and soil N, P, K, Ca, Mg, pH and organic matter contents were also analysed at end of the experiment. The results showed that there were significant ( $p<0.05$ ) increases in the vine length, leaf population, stem girth, tuber weight, tuber length, tuber girth, soil and leaf N, P, K, Ca, Mg; pH and organic matter of white yam cultivated under the different fertilizer treatments compared to the control treatment. Long yam bean plants used as soil amendment increased the yam vine length, stem girth, leaf population, tuber weight, tuber length and tuber girth by 81% 88.4%, 69.5%, 88.97%, 76% and 94% compared to the control. The same treatment (long yam bean plants) also increased the leaf population, tuber weight, tuber length and tuber girth of yam by 11%, 31%, 30% and 55% respectively compared to NPK fertilizer treatment. Long yam plants also increased the soil pH, O.M, K, Ca and Mg by 29%, 92%, 97%, 86%, 96%, 97% and 89% respectively compared to the control treatment. It increased soil pH, organic matter, K Ca and Mg by 31%, 87%, 1.42, 98% and 98.5% compared to NPK fertilizer. Long yam plants gave seed yield of 2.3 t/ha and produced yam tuber yield of 4900kg/ha amounting to \$6,050 compared to \$3453.00 and \$3,380.00 estimated on yam yields alone under poultry manure and NPK fertilizer treatments. Finally, the use of long yam bean plants as biological fertilizer source for yam production could substitute for 250kg/ha NPK fertilizer and 6t/ha poultry manure. [Journal of American Science. 2010;6(11):10-17]. (ISSN: 1545-1003).

**Keywords:** Long yam bean, soil amendment, white yam performance.

## 1. Introduction

One of the major problems facing tropical agriculture is the inherently low fertility status of the soils because of the presence of low activity clay minerals (1:1 Kaolinite). Soil organic matter status is identified as one of the major indicators of soil fertility Agboola and Obatolu, (1989) and it is now very important that agricultural practices and farming systems must be evolved to ensure stable soil organic matter levels. In-addition, agricultural production in low input systems in the tropics relies partly on nutrient recycling and the maintenance of soil fertility through biological processes.

Many researchers have advocated the use of green manuring and, the application of household and industrial wastes for crop production, even though the adoption of these practices has been very low because of the difficulty in transporting the bulk materials to the

field, the labour intensive nature of the materials and absence of immediate income or source of food (Ojeniyi, 1998; Moyin-Jesu 2003; Cherr et al., 2006; Brennan et al., 2009; Alluvione et al., 2010).

Therefore, there is need to look inwards for alternative sources of biological fertilizers using the traditional leguminous shrub crops such as pigeon pea (*cajanus cajan*) and long yam bean (*Sphenostylis stenocarpa*) to enrich the fertility of the soil grown to arable crops, serve as source of income and food to the farmers and livestock.

Having reviewed literature extensively, there is scarcity of research information on the use of long yam bean as soil amendments to increase the soil fertility, growth and yield of white yam.

Mulongoy and Kang, (1986) and Usman et al. (2006) reported that legumes have the potentials to improve soil fertility, thereby boosting subsequent crop

yield. They stated further that other benefits of legumes include maintenance and improvement of soil physical properties, providing ground cover to reduce soil erosion, increasing soil organic matter, cation exchange capacity and reduction of soil temperature.

Kang (1992); Graham and Vance (2003) also reported that legumes could be integrated into existing cropping systems either as cover crops, live mulch, and food or fodder crops through planted fallows of multiple cropping systems. Therefore, the role of traditional legumes in soil fertility must be properly investigated.

Yam (*Dioscorea* spp) is a tuber crop belonging to the family Dioscoreaceae and it is a tropical crop with many species originating in South, east Asia. Among the species of yam is white yam (*Dioscorea rotundata* L) which produces edible tubers and it serves as sources of food to man and livestock and generates income to farmers (Adeyemi, 1999).

The objectives of this study are as follows:

- (i) To determine the suitability of long yam bean with the convectional fertilizers (poultry manure and NPK fertilizers) as soil amendments on the growth and yield parameters of yam.
- (ii) To determine their effectiveness on the yam leaf and soil chemical composition after harvesting
- (iii) To determine the comparative advantage of long yam bean plants as soil amendment over the convectional fertilizers in term of cost/benefit ratio to farmers.

## **2. Materials and Methods**

### *Collection, processing and analysis of the treatments used*

The poultry manure was collected from over 10,000 poultry birds of Rhode Island breed in the livestock unit of Federal College of Agriculture, Akure. The long yam bean seed were obtained from four hectares farm in the institution while NPK fertilizer were purchased from Ondo State Agricultural Input and Supplies Company, Akure and it is of high grade.

The poultry manure was stacked or heaped to allow for proper mineralisation processes while the long yam bean seeds were soaked in a 100ml 0.01M dilute H<sub>2</sub>S04 acid solutions for 30 minutes to weaken the hard seed coat for quick germination. The determination of the nutrients in the poultry manure was done using wet digestion method based on 25 – 5 – 5ml of H<sub>2</sub>N0<sub>3</sub> – H<sub>2</sub>S04 – HCl0<sub>4</sub> acids. The filtrate was collected for the amount of %P, K, Ca and Mg. The % P was evaluated using vanado-molybdate colorimetry and read on spectronic 20 while the % k and Ca were read on flame photometer. Mg was determined on atomic absorption spectrophotometry. The percentage N was determined by microkjedahl (Jackson, 1964)

while the nutrients composition of NPK 15 – 15 – 15 fertilizer was obtained from the manufacturer's label' (240kg N, 240kgP and 240 kg K)

### *Soil Analysis Before Planting*

The soil samples were collected from 0-15cm depth, air-dried, sieved with 2mm sieve and utilized for routine soil analysis. The particle size distribution was determined by the hydrometer method (Bouyoucos, 1951). The soil pH (1:1 soil/water and 1:2 soil/0.01M CaCl<sub>2</sub>) was determined (Crockford and Nowell, 1956).

The organic matter (O.M) was determined by the Walkley and Black (1934) while the exchangeable bases (K, Ca, Mg and Na) were extracted with 1M NH<sub>4</sub>OAc pH7 and the amount of K, Ca and Na were determined on the flame photometer using appropriate element filters. The Mg content in the extract was read on atomic absorption spectrophotometer (Jackson, 1958). The exchangeable acidity (H<sup>+</sup> and Al<sup>3+</sup>) was measured from 0.01M KCl extracts by titrating with 0.1M HC1 (McLean, 1965) while percent N was determined using microkjedahl method (Jackson, 1964). Available P was extracted using Bray P1 extractant and the extracts measured with Murphy-Riley blue method (Murphy and Riley, 1962) on spectronic 20 at 8821Um while the soil bulk density was determined using core method (Ojeniyi, 1985).

### *Field Experiments*

The experiments were carried out at Akure in the rainforest zone of Nigeria and the soil is sandy clay loam, skeletal, kaolinitic, isohyperthermic oxic paleustalf (Alfisol) or Ferric Luvisol (FAO). The site had been continuously cropped to cereals and tuber crops for 10 years while the two experiments were conducted between October 1999 and March 2001 and January, 2003 on the same site.

The annual rainfall of the study area is 1300mm and it is well distributed throughout the year while the annual temperature ranged between 22°C and 28°C. These climatic conditions are considered adequate for growth and yield of white yam. The land was cleared, ploughed, harrowed and ridged while the plots were laid out at 4m x 4m (16m<sup>2</sup>) and yam setts prepared from white yam variety (*Dioscorea rotundata* L) were planted in early November each cropping year into the plots at a spacing of 1m x 1m. The plots were mulched immediately to prevent scorching and decay of the planted yam setts by heat.

There were four treatments namely poultry manure, long yam beans, NPK 15 – 15 – 15 fertilizer and a control (no fertilizer; no manure), replicated six times and arranged in a randomized complete block design. The poultry manure was applied at 6 t/ha, NPK 15 – 15 – 15 fertilizer was applied at 250kg./ha, long yam beans were planted at two seeds per hole at a

spacing of 1m x 0.5m between the middle rows of yam plots and the control treatment ( no fertilizer; nor manure nor long yam bean plants).

Manual weeding operation was first carried out in the third week after sprouting and it continued at every three weeks interval until the 16<sup>th</sup> week sprouting. Individual staking of the yam vines was done in the second week after sprouting in early March 2001 and 2002 and the mulching materials were removed in each cropping year when the rain was steady. The young yam vines were trailed on the stakes to prevent vines from creeping on the soil and proper drainage channels were made to prevent the applied treatments from being washed away by rain water. The yam vine length (cm), leaf population and stem girth (cm) were measured at weekly interval beginning from two weeks after application of treatments till 12 weeks after sprouting.

Harvesting of the yam tubers was done at 32 weeks after sprouting and the yield parameters such as tuber girth, tuber length(cm) and tuber weight (kg) were measured. The harvesting of the long yam bean pods started in November 2000 to February 2001 for the first experiment and between November 2002 and February 2003 for the second experiment. The weight of the shelled grains were measured and recorded for each experiment. At end of the harvesting of the long yam bean pods in the first experiments, all the plants were allowed to dry in-situ and ploughed into the soil at the commencement of the second experiment.

#### *Leaf analysis of the yam plants*

At 18 weeks after sprouting, leaf samples were taken from the top, middle and lower parts of the yam crop in each treatment using secateurs, properly cleaned, milled into smaller pieces and dry ashed in a muffle furnace for 6 hours at 450°C. The ash produced, was made into solution, filtered and filtrates analyzed for N, P, K Ca and Mg.

The percentage N was determined using micro-kjedahl method (Jackson, 1964) while the P content was determined using Vanado-molybdate colorimetry and read on Spectronic 20 at 4421Um. The K and Ca contents were determined on flame photometer using appropriate filters while the Mg content was read on atomic absorption spectro photometer.

#### *Soil analysis after the experiment*

At the end of each experiment, soil samples were taken from 0-15cm depth from each treatment plot, air-dried, sieved and analyzed for soil pH, N, P, K, Ca, Mg and SOM, and soil bulk density as described earlier.

#### *Statistical analysis*

The data obtained from the means of the two experiments for the growth, yield parameters, leaf and soil chemical composition were analyzed using ANOVA F-test and the overall treatment effects were compared using Duncan Multiple Range Test at 5% level.

### 3. Results

#### *Soil fertility status before planting of yam*

Table 1 presents the soil physical and chemical properties before planting of yam.

Table 1: Pre-planting soil analysis

SOIL PROPERTIES	
pH (H <sub>2</sub> O)	5.80
(CaCl <sub>2</sub> )	5.30
Organic Matter (%)	0.50
Total Nitrogen (%)	0.06
Available Phosphorus (ppm)	5.08
Sodium (cmol/kg)	0.14
Potassium (cmol/kg)	0.06
Calcium (cmol/kg)	0.11
Magnesium (cmol/kg)	0.14
Bulk Density	1.60

The soil is acidic and very low in organic matter. The low organic matter content of the soil also reflected in the low values of soil N, P, K, Ca, Mg and Na, which were below 10mg/kg P, 0.20cmol/kg K, Ca, Mg and Na (Agboola and Corey, 1973) and 0.15% N (Sobulo and Osiname, 1981) considered as soil critical levels for optimum crop production in South western Nigeria. The soil bulk density was 1.60g/cm<sup>3</sup>.

Table 2 presents the chemical analysis of the poultry manure and long yam bean used for the cultivation of yam. The poultry manure had high values of %N, P, K, Ca, Mg, and Na.

Table 2: Chemical composition of the organic fertilizers used for the experiment

Organic Material	N	P	K	Na	Ca	Mg
<b>Poultry manure</b>	3.90	0.75	0.48	0.10	0.54	0.35
<b>Long yam bean</b>	226 kg/ha (symbiotic N fixed)	3.70	3.00	0.30	0.80	1.00

Source: Boonche and Anecksamphant (1993)

*Growth and yield parameters of yam under different treatments.*

Table 3: The growth and yield parameters of yam under different fertilizer treatments

Treatments	Vine length (cm)	Stem girth (cm)	Leaf population	Tuber weight (kg/ha)	Tuber length (cm)	Tuber girth (cm)
<b>Control (No fertilizer application)</b>	40.20	0.40	12.00	540.20	9.00	1.00
<b>NPK 15-15-15</b>	210.00	3.41	44.00	3380.40	26.00	7.00
<b>Poultry manure</b>	189.70	2.10	32.00	3453.20	30.00	9.00
<b>Long yam bean</b>	210.40	3.30	39.00	4900.00	37.00	15.70
<b>LSD</b>	<b>4.30</b>	<b>0.63</b>	<b>2.10</b>	<b>12.20</b>	<b>3.50</b>	<b>2.00</b>

Table 3 presents the values of leaf area, vine length, leaf population and stem girth, tuber weight, tuber length and tuber girth of white yam under the different fertilizer treatments. There were significant increases ( $P<0.05$ ) in these parameters for yam compared to the control treatment. The long yam bean treatment increased the yam vine length, stem girth, leaf population, tuber weight, tuber length and tuber girth by 81%, 88.4%, 69.5%, 89%, 76% and 94% compared to the control treatment. The same treatment (long yam bean plants) also increased these parameters by 10%, 37%, 18%, 29%, 19.4% and 40% respectively compared to the poultry manure treatment.

Long yam bean plants increased the leaf population, tuber weight, tuber length and tuber girth of yam by 11%, 32%, 30% and 55% respectively. Except in stem girth and vine length where NPK fertilizer treatment increased these parameters by 3.8% and 0.2% respectively compared to the long yam plants treatment.

#### *Leaf Chemical composition of Yam under different Fertilizer treatments*

Table 4: Leaf composition of yam under different fertilizer treatments

Treatments	N	P	K	Ca	Mg
<b>Control (No fertilizer application)</b>	1.50	0.30	0.20	0.15	0.15
<b>NPK 15-15-15</b>	2.50	1.80	1.73	0.10	0.12
<b>Poultry manure</b>	2.70	1.98	1.20	0.32	0.36
<b>Long yam bean</b>	2.80	1.93	1.50	0.42	0.45
<b>LSD</b>	<b>0.10</b>	<b>0.12</b>	<b>0.20</b>	<b>0.10</b>	<b>0.09</b>

Table 4 gives Leaf Chemical composition of Yam under different Fertilizer treatments.

There were significant ( $p<0.05$ ) increases in the leaf N, P, K, Ca and Mg under the fertilizer treatments compared to the control treatment (Table 4). The long yam bean plants increased the yam leaf N, P, K, Ca and Mg 99.14%, 99.45%, 97.3%, 90.3% and 88.6% respectively compared to the control treatment. It also increased the same parameters N, K, Ca and Mg by 2.13%, 20%, 31.3% and 26% respectively compared to the poultry manure treatment except in leaf P where poultry manure increased the value by 2.52% compared to the long yam beans. Long yam bean plants increased the yam leaf N, P, Ca and Mg by 10.63%, 6.7%, 96.7% and 97.14% respectively compared to the NPK fertilizer treatment.

*Soil Chemical Composition of yam plot after harvesting.*

Table 5: Soil chemical composition of yam plots after harvesting under different fertilizer treatments

Treatments	Bulk Density	pH	Organic Matter	N	P	K	Ca	Mg
<b>Control (No fertilizer application)</b>	1.63	5.1	0.21	0.04	3.40	0.05	0.03	0.07
<b>NPK 15-15-15</b>	1.66	5.0	0.34	1.40	29.30	1.38	0.02	0.01
<b>Poultry manure</b>	1.36	6.7	2.38	1.34	25.20	0.93	0.75	0.56
<b>Long yam bean</b>	1.20	7.2	2.70	1.37	27.30	1.40	0.91	0.65
<b>LSD</b>	<b>0.15</b>	<b>0.3</b>	<b>0.31</b>	<b>0.03</b>	<b>1.50</b>	<b>0.32</b>	<b>0.01</b>	<b>0.09</b>

There were significant increases ( $p<0.05$ ) in the soil pH, O.M, N, P, K, Ca and Mg, compared to the control treatment (Table 5).

The long yam bean increased the soil pH, organic matter, N, P, K, Ca and Mg by 29%, 92%, 97%, 86%, 96%, 97% and 89% compared to the control treatment. Long yam bean increased soil pH, organic matter treatment. Long yam bean increased soil pH, organic matter, K, Ca and Mg by 31%, 87%, 1.42%, 98% and 98.5% respectively. The long yam bean decreased the soil bulk density by 25% and 27% compared to control and NPK fertilizer treatments respectively.

*Comparative advantage of long yam bean plants as soil amendment.*

Table 6: Comparative advantages of pigeon long yam bean bio-fertilizer over the conventional organic and inorganic fertilizers in term of utility parameters

Treatments	Yield of yam (kg/ha)	Yield of Long yam bean seeds (kg/ha)	Total Cost of Production (US Dollars)	Total Revenue (US Dollars)	Discounted Factor at 15%	Cost-Benefit Ratio
<b>Control (No fertilizer application)</b>	540.2	Nil	500.00	540.2	0.87	1.08
<b>NPK 15-15-15</b>	3,380.4	Nil	567.00	3,380.4	0.87	5.96
<b>Poultry manure</b>	3,453.2	Nil	583.00	3,453.2	0.87	5.92
<b>Long yam bean</b>	4,900.2	2,300.0	517.00	6,550.0	0.87	12.67

Table 6 presents the data on the comparative advantage of long yam bean plants as bio-fertilizer plants over the convectional organic and inorganic fertilizers (poultry manure and NPK fertilizer) used in the fertilization of yam. The long yam bean plants produced 2.3t/ha (2300 Kg) of long yam bean seeds, in-addition, to the 4,900 kg/ha of yam tuber weight recorded compared to the 3,453.20 kg/ha and 3,380.40kg/ha of yam tuber weight recorded for poultry manure and NPK 15 – 15 – 15 fertilizer treatments respectively

**4. Discussion**

For the control treatment, the least values of growth and yield parameters such as vine length, leaf population, stem girth, tuber weight, tuber length and

tuber weight of white yam compared to that of NPK 15 – 15 – 15 fertilizer, poultry manure and long yam bean plants might be due to the initial low nutrient status of the soil before application of the fertilizer treatments. This observation was in line with the work of Agboola (1982), which reported poor growth and yield responses in soils that did not receive fertilizer application.

The low soil nutrient status also accounted for the least values of yam leaf N, P, K, Ca and Mg; soil pH, N, P, k, Ca, Mg and organic matter. The low organic matter status of the soil is consistent with low N and P status (Agboola and Corey, 1973). The low organic matter status also increased the initial bulk density values to 1.63 and 1.66 Mgm-3 in control and

NPK fertilizer treatments respectively as a result of continuous cultivation.

The significant increases in the growth and yield of white yam due to application of poultry manure, use of long yam bean plants and NPK fertilizer were adduced to increased availability of nutrients in the soil. The application of poultry manure and the use of long yam bean plants as soil amendment increased soil organic matter, N, P, K, Ca and Mg status and reduced the soil acidity. Soil acidity (low pH) is known to affect the yields of crops adversely through inhibition of nitrogen fixation processes. (Aduayi, 1980).

The highest nutrient contents (SOM, N, P, K, Ca and Mg) supplied by the long yam bean plants into the soil were responsible for the shoot, and yield development. K had been reported to encourage photosynthesis and tuber formation in yam (Adu Daaph et al, 1994). This could explain why the long yam bean plant produced the best values of yam tuber weight (kg/ha), tuber length and tuber girth compared to NPK, poultry manure and control treatments. Long yam bean plant is a legume which fixes N into the soil and increases the level of soil organic matter. For-instance, Boonche and Anecksamphant (1993) reported that long yam bean plant fixed into the soil 220kg/ha symbiotic N, 4.3% P, 3.00% K and 0.70% Ca.

The reduction in the soil bulk density by the use of long yam bean plants might have positively influenced other soil physical properties such as porosity, water-infiltration, permeability and aeration. Hence, the improvement of soil physical condition is consistent with the work of Woomer and Muchena (1993) which reported that continuous productivity of tropical soils is associated with maintenance and improvement of soil physical characteristics.

The increase in vegetative growth of white yam such as vine length, stem girth and leaf population under long yam bean plants compared to that produced by poultry manure and NPK 15 – 15 – 15 fertilizer might be attributed to the ability of the long yam bean plants to fix nitrogen into the soil. This finding agreed with the work of Boonche and Anecksamphant (1993) who reported that roots of nitrogen fixing crops such as legumes have nodules where nitrogen fixation takes place. Thus, the 220kg N/ha/yr fixed into the soil by long yam bean plants would enhance its use for soil fertility maintenance.

The reduction in the SOM of plots fertilized with NPK fertilizer also affected the Ca and Mg contents of the soils and this is because of high nutrient interactions between P and K contents in the soil fertilized with NPK and Ca or Mg contents (P/Mg, K/ca and K/mg ratio) which reduced their uptake and this view was supported by Bear (1950).

The comparative advantage of long yam bean plants as soil amendment compared to the poultry manure and NPK fertilizer in terms of providing additional food and income for farmers could be the major ways of improving farmers' standard of living and ensured food security. Ali (1996) also reported that in semi-arid tropics of Asia, pigeon pea, and soya bean-based systems can replace other systems because of higher monetary returns, thus, long yam bean plants can also help in improving farmers income in tropical Africa and Asian countries.

Agboola (1982) reported that the main reasons why farmers in the tropics could not adopt the use of green manure such as *Calopogonium mucunoides* for soil fertility maintenance was that it was labour intensive and farmers did not usually get food or income in return for their cultivation.

Therefore, the adoption of long yam bean plants by farmers would fertilize the soil, provides food and income for the farmers. For-instance, the 2.3 t/ha of long yam bean seeds and yam tuber yield under long yam bean seeds and yam tuber yield under long yam bean plants treatment provided (\$6,550.00) compared to \$3,453.20 and \$3,380.40 estimated on yam yields alone under poultry manure and NPK fertilizer treatments (Table 6). The higher Benefit/Cost ratio of 12.67 under long yam bean treatment was as a result of the sales of the additional 2,300kg/ha of long yam bean seeds.

However, the cultivation of traditional legumes such as long yam bean plants, pigeon pea, lima beans and forth by farmers had gone down drastically such that these crops were nearly going into extinction. There is need for a strong extension package on the cultivation and use of traditional legumes such as long yam bean, lima beans and pigeon pea as soil amendments for food crops. This will help in bringing them into commercial production by farmers, instead of the conventional legumes produced from research centers which encouraged heavy use of agrochemicals because they were susceptible to pests and diseases attack.

The use of long yam bean plants as soil amendment for yam production as reflected in the best values of yam tuber weight, tuber length and girth proved that it was compatible with food crops as an intercrop. This observation agreed with the work of Adeyemi (1999) which reported yield advantages in cocoyam/cowpea/maize/cassava intercrop.

## 5. Conclusion

The research work has identified that the use of fast growing legume such as long yam bean as soil amendment increased yam vine length, stem girth, leaf population, tuber weight, tuber length and girth;

leaf and soil N, P, K, Ca and Mg; soil pH and SOM. Therefore, the use of long yam bean plants as biological fertilizer source for yam production could substitute for 250kg/ha NPK fertilizer and 6t/ha poultry manure.

This recommendation agrees with the fact that long yam bean plant is environmentally compatible with the farming system in the tropics, provides additional source of food and income for the poor resource farmers. In-addition, the high cost of purchase, scarcity of inorganic fertilizers and the labour intensive nature of gathering high quantities of manure for crop production did not help farmers in achieving sustainable food production.

The utility of long yam bean as protein source and income will benefit the farmers but the successful adoption of long yam bean plants and other legumes based technologies may depend on dissemination of information on their production and utilization through extension approaches to stakeholders.

#### **Corresponding Author:**

Dr. Emmanuel Ibikunoluwa Moyin-Jesu

Agronomy Department,  
Federal College of Agriculture  
Akure, Nigeria  
[moyinjesu2004@yahoo.com](mailto:moyinjesu2004@yahoo.com)

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13/05/2010

## Biochemical and Molecular Profiles of Gibberellic Acid Exposed Albino Rats

Hanan A.E.Soliman<sup>1</sup>; Mona M. Mantawy<sup>2</sup> and Hany M. Hassan<sup>3</sup>

<sup>1</sup>Chemistry Departement, Biochemistry Branch, Faculty of Science, Beni suef University, Egypt. <sup>2</sup>; Department of Medicinal chemistry , National Research Center, Dokki , Egypt and <sup>3</sup> Immunobiology and immunopharmacology unit , Animal Reproduction Research Inst., Giza , Egypt

**Abstract:** The present study casts the light on the influence of the plant growth regulator, Gibberellic acid (GA3), on antioxidant defense systems [glutathione peroxidase, superoxide dismutase (SOD), and catalase (CAT)], lipid peroxidation level (malondialdehyde = MDA), AST, ALT , alkaline phosphatase, creatinine, total protein , albumin globulin , total lipids , total cholesterol, calcium and glucose . Moreover, histopathological examination of kidney and liver was done. On the molecular level, the DNA damage was determined. The rats were received 75 ppm of GA3 in drinking water ad libitum for 50 days. Gibberellic acid (GA3) treatments caused different effects on the estimated parameters compared to control. Gibberellic acid exposure induced significant elevations of plasma AST, ALT, alkaline phosphatase, creatinine and malondialdehyde. However, Gibberellic acid produced non significant alterations in plasma total protein, albumin globulin, total lipids, total cholesterol, calcium and glucose. On the other hand, exposure elucidate significant reductions of catalase, superoxide dismutase and glutathione peroxidase in comparison to control group. The histopathological findings revealed that Kidney sections of Gibberellic acid treated rats suffered from areas of interstitial fibrosis which appear as segmental and global glomerular sclerosis tubulointerstitial injury. On the similar ground, liver section of Gibberellic acid treated rats revealed that Gibberellic acid induced liver fibrosis; fatty metamorphosis and necrosis. The total genomic DNA electrophoretic pattern of lymphocytes deprived from Gibberellic acid treated rats revealed strong and obvious DNA damage as represented by a lot of fragments migrated from the wells. As a conclusion, Gibberellic acid (75 ppm) produce hepatoneurotoxicity, subsequently has oxidative stress role and DNA damage in albino rats 50 days post treatment. [Journal of American Science. 2010;6(11):18-23]. (ISSN: 1545-1003).

**Keywords:** plant growth regulator; Gibberellic acid (GA3); antioxidant defense systems; superoxide dismutase (SOD); catalase (CAT); lipid peroxidation

### 1. Introduction

Gibberellic acids are a group of plant growth regulators that have been identified in different plants (MacMillian *et al.*, 1961) and they are used in agriculture as plant regulators to stimulate both cell division and cell elongation that affect leaves as well as stems (Taiz and Zeige, 1991). Gibberellic acid (actually a group of related substances called Gibberellic acids) was discovered as a metabolic by product of the fungus *Gibberella fujikuroi* (Riley, 1987). If gibberellic acid or one of its metabolites is applied to dwarf varieties of peas, broad beans or maize, growth is greatly accelerated (Jones, 1973).

In *Alstroemeria hybrida*, leaf senescence is retarded effectively by application of Gibberellic acids (Kappers *et al.*, 1997). Feeding toads, *Bufo regularis*, with Gibberellic acid A3 induced hepatocellular carcinomas in 16% of the animals. Moreover, it was showed that Gibberellic acid A3 induced breast and lung adenocarcinomas in mice (El-Mofty and Sakr, 1988). Gibberellic acid was found to induce chromosomal aberrations in human lymphocytes (Zalinian *et al.*, 1990) and mice (Bakr *et*

*al.*, 1999). The World Health Organization (1990) classified Gibberellic acid-A3 as a plant growth regulators related to pesticides.

Gibberellic acid (Gibberellic acid A3) is used extensively in Egypt to increase the growth of some fruits (such as strawberries and grapes) and some vegetables (such as tomatoes, cabbages and cauliflower)( Weaver *et al.*, 1961).

Recently, Kamel *et al.*(2009) recorded that Male rabbits treated with Gibberellic acid at all studied doses caused a significant increase in semen ejaculate volume, sperm concentration, total sperm out-put and sperm motility (%) and has direct androgenic-like action on testes compared to the control group.

The present investigation aimed to cast the light on the possible effects of Gibberellic acid on biochemical profile, histopathological image and DNA integrity of exposed rats.

### 2. Material and Methods

Twenty sexually mature male albino rats weighing  $170\pm10$  g were used. Animals were kept in the laboratory under constant temperature ( $24\pm2$  °C) for at least one week before and throughout the experimental work. They were maintained on a standard diet and water were available *ad libitum*.

Animals were divided into two groups. Ten rats in the first group were orally given Gibberellic acid-A3 (Berelex®, BDH chemical, Pool, UK) at 75 ppm in drinking water were continuously administered orally to rats *ad libitum* for 50 days (Celik *et al.*, 2007). Animals in the second group (10 rats) were served as controls. The treated animals and their controls were sacrificed by decapitation after the end of treatment.

For enzyme determination, Plasma were obtained by centrifugation of the blood samples and stored at  $-20^{\circ}\text{C}$  until assayed for the biochemical parameters. Transaminases (ALT, AST) and alkaline phosphatase activities were determined on the basis of King (1965).

Plasma creatinine levels were measured using the photometric determination according to the Jaffe method (Ecoline Mega, DiaSys Diagnostic Systems GmbH, Holzheim, Germany) described earlier (van Dokkum *et al.*, 2004).

Plasma total protein concentration as (g/dl) was measured by the Biuret method as described by Armstrong and Carr (1964). Albumin (A) concentration as (g/dl) was determined by the method of Doumas *et al.* (1971). Globulin (G) concentration as (g/dl) was calculated as the difference between total protein and albumin. Plasma total lipids (PTL) concentration as (g/dl) was estimated according to Frings *et.al.* (1972). Total cholesterol (TCh) concentration as (mg/dl) was determined according to Richmond (1973). Plasma glucose (PG) concentration as (mg/dl) was estimated according to the method of Trinder (1969). Serum calcium (SCa) concentration as (mg/dl) was measured according to the method of Sarkar and Chauhan (1967) using commercial kits (Stanbio kits).

Catalase activity was estimated by the method of Cakmak and Horst (1991). The reaction mixture contained 100 crude enzyme extract, 500  $\mu\text{l}$  10 mM H<sub>2</sub>O<sub>2</sub> and 1400  $\mu\text{l}$  25 mM sodium phosphate buffer. CAT activity of the extract was expressed as CAT units. Superoxide dismutase activity was determined with the reaction mixture contained 100  $\mu\text{l}$  1  $\mu\text{M}$  riboflavin, 100  $\mu\text{l}$  12 mM L-methionine, 100  $\mu\text{l}$  0.1 mM EDTA (pH 7.8), 100  $\mu\text{l}$  50 mM Na<sub>2</sub>CO<sub>3</sub> (pH 10.2) and 100  $\mu\text{l}$  75  $\mu\text{M}$  Nitroblue Tetrazolium (NBT) in 2300  $\mu\text{l}$  25 mM sodium phosphate buffer (pH 6.8), 200  $\mu\text{l}$  crude enzyme extract in a final volume of 3 ml. SOD activity was

assayed by measuring the ability of the enzyme extract to inhibit the photochemical reduction of NBT glass test tubes containing the mixture were illuminated with a fluorescent lamp (120 W); identical tubes that were not illuminated served as blanks. After illumination for 15 min, the absorbance was measured at 560 nm(Hernandez *et al.*, 2000). Glutathione peroxidase (GSH-Px) was measured by spectrophotometric method developed by Paglia and Valentine (1967). One unit of GSH-Px activity was defined as the amount of the enzyme that converted 1  $\mu\text{M}$  NADPH, substrate to NADP+ per minute. All the assays were carried out in triplicate using a spectrophotometer (Hitachi U-2000, Hitachi Ltd., Tokyo, Japan).

Malondialdehyde (MDA) was measured by colorimetric method (Stewart and Bewley, 1980).

#### Histological Examination

Rat kidneys were removed and portions fixed in 4% paraformaldehyde (PFA). Other kidney portions were frozen in optimum cutting temperature (OCT) compound (Miles, Elkhart, IN, U.S.A.) and stored at  $-80^{\circ}\text{C}$ . Fixed renal tissues were embedded in paraffin and cut into 4- $\mu\text{m}$ -thick sections. The sections were stained with periodic acid-Schiff (PAS) stain and Masson trichrome stain to reveal histological changes and areas of interstitial fibrosis.

Liver tissue sections were fixed in 100 mL/L formalin saline in phosphate buffer and processed in paraffin wax. Sections from blocks were stained with hematoxylin-eosin, histological analyses were performed blindly using light microscope.

#### Total genomic damage of DNA analysis

DNA was extracted from lymphocytes of blood samples using DNA extraction Kit (Fermentas life Sciences, Lithuania -Cat. #K0513).

Gel was prepared with 1.5% electrophoretic grade agarose (BRL) and 0.2% polyvinyl pyrrolidine (PVP, Sigma). The agarose and PVP were boiled with Tris Borate EDTA buffer (TBE buffer; 89 mM Tris, 89 mM boric acid, 2mM EDTA, pH 8.3). 0.5 mg ethidium bromide /ml distilled water (Sigma) was added to the gel at  $40^{\circ}\text{C}$ . Gel was poured and allowed to solidify at room temperature for 1h before samples were loaded ( 15  $\mu\text{l}$  of extracted DNA/well). Electrophoresis was performed for 2 hrs at 50 volt using IX TBE buffer as a running buffer. Gel was photographed using a polaroid camera while DNA was visualized using a 312 nm UV light under a transilluminator (Herolab, Germany) . The photographs were analyzed using Phoretex software version 3.0, UK to determine the degree of DNA damage (Cressman *et al.* ,1999) .

### Statistical analysis

All values were expressed as mean  $\pm$  standard error (SE). All statistical analyses were performed using SAS (version 8.02). Statistical differences among the experimental groups were assessed by one-way ANOVA. Tukey's test was used as a follow-up test and significance was defined at  $p<0.05$ .

### 3. Results

Table (1) revealed that Gibberellic acid-A3 exposure induced significant elevations of plasma

AST, ALT, alkaline phosphatase, creatinine and malondialdehyde. However, Gibberellic acid produced non significant alterations in plasma total protein, albumin globulin, total lipids, total cholesterol, calcium and glucose. On the other hand, Gibberellic acid exposure elucidate significant reductions of catalase, superoxide dismutase and glutathione peroxidase in comparison with control group.

Table (1): Effect of Gibberellic acid-A3 exposure on some biochemical parameters of rats.

Items	Control group	Exposed group
AST (iu/l)	$52.28 \pm 4.28$	$66.85 \pm 4.51^*$
ALT(iu/l)	$33.27 \pm 4.33$	$44.31 \pm 3.07^*$
Alkaline phosphatase (iu/l)	$103.85 \pm 12.36$	$137.08 \pm 5.88^*$
Creatinine (mg/dl)	$0.346 \pm 0.16$	$0.636 \pm 0.09^*$
Total protein (gm/dl)	$8.82 \pm 1.61$	$8.91 \pm 1.55$
Albumin (gm/dl)	$4.42 \pm 0.54$	$4.35 \pm 0.52$
Globulin (gm/dl)	$4.37 \pm 0.33$	$3.61 \pm 0.21$
Total lipids (gm/l)	$4.208 \pm 0.59$	$4.34 \pm 0.18$
Total cholesterol (mg/dl)	$72.58 \pm 5.78$	$75.89 \pm 5.48$
Glucose (mg/dl)	$138.24 \pm 10.95$	$145.35 \pm 7.51$
calcium (mg/dl)	$1.66 \pm 0.33$	$2.15 \pm 0.05$
Catalase (nmol/min/ml)	$18.77 \pm 1.91$	$15.04 \pm 0.33^*$
Superoxide dismutase (u/ml)	$105.28 \pm 10.58$	$85.28 \pm 5.71^*$
Glutathione peroxidase (nmol/min/ml)	$90.48 \pm 10.84$	$74.33 \pm 5.49^*$
Malondialdehyde (nmol/dl)	$4.33 \pm 1.63$	$6.85 \pm 0.35^*$

\*significant at  $p < 0.05$

### Total genomic damage of DNA:

Figure (1) shows the total genomic DNA electrophoretic pattern of lymphocytes deprived from control and Gibberellic acid treated groups. Gibberellic acid revealed strong and obvious DNA damage at the tested concentrations as represented by a lot of fragments migrated from the wells. On the other hand, control group did not reveal any damage of DNA.

### Histological Examination

Kidney sections of Gibberellic acid treated rats which stained with periodic acid-Schiff (PAS) stain and Masson trichrome stain to reveal histological changes and areas of interstitial fibrosis appear as segmental and global glomerular sclerosis tubulointerstitial injury (tubular dilatation, atrophy of tubular epithelial cells) (Photo , 1).

Histopathological examination of Hematoxylin and eosin-stained liver section of normal and Gibberellic acid treated rats with magnification -400 X, revealed that Gibberellic acid induced liver fibrosis; fatty metamorphosis and necrosis (Photo , 2).

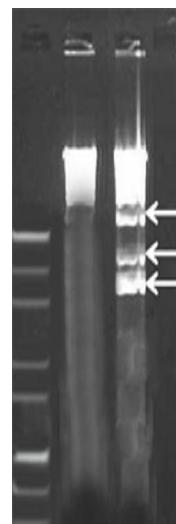


Fig (1): Effect of Gibberellic acid-A3 treatment on DNA damage of DNA of rats, arrows indicate that fragmented DNA bands in Gibberellic acid treated group.

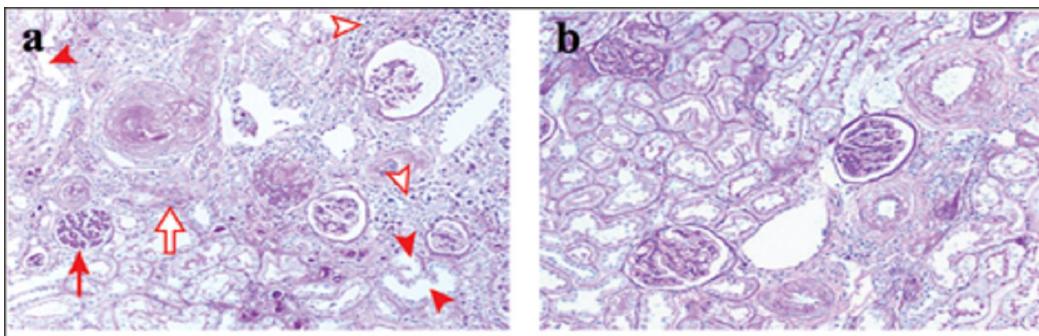


Photo (1): Rat renal tissue sections with PAS staining arrows indicating global glomerular sclerosis and tubulointerstitial injury: arrow heads; fibrosis: open arrow; and infiltration of inflammatory cells: open arrow heads) in Gibberellic acid treated (a) and control (b).

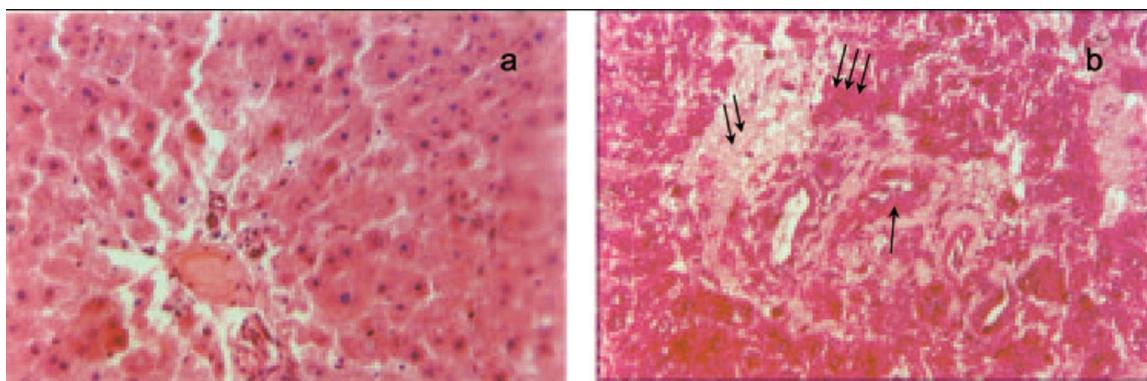


Photo (2): Liver tissue sections were stained with haematoxylin and eosine for light microscope observation  
 (a) Control (b) Gibberellic acid treated. ↓↓, fibrosis; ↓↓↓,

#### 4. Discussion

Gibberellic acid is a type of plant hormone which regulates growth. There are 126 known Gibberellic acids, divided into two classes, and many more may be discovered in the future. Plants produce these hormones naturally through biosynthesis as they grow, ensuring that they have the hormones they need to develop normally, and these hormones can also be applied to plants by gardeners and farmers to achieve specific desired outcomes (Fernandez and Rodriguez, 1979).

In the present study, Gibberellic acid induced significant elevations of plasma AST, ALT, alkaline phosphatase, creatinine and malondialdehyde. However, Gibberellic acid produced non significant alterations in plasma total protein, albumin globulin, total lipids, total cholesterol, calcium and glucose. On the other hand, Gibberellic acid exposure elucidate significant reductions of catalase, superoxide dismutase and glutathione peroxidase in comparison with control group.

The histopathological findings revealed that Kidney sections of Gibberellic acid treated rats suffered from areas of interstitial fibrosis appear as segmental and global glomerular sclerosis tubulointerstitial injury .

On the similar ground, liver section of Gibberellic acid treated rats with magnification -400 X, revealed that Gibberellic acid induced liver fibrosis; fatty metamorphosis and necrosis.

The previous findings were in accordance with the recorded findings of Sakr *et al.*(2003), who recorded that Gibberellic acid induced histopathological changes in the liver such as cytoplasmic vacuolization of the hepatocytes with pyknotic nuclei, blood vessel congestion and inflammatory leucocytic infiltrations. Histochemical observations revealed marked reduction in total carbohydrates and total protein contents in the hepatocytes. These changes proved to be time dependent.

On the same hand, Celik *et al.* (2007) recorded that gibberellic acid (GA3) has deleterious

effect on the antioxidant defense systems [reduced glutathione (GSH), glutathione reductase (GR), superoxide dismutase (SOD), glutathione-S-transferase (GST) and catalase (CAT)] and lipid peroxidation level (malondialdehyde = MDA) in various tissues of the rat were investigated during treatment as a drinking water 75 ppm of ABA and GA3 in drinking water were continuously administered orally to rats for 50 days. The lipid peroxidation end product MDA significantly increased in the lungs, heart and kidney of rats treated with GA3. The GSH levels were significantly depleted in the spleen, lungs and stomach of rats treated with GA3. SOD significantly decreased in the spleen, heart and kidney. While CAT activity significantly decreased in the lungs of rats treated with GA3. The drug metabolizing enzyme GST activity significantly decreased in the lungs of rats treated with ABA but increased in the stomach of rats treated with GA3. The authors concluded that GA3 produced substantial systemic organ toxicity in the spleen, lungs, stomach, heart and kidney during a 50-day period of subchronic exposure.

The present study discloses that the total genomic DNA electrophoretic pattern of lymphocytes deprived from control and Gibberellic acid treated groups. Gibberellic acid revealed strong and obvious DNA damage at the tested concentrations as represented by a lot of fragments migrated from the wells. On the other hand, control group did not reveal any damage of DNA.

These data on the same way of the findings of Hassab-Elnabi and Sallam (2002).

They recorded that higher concentration of Gibberellic acid induced total genomic damage of DNA. By the application of modified comet assay (single cell gel electrophoresis) technique, DNA damage was found at all applied doses. Also, Bakr *et al.* (1999) reported a significant increase in the incidence of total chromosomal aberrations induced by gibberellic acid in bone marrow cells of albino mice.

The illustrated results are fully agreed with that recorded by Zalinian *et al.* (1990). They reported that gibberellic acid induced chromosomal aberrations in human lymphocyte cultures. Gibberellic acid in this demonstration induced a significant damage of DNA. This result agreed with the data that declared by Abou-Eisha (2001). He showed that Gibberlic acid induces a dose-dependent increase in the level of DNA breakage in human blood cells , this increase attaining statistical significance at the highest concentrations tested (25, 100, 150 $\mu$ g/ml), which would confirm its genotoxicity.

So, the DNA damage may attributed to the direct attack of DNA by gibberellic acid causing alkali labile and single strand breaks and total genomic damage as revealed by our demonstration, or may be due to accumulation of nucleases as reported by Fath *et al.* (1999). The mechanism of gibberellic acid to induce DNA damage may be attribute to elevation of oxidative stress markers such as (ROS and GSH), Bcl-2 protein expression, mitochondrial membrane potential and caspase-3 activity. The sequel of these events lead to mitochondrial membrane depolarization and caspase-3 activation followed by apoptosis (Abou-Eisha , 2001).

From the recoded data , it could be concluded that Gibberellic acid (75 ppm) produce hepatonephrotoxicity , subsequently has oxidative stress role and DNA damage in albino rats 50 days post treatment.

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5/1/2010

# An Efficient Algorithm for Transforming XML Schema into Relational Schema

<sup>1</sup> Abad Shah, <sup>2</sup>Amjad Farooq, <sup>3</sup>Syed Ahsan

<sup>1,3</sup> Al-Khawarizmi Institute of Computer Science, University of Engineering and Technology, Lahore

<sup>2</sup> Department of Computer Science, University of Engineering and Technology, Lahore

[Amjadfaroquet@gmail.com](mailto:Amjadfaroquet@gmail.com)

**ABSTRACT:** The Web and XML have influenced all walks of life especially those that involve business activities over the Internet. People like to do their business activities and transactions from their homes to save time and money. Many business and commercial companies such as insurance companies and banks maintain their records using relational database management systems. But the traditional relational database technology is unable to provide all these new facilities to the users. To enable the traditional relational database technology to cope with the new challenges of the Web and XML technologies, we need a transformation between the XML technology and the relational database technology as a middleware. To achieve this objective, we already proposed and reported an algorithm. In this paper, we extend our previous work and present automation details, testing, and performance report of our proposed algorithm. The result shows that the implementation of the algorithm is more efficient than the existing algorithms for the same purpose [Journal of American Science. 2010;6(11):24-37]. (ISSN: 1545-1003).

**Keywords:** XML, web, rational database, transforming algorithm

## 1. Introduction

An electronic document contains regular and irregular structures and may not be completely understood by users (Suciu, 1999; Abiteboul & Vianu, 1997; Brayan, 1997). This type of document (or data) is referred to as *semistructured data* (Suciu, 1999; Abiteboul, 1997). Unlike the data in relational databases (RDBs), the semistructured data is stored without any schema or with a vague schema (Suciu, 1999; Buneman, 1997). There are many other sources of semistructured data, such as the Web, heterogeneous networking of integrated systems, file systems, electronic mail systems, digital libraries etc. (Abiteboul, 1997; Buneman, 1997).

The introduction of Extensible Markup Language (XML) as a standard data/information representation has facilitated the publication of electronic data on the Web (W3C). This language also provides a hierarchical format for the data exchange over the Web with structure (Laurent, 1999; Bray, 2002). Information in a XML document is represented as nested *element* structures (i.e. a tree structure), which start with a root element. An element can have an attribute or a sub-element (for further details about XML see (W3C; Bray et al., 2002). A XML document has an optional part, which is called *Document Type Declaration/Description (DTD)*. A DTD of a XML document is considered as the schema of the XML document (W3C; Bray et al., 2002; Men-Hin & Fu, 2001).

A relational database (RDB) has two main components, a schema and a set of operational data

files which are created according to the schema. As mentioned earlier, a DTD is considered as a schema of a XML document but there are noticeable differences between a RDB schema and DTD. A complete comparison between them is given in Table 1. The basic difference between them is their structural representations; a DTD represents a hierarchical structure whereas a RDB schema represents a relational (tabular) structure. We can consider XML documents schema analogous to the hierarchical data model schema.

XML is considered as the best tool for representing, transporting and exchanging information on the Web (Laurent, 1999; Bray et al., 2002). This language allows users to define and also display data on the Web. These features make XML powerful and different from Hypertext Markup Language (HTML) (Suciu, 1999; Comer, 2000). XML enables the user to define his own structures using the syntax of the elements in a DTD. A DTD describes the structure of information in a XML document in a hierarchical fashion (Bray et al., 2002). The structure of a DTD consists of elements which are further specified by attributes and/or sub-elements. Recursive and optional type of the sub-element can be defined using the operations \* (zero or more times), + (one or more times), ? (optional) and | (or). Many types of data value can be assigned to attributes, i.e. string-type or entity. The data value ANY means that an arbitrary declaration can be made by the programmer. An element in a XML document is uniquely identified by a special attribute ID. This

unique attribute of an element can be regarded as the primary key of the element. As it has been mentioned in Table 1, a DTD does not support the concept of the composite ID (or key). An attribute can be referenced in another element through a field called IDREF, and it is a type-less attribute. The concept of an IDREF is similar to the concept of a foreign key in relational databases. There is no concept of a root of a DTD (Bray et al., 2002).

Nowadays, many financial organizations want to empower their customers so that they can perform their financial activities from their homes through the Internet. For these financial organizations to provide their customers with this facility, it is essential and beneficial that the databases (which are mostly relational DB systems) should be presented and processed in the XML format. To provide this facility, we therefore need a technique that can process and transform a RDB and queries into a XML format and vice versa. This technique for the transformation is essential because most of the commercially available database management systems (DBMSs) are relational DBMSs.

To meet these requirements, we have proposed a transformation technique in the form of an algorithm and reported in (Shah et al., 2005). This technique integrates and handles heterogeneous RDBs in the same and uniform manner. Most of investigators agree that the currently available RDB technology

alone is not adequate to meet these objectives of using them on the Web without such transformation technique (Shanmug et al., 1999). Recently, some investigators have proposed a few algorithms for this purpose (Shanmug et al., 1999; Men-Hin & Fu, 2001; Williams, 2000; Mani & Lee 2002). In these transformation algorithms, most of the investigators have considered a DTD as a schema of the XML document, and they have used the tree data structure during the transformation. In our proposed algorithm, we didn't use tree data structure because the processes of creating and maintaining tree data structures are expensive and affect the performance of the transformation process as pointed out by Shanmugasundaram et al in (Shanmug et al., 1999). Also, there are many syntax options that are available for writing DTDs. Most of the existing transformation algorithms from DTD into RDB schema are unable to accept DTDs written in different ways (Men-Hin & Fu, 2001; Shanmug et al., 1999). In (Shah et al., 2005), we have used a different approach and proposed a simple algorithm that transforms any DTD of a XML document into RDB schema. In this paper, we extend that work and further report the implementation details and testing results of our algorithm. We also give its analytical analysis and performance comparison with the existing algorithms.

**Table 1:** Comparison between RDB schema and DTD

RDB Schema	DTD
Tabular format	Hierarchical format
It supports many data types.	It supports only character data types.
Schema and tuples of a relation are considered as two different entities, and they are stored in two different files.	XML document and its DTD can be stored in the same file or in two different files.
It is mandatory for a database.	It is optional for a XML document especially for small XML documents.
It is based on the rational data model.	It is not based on any such data model.
It supports the concept of a composite key.	The concept of composite key is not supported.
It supports the concept of foreign key.	Does not support any such concept.
A schema of a relation is defined before creating its instances (or tuples)	Since it is optional, it can, therefore, be defined after the creation of a XML document.

The remainder of this chapter is organized as follows. In Section 2, we describe and analyze the existing approaches for transforming a DTD of a

XML document into a relational database schema. In Section 3, we present our proposed approach for transforming a DTD into a relational database

schema, and in Section 4, we demonstrate the proposed approach in a case study. Finally, in Section 5, we give our concluding remarks and future direction of this work.

## 2. Related Work

Investigators have produced many different techniques for transforming DTDs into relational database schemas (Shanmug et al., 1999; Men-Hin & Fu, 2001; Eisenberg & Melton, 2002; Yan, 2001; Williams, 2000; Mani & Lee 2002). There are three (3) main issues that need to be handled during this transformation. These issues are: i) the complexity of the DTD element specifications, ii) the resolution of the conflict between arbitrary nesting in a DTD and relational schema, iii) set-valued attributes and recursion (Shanmug et al., 1999). In the following paragraphs, we give a brief description of the works of these investigators and give, in Table 2 (at the end of this chapter), a comparison of these transformation approaches and our proposed approach.

Shanmugasundaram et al initially proposed an approach in the form of algorithms for transforming a DTD of a XML document into a RDB schema. Men-Hin and Fu later proposed an improvement to the algorithms, (Men-Hin & Fu, 2001; Shanmug et al., 1999). Men-Hin and Fu proposed two algorithms both of which work in the same way, except that they differ mainly in their Step 2 and Step 5. In Step 2 of the improved algorithm they gave more rules for determining the roots. The transformation algorithm by Men-Hin and Fu works in six (6) steps, and they are briefly given below:

**Step 1: Simplifying the DTD:** This step simplifies DTDs of XML documents using the rules similar to regular expression rules. The information that is useful in constructing schema prototype trees is preserved in the DTDs. The value types (e.g. #IMPLIED, #FIXED etc) for the character data (CDATA) are removed from the DTDs.

**Step 2: Determining the Root node:** In this step, roots of the prototype trees are determined from the simplified DTDs using the set of rules that are suggested for this purpose.

**Step 3: Constructing Schema Prototype Trees:** The prototype trees are constructed from the roots that are determined in the previous step using a set of rules.

**Step 4: Generating a Relational Schema Prototype:** This step realizes a prototype relational database schema from the prototype tree using the following rules:

i) Regard all the necessary attributes and elements in the simplified DTD as the attributes that are treated in the entity- relationship (ER) Model.

ii) Inline all the necessary descendants of the schema prototype tree starting from the root. The necessary descendants refer to all the leaf nodes in the schema prototype tree, and the nodes marked with a “#” if they exist.

**Step 5: Discovering Functional Dependencies (FDs) and Candidate Keys:** In this step the traditional relational database design techniques are applied in order to produce suitable relational schemas. These design techniques reduce the redundancy and inconsistency in a relational database schema, and discover the functional dependencies and the candidate keys by analyzing the newly constructed relational database schema.

**Step 6: Normalizing the Relational Schema Prototypes:** This step applies the normalization rules on the relational database schema, after determining the FDs and candidate keys of a relational database schema in the previous step.

In the first algorithm of Men-Hin and Fu, hence functional dependencies are found in Step 5, first by analyzing the XML data, and then by applying the algorithm: Efficient discovery of functional and approximate dependencies using partitioning. Step 6 of this algorithm is time-consuming according to Men-Hin and Fu. Hence they modified this step to make the first algorithm more efficient (Men-Hin & Fu, 2001). The modified algorithm decomposes a DTD into small prototypes in Step 4: Tree Construction, and Step 5: Generating a Relational Schema Prototype. The reason for the decomposition is to minimize the cost of finding functional dependencies.

Both of these algorithms-the first and the modified algorithms- use the tree data structure in their transformation processes (or algorithms). The use of this data structure affects the performance of the transformation process because creating and maintaining the tree structure are costly procedures. Also, these two algorithms are unable to handle all types of DTDs as it has been mentioned in (Shanmug et al., 1999).

Eisenberg, and Melton in (Eisenberg & Melton, 2001; Eisenberg & Melton, 2002) gave an informal proposal for a bi-directional transformation between a XML document and a RDB. This transformation can do a complete or a partial transformation at schema level as well as tuple-level (or row-level). The partial transformation may however miss some semantics. This draft for the bi-directional transformations also suggests a transformation of the

data types. The authors did not give any proper formal algorithm for these transformations. It is therefore difficult to comment about the real effectiveness of these transformations.

Williams et al have proposed 18 steps for transforming DTD into a relational database schema and 11 steps for the reverse transformation (Williams, 2000). Both of these transformations do not use the tree data structure, but some steps in both of these transformations are unclear. For example, in Step 9 and Step 13 of the transformation of a DTD into a relational database schema, data types are assigned to attributes of a DTD without any explanation and justification. This type of vagueness in the transformation processes makes them difficult to understand and to draw any conclusion about their correctness and accuracy.

Mani, & Lee (Mani & Lee 2002) have proposed a process for transforming a DTD into a relational database schema using a regular tree grammar. The use of the regular tree grammar is helpful in maintaining semantics constraints in the transformation process. The theory of regular tree grammars provides a useful formal framework for understanding various aspects of XML schema languages. The two normal forms (NF1 and NF2) are used for the representation of regular tree grammars. NF1 is used in the XML document validation process, and to check whether a given XML schema satisfies the structural constraints imposed by the schema languages.

XML schema (or DTD) provides several unique data modeling features such as union type “+”, which does not exist in the traditional database models such as relational database model. In (Mani & Lee 2002), NF2 is used for representing the basic items of the conversion definition of the two schema languages, that is, a schema that supports union types (e.g., XML-Schema Language (Thompson, 2001), and a schema language that does not support union types (e.g., SQL). This conversion definition is used as the first step in this transformation process of XML schema into relational database schema. The entities and relationships, which form the basic items of data

modeling, are represented as elements and attributes of a DTD.

The process of mapping XML schema (or DTD) into RDB schema has several issues, and they have been pointed out in (Mani & Lee 2002). One of the most important among them is the semantic constraint which exists in the XML model. Since relational database schema cannot express these constraints in the XML schema languages, a useful and meaningful subset of those constraints should therefore be found in the mapping process. This process of finding the subset needs simplification of a XML schema. The concept of inlining technique is used for generating an efficient relational schema (Mani & Lee 2002), however; the inline technique that is presented in this work generates a huge number of relations. In addition, this work does not present any proposal for assigning data types to attributes of tables after or during the transformation process.

The transformation process of a XML DTD to relational data schema is the mapping of each element in the DTD to a relation, and it maps the attributes of an element to the attributes of the relation. However, there is no correspondence between elements and attributes of DTDs and entities and attributes of ER model. The attributes in an ER model are often represented as elements in a DTD.

```
<!ELEMENT author (name, address)>
<!ATTLIST author id ID #REQUIRED>
<!ELEMENT name (firstname , lastname)>
<!ELEMENT firstname (#PCDATA)>
<!ELEMENT lastname (#PCDATA)>
<!ELEMENT address ANY>
```

In the ER model, *author* would be taken as an entity and *firstname*, *lastname* and *address* would be taken as the attributes of the entity. But in defining a DTD there is no incentive to consider *author* as an element and *firstname*, *lastname*, and *address* as attributes. In the syntax of a DTD, if *firstname* and *lastname* were defined as attributes, then they could not be nested under *name* because DTD attributes cannot have a nested structure. A direct mapping of elements to relations therefore leads to an excessive fragmentation.

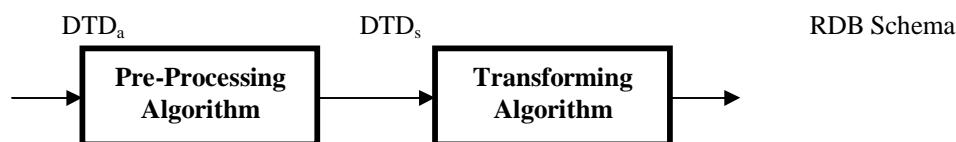


Figure 1: General view of the algorithm

### 3. The Proposed Algorithm

As mentioned earlier in (Shah et al., 2005), we have reported a transforming algorithm that works in two steps. In the first step it takes a DTD written in any form using the DTD syntax and transforms it into a *standard* form that is devised keeping in view the syntax of a relational database schema. The second step of the algorithm takes the standard DTD and transforms it into a relational database schema. Note that in our approach the transforming algorithm development, we did not include the processes of finding functional dependencies (FDs), assigning data types to attributes of relations after the transformation (note that DTD supports only one data type, i.e. PCDATA), and normalization of the

relation database schema. The reason for this is that these three processes are database design techniques and they depend on the perception of a database designer about the database schema (Elmasri & Navathe, 2000). These processes also need direct or indirect expert's intervention and decision. Therefore, in the proposed transforming algorithm, we have separated these three processes from the actual transformation process. Our decision is to separate the manual processes and the automated processes, and this has made our algorithm simpler and helped in achieving our objective of transforming any DTD into a relational schema. In this section, we present only those processes of the transforming algorithm that can be automated.

**Table 2:** Comparative study of our algorithm and existing algorithms

	<b>BI</b>	<b>SI &amp; HI</b>	<b>GSE</b>	<b>DSE</b>	<b>WIL</b>	<b>RTG</b>	<b>EIS</b>	<b>OPA</b>
<b>Data Structure Used</b>	Graph	graph	Tree	Tree	relational structure	regular tree grammars	relational structure	no such abstract data structure is used
<b>Type of conversion</b>	Structured	structured	Structured	Structured	Structured	structural &subset of semantics	mapping	Structural & semantic
<b>Operators Handling</b>	creates a relation for every element in the DTD	ensure that each element is represented only once in a relation	eliminates operators from DTD	preserves some operators to preserve some sub-elements occurrences	some rules are specified to handle them	support XML-Schema (not DTD)	support XML-Schema (not DTD)	Pre-processing algorithm processes them
<b>Advantage</b>	handles fragmentation problem*	the common elements are shared	actual data fields are available in relational schema	number of attributes of the schema is less than the algorithms basic inlining	preserves entities and definitions	maintains semantics constrains	bi-directional transformation	simple, direct mapping & maintains the semantics
<b>Disadv. F</b>	creates large number of relations	large number of joins in mapping for particular elements	number of possible minimal dependencies is exponential	works with limited number of elements and attributes	some rules of the mapping are vague such as assigning	a complex mapping process	miss some semantics	data types assigning is with human intervention
<b>Prfce. FF</b>	low	low	Low	low	didn't mention	didn't mention	didn't mention	high (expected)

Table Legends:

**BI:** Basic Inlining (Shanmug et al., 1999), **SI & HI:** Shared Inlining & Hybrid Inlining (Shanmug et al., 1999), **GSE:** Global Schema Extraction (Men-Hin & Fu, 2001), **DSE:** DTD-splitting Extraction (Men-Hin & Fu, 2001) **WIL:** (Williams, 2000), **RTG:** Regular Tree Grammar (Mani & Lee 2002), **EIS:** , **OPA:** Our Proposed Algorithm (Shah et al, 2005).

**F:** Disadvantages.

**FF:** Performance.

\*: A direct mapping of elements to relations leads to excessive fragmentation of attributes (for more details see [(Mani & Lee 2002)].

Our proposed transforming algorithm further consists of two algorithms (or steps): i) *Pre-Processing Algorithm*, ii) *Transformation Algorithm*. In Figure 1, we have given a general view of our transformation process. Pre-Processing Algorithm transforms any DTD that is written in any form into the standard DTD that is referred to as *DTD<sub>s</sub>* (see Figure 1). The main objective of Pre-Processing Algorithm is to transform a DTD into a simple uniform and standard form which is denoted as *DTDs* in Figure 1. The second algorithm, (i.e., Transformation Algorithm), transforms a *DTDs* in this standard form into a RDB schema (see Figure 1). In the next two paragraphs, we briefly describe the working of these two algorithms. The details of these algorithms can be seen in (Shah et al, 2005).

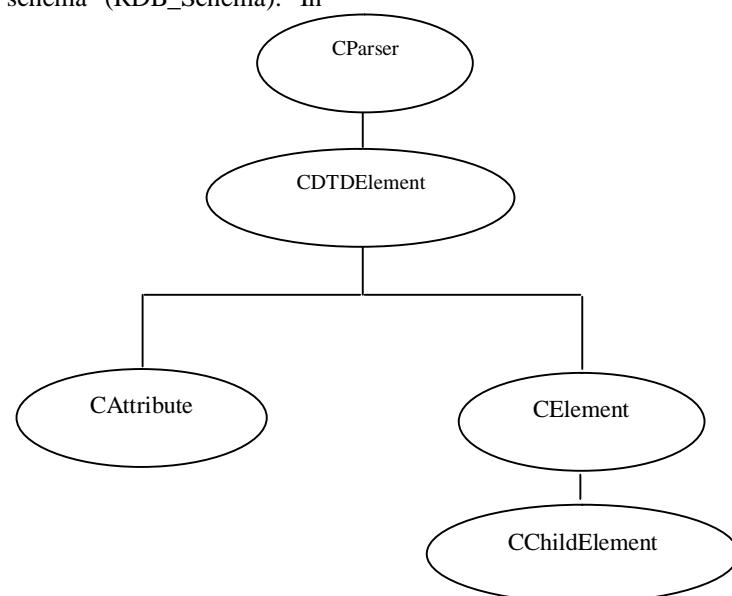
The main function of Pre-processing Algorithm is to enable the overall transformation process to handle *DTDs* which are written in different ways, and to transform them into a uniform and standard form. The output of this algorithm is the standard DTD denoted as *DTDs* (Shah et al, 2005) and it is used as the input to Transformation Algorithm as shown in Figure 1. The working of Pre-Processing Algorithm is given in Figure I-1 for more details see (Shah et al, 2005).

Transformation Algorithm takes the *DTD<sub>s</sub>* of a XML document input and transforms it into a relational database schema (RDB\_Schema). In

Figure I-2, we give the working of the algorithm. In this algorithm, there are two nested loops. The outer loop deals with elements of the *DTD<sub>s</sub>* and transforms them into corresponding tables/relations. The inner loop transforms every attribute of the element into the attributes of the corresponding relation. In Step (iii) of the algorithm (see Figure I-2), it transforms ID and IDREF attributes of an element into primary key and foreign key of the relation, respectively. Note that since the syntax of DTD does not allow the concepts of composite key, therefore, our proposed transformation process also does not support this concept.

## 1. Implementation Details

As we know from the previous section that the proposed transformation process consists of the two algorithms, and these algorithms are implemented on the platform Intel Pentium II, 400 MHz, Microsoft Windows 98, and using Visual C++ version 6.0. The implementation has two (2) modules corresponding to the two algorithms, namely the Pre-processing module and the Transformation modules. The design of the process is an object-oriented design and it consists of five base classes as show in Figure 2. The designs (or contents) of the classes are given in Appendix II.



**Figure 2:** Class-lattice of the transformation process

## 5. Testing and Analysis

In this section, we give description of the selected test cases and the test data (or instances) which are used to test our proposed transformation process/algorithm. Then we analyze the test results, and based on these results we compare our proposed algorithm with the existing transformation algorithms. We have taken five (5) different and typical test cases and one test data for each test case to test the proposed algorithm. In the next five sections, we describe the five test cases, give their test data, and report their test results.

### Test Case 1: Simple DTD

We consider DTD as a *simple DTD* if it contains the basic components/features of the DTD syntax. It does not contain the features such as nesting structures, ID and IDREF(s) attributes, or any referencing to any external entity. We have taken simple DTD as the first test case to the proposed algorithm. A test data (or instance) of this test case that is used for testing the algorithm, is given in Figure III-1 (see Appendix III). The execution of the algorithm for this test data was successful, and the result was as it was expected. The result/outputs of the test data is given in Figure III-2.

For the test case of the simple DTD, our finding is that its automatic transformation using our proposed algorithm into a relational schema is close to its manual transformation.

### Test Case 2: DTD Containing ID and IDREF

The concepts of ID and IDREF in DTD are important from database point of view. To test these concepts, we have taken a test case to test these concepts. For this purpose, we have picked a DTD of a Library system as an instance of this test case and it is shown in Figure III-3. After the execution of the algorithm with the instance (given in Figure III-3), the result/output is shown in Figure III-4. After testing this test case, we noticed that the results of our transformation algorithm and the existing algorithms are identical. Note that most of the existing algorithms are not automated.

### Test Case 3: DTD Containing Entity Reference and Operators

In a DTD, an entity referencing is a reference to a content of a named entity whether this entity is referencing to a separate external or an internal location, where the content is given in the declaration of the DTD. In this test case, we have picked a DTD as instance of the test case which has this characteristic. An instance (DTD) of this test case is given in Figure III-5. After the successful executing of the instance, the result is given in Figure III-6. The transformation algorithm has worked successfully for

the instance, and it has removed the entity declaration from the Catalog DTD as shown in Figure III-6.

### Test Case 4: DTD Containing Multiple Root Elements

Some DTDs may have irregular structure which means that these DTDs are missing their root elements. In other words, such type of DTDs have multiple root elements. Our next test case deals this type of DTDs. An instance of this test case is given in Figure III-7. Note that in the figure *Building* element has two parent elements and they are *owner* and *compound*. This instance was successfully executed, and the result of the transformation algorithm is the same as it was expected and given in Figure III-8.

### Test Case 5: DTD with Irregular Structure

Usually to write a DTD, there is no fixed format. In other words, DTD of a XML document can be written in many different ways. A DTD is called an *irregular DTD* if it has one element existing as a sub-element of two different main elements (Shah et al, 2005), an example is the element *person* in the Conference DTD, shown in Figure III-9, person element is a sub-element of the two main elements (*editor* and *author*). An instance of this test case is given in Figure III-9. After testing the instance, the result is shown in Figure III-10. From this test case an interesting result can be concluded, that is, if root element of a DTD contains an attribute of type ID, then the DTD could be a part of another DTD.

## DISCUSSION

In the previous section, we report testing result of our proposed and implemented transforming algorithm. These testing results show that this algorithm works successfully on more different types of DTDs as compared to many existing algorithms which are described in Section 2. Another main feature of our algorithm is its implementation because most of the existing algorithms are not implemented; therefore, it is hard to say about their test results and performance. We can conclude that the performance of our algorithm is better than the existing algorithms because our algorithm does not use the tree data structure during the transforming process. Our algorithm saves the heavy construction and maintenance of the tree structure.

During the testing of the algorithm, we have noted that the number of elements and attributes do not affect the working of the algorithm.

Now we summarize our findings of our algorithm, compare it with the existing algorithms and give our concluding discussion. In Table 2, we present the summary of main features of our

proposed and implemented algorithm, and the existing algorithms.

In the three algorithms, i.e., Basic Inlining, Hybrid Inlining, and Shared inlining, the evaluation is based on real DTDs which raise the performance concern as it has been mentioned in (Shanmug et al., 1999). This concern is due to a big number of relations generated by the algorithms. In Global-Schema extraction and DTD-Splitting, it has been pointed out in (Men-Hin & Fu, 2001) to the high cost of finding the functional dependencies because the cost of finding the possible minimal dependencies is exponential due to the number of attributes. The other algorithms did not mention their implementation and test results. As we have mentioned earlier that we have implemented our proposed algorithm and successfully tested it by taking different test cases. Whereas, most of the existing algorithms are not yet automated, and also they use graph or tree data structures during their transformation process (see Table 2). We did not use these data structure in our algorithm, which has caused better performance of our algorithm than the existing algorithms. By comparing other parameters in Table 2, it is obvious that our algorithm shows better results than the existing algorithms.

## 6. Concluding Remarks and Future Directions

In this paper, we have presented the extension of previous work that was reported in (Shah et al, 2005). Here, we have presented development details, testing results and analysis of our proposed algorithm. The proposed algorithm efficiently transforms the DTDs of a XML document (which are written in different ways using different syntax options) into a relational database schema. This transformation algorithm works in two steps/sub-algorithms: i) Pre-Processing Algorithm, and ii) Transformation Algorithm. The first step transforms DTD into a standard form of DTD which is closer to relational database schema. The second step does the actual transformation.

We have demonstrated and tested the working of our proposed algorithm by taking five different test cases. The results are encouraging. The main features of our proposed algorithm are that it is simpler and easy to understand, implemented and tested on different types of DTDs, and more efficient than the existing algorithms.

A possible extension of this work can be the reverse-directional transformation (i.e., RDB Schema into XML documents schema). These issues and future directions of this work are following:

- (i) Handling of IDREFS: It can be an interesting study to translate the concept of IDREFS into relational paradigm.
- (ii) Naming conflict between relations and attributes during the transformations.
- (iii) Assigning data types to attributes, because DTD supports only character data type and RDB schema supports multiple data types.

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## Appendix I

```

Algorithm Pre-processing (DTDa):DTDs
(i) Scan DTD_Name in DTDa and make it
DTDs_Name of DTDs
    and add <!DOCTYPE DTDs_Name [ ;
        /* DTDs is the standard DTD */
(ii) Find all the Root_elements
of DTDa;
    /* Root_element is
       the element that
       is not sub-element
       of any other
       element in the DTD
    */
(iii) Scan each Root_element of
      DTDa and make it
      Root_element of DTDs;
(iv) Find total number of main_elements
in the Root_element of
      DTDa, say that they are n;
      /* n is the total number of
main_elements */
(v) Transform the main_elements into
DTDs as follows
      /* main_element has the following
features:
         (i) a sub-element of the
Root_element,(ii)a sub-element of
another main_element, and/or (iii)
has at least one sub-element, or
(iv) has at least one attribute */
      <!ELEMENT Root_element
(main_element1,...,main_elementn)> ;

(vi) FOR i= 1 to n
      Find total number of sub-elements
in main_elementi
      say they are m;
      /* Root_element could be
main_element; for example if
it has at least one sub-
element or at least one
attribute */
      /* the sub-element has one of
the following features (i)it
has no sub-elements,
(ii)neither sub-element of any
other main_element nor sub-
element of the Root_element,
and (iii)is not
a main_element */

      IF m > 0 THEN

```

```

Add <!ELEMENT main_elementi
(#PCDATA)> , Add <!ATTLIST main_elementi
;

For j=1 to m
    Transform sub-elementj into
DTDsas sub-elementj CDATA #FIXED

END FOR LOOP /*inner
loop*/

attribute:

IF main_elementi has an
attribute of type ID THEN
Transform it in DTDs as
attribute_name ID
#REQUIRED
ELSEIF main_elementi has an
attribute of type
IDREF/IDREFS THEN
Transform it in DTDs
as
attribute_name IDREF
TYPE
/* TYPE is the type of the
attribute originally exist in
DTDa it could be (#REQUIRED,
#IMPLIED, #FIXED, or
defaultvalue) just transform it
as it is in DTDa */
ELSE Transform any other
attribute defined in DTDa,
into DTDs as it is defined
in DTDa
add'>

ELSE add main_elementi to DTDs
as its;
/* it means m = 0; that is
for the two cases:
(i)<!ELEMENT
main_elementi (#PCDATA)>
, and (ii)<!ELEMENT
main_elementi EMPTY> */

GOTO attribute;

END FOR LOOP /* outer loop */
(vii) add']';
(viii) RETURN (DTDs);
(ix) END Algorithm;

```

**Figure I-1:** Pre-Processing Algorithm

Algorithm **Transforming** (DTD<sub>s</sub>): RDB\_Schema

```

(i) Scan Root_element of DTDs make it as
Schema_name
    of RDB_schema;
(ii) Find total number of main_elements
    in DTDs (say they are n);
(iii) IF n = 0 THEN EXIT();
    ELSE
        FOR i = 1 to n
        {
            Transform main_elements into Tablei
            and give it the name of the
            element;
            Find total number of attributes
            in main_elementi (say they are
            m);
            IF m = 0 THEN i++
            ELSE
                FOR j = 0 to m
                    Scan attribute_namej;
                    IF attribute_namej is
                    of type ID THEN
                        make attribute_namej
                        as primary key of Tablei;
                    ELSEIF attribute_namej is of type
                    IDREF THEN
                        make attribute_namej as a
                        foreign key of Tablei;
                    ELSE make attribute_namej
                    a normal attribute;
                    Tablei; }
                END FOR LOOP; /* inner
                loop */
            END FOR LOOP; /* outer loop */
(iv) RETURN (RDB_Schema);
(v) END Algorithm

```

**Figure I-2:** Transformation Algorithm**Appendix II**

Here, due to the space problem we give design of sample classes of the case study.

**Class name: CParser**  
**Attributes:**  
 strPath : Char  
 m\_strError : Char  
 m\_bIsValid : BOOL = FALSE  
 m\_strDocTypeStart : Char  
 m\_strDocTypeEnd : Char  
 m\_strDocTypeName : Char

**Methods:**

1. Function ReadDTDFile(strDTDURL:Char )  
 :Char;  
 {
 x= DTDfile;  
 {
 get URL of x;  
 read x;
 };
 };

2. Function  
**ManipulateParamEntities**(strDTDFile:Char,p  
 ArrNames:CharArray\*=NULL,

```

pArrVals:CharArray*=NULL):Char
X = Entity;
{
  RemoveIGNORE(Char );
  RemoveComments (Char );
  Find x;
  If (!x) exit( );
  Else get x name;
  If (parameter x);
  Then if x is external entity
  {
    open the file;
    x = file content;
  }
  If x is external entity {get the
  value}
  X = value;
  Call ManipulateParamEntitis;
};

3. Function
RemoveIGNORE(strDTDURL:Char): Char
{
  Find "<![" ;
  Find "]]>" ;
  Remove inbetween;
}

4. Function
RemoveComments(strDTDURL:Char): Char
{
  Find "<! - " ;
  Find " - >" ;
  Remove inbetween;
}

5. Function
ConstructElementMap(strDTDFile:Char): Char
{
  x = Element;
  {
    find (x);
    get name;
    if name is not exist in the
    map (HashTable)
    Then Construct DTD Element
    object;
    Else (Error);
  }

6. Function
BuildChildAttributesArray
(strDTDFile:Char): BOOL
{
  For each list of attributes
  {
    Find "<ATTLIST ";
    Get the Element name of the
    attribute list;
    Find Element in the Map (Hash
    Table);
    If Element not found
    Display (Error);
  }
  For each attribute in the

```

```

attribute list
{
    get attribute name;
    Determine attribute type;
    Get default value;
    Construct Transformed
attribute Expression;
    Put in Element
ChildAttributeArray;
}
Repeat
}
7. Function      SaveOutputFile
(strOutputFileName : Char)
{
    Write "DOCTYPE";
    For (m_ElementMap)
    {
        write "<!ELEMENT";
        If (m_bIsEmpty)
            write "EMPTY>";
        Else write
"(#PCDATA)";
        For (
m_arrChildAttribute)
            get attributes;
            write "<!ATTLIST";
            write attribute
name
        }
        get child elements array of the
Main Element;
        If child element is sub-element
and m_bHasOR =False Then
        {
            Transform it to attribute
of type CDATA;
        }
        else attribute is of type
IMPLIED;
    }
}
8. Function SaveOutputMappingFile
(strOutputMappingFileName:Char)
{
    Write Relation_Name = DOCTYPE;
    Write Relation Tables
    Write "(";
    For (m_ElementMap)
    {
        read element ;
        If element has no child and no
attraibutes Then (continue);
        Else write (Element_Name)
        Write ")";
    }
}

Class name: CDTDElement
Attributes:

```

**m\_strChildElementsExpression : Char**  
**m\_strElementExpression : Char**  
**m\_bHasOR : BOOL = FALSE**  
**m\_bIsEmpty : BOOL = FALSE**

**Methods:**

1. Function  
**ChildElementArray**(pParser:  
*CParser\**):BOOL  
CEE= m\_strChildElementExpression;  
Or = "|";  
Emp = "EMPTY";  
Any = "ANY";  
PCdata = "#PCDATA";  
{  
For each Element  
{  
 get CEE;  
 Remove operators;  
 Remove "(";  
 Scan CEE;  
{  
 If (Emp) Then m\_bIsEmpty=  
true;  
 Continue;  
 If (Any or PCdata) continue;  
}  
 get the name of each  
elements in CCE  
 search for the name at the  
m\_ElementMap  
 If (name not found) Then  
display Error;  
 Exit();  
 Get CDTDElement of  
ChildElementArray  
 Put CChildElement object in  
m\_arrChildElements;  
 Continue;  
}
}
2. Function  
**CheckAttributeValidity**(pParse  
r:*CParser\**):BOOL  
For each attribute  
{  
 If attribute name is repeated  
Then (Error);
}

### Appendix III

```

<!DOCTYPE PEOPLE [
<!ELEMENT People (Person )>
<!ELEMENT Person (Name, Address, PhoneNumber,
FaxNumber, Email, Notes)>
<!ELEMENT Name (FirstName, MiddleName,
FamilyName, Title )>
<!ELEMENT Address (Street1, Street2, City, State,
Country, ZipCode) >

```

```
<!ELEMENT FirstName (#PCDATA)>
<!ELEMENT MiddleNAmE (#PCDATA)>
<!ELEMENT FamilyName (#PCDATA)>
<!ELEMENT Title (#PCDATA)>
<!ELEMENT Street1 (#PCDATA)>
<!ELEMENT Street2 (#PCDATA)>
<!ELEMENT City (#PCDATA)>
<!ELEMENT State (#PCDATA)>
<!ELEMENT Country (#PCDATA)>
<!ELEMENT ZipCode (#PCDATA)>
<!ELEMENT PhoneNumber (#PCDATA)>
<!ELEMENT FaxNumber (#PCDATA)>
<!ELEMENT Email (#PCDATA)>
<!ELEMENT Notes (#PCDATA)>]
```

**Figure III-1:** People DTD – test cases of the simple DTD

RELATION NAME: PEOPLE  
 RELATION TABLES: (Person,Name,Address)  
 TABLE: Person  
 ATTRIBUTES:  
   PhoneNumber (Char)  
   FaxNumber (Char)  
   Email (Char)  
   Notes (Char)  
 TABLE: Name  
 ATTRIBUTES:  
   FirstName (Char)  
   MiddleName (Char)  
   FamilyName (Char)  
   Title (Char)  
 TABLE: Address  
 ATTRIBUTES:  
   Street1 (Char)  
   Street2 (Char)  
   City (Char)  
   State (Char)  
   Country (Char)  
   ZipCode (Char)

**Figure III-2:** Relational schema of the People DTD<sub>s</sub>

```
<!ELEMENT Library (Books+, Publishers+,  

  Borrowers+, Loans+)>
<!ELEMENT Books (#PCDATA)>
<!ATTLIST Books LCNo ID  

#REQUIRED  

#REQUIRED  

#FIXED  

#FIXED>
<!ELEMENT Publishers (#PCDATA)>
<!ATTLIST Publishers PName ID  

#REQUIRED  

#FIXED
```

PCity	CDATA
#FIXED>	
<!ELEMENT Borrowers (#PCDATA)>	
<!ATTLIST Borrowers CardNo ID #REQUIRED	
Name	CDATA
#FIXED	
Addr	CDATA
#FIXED	
City	CDATA
#FIXED>	
<!ELEMENT Loans (#PCDATA)>	
<!ATTLIST Loans CardNo ID #REQUIRED	
LCNo	IDREF
#REQUIRED	
Date	CDATA
#FIXED>	

**Figure III-3:** DTD of the library system

RELATION NAME: LIBRARY  
 RELATION TABLES:  
 (BOOKS,PUBLISHER,BORROWERS,LOANS)  
 TABLE: BOOKS  
 ATTRIBUTES:  
   LCNoB (Char) [Primary Key]  
   Pname (Char) [Foreign Key] [Not Null]  
   TITLE (Char)  
   AUTHOR (Char)  
   PName (Char)  
 TABLE: PUBLISHER  
 ATTRIBUTES:  
   PNAME (Char) [Primary Key]  
   PADDR (Char)  
   PCITY (Char)  
 TABLE: BORROWERS  
 ATTRIBUTES:  
   CARDNoB (Char) [Primary Key]  
   NAME (Char)  
   ADDR (Char)  
   CITY (Char)  
 TABLE: LOANS  
 ATTRIBUTES:  
   CARDNoL (Char) [Primary Key]  
   LCNoL (Char) [Foreign Key] [Not Null]  
   Date (Char)

**Figure III-4:** RDB schema of Library DTD<sub>s</sub>

```
<!DOCTYPE CATALOG [
<!ELEMENT CATALOG (PRODUCT+)>
<!ELEMENT PRODUCT (SPECIFICATIONS+,  

  OPTIONS?, PRICE+, NOTES?)>
<!ELEMENT SPECIFICATIONS (#PCDATA)>
<!ELEMENT OPTIONS (#PCDATA)>
<!ELEMENT PRICE (#PCDATA)>
<!ELEMENT NOTES (#PCDATA)>
<!ELEMENT CATEGORY (#PCDATA)>
```

```

<!ELEMENT PARTNUM (#PCDATA)>
<!ELEMENT PLANT (#PCDATA)>
<!ELEMENT INVENTORY (#PCDATA)>
<!ELEMENT SPECIFICATIONS (#PCDATA)>
<!ATTLIST PRODUCT NAME CDATA #IMPLIED>
<!ATTLIST CATEGORY TYPE (HandTool | Table |
Shop-Professional) "HandTool">
<!ATTLIST PARTNUM Num CDATA #IMPLIED>
<!ATTLIST PLANT Branch (Pittsburgh | Milwaukee |
Chicago) "Chicago">
<!ATTLIST INVENTORY Status (InStock | Backordered |
Discontinued) "InStock">
<!ATTLIST SPECIFICATIONS Weight CDATA
#IMPLIED>
<!ATTLIST OPTIONS Finish (Metal | Polished | Matte)
"Matte">
<!ATTLIST OPTIONS Adapter (Included | Optional |
NotApplicable) "Included">
<!ATTLIST OPTIONS Case (HardShell | Soft |
NotApplicable) "HardShell">
<!ATTLIST PRICE Msrp CDATA #IMPLIED>
<!ATTLIST PRICE WholeSale CDATA #IMPLIED>
<!ATTLIST PRICE Street CDATA #IMPLIED>
<!ATTLIST PRICE Shipping CDATA #IMPLIED>
<!ENTITY AUTHOR "John Doe">
<!ENTITY COMPANY "JD Power Tools, Inc.">
<!ENTITY EMAIL "jd@jd-tools.com">]

```

**Figure III-5:** The catalog DTD

**RELATION NAME: CATALOG**

**RELATION TABLES:** (PRODUCT,SPECIFICATIONS, OPTIONS,PRICE, CATEGORY, PARTNUM, PLANT, INVENTORY)

**TABLE: PRODUCT**  
**ATTRIBUTES:**  
Name (Char)  
NOTES (Char)

**TABLE: SPECIFICATIONS**  
**ATTRIBUTES:**  
Weight (Char)

**TABLE: OPTIONS**  
**ATTRIBUTES:**  
Finish (Char) [Not Null]  
Adaptor (Char)  
Case (Char)

**TABLE: PRICE**  
**ATTRIBUTES:**  
Msrp (Char)  
WholeSale (Char)  
Street (Char)  
Shipping (Char)

**TABLE: CATEGORY**  
**ATTRIBUTES:**

AA (Char) <b>TABLE: PARTNUM</b> <b>ATTRIBUTES:</b> Type (Char)
<b>TABLE: PLANT</b> <b>ATTRIBUTES:</b> Branch (Char) [Not Null]
<b>TABLE: INVENTORY</b> <b>ATTRIBUTES:</b> Status (Char)

**Figure III-6:** RDB schema for the catalog DTDs

```

<!DOCTYPE HOUSING [
<!ELEMENT OWNER (BUILDING+)>
<!ELEMENT COMPOUND
(BUILDING+)>
<!ELEMENT BUILDING (ROOM+)>
<!ELEMENT ROOM
(BED*,CHAIR*,(CENTRAL_AC
,(EXT_AC|(FAN,HEATER)+))?)>
<!ELEMENT BED EMPTY>
<!ELEMENT CHAIR EMPTY>
<!ELEMENT CENTRAL_AC (#PCDATA)>
<!ELEMENT EXT_AC (#PCDATA)>
<!ELEMENT FAN (#PCDATA)>
<!ELEMENT HEATER (#PCDATA)>
<!ATTLIST OWNER NAME ID #REQUIRED>
<!ATTLIST OWNER AGE CDATA #IMPLIED>
<!ATTLIST COMPOUND ADDRESS ID
#REQUIRED>
<!ATTLIST BUILDING BUILDING_NO ID
#REQUIRED>
<!ATTLIST ROOM ROOM_NO ID #REQUIRED>
<!ATTLIST CENTRAL_AC ELECTRIC_POWER
CDATA #IMPLIED>
<!ATTLIST CENTRAL_AC HORSE_POWER
CDATA #IMPLIED>
<!ATTLIST EXT_AC ELECTRIC_POWER CDATA
#IMPLIED>
<!ATTLIST EXT_AC HORSE_POWER CDATA
#IMPLIED>
<!ATTLIST FAN ELECTRIC_POWER CDATA
#IMPLIED>
<!ATTLIST FAN SPEED CDATA #IMPLIED>
<!ATTLIST HEATER ELECTRIC_POWER CDATA
#IMPLIED>

```

**Figure III-7:** Housing DTD

<b>RELATION NAME: HOUSING</b> <b>RELATION TABLES:</b> (OWNER,COMPOUND,BUILDING,ROOM,CENTRAL_AC,EXT_AC,FAN,HEATER)
---

TABLE: OWNER
ATTRIBUTES:
NAME (Char) [Primary Key]
AGE (Char)
TABLE: COMPOUND
ATTRIBUTES:
ADDRESS (Char) [Primary Key]
TABLE: BUILDING
ATTRIBUTES:
BUILDING_NO (Char) [Primary Key]
TABLE: ROOM
ATTRIBUTES:
ROOM_NO (Char) [Primary Key]
BED (Char)
CHAIR (Char)
TABLE: CENTRAL_AC
ATTRIBUTES:
ELECTRIC_POWER (Char)
HORSE_POWER (Char)
TABLE: EXT_AC
ATTRIBUTES:
ELECTRIC_POWER (Char)
HORSE_POWER (Char)
TABLE: FAN
ATTRIBUTES:
ELECTRIC_POWER (Char)
SPEED (Char)
TABLE: HEATER
ATTRIBUTES:
ELECTRIC_POWER (Char)

**Figure III-8:** RDB Schema after the transformation

eids (Char) [Foreign Key]
TABLE: paper
ATTRIBUTES:
id (Char) [Primary Key]
title (Char)
TABLE: contact
ATTRIBUTES:
aid (Char) [Foreign Key] [Not Null]
TABLE: author
ATTRIBUTES:
id (Char) [Primary Key]
TABLE: person
ATTRIBUTES:
id (Char) [Primary Key]
email (Char)
phone (Char)
TABLE: name
ATTRIBUTES:
first_name (Char)
last_name (Char)
TABLE: cite
ATTRIBUTES:
id (Char) [Primary Key]
format (Char)

**Figure III-10:** RDB schema of the conference

5/5/2010

**Figure III-9:** DTD of there conference

ELATION NAME: Conference
RELATION TABLES:
(conf,date,editor,paper,contact,author,person,name,cite)
TABLE: conf
ATTRIBUTES:
id (Char) [Primary Key]
title (Char)
TABLE: date
ATTRIBUTES:
year (Char) [Not Null]
mon (Char) [Not Null]
day (Char) [Not Null]
TABLE: editor
ATTRIBUTES:

## Effect of injector types, irrigation and nitrogen levels on II- Garlic yield, water and nitrogen use efficiency.

Tayel, M.Y., \*Shaaban, S.M., Ebtisam I. El-Dardiry and Sabreen Kh.

Water Relations and Field Irrigation Dept., National Research Centre, Dokki, Cairo, Egypt.

[\\*shaabansm@yahoo.com](mailto:shaabansm@yahoo.com)

**Abstract:** Field experiments were conducted during two consecutive growing seasons in split split plot design on a clay loam soil at Shalaquan, Qalubia Governorate, Egypt. Experiments investigated the effect of three injectors types by-bass pressurized mixing tank ( $J_1$ ), venturi ( $J_2$ ) and piston pump ( $J_3$ ); three rates of irrigation 50, 75, 100% of  $ET_c$  ( $I_1, I_2, I_3$ ); three nitrogen levels 60, 90, 120 kg fed $^{-1}$  ( $N_1, N_2, N_3$ ) on garlic yield, water use efficiency (WUE) and nitrogen use efficiency (NUE). The main results could be summarized as follows; the maximum and minimum garlic yields (6.34, 2.38 ton fed $^{-1}$ ) were obtained with treatment  $J_3 I_2 N_3$  and  $J_1 I_1 N_1$ , respectively. Maximum value of WUE was 3.29 kg garlic m $^{-3}$  of irrigation water as recorded with the treatment  $J_3 I_1 N_3$ , while the minimum value was 1.30 kg garlic m $^{-3}$  of irrigation water as recorded with the treatment  $J_1 I_3 N_1$ . The maximum and minimum values of NUE in kg garlic kg $^{-1}$  N were 83.22 and 29.17 for  $J_2 I_2 N_1$  and  $J_1 I_1 N_3$ , respectively. A positive linear relationship was found between WUE and NUE. [Journal of American Science. 2010;6(11):38-46]. (ISSN: 1545-1003).

**Keywords:** Field experiments; clay loam soil; water use efficiency (WUE); nitrogen use efficiency (NUE)

### 1. Introduction

The use of modern irrigation systems becomes very important to save both water and chemicals. One of the most influencing operations for both production and costs is fertilizer application. Any improvement which takes place on this factor would, no doubt, have a considerably effect on production.

The academic and applied researches emphasized that, lots of fertilizers are lost through leaching with drainage water; this phenomenon is highly remarked under the conventional methods of fertilizing. So the application of fertilizer via the modern irrigation methods will be very effective. El-Gindy [1], studied the optimization of water use for pepper crop, he found that the drip irrigation method increased the pepper yield by 64.0% and water use efficiency over the furrow one. El-Adl [2], recorded maximum water use efficiency for peas crop in the treatment (surge drip irrigation, fertigation, and 75 % of  $ET_c$ ). WUE was 3.78 kg green pods and 0.693 kg dry grain m $^{-3}$  of irrigation water. On the contrary, the same author [3] in his study on peanut crop, found that the maximum value of WUE was 0.42 kg seed m $^{-3}$  of irrigation water for the treatment (irrigation every day with 100% of  $ET_c$  and traditional fertilization). Morad et. al. [4] revealed that FUE kg N unit $^{-1}$  was significantly affected by changing the doses of nitrogen; increasing dose from 90, 120 and up to 150 unit of nitrogen caused a considerable decrease in FUE kg N unit $^{-1}$ . These decreases were 13.72 and 21.93 %, respectively.

Panchal et. al. [5] studied the effect of irrigation rates as irrigation water/ cumulative pan evaporation ratios (IW/CPE of 1.0, 1.2 or 1.4), N (25,

50 or 75 kg ha $^{-1}$ ) and P $_2O_5$  (25, 50 or 75 kg ha $^{-1}$ ) on garlic yield. They found the bulb yields were highest at IW/CPE ratios of 1.2 or 1.4, N at 50 or 75 kg N ha $^{-1}$ , and P $_2O_5$  at 50 or 75 kg ha $^{-1}$ . In a study using garlic (cv. G1) grown on a clay loam soil, 5 levels of irrigation as ID/CPE of (0.5, 0.75, 1.00, 1.25 and 1.50) and 3 N levels (50, 100 and 150 kg N ha $^{-1}$ ) were compared [6]. They found that the highest yield was 157.33 q ha $^{-1}$  at 1.5 ID/CPE. These rates of irrigation and N also resulted in the greatest bulb diameters and weight of 10 bulbs. Carvalho et. al. [7] planted garlic, under the conditions of 3 rates of applied N (0-120 kg ha $^{-1}$ ) and 4 rates of K (0-160 kg K $_2O$  ha $^{-1}$ ) at irrigation rates corresponding to 60, 100 or 140% of maximum evapotranspiration (401.5 -716.5 mm). Rainfall during the growing season was relatively high and no significant effects of irrigation were observed. Although various effects of N and K on emergence and morphology were noted, total and marketable bulb yields were affected only by nitrogen. They added that the highest yields (2400-4440 kg ha $^{-1}$ ) were obtained with 70-76 kg N ha $^{-1}$ . Sadaria et. al. [8] studied the effects of irrigation (at irrigation water cumulative pan evaporation ratios (IW/CPE) of 1, 1.2 and 1.4). The highest bulb yields (5594 kg ha $^{-1}$ ) were obtained at IW/CPE= 1.4 the increase in productivity obtained in this treatment was significantly higher than that at IW/CPE= 1.2. It is suggested that higher water availability at 1.2 and 1.4 than at 1.0 IW/CPE increased nutrient availability, and therefore increased growth and productivity. The different N treatments tested had no significant effects on bulb yield, and the effects of P treatments were not clear. Tayel et. al. [9] conducted a

field experiment on onion (*Allium cepa*; Giza 20), potato (*solanum tuberosum*, sponta Holand) and peas (*Pisum Sativum*, Master B- Short) under different irrigation system; surface drip (SDI), sub surface drip (SSDI), uncontrolled surface irrigation (UCSI), and controlled surface irrigation (CSI). They found that WUE varied from 6.45 (SDI) to 10.37 (CSI) and from 2.47 (SSDI) to 5.08 (CSI) for onion and peas crop, respectively at Bahtem and Shalaqan sites. Data indicated that UCSI has the maximum value of WUE ( $10.2 \text{ kg m}^{-3}$ ) of irrigation water while CSI has the minimum one ( $4.96 \text{ kg m}^{-3}$ ) of irrigation water for onion crop. Tayel et. al. [10] studied the effect of different irrigation systems (drip, low head bubbler; gated pipe) on yield and both water and fertilizers use efficiency by grape (thompson seedless) grown in silty clay loam soil in Egypt. They found that nitrogen use efficiency ranged from 32.1 to 35.1  $\text{kg yield kg}^{-1}$ , whereas, water use efficiency varied from 0.51 to 1.07  $\text{kg yield m}^{-3}$  of irrigation water. They found high

significant linear relation ( $R^2 = 0.896^{**}$ ) between the yield (y) and nitrogen use efficiency (NUE),  $y = 0.0067 \text{ NUE} + 18.972$ . Also, high significant linear equation ( $R^2 = 0.911^{**}$ ) between WUE and NUE was found,  $\text{WUE} = 10.515 \text{ NUE} + 25.538$ . Abdel- Baset [11] found that the max yield of garlic (19.9 ton fed $^{-1}$ ) and the maximum WUE ( $12.57 \text{ kg m}^{-3}$ ) were achieved in the irrigation treatment 81.2 and 62.5 of ETc, respectively. The main objective of this work was to study the effect of injector types, irrigation and nitrogen levels on garlic yield, water and nitrogen use efficiency.

## 2. Material and Methods

Experiments were carried out at the Experimental Farm of the Faculty of Agriculture, Ain Shams University, at Shalaquan village, Qalubia Governorate as follow:

### 1. Soil

Soil samples were taken randomly to determine some physical and chemical properties as shown in table (1)

Table (1 ): Some soil physical and chemical properties of the experiment site.

Sample depths (cm)	Particle Size Distribution %			Texture class	w % at			BD (g cm $^{-3}$ )	pH 1:2.5	ECe ds m $^{-1}$ 1:5
	Sand	Silt	Clay		FC	WP	AW			
0-15	28.3	41.4	30.3	CL	35.5	19.2	16.3	1.25	7.9	0.26
15-30	28.2	41.2	30.6	CL	35.2	19.4	15.8	1.26	7.8	0.25
30-45	28.4	38.5	33.1	CL	34.7	19.8	14.9	1.28	7.6	0.26
45-60	29.1	37.3	33.6	CL	34.7	20.1	14.6	1.30	7.2	0.28

FC : Field capacity  
BD : Bulk density

WP : Welting point  
CL : Clay loam

AW : Available water

### 2. Fertilizer Injectors

Three injector types: By-bass pressurized mixing tank ( $J_1$ ), venturi ( $J_2$ ) and piston pump ( $J_3$ ) were used for nitrogen fertilizer injection in the form of  $(\text{NH}_4)_2\text{SO}_4$  solution.

### 3. Experiment layout

Field experiments were conducted during two consecutive growing seasons in split split plot design with three replications. Super phosphate 15.5%  $\text{P}_2\text{O}_5$  and potassium sulphate 48-52%  $\text{K}_2\text{O}$  were added in soil at rate of  $100 \text{ kg fed}^{-1}$  before transplanting and during the vegetative growth in two equal doses, respectively. Chinese garlic (*Allium Sativum*) variety (Cloves cv.) was planted at the second week of September. The main plots were devoted to irrigation treatment. Three irrigation rates

occupied the sub-plots. Three levels of N-fertilizer namely  $60, 90, 120 \text{ Kg N fed}^{-1}$  (one fed.  $4200 \text{ m}^2$ ) were added. Since three methods for fertilizer application (by-bass fertilizer tank, venturi, and piston pump) were used, the layout mentioned above was repeated three times. Garlic was drip irrigated. Interval between irrigation was 4 days. Total growing period of marketable yield was 195 days,

### 4. Examined parameters:

Water use efficiency (WUE)  $\text{kg garlic m}^{-3}$  of irrigation and nitrogen use efficiency (NUE)  $\text{kg garlic per kg N}$  were calculated according to Israelson and Hanson [12] as follows:

$$\text{WUE} = Y / W \text{ and } \text{NUE} = Y / C$$

where:

$Y$  = total crop yield,  $(\text{Kg fed}^{-1})$

$W$  = total water applied and  $(\text{m}^3 \text{ fed}^{-1})$

$C$  = amount of nitrogen fertilizer applied  $(\text{kg N fed}^{-1})$

were 50, 75, and 100 % of  $\text{ET}_c$  (1423, 2134 and 2846  $\text{m}^3 \text{ fed}^{-1}$ ). On the other hand, N-fertilizer treatments

### 3. Results and Discussion

The female partner was of average height and 1. Garlic yield:

Tables (2) and Figure (1) indicate the effects of injector types; by-bass pressurized mixing tank ( $J_1$ ), venturi ( $J_2$ ); and positive displacement pump ( $J_3$ ) irrigation treatments 50, 75; 100% of  $ET_c$  ( $I_1, I_2, I_3$ ) and nitrogen treatments 60, 90; 120 kg fed<sup>-1</sup> ( $N_1, N_2, N_3$ ) on garlic yield.

All the main effects of injector types, irrigation treatments and nitrogen treatments on garlic yield are significant at the 5% level on garlic yield. They can be put in the following ascending orders according to the yield  $J_1 < J_2 < J_3$ ,  $I_1 < I_3 < I_2$  and  $N_1 < N_2 < N_3$ . These results agree with those of [13].

All the first interactions:  $J_1 \times I$ ,  $J_1 \times N$ ,  $J_2 \times I$ ,  $J_2 \times N$ ,  $J_3 \times I$ ,  $J_3 \times N$  and,  $I \times N$  have significant effect at the 5% level on garlic yield in ton fed<sup>-1</sup>. The maximum yields (6.10, 6.04; 5.78) ton fed<sup>-1</sup> and the minimum ones (2.96, 3.23; 3.33) ton fed<sup>-1</sup> were obtained in the following interactions: ( $I_2 \times N_3$ ,  $I_3 \times N_3$ ;  $I_2 \times N_2$ ) and ( $J_1 \times I_1, I_1 \times N_1$ ;  $J_1 \times N_1$ ), respectively.

With respect to the second interactions they have significant effects at 5% level on garlic yield in ton/fed. The highest values of yields were 6.34, 6.26; 6.19 and the lowest ones were 2.38, 3.00; 3.36 ton/fed, in the following interactions: ( $J_3 \times I_2 \times N_3$ ,  $J_3 \times I_3 \times N_3$ ;  $J_3 \times I_2 \times N_2$  and ( $J_1 \times I_1 \times N_1$ ,  $J_1 \times I_1 \times N_2$ ;  $J_2 \times I_1 \times N_1$ ), respectively.

Data obtained could be due to one or more of the following reasons:

- 1-  $J_1$  and  $J_2$  decrease pressure within the irrigation system,
- 2- Nitrogen concentration is not constant with time in the case of injector  $J_2$
- 3- Nitrogen concentration decreased with time in the case of injector  $J_1$
- 4- Injector  $J_3$  does not decrease pressure within irrigation system and it's piston movement increases N solubility and decreases both precipitation and emitters clogging, [14],
- 5- Increment N levels increases plant growth and emitters clogging, and
- 6- Increasing irrigation rates increases emitters flushing and salt removal from root zone, and decreases both soil aeration and emitters clogging].

### 2. Water use efficiency

Table (2) and Figure (2) show that effects of injectors types ( $J_1, J_2, J_3$ ), irrigation treatments ( $I_1, I_2$ ;  $I_3$ ) and nitrogen treatments ( $N_1, N_2, N_3$ ) on the water use efficiency (WUE).

$I_3$ ) and nitrogen treatments ( $N_1, N_2, N_3$ ) on the water use efficiency (WUE).

Table (2) illustrates the main effects of injector types, irrigation rates and nitrogen levels on WUE. The three parameters have significant effect at 5% level on WUE. According to WUE values, obtained the studied parameters could be written in the following ascending orders:  $J_1 < J_2 < J_3$ ,  $I_3 < I_1 < I_2$  and  $N_1 < N_2 < N_3$ . Data obtained could be due to the effects of the following reasons on yield:

- 1- The decrease in nitrogen concentration with time when  $J_1$  is used,
- 2- The fluctuation in nitrogen concentration with time when  $J_2$  is used;
- 3- Increment of N concentration increases drippers clogging and vice versa.
- 4- Increasing irrigation rates to some extent ( $I_2$ ) increases yield and decreases drippers clogging.

In the first interactions, maximum values of WUE were 3.05, 2.93; 2.86 kg garlic m<sup>-3</sup> of irrigation water) and the minimum ones were 1.55, 1.60; 1.70 kg garlic m<sup>-3</sup> of irrigation water were obtained in the following interactions: ( $J_3 \times I_1$ ,  $I_1 \times N_3$ ;  $I_2 \times N_3$ ) and ( $I_3 \times N_1$ ,  $J_1 \times N_1$ ;  $J_1 \times I_3$ ), respectively.

Concerning the second interactions they led to significant effects on WUE in kg garlic m<sup>-3</sup> of irrigation water. The maximum values of WUE were 3.29, 3.07; 3.03 kg garlic m<sup>-3</sup> of irrigation water) and the minimum ones were 1.30, 1.66; 1.67 kg garlic m<sup>-3</sup> of irrigation water were due to the following interactions: ( $J_3 \times I_1 \times N_3$ ,  $J_3 \times I_1 \times N_2$ ;  $J_2 \times I_1 \times N_3$ ) and ( $J_1 \times I_3 \times N_1$ ,  $J_3 \times I_3 \times N_1$ ;  $J_1 \times I_1 \times N_1$ ), respectively.

### 3. Nitrogen use efficiency

Illustrated data in Figure (3) show the main effect of injector types, irrigation rates and nitrogen levels all have significant effects on NUE at 5% level. According to the values of NUE, the parameters under investigation could be put in the following ascending orders:  $J_1 < J_2 < J_3$ ,  $I_1 < I_3 < I_2$  and  $N_3 < N_2 < N_1$ . Reasons for this have been previously discussed under yield and water use efficiency.

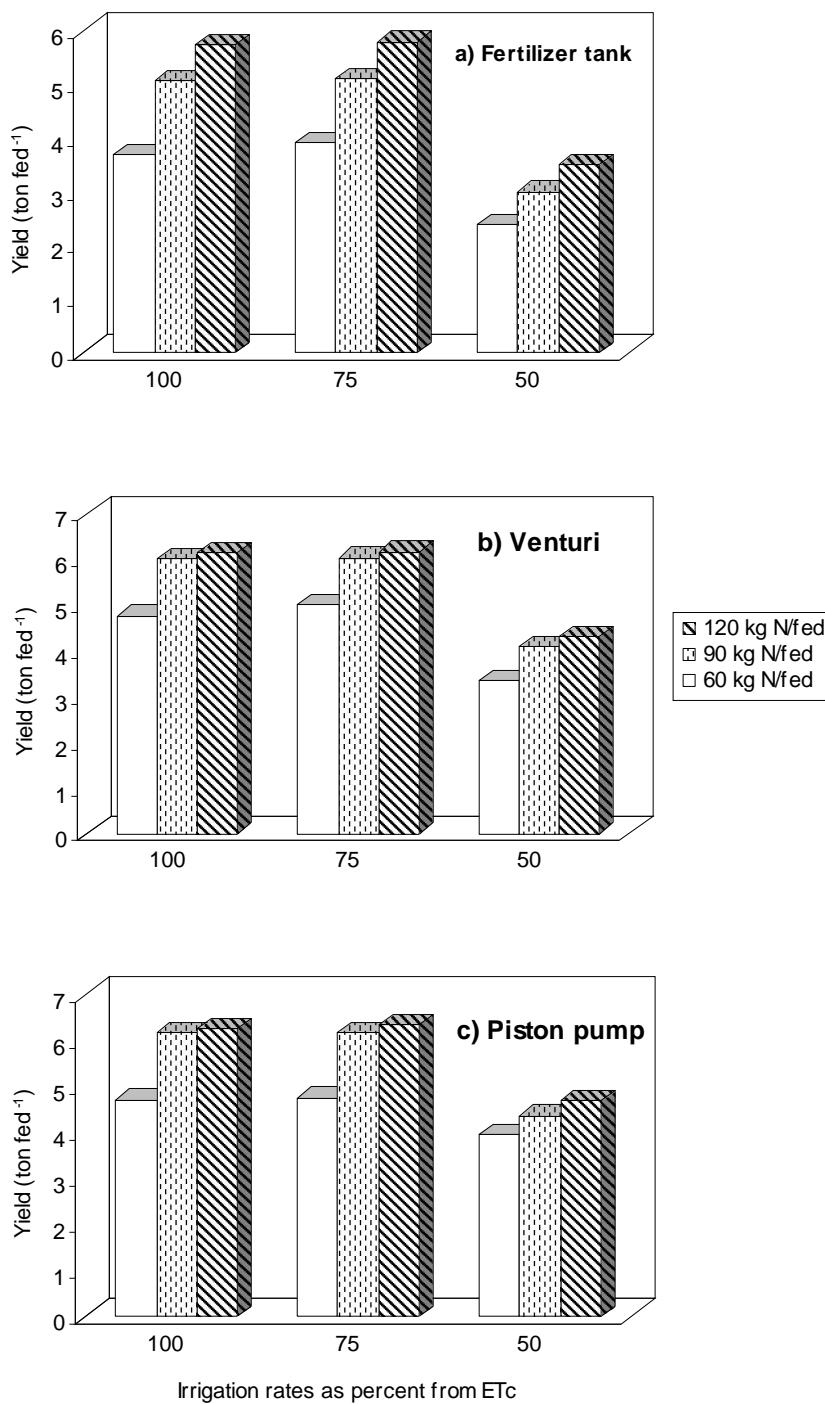
The maximum values of NUE for first interactions in kg garlic kg<sup>-1</sup> nitrogen were 75.9, 74.6; 73.4 and the minimum ones were 34.1, 34.7; 41.8 in the following interactions: ( $I_2 \times N_1$ ,  $J_3 \times N_1$ ;  $I_3 \times N_1$ ) and ( $J_1 \times I_1$ ,  $I_1 \times N_3$ ;  $J_1 \times N_3$ ), respectively.

In the case of second interactions, the maximum values of NUE in kg garlic kg<sup>-1</sup> N were 83.2, 79.7; 79.3 whereas the minimum ones 29.2, 33.3; 35.9 were achieved in following interactions.

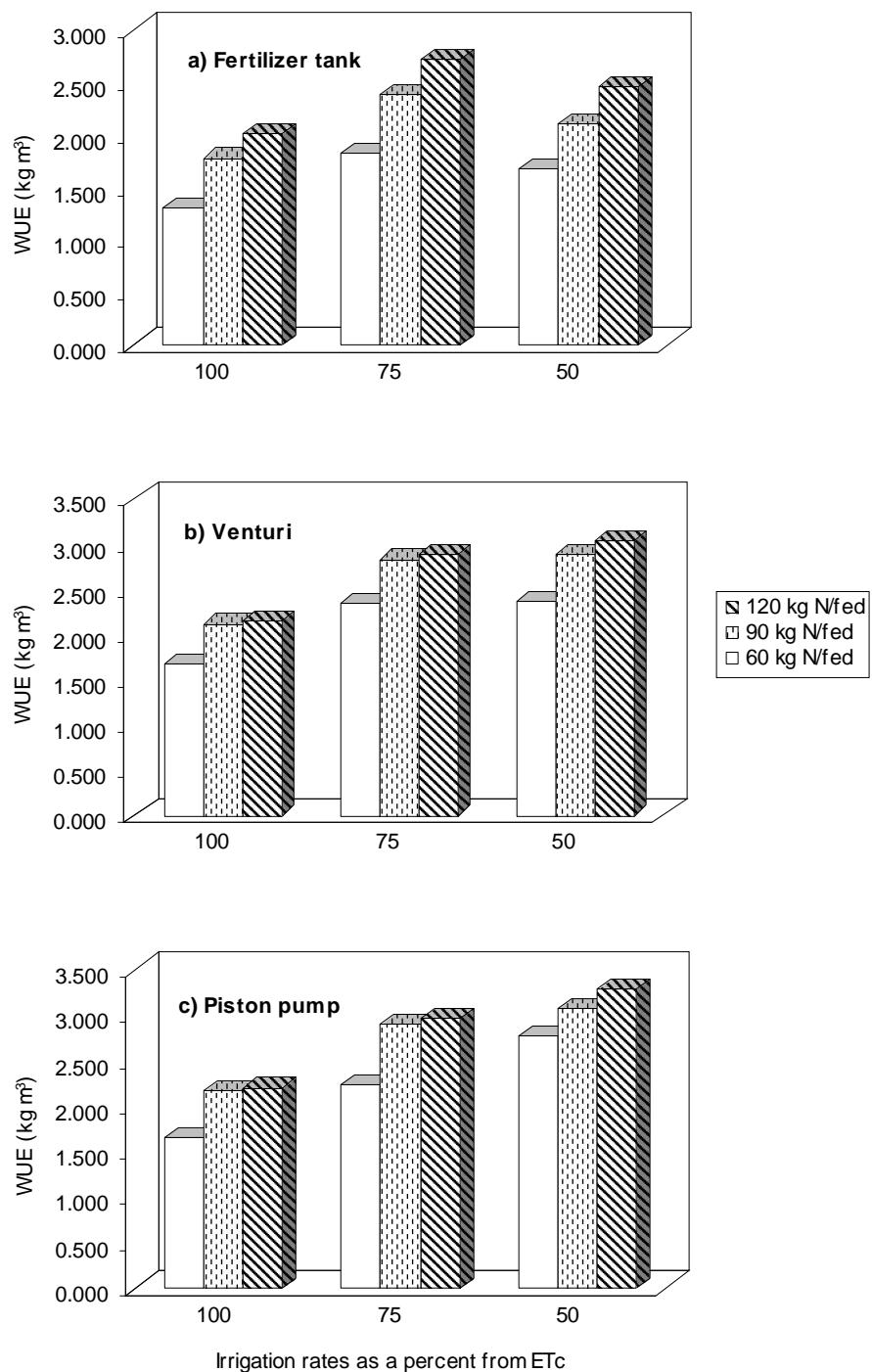
Table (2): Effect of irrigation and nitrogen fertilization levels on garlic yield, WUE\* and NUE\*\* under different injector types

Injector Types (J)	Irrigation rates (I) % of ETc	Nitrogen level (N) Kg fed <sup>-1</sup>	Yield Ton fed <sup>-1</sup>	WUE*, Kg m <sup>-3</sup>	NUE** (Kg kg <sup>-1</sup> )	
a) Fertilizer tank (J <sub>1</sub> )	100 (I <sub>3</sub> )	120 (N <sub>3</sub> )	5.73	2.01	47.75	
		90 (N <sub>2</sub> )	5.07	1.78	56.33	
		60 (N <sub>1</sub> )	3.70	1.30	61.67	
	Mean		4.83	1.70	55.25	
	75 (I <sub>2</sub> )	120 (N <sub>3</sub> )	5.80	2.72	48.33	
		90 (N <sub>2</sub> )	5.10	2.39	56.67	
		60 (N <sub>1</sub> )	3.90	1.83	65.00	
	Mean		4.93	2.31	56.67	
	50 (I <sub>1</sub> )	120 (N <sub>3</sub> )	3.50	2.46	29.17	
		90 (N <sub>2</sub> )	3.00	2.11	33.33	
		60 (N <sub>1</sub> )	2.38	1.67	39.67	
Mean		2.96	2.08	34.06		
Total mean		4.24	2.03	48.66		
b) Venturi (J <sub>2</sub> )	100 (I <sub>3</sub> )	120 (N <sub>3</sub> )	6.13	2.15	51.08	
		90 (N <sub>2</sub> )	6.03	2.12	67.00	
		60 (N <sub>1</sub> )	4.77	1.68	79.72	
	Mean		5.64	1.98	65.93	
	75 (I <sub>2</sub> )	120 (N <sub>3</sub> )	6.17	2.89	51.42	
		90 (N <sub>2</sub> )	6.04	2.83	67.11	
		60 (N <sub>1</sub> )	4.99	2.34	83.22	
	Mean		5.73	2.69	67.25	
	50 (I <sub>1</sub> )	120 (N <sub>3</sub> )	4.31	3.03	35.92	
		90 (N <sub>2</sub> )	4.10	2.88	45.56	
		60 (N <sub>1</sub> )	3.36	2.36	56.00	
Mean		3.92	2.76	45.83		
Total mean		5.10	2.48	59.67		
c) Piston Pump (J <sub>3</sub> )	100 (I <sub>3</sub> )	120 (N <sub>3</sub> )	6.26	2.20	52.17	
		90 (N <sub>2</sub> )	6.18	2.17	68.67	
		60 (N <sub>1</sub> )	4.72	1.66	78.67	
	Mean		5.72	2.01	66.50	
	75 (I <sub>2</sub> )	120 (N <sub>3</sub> )	6.34	2.97	52.83	
		90 (N <sub>2</sub> )	6.19	2.90	68.78	
		60 (N <sub>1</sub> )	4.76	2.23	79.33	
	Mean		5.76	2.70	66.98	
	50 (I <sub>1</sub> )	120 (N <sub>3</sub> )	4.68	3.29	39.00	
		90 (N <sub>2</sub> )	4.37	3.07	48.56	
		60 (N <sub>1</sub> )	3.95	2.78	65.83	
Mean		4.33	3.05	51.13		
Total mean		5.27	2.59	61.54		
LSD at 0.05		J =	0.0034	0.28	3.10	
		I =	0.0019	0.21	2.72	
		N =	0.0025	0.0082	2.42	
		J x I =	0.0032	0.36	4.70	
		J x N =	0.0043	0.14	4.19	
		I x N =	0.0043	0.14	4.19	
		J x I x N =	0.0074	0.25	7.26	

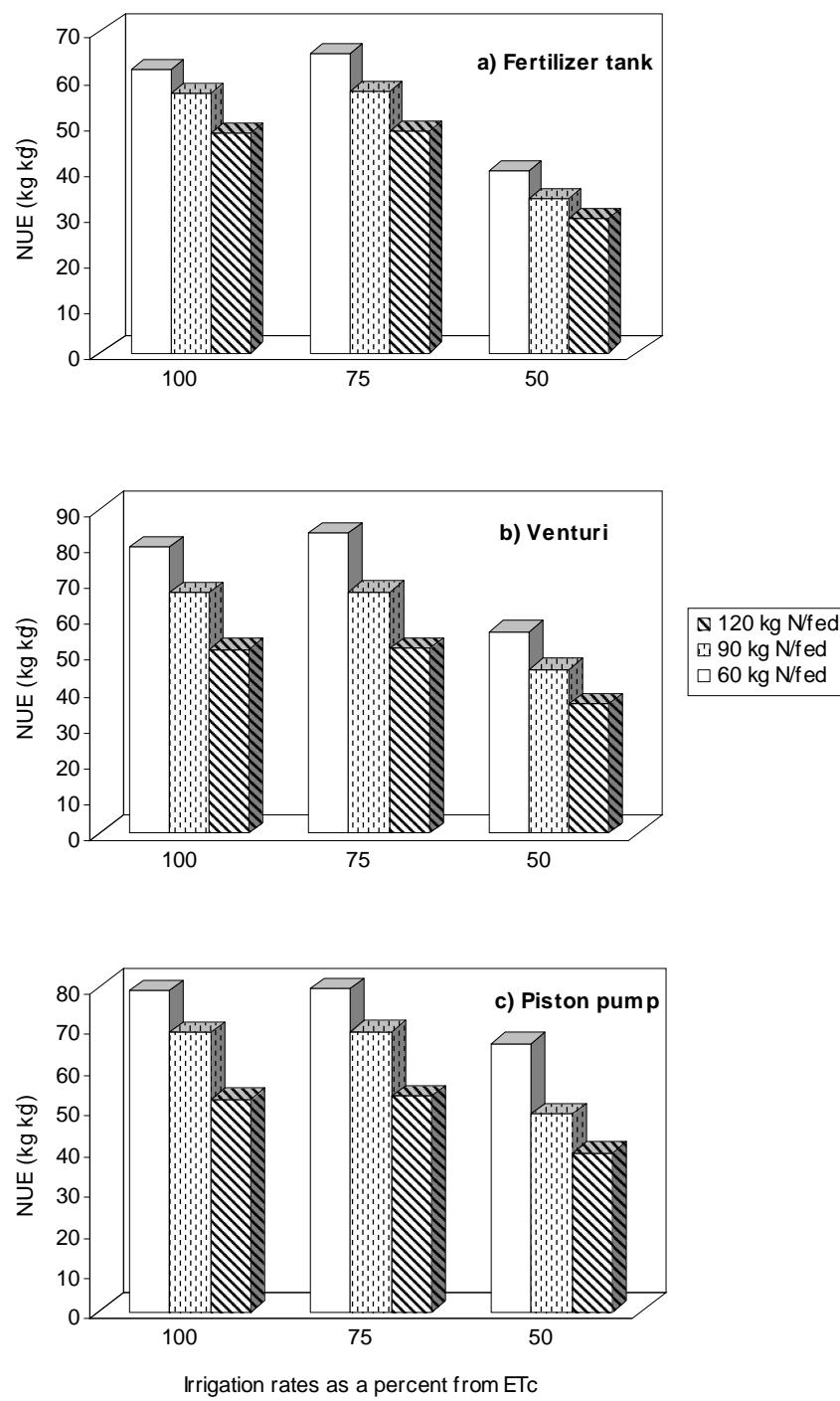
WUE\*: water use efficiency. NUE\*\*: nitrogen use efficiency.



**Figure (1 ):** Effect of irrigation and nitrogen fertilization levels on garlic yield under different injection methods.



**Figure (2): Effect of irrigation and nitrogen fertilization levels on water use efficiency under different injection methods.**

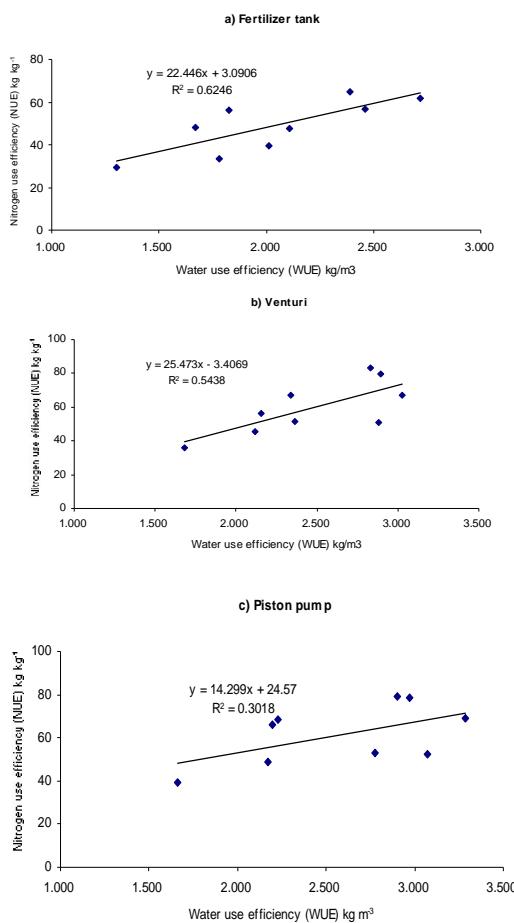


**Figure (3): Effect of irrigation and nitrogen fertilization levels on nitrogen use efficiency under different injection methods.**

$(J_2 \times I_2 \times N_1, J_2 \times I_3 \times N_1, J_3 \times I_2 \times N_1)$  and  $(J_1 \times I_1 \times N_3, J_1 \times I_1 \times N_2, J_2 \times I_1 \times N_3)$ , respectively.

#### 4. The relationship between water and nitrogen use efficiency

Figure (4) describes the relationship between water and nitrogen use efficiency under different injectors types indicating that there was a positive linear relationship between WUE and NUE which could be written as follow equations:  
 $NUE = 22.446 \text{ WUE} + 3.0906, R^2 = 0.6246^{**}$   
 $NUE = 25.473 \text{ WUE} + 3.4069, R^2 = 0.5438^*$  and  
 $NUE = 14.299 \text{ WUE} + 24.57, R^2 = 0.3018$   
for fertilizer tank ( $J_1$ ), venturi ( $J_2$ ), and piston pump ( $J_3$ ), respectively. Data on hand, indicate highly positive significant of correlation coefficient at 1% for  $J_1$ , positive significant at 5% for  $J_2$  and no significant for  $J_3$ . This means that any increase in WUE is followed by significant increase in NUE. These results are agreeable with those obtained by [9].



**Figure (4): The relationship between water and nitrogen use efficiency under different injection methods.**

#### 5. Conclusion

From the above mentioned presentation, it can be concluded that the highest and the lowest garlic yield (6.34; 2.38 ton fed<sup>-1</sup>) was obtained with treatment  $J_3 \times I_2 \times N_3$  and  $J_1 \times I_1 \times N_1$ , respectively. The maximum value of WUE was 3.29 kg garlic m<sup>-3</sup> of irrigation water as recorded with the treatment  $J_3 \times I_1 \times N_3$ , while the minimum value was 1.30 kg garlic m<sup>-3</sup> of irrigation water as recorded with the treatment  $J_1 \times I_3 \times N_1$ . The maximum value of NUE in kg garlic kg<sup>-1</sup> nitrogen was 83.2 and the minimum ones 29.2 in the following interactions between  $J_2 \times I_2 \times N_1$  and  $J_1 \times I_1 \times N_3$ , respectively. A positive linear relationship was found between WUE and NUE.

#### Corresponding Author

Shaaban, S.M  
Water Relations and Field Irrigation Dept., National Research Centre, Dokki, Cairo, Egypt.  
[shaabansm@yahoo.com](mailto:shaabansm@yahoo.com)

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5/5/2010

# Software Cost Estimation through Entity Relationship Model

Arshid Ali <sup>1</sup>, Salman Qadri <sup>2</sup>, Syed Shah Muhammad <sup>2</sup>, Jalil Abbas <sup>3</sup>, Muhammad TariqPervaiz <sup>2</sup>,  
Sarfraz Awan <sup>2</sup>

<sup>1</sup>. Department of Computer Science, GCU Faisalabad, Pakistan

<sup>2</sup>. Department of Computer Science, Virtual University of Pakistan, Lahore, Pakistan

<sup>3</sup>. Department of Computer Science, University of Central Punjab, Lahore, Pakistan

[sayyed\\_qadri@hotmail.com](mailto:sayyed_qadri@hotmail.com)

**Abstract:** Software Cost Estimation is essential for efficient control and management of the whole software development process. Today, Constructive Cost Model (COCOMO 11) is very popular for estimating software cost. In Constructive Cost Model lines of code and function, points are used to calculate the software size. Actually, this work represents the implementation stages but in early stages in software development, it was not easy to estimate software cost. The entity relationship model (ER Model) is very useful in requirement analysis for data concentrated systems. This paper highlights the use of Entity Relationship Model for software cost estimation. Pathway Density is ushered in. By using the Pathway Density and other factors, many regression models are built for estimating the software cost. So in this paper, Entity Relationship Model is based on estimated cost of software. [Journal of American Science. 2010;6(11):47-51]. (ISSN: 1545-1003).

**Keywords:** ER Model, Cost Estimation, Entity

## 1. Introduction

Software cost estimation is critical process for software development. It is important for efficient control and management of the whole software development process. A number of models, such as Farr and Zagorski Model, Woerton Model have been anticipated [1]. Now days Constructive Cost Model (COCOMO11) is popular cost Estimation model. COCOMO 11 is divided into three sub models:

- Applications Composition
- Early design strategy
- post-architecture strategy

They can be blended in various ways to deal with the current and likely future software practices market place [4] it accesses the software effort based on the same model:

$$E = a (EDSI)^b \times EAF$$

Where E is an effort estimate, expressed in person-months. EDSI refers to the number of Estimated Delivered Source Instructions. The parameters  $a$  and  $b$  are determined by the application complexity mode. EAF (Effort Adjustment Factor) is equal to one for the basic sub-model, and equals the product of fifteen cost factors for the intermediate and advanced sub-models [3, 4].

COCOMO II uses Function Points or Lines of Code for estimating the size of a software system. Intuitively, lines of code cannot be obtained or estimated at the early stage of the software development. Function Point appears to be requirements oriented. However, Function Point

counts the number of files updated and reports printed, etc, which are actually the result of design. As a result, it also confronts many problems [2]. This research proposes the use of the popular data model, ER model, for the estimation of software cost. ER model is usually constructed in the requirements analysis stage. The organization of this paper is as follows: section 2 shows the background of ER model and the factors we want to use in our research. Section 3 proposes a new term, Path Complexity and how to count the value of Path Complexity. Section 4 illustrates the new estimating model for software cost that is based on the multiple linear regression technique and gives the conclusion.

## 2. Background

The ER Diagram was brought to limelight by Professor Chen in 1976 [8] and adapted in the Information Engineering approach. The ER Diagram originally used in the database field and now is being used in Object-Oriented Analysis. An ER model is constructed to show the ideal organization of data, independent of the physical organization of the data and where and how data are used. Currently, data-intensive systems constitute a main domain in software. These systems maintain a large amount of structured data in a database built through a database management system (DBMS). Although UML (Unified Modeling Language) has gained its popularity as a standard software modeling methodology, ER model is still used to model the data conceptually in the requirement capturing and

analysis stages. Moreover, most of the design and development activities are based on the ER model. Therefore, ER model seems to have the most readily available information from requirements capturing and analysis stages. After studying the ER Diagram, we find that ER diagram is a non-directional graph[6]. It is easy to sketch an ER Diagram onto a directional graph by considering the entity as the vertex in a graph, and considering the relationship as the edge in a graph. From our observation, software effort is effected by the number of entities, relationships and attributes. In addition, it can be seen that more complex structure of ER Diagram is, mere effort that will be spent on the software system. Thus, we want to find out some relationships with software effort. In this research, we use the following metrics in the estimating model [7, 8]:

**NOE:** the number of entities in an ER Diagram.

**NOR:** the number of relationships in an ER Diagram.

**NOA:** the number of attributes in an ER Diagram.

**NOP:** the number of Path Complexity of an ER Diagram.

### 3. Complexity Metrics:

The ER Diagram shows the whole structure of the Database, where one entity can access the other entities through the relationship and get the related data. More ways one entity can access the other entities, more things we should consider about it. This can reflect the whole system complexity. Thus, we want to quantify the data that can be used in the software system. It is a problem of complexity metrics of the software product. Path Complexity is proposed as a complexity metrics to measure software effort [5]. In order to quantify the whole system data, we should first know through how many paths one entity could influence the other entities, and the length of each path. Because an ER Diagram can be converted into a graph, we can calculate these data by using graph theory path.

#### Definition 1:

Path Complexity of a vertex is:

$$P_i = \frac{1}{n} \sum_{j \neq i} l_{ij}$$

Where

The  $i$ th vertex is not the same to the  $j$ th vertex;

$P_i$  is the Path Complexity of the  $i$ th vertex;

$n$  is the number of the paths through which  $i$ th vertex can access the  $j$ th vertex;

$l$  is the length of each path through which  $i$ th vertex can access the  $j$ th vertex.

#### Definition 2

Path Complexity of an ER Diagram is:

$$P = \max_{v \in G} P_v$$

Where

$P$  is the Path Complexity of the whole ER Diagram;

$P_v$  is the Path Complexity of the  $v$ th vertex in the ER Diagram. Algorithm *Search Path* ( $G, s$ ) given by following is used to count how many paths we can get from a fixed vertex in a connected graph to the other vertices in the same graph, and the length of each. We assume that the input graph  $G = (V, E)$  is a connected graph and it can be represented using adjacency matrix. Each vertex  $u$  in the connected graph has a timestamp  $time[u]$ , it records how many edges it has in one path from  $s$  (the beginning vertex) to  $u$  (the destination vertex). We use  $P[u]$  to record a set of vertices that are ahead of the vertex  $u$  through a path.  $N(G)$  is a vertex set to present the vertices left in a connected graph

*Search Path*

```

1 for each component G
2 while ( ) (G N)
3 do select s N(G) as the Beginning Vertex
4 for each t } { } (s G N
5 set t = EndVertex
6 for each vertex u ) (G V
7 time[u] = 0
8 P[u] NIL
9) (s Search)
(s Search

1 for each vertex u Adj[s]
2 P[u] P[s] + s
3 time[u] time[s] +1
4 if u = t
5 print time[u]
6 else if u P[u]
7 break
8 else) (s Search)

```

#### 3.1 Proposed Model

We proposed a model to estimate software effort (shown in Figure 1). It contains an Adjacency Matrix Generator, A Path Complexity Generator, a Metric Generating Tool and a Statistical Module.

Among the existing techniques used in software estimation, regression-based techniques are the most popular ways of building models. After comparing four techniques (regression, neural networks, Case-Based Reasoning, Rule induction)[3] shows a result that regression and Case-Based Reasoning perform better than the other techniques [4] also compares the methods used in regression, neural networks and genetic programming. We winds up that although neural networks and genetic programming can improve the estimations of regression, the results are

not very impressive. Thus in its multiple linear regression model was adopted. All system data used in this project are actual industry data. Several software development companies in Singapore and Pakistan were considered, and provided twelve software systems' data. These projects cover multiple application domains including freight management, quotation, billing or order processing. We got data of NOE, NOR, NOA and NOP from those software systems. And we use Man-Day to measure software cost. (Shown in Figure1).

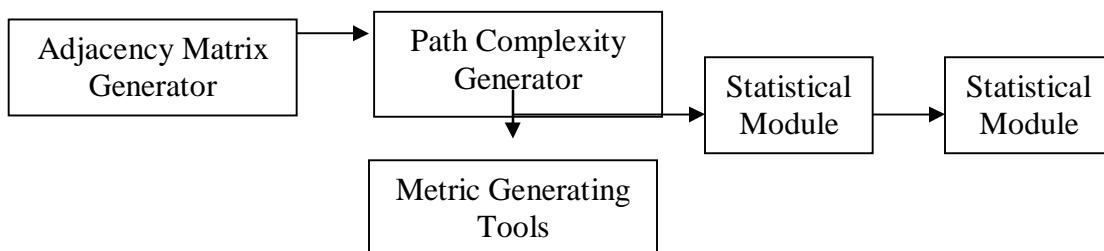


Figure 1: Complexity path definition

Factors and software cost, we use the coefficient of correlation  $r$ . The closer  $r$  is to 1, the stronger the positive linear relationship is. The values of  $r$  to NOE, NOR, NOA and NOP are respectively 0.9395, 0.9729, 0.9474 and 0.8842. According to these values, all these factors have the strong linear relationships with software cost. We got the multiple regression equation are  $Y = 23.01 + 3.80 \text{ NOV} + 1.03\text{NOR} - 0.01 \text{ NOA} - 0.49 \text{ NOP}$  to check the accuracy of this multiple linear regression model, we use the multiple coefficient of determination  $R^2$  and the F-statistic.  $R^2$  can evaluate the strength of the multiple regression relationship. In this project, the value of  $R^2$  is 0.8766. It shows this model has a high regression relationship. In order to use the F-statistic, the hypotheses were set as below, and

alpha =0.05:

$H_0$ : all the regression coefficients are zero

$H_1$ : not all the regression coefficients are zero

After F-statistic, it gives a p-value of 0.0171; this value is much smaller than, so  $H_0$  was rejected. It indicates that it is highly unlikely that all of the regression coefficients are zero. Therefore, we can jump to a conclusion that this multiple regression model is reasonable. The comparison of estimating cost and actual cost is shown in figure 2. Here, the authors are grateful to the support and help of AKEMCO Technology Pvt Ltd, IPACS e-Solutions(S) Pvt Ltd and Singapore Computer Systems Ltd. to provide the system data. However, only twelve projects are not enough, more system data will be collected in the future work.

	NOE	NOR	NOA	ODP	Predictive Cost	Actual Cost
1	6	5	112	9.33	45	48
2	6	5	75	8.33	46	38
3	21	22	512	63.17	90	81
4	3	2	86	2.67	34	29
5	3	2	86	2.67	34	34
6	25	33	656	118.03	88	92

7	4	3	66	5.00	43	33
8	8	7	212	19.00	49	70
9	14	14	126	31.43	74	80
10	16	17	441	33.93	80	85
11	64	69	1524	204.15	324	322
12	38	38	779	84.36	225	235

Figure 2 Predictive Cost and Actual Cost

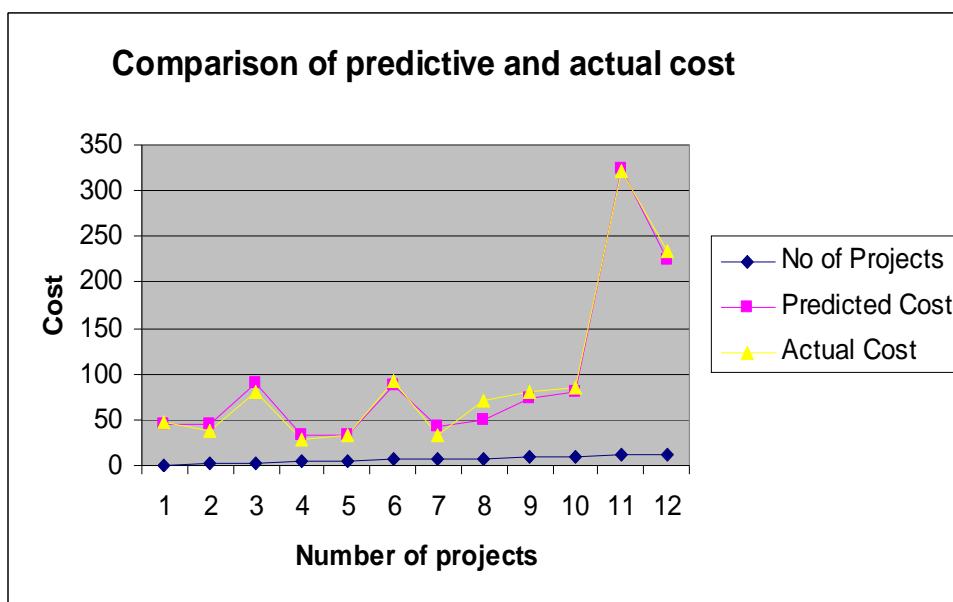


Figure 3. Comparison of Predictive and Actual Software Cost

This work is exploring a software cost estimation model, especially for software development industries [10] and it is best software cost estimation model for large-scale development and in house development. It is very plain model for software costing rather than the difficult techniques like COCOMO MODEL, which is not efficient in the environment of Pakistan. We can estimate a cost of a project from scratch by using this simple model. our aim to provide a model and a user-friendly tool to do those estimations in order to assist managers assessing the worthiness of the investment they are going to undertake. We believe that massively collected software project data present an interesting aspect of cost modeling, providing a unique opportunity to design helpful tools for software managers that wish to benchmark their projects and

are interested in developing knowledge concerning software measurement and estimation [9].

#### 4. Future Work:

This research focuses on the cost estimation technique and finds the best way cost estimation through ER Diagram for large-scale project and small projects. For future Extension, this area requires the improvement by using agile software cost methodology implementation technique to finds out the software cost estimation for better and more efficient way.

#### Acknowledgements:

Authors are grateful to the Department of Computer Science, Virtual University of Pakistan, for support to carry out this work.

**Corresponding Author:**

Syed Shah Muhammad  
Department of Computer Science,  
Virtual University of Pakistan,  
Lahore, Pakistan.  
E-mail: [sayed\\_qadri@hotmail.com](mailto:sayed_qadri@hotmail.com)

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4/4/2010

# Web-Ontology Design Quality Metrics

<sup>1</sup>Amjad Farooq, <sup>2</sup>Syed Ahsan, <sup>2</sup>Abad Shah

<sup>2</sup> Al-khawarizmi Institute of Computer Science

<sup>1</sup>Department of Computer Science and Engineering  
University of Engineering and Technology, Lahore  
[amjafarooq@uet.edu.pk](mailto:amjafarooq@uet.edu.pk)

**Abstract:** Semantic Web is an extension of current web in which the web resources are equipped with formal semantics about their interpretation for the machines. These web resources are integrated in the form of web information systems, and their formal semantics are normally represented in the form of web-ontologies. Using the database terminology, we can say that web-ontology of a semantic web system is schema of that system. Since web-ontology is an integral element of semantic web systems, therefore, design quality of a semantic web system can be measured by measuring the quality of its web-ontology. The key consideration is that after completing design of a web-ontology, it is appropriate time to assess its quality so that in case, the design is of low quality, it can be improved before its instantiation. This can save a considerable amount of cost and effort for developing high quality semantic web systems. Metrics are considered as suitable tools for evaluating quality. In this paper, we propose certain metrics for web-ontology quality evaluation. These metrics may contribute in developing a high quality semantic web system. [Journal of American Science. 2010;6(11):52-58]. (ISSN: 1545-1003).

**Keywords:** Semantic web; Ontology metrics; quality measurement

## 1. Introduction

Web-ontologies are backbone of a new type of the Web, called the semantic Web. These provide declarative knowledge in a machine processable way. Within the web community, a web-ontology is a formal description of descriptive knowledge of a domain, coded in W3C recommended logic-based languages such as Web Ontology Language (OWL) (Peter et al., 2004). Web-ontologies are an integral part of a semantic web system as schema is an integral part of a database system. It is also obvious that performance of information or knowledge retrieval from semantic web systems depends on the design quality of its ontologies. Therefore, it is very much desirable that the design quality of ontologies should be measured as early as possible during the development of semantic web systems but is very difficult task (Parsia et al., 2005). In our opinion, after completing design of a web-ontology, it is appropriate time to assess its quality so that in case the design is of low quality, it can be improved before its instantiation. This can save a considerable amount of cost and effort for a developing semantic web system of good performance. In this paper, we attempt to achieve this objective and we propose design metrics for ontologies of a semantic web system.

The remainder of this paper is organized as follow. Current status of web-ontology metrics and overview of the related work are given in Section 2.

In Section 3, we propose design metrics for a web-ontology, and these metrics are validated in Section 4. Finally, the paper is concluded with recommendations for the future direction in Section 5.

## 2. Related Work

The AI-community has done a lot of work in the area of ontology as reported in literature such as in (Lozano-Tello et al., 2004), but the web-community has just started work on this area few years ago, especially when the idea of Semantic Web (Lee et al., 2001) was envisioned. Some design metrics for web-ontology have been proposed as reported in literature (Yang et al, 2006; Michael et al., 2005; Burton-Jones et al., 2005), but this area is still in its preliminary stage because little work has been done in this area. Coupling metrics have been suggested for design of web-ontology-based systems (Orme et al., 2006). These metrics are number of external classes, reference to external classes, and referenced includes. It has argued that system quality can be improved if the coupling is measured early in web-ontology-based system's development cycle.

Web-ontology cohesion metrics have been proposed to measure the modular relatedness of web-ontologies (Yao et al., 2005). These metrics compute the number of root classes, number of leaf classes and average depth of inheritance tree (or class-hierarchy). These can be helpful in determining web-ontology structure, its cost estimation and maintenance. These metrics are validated

theoretically and also empirically using the validation standards (Briand et al., 1996; Kitchenham et al., 1995).

Semantic metrics, conceptual metrics and web-ontology metrics, for semantic web systems, have been discussed, and compared (Etzkorn, 2006). And it is concluded that more work is needed to validate these metrics in different application areas and their role in the maintenance of those web-ontology-based software systems.

Web-ontology instance metrics have been proposed (Michael et al., 2005). These instance metrics can be used in measuring quantity and importance of data-placement in a web-ontology, and they reflect normalization and efficiency of web-ontology. The instance metrics are further divided into two types which are: knowledgebase metrics, and class metrics. Schema metrics are also proposed for evaluating different characteristics of web-ontology. These schema metrics are relationship-richness, attribute-richness and inheritance-richness metrics. These metrics are used to measure design quality of web-ontology.

As web-ontology is just like knowledge-intensive software therefore all generic metrics for software are also applicable for web-ontology. Some of them are: suitability, accuracy, interoperability, compliance, traceability, understandability, learnability, stability, customizability, user-friendliness, reusability, analyzability, changeability, testability and manageability (Hakkarainen et al., 2005; Korotkiy, 2005; Norman et al., 2003; Paslaru et al., 2005).

In (Baumeister and Seipel, 2005), authors have proposed metrics to measure quality of taxonomy and design of web-ontology. They have focused on inconsistency, incompleteness and redundancy attributes of taxonomy metric and lazy concepts, chains of inheritance, property clumps and lonely disjoints attributes of design metric. While aligning ontologies, the level of similarity among two entities can be measured by metric proposed (Stoilos et al., 2005), in this metric the similarity between two ontologies has been computed, based on their commonalities as well as to their differences.

### 3. Proposed Design Metrics

In this section we propose design metrics for web-ontology by keeping certain recommended guidelines like a metric may reach its highest value for perfect quality for excellent case and vice versa that is it may reach its lowest level when for worst case. It should be monotonic, clear, and intuitive. It must correlate well with human judgments and it should be automated if possible (King, 2003). The proposed metrics may give information about how

much knowledge can be derived from a given web-ontology; how much it is relevant to a user's specific requirements and how much it is easy to reuse, manage, trace and adapt. These metrics are named as *Knowledge Enriched (KnE)*, *Characteristics Relevancy (ChR)* and *Domains modularity (DoM)*.

#### 3.1 Knowledge Enriched metric

Knowledge Enriched (KnE) metric quantifies the reasoning capability of a web-ontology, and it based on two sub-metrics so-called *Isolated Axiom Enriched (IAE)* metric and *Overlapped Axiom Enriched (OAE)* metric. The axiom mostly consists of three parts: predicate, resource and object. If none of these is common with any other axiom of same domain then that axiom is termed as isolated axiom. Similarly two axioms are said overlapped if those have some common parts. There may be several transitively overlapped axioms in any domain. This metric determines the percentage of IAE and OAE, and if the former is more than the later one, then the web-ontology can be considered less knowledge enriched. IAE is formally defined as the ratio of total number of isolated axioms (tIAs) to the total number of domain axioms (tDAs).

$$IAE = \frac{\sum_{i=1}^n tIAs}{tDAs} \quad \text{for all } 1 \leq i \leq n \quad (1)$$

In Equation (1),  $n$  is total number of sub-domains of web-ontology. Similarly, the OAE metric is formally defined as ratio of total number of overlapped axioms (tOAs) to the total number of domain axioms. Mathematically it can be written as follows:

$$OAE = \frac{\sum_{i=1}^n tOAs}{tDAs} \quad \text{for all } 1 \leq i \leq n \quad (2)$$

In Equation (2),  $n$  is total number of sub-domains of web-ontology. Finally, the KnE metric is the difference of total number of overlapped axioms and the total number of isolated axioms. Mathematically it may be written as follows:

$$KnE = OAE - IAE \quad (3)$$

From equation (1) and equation (2)

In Equation (3) if the resultant value is positive, then the web-ontology is more knowledge enriched, if it is zero, then the web-ontology is average knowledge enriched, and if it is negative, then the web-ontology is less knowledge enriched.

#### 3.2 Characteristics Relevancy metric

Characteristics Relevancy (ChR) metric gives us an idea about how much a given web-ontology is close to a user's specific requirements and the degree of reusability of the web-ontology. Formally, it is defined as the ratio of the number of relevant attributes (nRAs) in a class to the total number of attributes (TnAs) of that class. Mathematically, it is written in Equation (4) as follows:

$$\text{ChR} = \frac{\sum_{i=1}^n \text{nRAs}_i}{\text{TnAs}}$$

for all  $1 \leq i \leq n$  ..... (4)

Where  $n$  in above equation is the total number of classes in the given web-ontology. ChR metric reveals the percentage of relevant attributes in the web-ontology, and this number gives insights how much a web-ontology is relevant.

### 3.3 Domain Modularity metric

Domain modularity (DoM) metric measures the component-orientation feature of a web-ontology. This metric indicates the grouping of knowledge in different components of web-ontology. The web-ontology is better manageable, traceable, reusable

and adaptable, if it is designed in components (sub-domains). Formally, the DoM metric is defined as the number of sub-domains (NSD) contained in a web-ontology. This metric also depends on the coupling and cohesion levels of sub-domains, and it is directly proportional to its cohesion level and inversely proportional to its coupling level.

$$\text{DoM} = \text{NSD} + \sum_{i=1}^n \text{DCoh}_i + 1 / \sum_{i=1}^n \text{DCoup}_i$$

for all  $1 \leq i \leq n$  ..... (5)

In Equation (5), DCoh represents level of domain cohesion and DCoup represents the level of coupling among sub-domains of web-ontology domain. DoM metric is a real number representing degree of partial reusability of a given web-ontology.

### 4. Case Study

We have taken a web-ontology of university domain for validating our proposed metrics KnE, ChR and DoM as described in previous sections.

<pre> &lt;rdf:RDF xmlns="http://www.owl-ontologies.com/uet-1.owl#"   xmlns:base="http://www.owl-ontologies.com/uet-1.owl"   xmlns:xsd="http://www.w3.org/2001/XMLSchema#"   xmlns:rdfs="http://www.w3.org/2000/01/rdf-schema#"   xmlns:rdf="http://www.w3.org/1999/02/22-rdf-syntax-ns#"   xmlns:owl="http://www.w3.org/2002/07/owl#"&gt;   &lt;owl:Ontology rdf:about=""&gt;     &lt;owl:versionInfo rdf:datatype="&amp;xsd:string"&gt;       &gt;Web-ontology for Research Activity     Management Domain&lt;/owl:versionInfo&gt;     &lt;rdfs:comment       rdf:datatype="&amp;xsd:string"&gt;&lt;/rdfs:comment&gt;     &lt;owl:Ontology&gt;     &lt;owl:Class rdf:ID="Author"/&gt;     &lt;owl:Class rdf:ID="Director"/&gt;     &lt;owl:Class rdf:ID="DuptyDirector"/&gt;     &lt;owl:Class rdf:ID="Faculty"/&gt;     &lt;rdfs:subClassOf rdf:resource="#Person"/&gt;     &lt;owl:Class&gt;     &lt;owl:DatatypeProperty rdf:ID="hasAffiliation"&gt;       &lt;rdfs:domain rdf:resource="#Researcher"/&gt;       &lt;rdfs:range rdf:resource="&amp;xsd:string"/&gt;     &lt;owl:DatatypeProperty&gt;     &lt;owl:ObjectProperty rdf:ID="hasArea"&gt;       &lt;rdfs:domain rdf:resource="#ResearchGroup"/&gt;       &lt;rdfs:range rdf:resource="#ResearchArea"/&gt;     &lt;owl:ObjectProperty&gt;     &lt;owl:ObjectProperty rdf:ID="hasAuthor"&gt;   </pre>	<pre>     &lt;/owl:unionOf&gt;     &lt;/owl:Class&gt;   &lt;/rdfs:domain&gt;   &lt;rdfs:range rdf:resource="&amp;xsd:string"/&gt;   &lt;owl:DatatypeProperty&gt;   &lt;owl:DatatypeProperty rdf:ID="hasId"&gt;     &lt;rdfs:domain&gt;     &lt;owl:Class&gt;     &lt;owl:unionOf       rdf:parseType="Collection"&gt;         &lt;owl:Class rdf:about="#Author"/&gt;         &lt;owl:Class rdf:about="#Director"/&gt;         &lt;owl:Class           rdf:about="#DuptyDirector"/&gt;         &lt;owl:Class           rdf:about="#PaperCategory"/&gt;         &lt;owl:Class rdf:about="#Person"/&gt;         &lt;owl:Class           rdf:about="#ResearchArea"/&gt;         &lt;owl:Class rdf:about="#Researcher"/&gt;         &lt;owl:Class           rdf:about="#ResearchPaper"/&gt;         &lt;/owl:unionOf&gt;         &lt;owl:Class&gt;       &lt;/rdfs:domain&gt;       &lt;rdfs:range rdf:resource="&amp;xsd:int"/&gt;     &lt;owl:DatatypeProperty&gt;     &lt;owl:DatatypeProperty rdf:ID="hasName"&gt;       &lt;rdfs:domain&gt;       &lt;owl:Class&gt;       &lt;owl:unionOf         rdf:parseType="Collection"&gt;   </pre>
--	---

Figure 1: A Code Slice Of Sample Web-Ontology

First of all we verified its syntactical correctness using w3c validation service. Its RDF graph has show in figure 2. It was found syntactically correct.

### KnE - Knowledge Enriched metric

We are working to automate KnE metric, but now it is performed manually. With the help of w3c validation service, we transform web-ontology into list of triples in order to count total axioms, isolated axioms and

overlapped axioms present in web-ontology. Its sample is shown in figure 3. There were found 149 total axioms, 96 overlapped axioms and 53 isolated axioms.

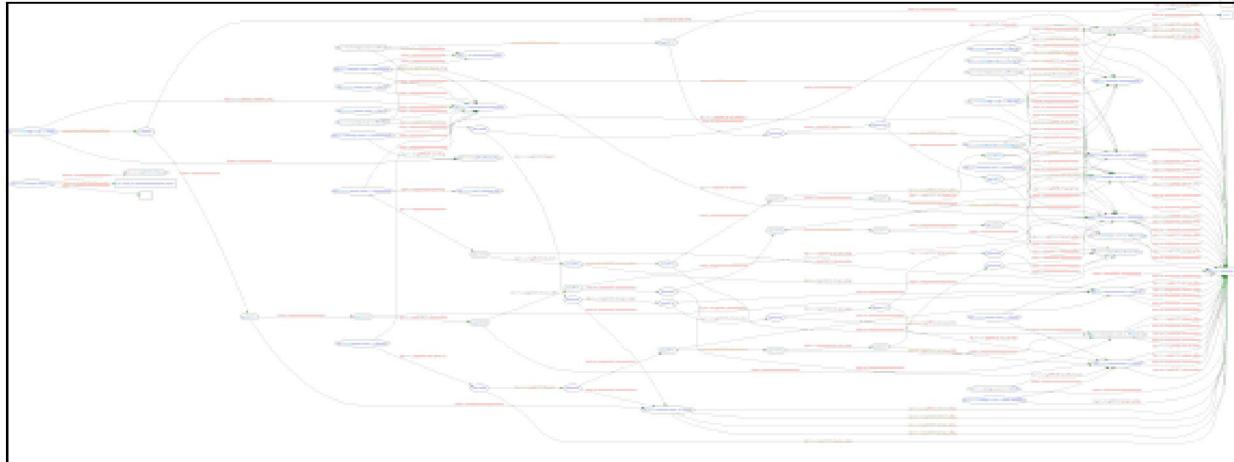


Figure 2: RDF –Graph of Sample Web-Ontology.

N#	Subject	Predicate	Object
1	<a href="http://www.owl-ontologies.com/uet-1.owl">http://www.owl-ontologies.com/uet-1.owl</a>	<a href="http://www.w3.org/1999/02/22-rdf-syntax-ns#type">http://www.w3.org/1999/02/22-rdf-syntax-ns#type</a>	<a href="http://www.w3.org/2002/07/owl#Ontology">http://www.w3.org/2002/07/owl#Ontology</a>
2	<a href="http://www.owl-ontologies.com/uet-1.owl">http://www.owl-ontologies.com/uet-1.owl</a>	<a href="http://www.w3.org/2002/07/owl#versionInfo">http://www.w3.org/2002/07/owl#versionInfo</a>	"Web-ontology for Research Activity Management Domain"^^ <a href="http://www.w3.org/2001/XMLSchema#string">http://www.w3.org/2001/XMLSchema#string</a>
3	<a href="http://www.owl-ontologies.com/uet-1.owl">http://www.owl-ontologies.com/uet-1.owl</a>	<a href="http://www.w3.org/2000/01/rdf-schema#comment">http://www.w3.org/2000/01/rdf-schema#comment</a>	""^^ <a href="http://www.w3.org/2001/XMLSchema#string">http://www.w3.org/2001/XMLSchema#string</a>
4	<a href="http://www.owl-ontologies.com/uet-1.owl#Author">http://www.owl-ontologies.com/uet-1.owl#Author</a>	<a href="http://www.w3.org/1999/02/22-rdf-syntax-ns#type">http://www.w3.org/1999/02/22-rdf-syntax-ns#type</a>	<a href="http://www.w3.org/2002/07/owl#Class">http://www.w3.org/2002/07/owl#Class</a>
5	<a href="http://www.owl-ontologies.com/uet-1.owl#Director">http://www.owl-ontologies.com/uet-1.owl#Director</a>	<a href="http://www.w3.org/1999/02/22-rdf-syntax-ns#type">http://www.w3.org/1999/02/22-rdf-syntax-ns#type</a>	<a href="http://www.w3.org/2002/07/owl#Class">http://www.w3.org/2002/07/owl#Class</a>
6	<a href="http://www.owl-ontologies.com/uet-1.owl#DputyDirector">http://www.owl-ontologies.com/uet-1.owl#DputyDirector</a>	<a href="http://www.w3.org/1999/02/22-rdf-syntax-ns#type">http://www.w3.org/1999/02/22-rdf-syntax-ns#type</a>	<a href="http://www.w3.org/2002/07/owl#Class">http://www.w3.org/2002/07/owl#Class</a>
7	<a href="http://www.owl-ontologies.com/uet-1.owl#Faculty">http://www.owl-ontologies.com/uet-1.owl#Faculty</a>	<a href="http://www.w3.org/1999/02/22-rdf-syntax-ns#type">http://www.w3.org/1999/02/22-rdf-syntax-ns#type</a>	<a href="http://www.w3.org/2002/07/owl#Class">http://www.w3.org/2002/07/owl#Class</a>
8	<a href="http://www.owl-ontologies.com/uet-1.owl#Person">http://www.owl-ontologies.com/uet-1.owl#Person</a>	<a href="http://www.w3.org/2000/01/rdf-schema#subClassOf">http://www.w3.org/2000/01/rdf-schema#subClassOf</a>	<a href="http://www.owl-ontologies.com/uet-1.owl#Person">http://www.owl-ontologies.com/uet-1.owl#Person</a>

Figure 3: Partial List of Triples

Although a university ontology is a multi-domain (i.e. there are different sub-domains in university domain) ontology, but this web-ontology file has shown that there is one domain (all sub-domains were merged), this means that n=1 in equation 1.

$$\text{IAE} = 50 / 200 = 0.25, \text{ from equation 1.}$$

$$\text{OAE} = 150 / 200 = 0.75$$

$$\begin{aligned} \text{KnE} &= \text{OAE} - \text{IAE} \\ &= 0.75 - 0.25 \\ &= 0.50 \end{aligned}$$

This indicates that web-ontology may be considered a knowledge-enriched ontology, with respect to criteria given in section 3.1.

#### ChR - Characteristics Relevancy metric

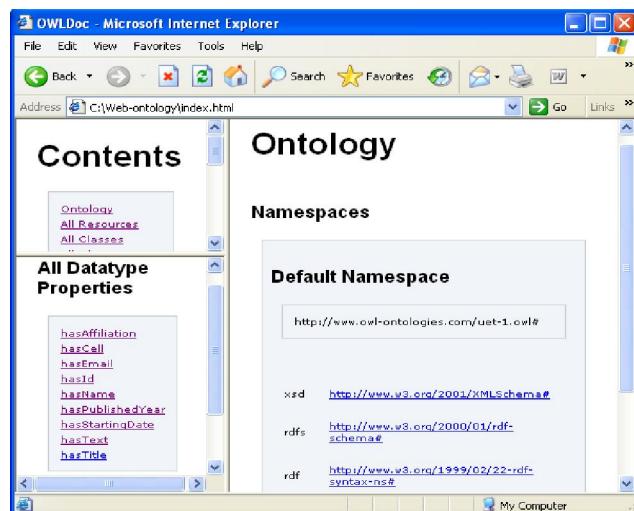


Figure 4: Web-Ontology Document Used For Counting Characteristics

We took a list of requirements from a concerned person from different universities, and then checked web-ontology for how much it was relevant to that user's specific requirements. There were 57 characteristics in the requirement-list, then determined, their presence in the web-ontology using web-ontology documentation, partially shown in figure 3. It was found that there were total 85 characteristics available in web-ontology, and only 22 were found relevant so by formula:  $ChR = 22 / 85 = 0.26$

This means that although available characteristics are more than the required list, but only 26 percents are relevant. It was concluded, that web-ontology is not appropriate for that user's requirements.

#### DoM - Domain Modularity metric

As we know that a university domain consists of multiple sun-domains. A separate web-ontology should be developed for each sub-domain then integrates all of them to develop a multi-domain ontology. But the sample web-ontology has been developed by merging all sub-domains as we have examined that no web-ontology has imported in sample web-ontology.

This means that whenever we need a web-ontology of any sun-domain of university domain, we have to use complete web-ontology. We have concluded that partial re-usability of the sample web-ontology is very poor. The coupling and cohesion level can be determined by using existing relevant metrics.

```
<?xml version="1.0"?>
<!DOCTYPE rdf:RDF [
  <!ENTITY owl "http://www.w3.org/2002/07/owl#" >
  <!ENTITY xsd "http://www.w3.org/2001/XMLSchema#" >
  <!ENTITY rdfs "http://www.w3.org/2000/01/rdf-schema#" >
  <!ENTITY rdf "http://www.w3.org/1999/02/22-rdf-syntax-ns#" >
]>
<rdf:RDF xmlns="http://www.owl-ontologies.com/uet-1.owl#"
  xmlns:base="http://www.owl-ontologies.com/uet-1.owl"
  xmlns:xsd="http://www.w3.org/2001/XMLSchema#"
  xmlns:rdfs="http://www.w3.org/2000/01/rdf-schema#"
  xmlns:rdf="http://www.w3.org/1999/02/22-rdf-syntax-ns#"
  xmlns:owl="http://www.w3.org/2002/07/owl#">
  <owl:Ontology rdf:about="">
    <owl:versionInfo rdf:datatype="&xsd:string">
      <a web-ontology for university domain</owl:versionInfo>
    <rdfs:comment rdf:datatype="&xsd:string"></rdfs:comment>
  </owl:Ontology>
  <owl:Class rdf:ID="Author"/>
```

Figure 5: Code Slice Indicating Zero Imported Web-Ontology

#### 5. Theoretical Analysis of Proposed Metrics

In the previous section, we have proposed design metrics for web-ontologies. In this section, we take web-ontology design schema of a university coded in OWL, and evaluate proposed metrics of the web-ontology using the same theoretical standards given in (Kitchenham et al., 1995).

According to (Baumeister and Seipel, 2003), an entity is the item being observed and an attribute is a property of that entity. To measure an attribute-value, its measuring unit should be specified. Measurement scales are nominal, ordinal, interval, or ratio. It has claimed that a valid metric must have validity of attribute, unit, instrument and protocol. Attribute validity states that the entity being evaluated has attributes. Unit validity states that the unit used should be appropriate for the attribute. Instrumental validity states that the underlying model should be valid and the measurement instrument should be regulated. And the protocol validity states that the protocol used for the measurement is consistent, unambiguous and prevents problems. In Short, the concepts necessary to be there in a valid metric are entity, attribute, measuring unit and scale type. A metric satisfying the validity constraints such as attribute validity, unit validity, instrumental validity and protocol validity, is called a valid metric. We have analyzed our metrics against all these constraints.

#### Analysis of Knowledge Enriched metric

*Web-ontology* is an entity for proposed metric; the attribute of this metric is the *axiom* to be counted; Unit is *number of attributes and Data Scale*

is absolute value. *Attribute validity*: The entity (the web-ontology being analyzed) has number of attributes (isolated and linked axioms). *Unit Validity*: The attribute is measured by counting the number of isolated and linked axioms respectively. *Instrumental Validity*: The instrument is valid as long as the axioms computing tool parse and count the number of isolated and linked axioms respectively. *Protocol Validity*: The computation performed according to equations given in this paper is consistent, unambiguous and error free.

#### **Analysis of Characteristics Relevancy metric**

For this metric the *attribute* to be counted, is treated as a concept or entity; *Relevancy* to the user specific needs is an attribute for this metric; unit is *percentage* of correct matches and data scale is *ratio*. *Attribute validity*: The entity (the attribute being analyzed) has attribute (i.e. relevancy). *Unit Validity*: The relevancy is measured by computing percentage of relevant attribute to the user needs. *Instrumental Validity*: We have implemented our model in java modules and it is working correctly, we have also verified it manually. *Protocol Validity*: The formal description of this metric given in this paper is consistent, unambiguous and error free.

#### **Analysis of Domain Modularity metric**

*Web-ontology* is an entity of this metric; attribute of this metric, is *sub-domain* to be counted; Unit is *number* of sub-domains; and Data scale is *absolute value*. *Attribute validity*: The entity (the web-ontology being analyzed) is number of sub-domains used for making the main web-ontology. *Unit Validity*: The sub-domains are counted in numbers. *Instrumental Validity*: We have implemented our algorithm for counting sub-domains, in java modules and it is working correctly, we have also verified it manually. *Protocol Validity*: The equation given in the proposed metric is performing the right calculation.

#### **6. Conclusion and Future Directions**

In this paper, we have proposed a set of quality metrics to evaluate design of ontologies. These proposed metrics may be helpful in evaluating design quality of ontologies and improving their design. In this way we may save a considerable amount of development time and cost of good quality semantic web applications. We feel that serious efforts and attentions are needed towards web-ontology design metrics to improve the quality of semantic web applications. It is expected that in the future most software development work will be the development of semantic web applications. By understanding the urgency and importance of this work, we are actively working in this direction.

#### **Acknowledgement**

This research work has been supported by the "Higher Education Commission of Pakistan", and the University of Engineering and Technology, Lahore.

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5/5/2010

# Determining Regression Models of Almond and its Kernel Mass Based on Geometric Properties (Shahrud 12 and Mama'e Varieties)

A.Mohamadi<sup>1</sup>, M.A.Ghazavi<sup>2</sup>, B.Hosseinzadeh<sup>2</sup>

<sup>1</sup>Payame-noor university, Farsan, Iran

<sup>2</sup> Department of mechanical farm machinery, University of Shahrekord, Shahrekord 115 Iran

[bahram\\_hs@yahoo.com](mailto:bahram_hs@yahoo.com)

**Abstract:** Almond (*Prunus amygdalus*) belongs to Rosaceae family and sub-family of Pomoideae. Physical traits of agricultural products are main parameters in designing of grading, conveying, processing, and packing systems. In this study the physical traits such as dimensions, mass, volume, sphericity, geometric average of Mama'e and Shahrud 12 almonds and their kernels were measured and calculated. The average amounts of length, width, and thickness for both almond varieties were 37.41, 23.21, and 16.63 mm, respectively, and for almonds' kernel were 28.05, 13.4, and 7.82 mm, respectively. Results from modeling of almond and its kernel masses based on dimensions and volume showed that there exists a great correlation coefficient between the samples actual volumes and masses, but since determining actual volume of almond and its kernel is a time-taking task, it was suggested to use calculated volume and presuming that the cross-sectional area of the almond is oval. Also the mass model based on the thickness had the highest determination coefficient and lowest regression error which was the best option for industrial and economical applications. [Journal of American Science. 2010;6(11):59-64]. (ISSN: 1545-1003).

**Keywords:** Almonds, Physical Properties, Mass Modeling, Dimensional Models, Volumetric Models

## 1. Introduction

According to the statistics released by FAO (2004), Iran with 80000 tonnes of almonds is fourth throughout the world which implies that with proper climatic conditions and development of various main and wild types of almond, Iran is a favorite place to grow almond and by paying special attention to this product, quiet a considerable wealth would be earned [FAO,2007].

Recognizing the physical and mechanical properties of agricultural produce has always been on the center of attention and interest of agriculture researchers. This especially in relation with designing of machineries and equipments which are used during harvest, transport, storing, and process of agricultural products is of utmost importance. Among physical properties of agricultural products, dimensions, mass, volume, projected area, and surface area have the most importance in grading systems. Therefore, dimensional grading of products decreases the packaging and transportation costs and allows using of proper packaging models [Peleg,1958].

Post harvest operations for almond generally consist of 3 phases of breaking the almond, slicing the kernel, and packaging. In Iran these operations often is done in small workshops and manually. Lack of standard principles for packaging and not considering consumers tastes have led to problems in importing section. Therefore, in order to design machinery to process almond, determining some of its physical and mechanical properties is essential. Otherwise, incoherence between machinery and

product causes losses of kernel and a decrease in final product quality [Khaza'e,2003].

Due to dissimilarity in almond dimensions, the almond breaking device should favor an adjusting ability for almonds with different dimensions. Therefore, in order to determine the working range of the device, knowledge about the averages of all the three dimensions of almond is necessary. Also for designing slicing devices and almond's kernel grading machineries, determining of all the three dimensions of almonds kernel and other center-inclination parameters are necessary.

Based on this, several studies on determining the physical and mechanical properties of different products have been carried out, which some of them are pointed out in the following.

Aydin studied the mechanical and physical properties of a kind of almond which grows in Turkey. Average amounts for length, width, and thickness for almond were 25.49, 12.03, and 12.17 mm, respectively, and for the kernel were 21/19, 11.34, and 6.38, respectively. Also averages for mass, volume, geometric average, and sphericity were 2.64 g, 2.61 cm<sup>3</sup>, 18.13 mm, and 69.59% for almond, respectively, and for the kernel were 0.73 g, 0.82 cm<sup>3</sup>, 11.42 mm, and 55.17%, respectively [Aydin,2003].

Turkan et al measured the average length, width, and thickness of a Golkan 23-101 almond cultivar to be 36.60, 19.24, and 11.47 mm, respectively, and 31.10, 18.31, and 11.04 mm for Nanparil cultivar, respectively. Also averages for mass, volume, geometric average, sphericity, and

surface area for Golkan 23-101 cultivar were 3.02 g, 4.24 cm<sup>3</sup>, 20.03 mm, 54%, and 12.61 cm<sup>2</sup>, respectively, and for Nanparil cultivar were 1.72 g, 3.33 cm<sup>3</sup>, 18.50 mm, 59%, and 12.80 cm<sup>2</sup>, respectively [Turkan et al,2007].

Moradi studied some of qualitative and quantitative properties of both Shahrud 12 and Mama'e cultivars of almond. He measured length, width, and thickness averages of Mama'e cultivar 22, 35, and 14.7 for almond, respectively, and for its kernel 26.7, 13, and 7.25 mm, respectively. Also length, width, and thickness averages for Sharud 12 almond were 37.6, 21.9, and 16.2 mm, respectively, and for its kernel were 25.9, 12, and 7.14 mm [Moradi,2002].

Aydin evaluated physical properties of hazelnut as a function of its moisture content. The objective of the study was to determine and evaluate dimensions, weight, unit volume, sphericity, density, porosity, projected area, limit velocity, breakage resistance, and static and dynamic friction coefficients of full hazelnut and its kernel. Moisture content of samples ranged between 2.87% to 19.98% (based on dry weight) [Aydin,2002].

Balasubramanian (2001) studied the physical properties of raw cashew nut due to lack of information on this field and the possibility of applying the results for designing process machineries. In this study average amounts of main dimensions (length, width, and thickness), weight ratio, equal diameter, and sphericity at 8.46% of moisture content and weight of a thousand seeds, porosity, bulk density, actual density, and friction coefficient at moisture range of 3.15 to 20.05% (5 levels of moisture content) were determined [Balasubramanian,2001].

Craig and Debra in addition to study physical properties of three almond kernels namely Nanparil, 23.5-16, and 23-122, also obtained regression models for kernel mass of three almond cultivars based on length, width, and thickness. According to their report it was determined that kernel mass favors the most correlation with length and lowest correlation with thickness [Craig,2006].

In this study some of the physical properties of two almond cultivars (Mama'e and Sharud 12) such as dimensions, mass, volume, diameter, geometric average, and sphericity degree were studied. Also regression models for almond and its mass based on geometric properties were determined.

## 2. Material and Methods

In this study tests were carried out on two almond cultivars of Saman region, Shahrud 12 and Mama'e. After cleaning and separating samples from their husks, the samples were packed. The package containing samples were held inside a refrigerator at

5°C in order to keep the moisture conditions and maintaining them for tests.

In order to determine almond dimensions, 3 perpendicular axes were defined. The longest dimension was considered as length (L). The longest dimension perpendicular to the length axis was considered as sample width (W) and the dimension perpendicular to length and width axes, was defined as thickness (T).[Eshaghbeigi et al,2008] Therefore, 120 almonds were selected and by a digital caliper with 0.01 accuracy length, width, and thickness of each almond were measured and then by breaking it and taking out its kernel, length, width, and thickness of the kernels were also measured, and in order to measure almond and kernel masses a digital weight with 0.1 g accuracy was used.

For determining the volume of almond and its kernel (V), platform weight method was used. In this method a bulb containing some water was placed on a weight with platform (0.1 g accuracy) and its mass was measured ( $M_{bw}$ ). Then the sample was floated in the water so that it would have no contact with bottom and edges of the bulb. This can be done by means of a nylon string (in case the sample is heavier than water) or a thin metal string (in case the sample is lighter than water).

In this case, the weight of bulb with water and the floated sample were determined ( $M_{bws}$ ). The difference in weight is caused by Archimedes force and the volume can be calculated by dividing the Archimedes force to water density.

$$V = (M_{bws} - M_{bw}) / \rho_w \quad (1)$$

Since the seed shape and other granule agricultural crops are usually irregular, seeds size is determined as geometric diameter. Geometric diameter can be calculated from equation 2.

$$D_g = (L * W * T)^{1/3} \quad (2)$$

If almond volume is presumed to be equal to an oval with three L, W, and T axes so that the peripheral sphere will have the longest axis of ellipsoid (L), then the sphericity coefficient can be calculated as follow.

$$Sp = \frac{D_g}{L} \quad (3)$$

In this study to estimate almond and its kernel masses two kind of models were used:

a) A model that predicts the mass with one or a combination of two or three dimension parameters (length, width, and thickness) as equation (4).

$$M = F(L, W, T) \quad (4)$$

b) A model that predicts mass based on real volume or calculated mass based on the extended sphere and ellipsoid as equation (5).

$$M = F(V_x) \quad (5)$$

In order to estimate crops volume through similarity of geometric shapes, equations (6) and (7) are respectively used for bodies like extended sphere and ellipsoid.

$$V = \frac{4\pi}{3} ab^2 \quad (6)$$

$$V = \frac{4\pi}{3} abc \quad (7)$$

In above equations a, b and c are half of diameters at direction of three main axes (X, Y, Z), respectively.

The measured data were transferred to Excel software and were categorized and saved in separate files. Average amounts and conditioning operations were done through Excel and regression equations on data were performed by MiniTab(12 version) software.

### 3. Results And Discussion

#### 3-1. Physical Properties of Almond its Kernel

Average amounts for dimensions, mass, volume, geometric diameter, and sphericity for both almond cultivars and kernels at moisture rage of 9-10% (on dry basis) are shown in tables 1 and 2.

Ranges of changes for dimensions i.e. length, width, and thickness for Mama'e almond were 34.65-45.35, 18.51-29.26, and 14.08-24.56 mm, respectively, and for Shahrud 12 were 28.93-40.11, 20.47-27.55, and 14.57-18.23 mm, respectively. Also ranges of changes of length, width, and thickness for Mama'e almond kernel were 23.67-34.5, 9.46-15.91, and 5.44-16.95 mm, respectively, and for Shahrud 12 almond kernel were 28.93-40.11, 20.47-27.55, and 14.57-18.23 mm, respectively.

#### 2-3 Determining Regression Models of Almond Mass Based on Geometric Properties

##### a) Dimensional Models

In table (3) seven models to show mass based on almond dimensions are shown. As it appears for both cultivars, model number 7, which models mass based on three perpendicular dimensions, has the highest determination coefficient ( $R^2$ ) and lowest regression standard error (RSE) among all the other models. Since in this model all the three dimensions should be measured is used for grading and sorting machineries which require high accuracy and the high costs have economic justifications. Among models 1, 2, and 3 which are single-variable models, model number 3 which predicts mass based on thickness is the best model. Due to above mention points equation (8) is suggested for predicting almond mass based on

$$\text{thickness for both cultivars.} \quad M = 4.27 + 0.525T \quad R^2 = 0.76 \quad (8)$$

##### b) Volumetric Models

Models that predict almond mass based on volume are indicated in table (4). Model 1 has the highest determination coefficient ( $R^2$ ) and lowest RSE rather than all the other models. Since determination of actual volume of almond is performed by expensive and complex machineries, therefore, it has fewer usages in practical applications; instead, one can use volumes calculated from dimensions, ( $V_{psp}$ ) and ( $V_{ellip}$ ). Results from table (4) show that mass predicting model based on presumed volume as an ellipsoid volume ( $V_{ellip}$ ) for all the observations has the highest  $R^2$  and lowest RSE. Therefore, this volume is suggested for determining almond mass in machineries, also mass model based on ellipsoid volume for all observations is determined from the following equation:

$$M = 0.563 + 0.000518V_{ellip} \quad R^2 = 0.75 \quad (9)$$

#### Determining Regression Models of Almond Kernel Mass Based on Geometric Properties

##### a) Dimensional Models

In table (5) seven models that estimate kernel mass based on geometric dimensions are shown. As it seems, model number 7 which predicts mass based on three perpendicular dimensions, has the highest  $R^2$  and lowest RSE rather than the other models.

Almond kernel mass model based on model number 7 (for total observations) is shown by equation (10).

Indeed, since this model increase the costs and complexity of machineries is cost worthy.

$$M = -2.2 + 0.114 T + 0.121 W + 0.04 L \quad R^2 = 0.91 \quad (10)$$

Regarding the results obtained from table (5), among single-variable models, model 3 that predicts mass based on thickness (equation 11) for Mama'e kernel, model 1 that predicts mass based on length (equation 12) for Shahrud 12, and model 3 that predicts mass based on thickness (equation 13) for total observations, have the highest  $R^2$  and lowest RSE. Also among bivariate models for both observed cultivars, model 6 that predicts mass based on width and thickness has the highest  $R^2$ .

$$M = 0.36 + 0.128 T \quad R^2 = 0.53 \quad (11)$$

$$M = -1.67 + 0.114 L \quad R^2 = 0.86 \quad (12)$$

$$M = 0.252 + 0.151 T \quad R^2 = 0.60 \quad (13)$$

##### b) Kernel Mass Model Based on Volume

Models that estimate kernel mass based on volume are shown. Model 1 has the highest  $R^2$  and

lowest RSE rather than the other models. Results indicate that calculated volume based on ellipsoid ( $V_{\text{ellip}}$ ) has a higher determination coefficient with mass; therefore, it is suggested to use ellipsoid

volume to predict kernel mass in machineries. Mass model based on ellipsoid volume for total observations is determined by equation (14).  
 $M=0.365+.000691V_{\text{ellip}} R^2=0.82$  (14)

**Table 1 – Average amounts for some of physical properties of Shahrud 12 and Mama'e cultivars**

Volume (mm <sup>3</sup> )	Mass g	Sphericity %	Geometric Average mm	thickness mm	width mm	length Mm	Cultivar
4167/5	4/21	68	23/73	16/12	/79 23	34/87	Shahrud 12
/55 4877	4/74	62/3	24/9	17/15	/62 22	39/97	Mama'e

**Table 2 – Average amounts for some of physical properties of Shahrud 12 and Mama'e cultivars kernels**

Volume (mm <sup>3</sup> )	Mass g	Sphericity %	Geometric Average mm	thickness mm	width mm	length Mm	Cultivar
1363/23	1/38	51	13/78	7/02	13/98	26/73	Shahrud 12
1451/53	1/47	50	14/72	8/59	12/84	29/29	Mama'e

**Table 3 – Mass modeling of almond using dimensions**

Total Observations	Shahrud 12	Mama'e	Statistical Parameters	Model	#
0/53	0/68	0/62	R <sup>2</sup>		
0/49	0/33	0/49	R.S.E	M= al +b	1
0/6	0/59	0/26	R <sup>2</sup>		
0/44	0/38	0/7	R.S.E	M= aw +b	2
0/76	0/71	0/63	R <sup>2</sup>		
0/42	0/31	0/51	R.S.E	M= aT +b	3
0/7	0/83	0/68	R <sup>2</sup>		
0/39	0/23	0/46	R.S.E	M= al +bw+c	4
0/72	0/75	0/82	R <sup>2</sup>		
0/38	0/3	0/37	R.S.E	M= al +bT+c	5
0/71	0/79	0/73	R <sup>2</sup>		
0/39	0/29	0/46	R.S.E	M= aw +bT+c	6
0/84	0/83	0/85	R <sup>2</sup>		
0/29	0/26	0/33	R.S.E	M= al +bw+cT+d	7

**Table 4 – Almond mass modeling using volume**

Total Observations	Shahrud 12	Mama'e	Statistical Parameters	Model	#
0/87 0/27	0/92 0/18	0/98 0/11	R <sup>2</sup> R.S.E	M= av <sub>m</sub> +b	1

0/75 0/4	0/54 0/44	0/75 0/41	$R^2$ R.S.E	$M = av_{ellip} + b$	2
0/54 0/45	0/79 0/26	0/33 0/66	$R^2$ R.S.E	$M = av_{psp} + b$	3

**Table 5 – Almond kernel mass modeling using dimensions**

Total Observations	Shahrud 12	Mama'e	Statistical Parameters	Model	#
0/46	0/86	0/45	$R^2$		
0/23	0/08	0/28	R.S.E	$M = al + b$	1
0/40	0/70	0/27	$R^2$		
0/18	0/11	0/32	R.S.E	$M = aw + b$	2
0/60	0/40	0/53	$R^2$		
0/18	0/15	0/22	R.S.E	$M = aT + b$	3
0/77	0/91	0/57	$R^2$		
0/12	0/06	0/20	R.S.E	$M = al + bw + c$	4
0/65	0/86	0/90	$R^2$		
0/17	0/07	0/10	R.S.E	$M = al + bT + c$	5
0/84	0/77	0/90	$R^2$		
0/11	0/10	0/11	R.S.E	$M = aw + bT + c$	6
0/91	0/92	0/93	$R^2$		
0/09	0/06	0/09	R.S.E	$M = al + bw + cT + d$	7

**Table 6 – Almond kernel mass modeling using volume**

Total Observations	Shahrud 12	Mama'e	Statistical Parameters	Model	#
0/96	0/93	0/98	$R^2$		
0/6	0/05	0/06	R.S.E	$M = av_m + b$	1
0/82	0/81	0/92	$R^2$		
0/11	0/07	0/10	R.S.E	$M = av_{ellip} + b$	2
0/47	0/85	0/42	$R^2$		
0/18	0/07	0/26	R.S.E	$M = av_{psp} + b$	3

#### 4. Conclusions

In this study, physical properties such as dimensions, mass, volume, sphericity, and geometric average were measured and calculated for Mama'e and Shahrud 12 almonds and kernels. The average amounts of length, width, and thickness for both almond cultivars were 34.41, 23.21, and 16.63 mm, respectively, and for both cultivars' kernels were 28.05, 13.40, and 7.82 mm, respectively. Mama'e dimensions were larger than Shahrud 12 and consequently had larger geometric diameter and volume.

Shahrud 12 cultivar had higher sphericity coefficient than Mama'e. At moisture range of 10-11 percent, 90% of Shahrud 12 masses were between

4.11 to 4.31 mm and also 90% of Mama'e cultivar's kernel masses were between 1.41 to 1.42 mm.

At moisture range of 7-8 percent, 90% of Shahrud 12 kernel masses were between 1.35 to 1.42 mm and also 90% of Mama'e cultivar masses were between 4.59 to 4.89 mm.

Results from modeling of almond and kernel masses based on dimensions and volume showed that there is great correlation between actual volume and mass of samples, which indicates the uniformity of almond's density. But since determination of actual volume of almond and kernel is a time-taking task, it is suggested to use calculated volume while presuming that the cross-section area of the almond is an ellipsoid ( $V_{ellip}$ ). Also mass model based on

thickness is suggested as the best option for industrial and economic applications in designing and manufacturing of machineries specified for breaking and grading.

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5/6/2010

# Regional assessment of groundwater vulnerability in Tamtsag basin, Mongolia using drastic model

Fanomezantsoa Hasiniaina<sup>1</sup>, Jianwei Zhou, Luo Guoyi

<sup>1</sup>School of Environmental Studies, China University of Geosciences (Wuhan)

Lumo Road 388, Wuhan City, 430074 Hubei Province, P.R. China.

[hasiniainaf@hotmail.com](mailto:hasiniainaf@hotmail.com)

**ABSTRACT:** Groundwater is one of the most valuable natural resources and for that reason, its protection and management is vital for human evolution, socio-economic development and ecological diversity. Because of the known health and economic impacts associated with groundwater contamination, steps to assess groundwater vulnerability must be taken. This study aimed to assess groundwater pollution potentials of the north-eastern part of the deep confined aquifer of block XIX, Tamtsag basin, Mongolia. The normal DRASTIC model was applied to the study area with the help of GIS. DRASTIC parameters were calculated from geological data, soil and elevation contour maps, and groundwater level data of the study area. ArcInfo/GIS was used to demarcate vulnerable zones based on their vulnerability index. Finally, a sensitivity analysis of the parameters constituting the model was performed in order to evaluate the relative importance of the each DRASTIC model parameters. The aquifer vulnerability map revealed that only 2% of the study area is under moderate vulnerability to contamination, the remaining zone was determined to be in a low risk category. GIS greatly facilitated the implementation of the sensitivity analysis applied on the DRASTIC vulnerability index which otherwise could have been impractical. Appropriate methods for keeping groundwater resource sustainability in the study area have been suggested. [Journal of American Science. 2010;6(11):65-78]. (ISSN: 1545-1003).

**Keywords:** Groundwater vulnerability / DRASTIC / Tamtsag basin

## 1. INTRODUCTION

Mongolia is a landlocked country between the Russian Federation and the People's Republic of China with over 3 million population and 1,565 thousands square kilometers territory. The country is rich in underground mineral resources. Currently, there are more than 80 proven minerals, including coal, copper, tungsten, fluorite, gold, silver, molybdenum, aluminum, tin, iron, lead, zinc, uranium, manganese, phosphorous, salt, petroleum and so on. The animal husbandry is a traditional economic sector and is the foundation of the national economy; the mining industry also has great potential. Scarcity of arable land and harsh climate make Mongolia unsuitable for agriculture production despite its large territory (seventeenth largest country in the world).

Mongolia belongs to not rich country in terms of water resources. The country's total water resource (30% of which is groundwater) was estimated to be 609.5 cubic kilometer in 2000 (Batsukh N.). Domestically, surface and ground water resources

play vital roles in Mongolia's economy, supporting agriculture, forestry, fishery, livestock production, industrial and domestic water demand and sanitation operations. Water demands are mainly met from the groundwater sources: 80% of the total water consumption. Mongolia's freshwater ecosystem is under increasing threats of degradation and resource depletion. Water shortage and scarcity is becoming inevitable with alarming numbers of dried-out rivers and lakes (WWF Mongolia Program Office). In 2005, UNDP commissioned a "Study on Economic and Ecological Vulnerability and Human Security for Mongolia", which pointed out water shortage as a major socio-economic problem that may soon create serious economic challenges throughout the country.

Despite its limited and finite nature, Mongolia's water has been subject to both natural and anthropogenic factors. Global climate change, which adversely impacts the natural dynamics of freshwater ecosystems, is one of leading natural factors. In some areas, water levels rise due to glacier and permafrost melting. In other, arid areas, lower water tables are

due to drought and loss of water retention capacity in riparian areas that have been heavily deforested. Anthropogenic activities causing excessive extraction and depletion of water resource include mining/gravel extraction; deforestation and wasteful irrigation systems. Socio-economic implications of water scarcity are gravest for those vulnerable to the poverty trap and water scarcity also escalates adverse change on an ecosystem level (WWF Mongolia Program Office). Mining also uses vast quantities of groundwater (rivers and underground water) which reduces the water table. If this process continues in long term, there might be a possibility that the future generation would be facing with scarcity and its ecological balance (Dolgorsuren G.)

In his book “Groundwater inventory”, A. Zaporozec stipulates that groundwater is one of the most valuable natural resources, because it:

- represents some 98 percent of the planet's freshwater resources (polar ice excluded),
- is extensively used for low-cost rural water supply,
- is increasingly developed for both large- and small-scale irrigation,
- is generally reliable in periods of drought because of its large storage capacity,
- is cheap to develop because of its widespread occurrence and its generally good natural quality.

Moreover, groundwater is the main source of water consumption of natural vegetation in arid regions. All ground water is vulnerable (The USA National Research Council, 1993). Even owing a function of self- remediation; it will be very difficult to be remediated once it was polluted. Therefore, a sustainable groundwater management should be based on prevention of contamination. An assessment of both the existing and potential sources of contamination and the spatial extent of the existing groundwater contamination is needed before considering methods to prevent future groundwater quality problems (A. Zaporozec et al. 2002)

This study aimed at assessing the vulnerability of groundwater to contamination in the vicinity of an oilfield exploration in Block XIX of Tamtsag Basin, Eastern Mongolia using a DRASTIC model (Aller et al., 1987) combined with a Geographic Information System (GIS). Many papers on the effects of oilfield on groundwater are available in the scientific literature, as are several comprehensive reviews. In their study of the impact of oilfield exploitation on eco-environment of the Daqing lakes, Yu S. et al. (2003) demonstrated that oilfield exploitation may harm its vicinity. Their paper stated that the impacts

became more evident with passage of time, and the intensity varied with areas. Actually, in any industrial activity, equipment can fail and employees may err; and groundwater pollution may result. Thus, it is important to evaluate sensitive areas to contamination is essential in order to prevent and control groundwater contamination.

The term ‘vulnerability of groundwater to contamination’ was introduced by Jean Margat in the late 1960s (Vrba and Zaporozec, 1994). He used the term “vulnerability” to mean the degree of protection that the natural environment provides against the ingress of pollutants to groundwater. Vulnerability assessment has been recognized for its ability to delineate areas that are more likely than others to become contaminated as a result of anthropogenic activities at/or near the earth’s surface (Babiker et al., 2007) The concept of groundwater vulnerability is based on the assumption that the physical environment may provide some degree of protection to groundwater against natural impacts, especially with regard to contaminants entering the subsurface environment. Consequently, some land areas are more vulnerable to groundwater contamination than others (Napolitano, 1995). Vulnerability assessment and vulnerability maps represent an important preliminary tool in decision-making pertaining to the management of groundwater quality. They provide a useful framework within which to designate priorities for the implementation of pollution protection and control measures. The vulnerability maps also serve to inform and educate the public, because non-professional people can readily understand their concept. They also create public awareness about potential pollution problems of groundwater, a situation needed for effective implementation of future protection programs (Rubhera, 2002).

## 2. STUDY AREA

The study area ( $116^{\circ}04'31''$  to  $116^{\circ}21'34''$  E and  $46^{\circ}50'01''$  to  $47^{\circ}04'17''$  N.) is located in the northeastern part of block XIX, Tamtsag basin. Situated in the eastern part of Mongolia, with an area of 381km<sup>2</sup>; it is located in the high plain zone with the altitude of 600 ~ 730m. The landform is relatively flat, the topography gradually rises from west to east, and it's in an undulant plain landform from a macroscopic view. There are no rivers in the study area but some small lakes in the northwestern part which are all dry in arid seasons.

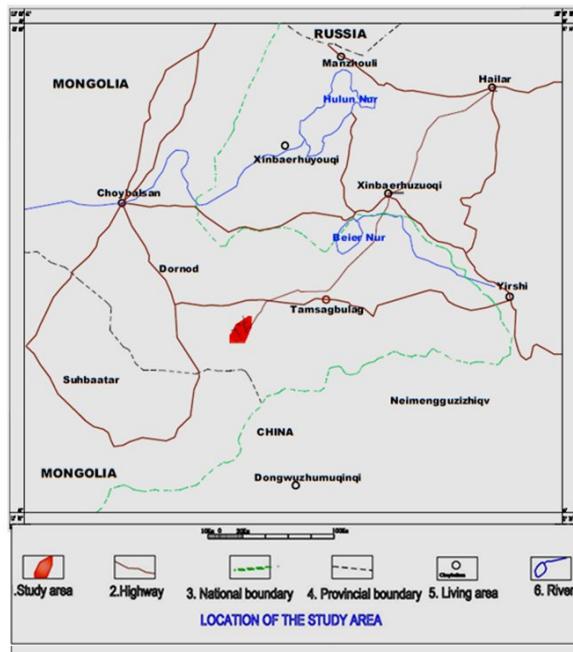


Figure 1 Location map of the study area

Climatically, the zone belongs to an arid area with an annual average precipitation of 276.4mm; an annual evaporation is 1518.7mm and an annual average temperature of -6.6°C ~ 3.9°C. It's a typical arid and semi-arid continental climate. Geologically, the top of the strata lithology is constituted by (1) a quaternary silty sand soil and silty clay, with partial sandy layer at the top; (2) a quaternary sandstone as water bearing layer and (3) cemented and loose sandstone, medium-sized coarse sandstone and fine sandstone.

Most of the working area is covered by sand soil, and the area has a very high sandy degree. It is also characterized by high alkali content. In the eastern part of the project zone, there exists an approximately south-north tectonic fracture. As a result, there is a huge water-rich difference on both sides of the fault zones which reflects the water-rich distribution of underground water. A water-rich belt of about 12-km-long and 8-km-wide is situated in the western part of the study area. A representation of the West-East (AA') cross-section and the South-North cross-section (BB') of the aquifer is illustrated in figure 2.

### 3. METHODS AND APPROACHES

#### 3.1. Model theory

The DRASTIC model was developed in USA for the purpose of protecting the groundwater resources (Aller et al., 1985; 1987). DRASTIC is an empirical

groundwater model that estimates groundwater contamination vulnerability of aquifer systems based on the hydrogeological settings of that area (Aller, et al., 1985, 1987).

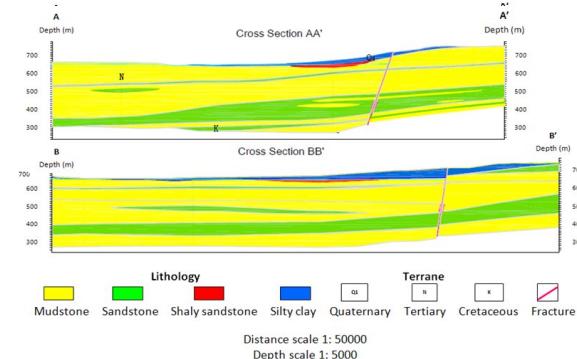


Figure 2 Simplified Cross Sections of the aquifer

The DRASTIC hydrogeologic vulnerability ranking method uses a set of seven hydrogeologic parameters to classify the vulnerability or pollution potential of an aquifer. The parameters are:

- Depth of groundwater (D);
- Recharge rate (R);
- the Aquifer media (A);
- the Soil media (S);
- Topography (T);
- the Impact of the vadose zone (I); and
- the hydraulic Conductivity of the aquifer (C)

Table 1 DRASTIC parameters assigned weights  
(Aller et al., 1987)

Factor	Weight
D Depth to top the of the Aquifer	5
R Net Recharge	4
A Aquifer Media	3
S Soil Media	2
T Topography	1
I Impact of the Vadose Zone	5
C Hydraulic Conductivity of the Aquifer	3

Each parameter is assigned a relative weight from one to five based on its relative susceptibility to pollutant (Shamsuddin, 2000) (Table 1). Similarly, parameter rankings are assigned on a scale of one to ten and are based on its significance to pollution potential in an assessed area (Dickerson, 2007) (Table 2 and 3). The set of variables that are considered for the DRASTIC model can be grouped according to three main categories: land surface factors, unsaturated zone factors and aquifer or saturated zone factors. The aquifer media properties and the hydraulic conductivity are the critical factors

identified for the saturated zone. The depth to water and the properties of the vadose zone characterize the water/contaminant path down to the saturated zone (Dirk et al., 1997). In soil and the unsaturated zone,

some mechanisms may affect the contaminant concentration much more than in the saturated zone (Gogu et al., 2000).

Table 2 Typical ranges and ratings of D, R and A

Depth to water (ft)		Net recharge (Inch)			Aquifer media		
Range	Rating	Range	Rating	Type	Rating	Typical Rating	
0-5	10	0-2	1	Massive Shale	1-3	2	
5-15	9	2-4	3	Metamorphic/igneous rocks	2-5	3	
15-30	7	4-7	6	Weathered metamorphic/igneous	3-5	4	
30-50	5	7-10	8	Thin bedded sandstone, limestone, shale sequence	5-9	6	
50-75	3	>10	9	Massive Sandstone	4-9	6	
75-100	2			Massive Limestone	4-9	6	
>100	1						

Table 3 Typical ranges and ratings of S, T, I and C

Soil media		Topography (percent slope)			Vadose zone media		Hydraulic Cond. (GPD/FT <sup>2</sup> )	
Range	Rating	Range	Rating	Range	Rating	Typical Rating	Range	Rating
Thin or absent	10	0-2	10	Silt, clay	2-6	3	1-100	1
Gravel	10	2-6	9	Shale	2-5	3	100-300	2
Sand	9	6-12	5	Limestone	2-7	6	300-700	4
Peat clay	8	12-18	3	Sandstone	4-8	6	70-1000	6
Shrinking or aggregated clay	7	>18	1	Bedded limestone, sandstone, shale	4-8	6	1000-2000	8
Sandy Loam	6			Sand and gravel with significant silt and clay	4-8	6	>2000	10
Loam	5			Metamorphic/igneous	2-8	4		
Silt loam	4			Sand and gravel	6-9	8		
Clay loam	3			Basalt	2-10	9		
Humus	2			Karst limestone	8-10	10		
Non-shrinking and non-aggregated clay	1							

The DRASTIC Index was computed by summing the weighted factors of each subdivision of the area. Generally, higher DI value indicates greater susceptibility to groundwater pollution

$$\text{DrasticIndex} = D_r D_w + R_r R_w + A_r A_w + S_r S_w + T_r T_w + I_r I_w + C_r C_w \quad (1)$$

Using the above equation, DRASTIC index values were obtained. According to the ranges, the degree of vulnerability of each area was concluded; a groundwater vulnerability map was then designed to show the vulnerability toward contamination of each area.

### 3.2. Data acquisition

Information about the seven parameters and all the necessary data were obtained from Daqing Tamtsag Co.,Ltd of China Petroleum. The data, in text (.doc), table (.xsl), drawing (.dwg), and ESRI shapefile (.shx, .dbf and .shp) formats include:

- Hydrogeological Reconnaissance report
- Geographical Prospecting Report on Underground Water Resources
- Hydrogeological Map of Hydrogeological Survey for Block 19 in Tamtsag Basin
- Underground Water Level Contour Map
- Underground Water Chemical Type diagram
- Well logs
- Aquifer roof elevation isoline diagram

These data are the result of different survey made by the Mining Group Co., Ltd. of Heilongjiang Province on the demand of Petrochina Daqing Tamtsag of China Petroleum. The works were undertaken between June 2007 and November 2008. Some of the data were ready to use but some others were in Chinese and needed a translation to English. Due to time constraint and the location of the study area, it was not possible to go to the site. However, a travel to Daqing was done in order to acquire technical advisory from knowledgeable individuals working for the Daqing Company. During the data manipulation, a close contact with a technical advisor from the company was necessary for the confirmation of the estimation of certain parameters.

Other information includes the Soil Map of the People's Republic of Mongolia and the Atlas of Mongolia which were published by the European Commission Joint Research Center (JRC) and the U.S Northern Circumpolar Soils Map respectively.

### **3.3. Development of the DRASTIC parameters**

#### **Factor 1: Depth to water**

Depth to water refers to the distance the contaminant travels before reaching the aquifer. Hence, it gives an insight of the contaminant's contact time with the surrounding media. Due to the presence of confining clay layers, the aquifer in the study area is classified as a confined aquifer. For a confined aquifer depth to water refers to the depth to the top of aquifer (DTTA). Because of the low permeability of the confining media, the contaminants travel to the aquifer is retarded; potential pollutants released from the ground surface cannot reach the aquifer easily. Therefore, confined aquifers have more natural protection from contaminants and are less vulnerable to pollution than unconfined aquifers. As the degree of confinement decreases, the pollution potential of the aquifer increases.

The depth to the top of the aquifer feature was obtained by combining the contour map of the ground elevation with that of the top of the aquifer (Eq.2).

DTTA = Groundwater elevation – Top of the Aquifer elevation <sup>(2)</sup>

The resulting map has shown that the DTTA varies between 179.7m and 280.6m which implies that most of the underground water in the study area is deep water.

#### **Factor 2: Net recharge**

Net Recharge represents the amount of water per unit area of land penetrating the ground surface and reaching the water table. It is thus influenced by the amount of surface cover, the slope of the land surface, the permeability of the soil and the amount of water that recharge the aquifer. The dispersion and dilution of contaminants depend greatly on the volume of water available in the vadose zone as well as in the saturated zone and thus on the net recharge. Additionally, the recharge water has the ability to carry contaminants to the water table and within the aquifer. Hence, a great recharge corresponds to a high potential for groundwater pollution. Net recharge is thereby an important factor in the contamination attenuation and is given a weight of 4. Regarding to the net recharge, the pollution potential of an area with confined aquifer is lesser than that of an unconfined one because of the presence of a confining layer.

The primary source of ground water is precipitation which infiltrates through the surface of the ground and percolates to the water table. Because the deep underground water in the project zone is isolated by the aquiferous stratum roof, the vertical infiltration of the local atmospheric precipitation replenishment cannot be directly received. Thus, the aquifer mainly receives the lateral flow replenishment from the upper reaches. The replenishment source of the Quaternary underground water of the study area and the replenishment mainly come from the jacking from the deep underground water to the top aquiferous stratum. The methodology prescribes that in this case, the recharge is negligible. Low recharge values were thereby chosen for aquifer.

Values of the net recharge are more difficult to obtain than the values of the six other parameters. As suggested by the model, more accurate estimates of net recharge should be done based on the available features which are believed to be important to the recharge component. The values were thus generated using the estimation formula that Piscopo established in 2001 and that Al-Adamat et al. applied in 2003 for their study of the Azraq basin, Jordan. Nevertheless, for the purpose of this study, the weighting factor of the normal DRASTIC was kept as all the parameters constituting the model were used. Because the major underground water resource volume in the zone is replenished with lateral runoff, the recharge map was constructed according to the following formula:

$$\text{Recharge value} = \text{Slope}(\%) + \text{Rainfall} + \text{Soil permeability} \quad (3)$$

Table 4 Range and factors of the features controlling the recharge value (Piscopo, 2001)

Slope	Rainfall		Soil permeability	
Slope (%)	Factor	Rain (mm)	Factor	Range
<2	4	>850	4	High
2–10	3	700–850	3	Mod-high
10–33	2	500–700	2	Moderate
>33	1	<500	1	Slow Very slow

First, a digital elevation model of the study area was generated from the 1m elevation contour map. Using the “surface analysis” function of the 3D analyst tool, the slopes values were deducted from the DEM and then classified according to the criteria cited in table 4. The slope values of the study area vary between 2 and 4% while the infiltration replenishment by precipitation is very slow, the annual evaporation being 5-7 times of the annual precipitation. The average rainfall of the study under investigation is 276.4mm (<500mm). With careful attention to the specific features of the study area cited in the above paragraphs, the soil permeability was estimated based on the typical classification of permeability given in table 5.

The next step consisted of classifying the soil permeability map into four classes: very slow (26%), slow (53%), moderate (16%) and high (5%). The thus obtained map was converted into grid coverage; then, a raster addition was performed using the 3D analyst tool of ArcGIS. The result which ranges from 3 to 10 was classified into ranges according to table 2. The final map of the net recharge was obtained by assigning the rating values as new values for each reclassified range.

The aquifer media has been chosen as the starting parameter because on it depend the values chosen for the other parameters. The aquifer medium determines the materials with which, the contaminant is in contact in the aquifer. Hence, it plays a significant role in the concentration attenuation process. Besides, it governs the groundwater flow system and consequently, affects the route and path length that the contaminant follows. These factors are important because they give an insight into the chance for the attenuation processes to occur. The pathways for groundwater flow are strongly influenced by the grain size of the medium, fractures or openings within the aquifer. The presence of a fracture implies a higher contamination potential because of the degree of secondary permeability it provides. Larger grain size and more fractures or openings imply a higher permeability and thus, a lower pollution attenuation capacity. Similarly, the presence of clay materials in the aquifer lowers the pollution potential. Hence, the rating for each aquifer

medium was evaluated based on the specific features of the aquifer.

Table 5 Typical values of permeability (Cashman et al., 2001)

Soil type	Typical classification of permeability	Permeability (m/s)
Clean gravels	High	$>1 \times 10^{-3}$
Clean sand and sand/gravel mixtures	High to moderate	$1 \times 10^{-3}$ to $5 \times 10^{-4}$
Fine and medium sands	Moderate to low	$5 \times 10^{-4}$ to $1 \times 10^{-4}$ $1 \times 10^{-4}$ to $1 \times 10^{-6}$
Silty sands	Low	$1 \times 10^{-6}$
Sandy silts, very silty fine sands and laminated or mixed strata of silt/sand/clay	Low to very low	$1 \times 10^{-5}$ to $1 \times 10^{-8}$
Fissured or laminated clays	Very low	$1 \times 10^{-7}$ to $5 \times 10^{-9}$
Intact clays	Practically impermeable	$>5 \times 10^{-9}$

### Factor 3: Aquifer media

Based on the geological description of the study area, there are two major aquiferous rock formations: Pre-Quaternary debris rocks and Quaternary loose rocks. The aquiferous stratum consists of medium-fine sands, with a thickness of 4-8 m. The Pre-Quaternary debris rocks are generally distributed in the study area; they consist of middle fine sandstone, medium coarse sandstone and medium sandstone. The map for the Aquifer media ranking was obtained from an interpolation of the lithology of the aquifer of each borehole. The typical ratings for aquifer media given in table 2 were not used; the rating for each medium was adjusted based on the characteristic of the zone. Higher ratings were chosen to indicate the presence of the North-South tectonic fault situated at the eastern part of the study area, the amount of fines and the clay within the aquifer. Conversely, lower ratings were assigned to the fine textured media

### Factor 4: Soil media

Soil is the first media the contaminant passes through when it percolates into the ground. Therefore, soil media influence strongly the recharge which percolates into the ground and hence, the contaminant movement. Several attenuation

processes can happen within the soil media, namely filtration, biodegradation, sorption and volatilization. These processes depend greatly on the thickness of the media and the material the contaminant is in contact with (type, texture...). Fine textured materials such as silts and clays restrict contaminant migration as they decrease the soil permeability. Similarly, a thick media offers greater chance for the attenuation processes to occur.

Soil ranking distribution was inferred from the soil map of the People's Republic of Mongolia, the Atlas of Mongolia and the written descriptions of the soil cover.

Most of the working area is covered by sand soil with different thicknesses in some areas, and the area has a high sandy degree. According to strata lithologic array, the top is the quaternary silty sand soil and silty clay with partial sandy layer, with the tertiary and cretaceous mudstone, sandstone, medium-sized coarse sandstone and interbedded fine sandstone at the bottom. These horizons of the soil profile were evaluated and the most significant textural layers which can affect the pollution potential were chosen for each zone. For the area where clay layer is the most significant soil texture, the shrink/swell potential was evaluated. The shrink/swell potential is important as it influences the transport of contaminants. Because the soil complexes were not particularly detailed, it was not possible to determine the degree of the shrink/swell potential. However, the drilling samples showed that the surface soils of the drilling samples have a very high hardness and are in a compact state. Thereby, a DRASTIC range of shrinking and aggregated clay was assigned to the clayey areas, as recommended by the methodology for soil with a moderate shrink/swell potential.

#### **Factor 5: Topography**

Even if topography is given the lowest weight (1), it has a relative significance as it controls the time during which contaminants remain on the surface. Topography expresses the slope and slope variability of the land surface. A high degree of slopes increases the runoff capacity. As the infiltration probability of contaminant is lessened, the groundwater pollution potential decreases. The topographic unit of the study area is a plateau area, and it belongs to a relatively flat area in Mongolia. It is characterized by a wavy plain relief: The overall topography of the project zone is high in the eastern and southwestern parts have a high topography while the northwestern part is low. The altitude varies between 618 and 717 m and the slopes range from 0

to 4.8%. Since the study area is relatively flat, the range 0 to 2% slope is predominant.

The mapping of the topography was the easiest process because the data required for this parameter were easy to find and didn't need many modifications. The slope map was generated using 3D analyst tool of Arcmap.

#### **Factor 6: Impact of the vadose zone**

The vadose zone is the portion of the subsurface in which granular openings are unsaturated or discontinuously saturated. The behavior of contaminants in the vadose zone is a key element in pollution attenuation as the media is the home to many natural organisms which break down many polluting substances into secondary by-products both harmful and harmless. Various attenuation processes may occur between the soil horizon and the water table; namely: biodegradation, neutralization, mechanical filtration, chemical reaction, volatilization and dispersion. The type of vadose zone is thereby of great importance because it determines the contact time for reaction to occur as it influences the path length and the routing of contaminants.

Regardless the presence of other layers composing the media, confining layer was chosen as the vadose zone media since the purpose of the study was to evaluate the confined aquifer. This is highly important because the confining layer is the media which most significantly impacts pollution potential.

#### **Factor 7: hydraulic conductivity**

The hydraulic conductivity of an aquifer is a measure of the aquifer's ability to transmit water when submitted to a hydraulic gradient. It is a critical factor because it controls the velocity of groundwater flow; which in turn controls the velocity of contaminant flow within the aquifer. An aquifer with high conductivity is vulnerable to substantial contamination as a plume of contamination can move easily through the aquifer (Rahman A., 2007). Hence, areas with high hydraulic conductivity values are more susceptible to contamination.

Values for hydraulic conductivity estimates were based on well yields and aquifer characteristics because the maps of hydraulic conductivity for the study area were not available in published reports. Thus, the hydraulic conductivity maps were generated using two components of conductivity: transmissivity and saturated thickness based on the formula  $T = K \cdot b$  where  $T$  represents the

transmissivity, K is the hydraulic conductivity and b the thickness of the aquifer

The procedure consisted of digitizing the contour lines of the aquifer thickness, hence creating a "DEM-like" surface image. The transmissivity maps were interpolated from pumping test data of some points of reference. The obtained values were divided by the aquifer saturated thickness on pixel-by-pixel basis using the Raster math tool of 3D analyst tool in ArcView. Generally, the study area has a low hydraulic conductivity ( $0.3 \times 10^{-6} \sim 5.3 \times 10^{-5}$  m/s). However, the central part of the aquifer has a higher conductivity compared to the rest of the study area.

### 3.4. Vulnerability mapping

A new raster data file for the DRASTIC Index was created according to Eq. 1 using the weighted sum overlay in spatial analyst tools using the 7 individual raster files created above. The first step was to consider the parameters one by one in order to calculate their respective index values. For each parameter the rating values were multiplied to the appropriate DRASTIC weight (table 1).

For all the parameters except the DTTA and the Impact of the vadose zone, the obtained index values were in raster format. For the DTTA, the rating value of the whole study area was equal to 1 and based on the weighting system; the result of multiplying Dr by Dw is equal to 5. Similarly, the net recharge index was obtained by multiplying  $I_r$  by  $I_w$ . 5 was obtained as a result and was also added to the total DRASTIC index as a constant value for all locations in the study area. In sum, a constant value of 10 was added to the final raster grid coverage. For the five remaining parameters, the values for each overlay were summed on pixel-by-pixel basis by running the model in ArcView GIS. The final raster for the Overall Vulnerability Index was created using the raster calculator in Spatial Analyst tools by combining the seven hydrogeological data layers as illustrated in figure 3.

Referring to Aller et al. (1987), the DRASTIC indexes were classified according to the ranges given in table 6. The final step of the vulnerability mapping consists of reclassifying the DRASTIC indexes and assigning to each group its degree of vulnerability; thereafter, groundwater vulnerability map was developed.

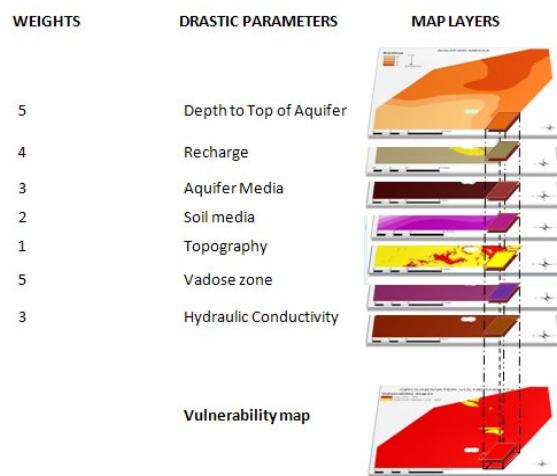


Figure 3 Vulnerability mapping

### 3.5. Sensitivity analysis

Sensitivity analysis (S.A) is a significant component of any modeling project as it allows evaluation of the accuracy of the result (Baker et al., 2005).

#### Map removal S.A

The map removal sensitivity analysis was performed to evaluate whether it was necessary to use all the parameters incorporated in the DRASTIC model. The sensitivity measure expressed in terms of variation index S is given by the formula:

$$S = \left( \frac{\left| \frac{V - V'}{N} - \frac{n}{V} \right|}{V} \right) \times 100 \quad (4)$$

where V is the unperturbed vulnerability index which represents the actual index used in the primary suitability using N parameters.  $V'$  is the perturbed vulnerability index while a lower number of parameters (n) were used.

The analysis comprised two studies. The first one was performed by removing only one layer at a time, considering each parameter constituting the DRASTIC model. This process aimed at evaluating the sensitivity of the vulnerability values upon the removal of the defined parameter. The second analysis consisted of removing a layer which compels less variation in the final vulnerability index. The thus obtained result was then used for the next removal analysis, and the same steps were followed until only one layer was left. For each new step, the layers assumed to be the most effective were considered each time (Babiker et al., 2005) while the least effective were removed. The computation was

done taking into account every sub-areas of the study area. The sensitivity value S was calculated for each grid cell using the Raster math tool of GIS according to the above formula (Eq. 4).

#### Single parameter S.A:

The next step of the analysis was to compare the effective weight of each parameter in each subarea with the theoretical weight assigned to it by the DRASTIC method. For each grid square element, the effective weight  $W_{pi}$  (in %) was calculated using a theory developed by Lodwick et al. (1990) and effectively used by Napolitano and Fabbri (1996), Rahman (2008), Babiker et al. (2005) among others.

$$W_{pi} = 100 * \left( \frac{P_{ri} * P_{wi}}{DI} \right) \quad (5)$$

Where  $P_{ri}$  and  $P_{wi}$  are the ratings and the weights respectively of the layer P assigned to the subarea i, and D.I is the vulnerability index. Based on this formula, the effective weight of each subarea was computed using GIS. In the same way as the first analysis, the analysis covered the whole study area and a statistical analysis was performed using the proUCL software for the display and the analysis of the obtained results.

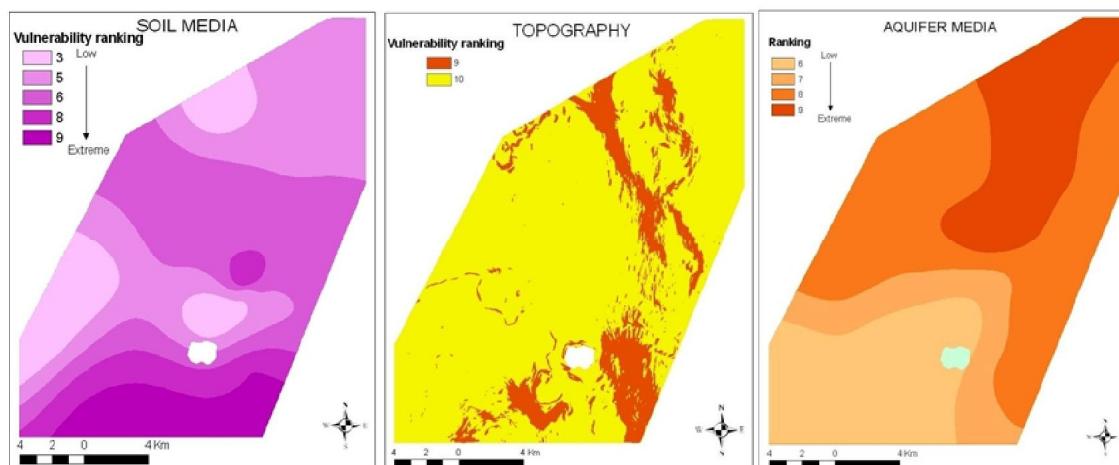


Figure 4 Examples of the rated maps of the DRASTIC parameters

The final integrated map of the groundwater vulnerable zones is presented in figure 5. The resulting DRASTIC values in the study area lay between 58 and 88. According to table 6, two classes of the vulnerability toward pollutants could be identified in the zone. In term of areal extent, almost 98 percent of the area was determined to have a low vulnerability toward contaminants. This can be associated to the presence of the confining layer as it is the media which significantly impacts pollution potential. Only a slight 2 percent, which is located in

## 4. RESULTS AND DISCUSSION

### 4.1. Groundwater vulnerability map

Figure 4 contains examples of the rated maps used to compute the DRASTIC vulnerability index. Regarding to the net recharge, about 72% of the study area has a low contamination risk, 27% is moderately vulnerable and only the remaining 1 % belongs to high vulnerable class. The high vulnerable class is situated in the shallower aquifer in the north-western area. With regards to the aquifer media, the major part of the study area has a relatively high vulnerability index. The northern part of the study area has a relatively high VI. It can be attributed to the coarser grain size of the unit that serves as an aquifer. The vulnerability index associated with the aquifer media indicates that GW resources surrounding the tectonic fault are susceptible to pollution to a high degree.

With a special consideration of the soil media, about 80% of the study area has low or moderate vulnerability toward contamination while almost the whole area is highly vulnerable regarding the topography slope.

the water-rich area, are more susceptible to pollutants. These zones are located in the north-western part and at the middle-eastern part of the study area. The vulnerability map offers the possibility to select priorities for restoration and remediation actions in regional planning. It is important as the toxicological index of the area under investigation has shown that the content of Na, Mg, Cl and Mn in the underground water are seriously over standard. It highlights need of establishing underground dynamic observation points to control the behavior of these chemicals.

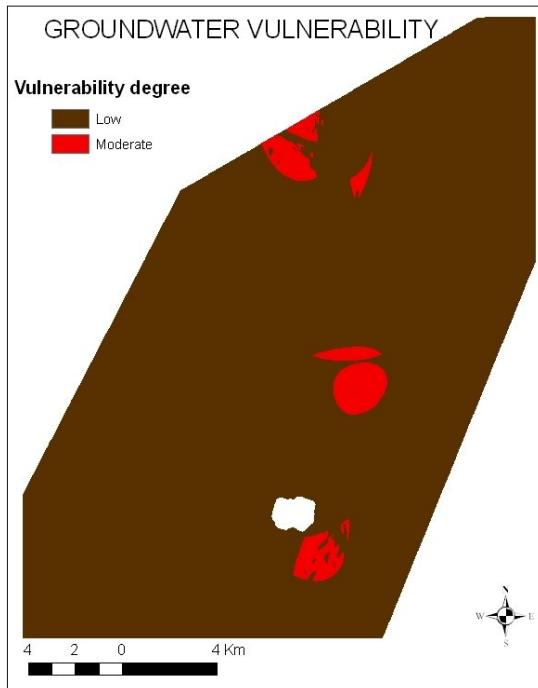


Figure 5 Groundwater vulnerable zones

#### 4.2. Summary of the DRASTIC parameters

A statistical summary of the seven parameters incorporated in DRASTIC model and which were used for the study is presented in Table 7. It shows that aquifer media has the largest impact on the intrinsic vulnerability of groundwater as it has the highest mean (22.8). Then, it is followed by the net recharge (with a mean of 14.24), the soil media (mean = 11.54) and the topography (9.15). The result also reveals that impact of vadose zone and DTTA which have both a mean value of 5 have low contribution to vulnerability index while hydraulic conductivity contributes the least as it has smallest mean value (3.02). Regarding the contribution to the variation of the vulnerability index; a small variation of the net recharge would greatly affect the values of the vulnerability indexes. This is exhibited by the high value of the coefficient of variation associated to this parameter (70.26%). Changes in soil media and aquifer media values (mean = 38.5 and 17.21 respectively) would have moderate impact on the system whereas variation in hydraulic conductivity would barely impact the vulnerability map (mean value = 0.47). Depth to aquifer and the impact of vadose zone both having fix value do not impact the variation of the sensitivity measure.

Table 7 Statistical summary of the DRASTIC parameters map

	D	R	A	S	T	I	C
Min.	5	4	18	6	9	5	3
Max.	5	32	27	18	10	5	6
Mean	5	14.24	22.8	11.54	9.15	5	3.02
SD	0	2.99	3.87	4.77	0.71	0	2.12
CV (%)	0	70.26	17.21	38.5	7.44	0	0.47

SD = Standard Deviation CV = Coefficient of Variation

#### 4.3. Map removal sensitivity analysis

The map removal sensitivity analysis was performed with the aim of establishing the significance of the parameters used for the DRASTIC model. The summary statistics of the map removal sensitivity analysis is displayed in table 8 and 9. Table 8 reveals that topography is the layer that affect least the variation in the final vulnerability index as the variation index has the least average value after its removal (= 0.23%). It is mainly due to the low contamination risk associated with topography (the mean rating value = 1). In contrast, a high variation of the vulnerability index is expected upon the removal of the aquifer media as this layer has the highest variation index (2.99%). It is mainly due to the presence of the tectonic South-North fault located in the eastern part of the study area which has a significant influence on pollution potential. In addition to the relatively high theoretical weight (3), aquifer media has high rating values in almost every subarea. Hydraulic conductivity removal also influences greatly the variation of the vulnerability assessment. It can be explained by the fact that the main recharge of the aquifer comes from lateral replenishment. The hydraulic gradient has therefore a significant impact on the fate of the travel of a plume of contaminant. The vulnerability index also seems to be sensitive to the removal of the impact of the vadose zone and the DTTA (their average variation indexes are both equal to 1.19). It could be attributed to the high theoretical weight (= 5) assigned to both of these layers. Moreover, the high mean value for the impact of the vadose zone confirms the fact that the confining layer is the media which most significantly impact pollution potential and thus the vulnerability of the aquifer; its removal will greatly impact the sensitivity measure of the area. The removal of the net recharge and soil media also have contributed to the variation of the sensitivity value of the aquifer; their mean value being 0.99 and 0.61 respectively.

Table 8 Statistical summary of one map removal sensitivity analysis

Parameters removed	Variation index (in percent)			
	Min	Max	Mean	SD
<b>D</b>	0.94	1.43	1.19	0.15
<b>R</b>	0.21	3.97	0.99	1.01
<b>A</b>	1.51	4.65	2.99	0.76
<b>S</b>	0	1.9	0.61	0.52
<b>T</b>	0	0.66	0.23	0.17
<b>I</b>	0.94	1.43	1.19	0.15
<b>C</b>	1.06	1.79	1.66	0.17

The variation of the sensitivity measure upon the removal of one or more maps from the computation is contained in table 9. It appears from the table that after the removal of the topography layer, the variation index has the least average value. This average variation index changes as more layer data are removed from the computation. However, there is no consistency on the trend of the mean variation index according to the number of parameters removed. This clearly demonstrates that all the seven parameters used to compute the DRASTIC model are all essentials in determining the vulnerability index.

Table 9 Statistical summary of the map removal sensitivity analysis

Parameters used	Variation index (in percent)			
	Min	Max	Mean	SD
D, R, A, S, I, C	0	0.66	0.23	0.17
D, R, A, I, C	0.03	2.26	0.71	0.62
D,A,I,C	0.05	4.22	1.63	1.14
A,I,C	0.06	3.94	0.74	1.29
A,C	0.64	9.15	3.55	3.02
A	6.39	10.75	9.72	1.82

#### 4.4. Single parameter sensitivity analysis

The single parameter sensitivity analysis compares the theoretical weight assigned to a parameter by the DRASTIC model with its real (or effective) weight. The result summarized in table 10 indicates the importance that should be accorded to some factors, namely the aquifer media; soil media and topography. With an average weight of 31.83 against a theoretical weight equal to 23.38, aquifer media mostly influences the vulnerability index. This is in agreement with the result from the map removal analysis which also states that aquifer media is the layer that compel most the variation of the final V.I. The effective weights of soil media together with topography exceed the theoretical weight imposed by DRASTIC. Their mean effective weights (%) are 15.93 and 12.42 respectively while their respective theoretical weight assigned by DRASTIC are both less than 10%. This reflects the importance of the aquifer media, soil media and topography layers in the model and the need to get accurate, detailed and

representative information about these factors. The net recharge almost conserves the weight that is assigned by the DRASTIC model: its real weight is just slightly greater than the theoretical weight. It is mainly due to the fact that in some portions of the aquifer, groundwater replenishment mainly comes from the jacking from the deep underground water to the top aquiferous stratum. Recharge of aquifer is thereby negligible in some parts of the study area. DTTA is the least important parameter as it exhibits a very low effective weight compared to the theoretical weight. This agrees with the basic assumption that depth to water is less important for confined aquifers.

Table 10 Statistical summary of the single parameter sensitivity analysis

Parameters	Theoretical weight	Theoretical weight (%)	Effective weight (%)			
			Mean	Min	Max	SD
<b>D</b>	5	21.7	6.68	5.68	8.62	1.12
<b>R</b>	4	17.4	19.52	5.97	38.09	9.52
<b>A</b>	3	13.0	31.83	23.38	42.19	5.72
<b>S</b>	2	8.7	15.93	6.82	25.71	5.98
<b>T</b>	1	4.3	12.42	10.34	17.24	2.45
<b>I</b>	5	21.7	6.68	8.62	5.68	1.29
<b>C</b>	3	13.0	3.91	3.53	7.89	1.58

#### 4.5. Conclusion and recommendations

DRASTIC system and GIS were used to analyze the Regional groundwater pollution susceptibility of a part of block 19 of Tamtsag Basin, in Mongolia. Topography, well, geology, soil databases were designed and constructed for the application of the DRASTIC model. Using these databases, hydrogeologic factors such as depth to water, net recharge, aquifer media, soil media, slope, hydraulic conductivity were extracted. The DRASTIC vulnerability index, which is defined as a linear combination of seven hydrogeological factors was computed with the help of GIS. The aquifer vulnerability map indicated that only 2% of the study area is under moderate vulnerability to contamination. The remaining zone was determined to be in a low risk category. GIS greatly facilitated the implementation of the sensitivity analysis applied on the DRASTIC vulnerability index which otherwise could have been impractical. The single-parameter sensitivity analysis has shown that aquifer media, soil media and topography are the most significant environmental factors which dictate the high vulnerability of the study area. This highlights the importance of obtaining accurate, detailed, and representative information about these factors. The map removal sensitivity analysis indicated that the vulnerability index is highly sensitive to the removal of aquifer media, hydraulic conductivity and the impact of vadose zone layers but is least sensitive to the removal of the topography layer. The analysis has

also demonstrated that all the seven parameters used to compute the DRASTIC model are all essentials in determining the vulnerability index.

Often only a portion of the groundwater in storage may be exploited without creating undesirable effects. Ground water pollution vulnerability maps, risk maps, groundwater quality maps etc may be used to assist planners, managers, and local officials in evaluating the potential for contamination from various sources of pollution. These maps are useful as preliminary screening tools for policy and decision. To keep groundwater resource sustainability, a reasonable management of the resource should be put forward based on the groundwater resource evaluation and groundwater vulnerability assessment. Therefore, the present vulnerability maps should be regarded as an important tool in prioritization of areas for monitoring purposes. Some precautionary measures should be taken for the more vulnerable zones and detailed study of the groundwater pollution should be carried out if necessary. Additionally, the study suggests that special consideration such as a denser monitoring system should be given to the zones with higher vulnerability. Knowing the vulnerable areas, users can recommend settings that are suitable for the areas which are critical to groundwater contamination.

Groundwater pollution susceptibility assessment is also necessary for systematic management and protection of groundwater resources in the study area for further works and projects (land use, well construction and abandonment for instance). It is suitable to evaluate the impact of a potential pollution source on the aquifer, not only for the oilfield exploitation but also for industries, storage areas, livestock rearing establishments, and any new development proposals in any locality within the same area of study. Although the vulnerability map showed the dominance of "low" vulnerability class, the results suggest that great care should be taken when siting developments in the moderate vulnerability areas. Without attention, prospecting and exploiting tasks would change the hydraulic balance between the various natural strata; which will cause a connection of underground water of different qualities. This can be a shortcut to artificial pollution.

Due to the large amount of salts in the underground water, negligence in water pollution and prevention might cause a serious soil compaction, land salinization and also a potential harm to the surface soils.

#### **ACKNOWLEDGEMENTS:**

Authors are grateful to the China Scholarship Council for granting a research scholarship to the first author.

#### **Corresponding author:**

FANOMEZANTSOA Hasiniaina

School of Environmental Studies, China University of Geosciences (Wuhan)

Lumo Road 388, Wuhan City, 430074

Hubei Province, P.R. China.

E-mail:[hasiniainaaf@hotmail.com](mailto:hasiniainaaf@hotmail.com)

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5/8/2010

# Structure Of Whey Protein Consequence For Dairy Industry (Review)

Khorshid,M.A.and Fatma,A.M.

Dairy Department, National Research Center, Dokki , Cairo , Egypt  
Email:[khorshid88@hotmail.com](mailto:khorshid88@hotmail.com)

**Abstract:** Milk proteins play a range of roles which make dairy products and products containing dairy components are valuable. Theses include nutrition, physical functionality and breakdown under controlled condition to produce nutritional, functional or flavour full products. This article reviews the structure of whey protein consequence for dairy industry. [Journal of American Science. 2010;6(11):79-84]. (ISSN: 1545-1003).

**Keywords:** Milk proteins; dairy components; nutrition; review; dairy industry.

## Introduction

Milk proteins play a range of roles which make dairy products and products containing dairy components are valuable. Theses include nutrition, physical functionality and breakdown under controlled condition to produce nutritional, functional or flavour full products. There is also undesirable behavior include fouling of heated surface, gelling in processing equipment during manufacture of some products. All of these behaviors related to the structure, and possible changes in structure during processing of the component milk proteins, an understanding of the structure of milk proteins, and how those structure can change under processing conditions, is therefore, an important enabling tool for the dairy processing industry.

Complete three dimensional structural information about a protein can be obtained experimentally in one of two ways: X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy. There are more than 12000 sets of crystal structure coordinates for a wide variety of protein in the protein Data Bank, the so-called PDB coordinates (Berman et al .,2000), together with over 2000 sets of coordinates derived from NMR data. Not all of these sets of data are unique; many are of the same protein but with different small-molecule ligands, crystallized under different conditions of temperature and/ or pH, from different species, or with one or two amino acid residues mutated. However, each set of coordinates in the data base represents a unique structure determination. In spection of this vast data base reveals that there are relatively few families of proteins, probably about 1000, that are distinctly different in structure. Thus, most but not all, new structure can be assigned to an already known family of structures (Murzin et al., 1995, Thornton et al., 1999) which in turn makes the determination of new structure easier. Theoretical methods of protein structure prediction can be employed. Prediction

methods are still being developed and, at the present time, their success depends upon the closeness of the unknown protein sequence to that of a known structure. Generally, the closer the amino acids sequence is to that of known molecular structure, the better is the prediction. (Moult, et al., 1999).

## Milk Whey Proteins

### (1) - Lactalbumin :

Two genetic variants, A and B of this protein exist. They differ by a single substitution, A having Gln. And Behaving Arg. At position 10. The B variant is the only one in the milk of European breeds; both A and B occur in Indian cattle. Some minor forms of bovine - lactalbumin are revealed by electrophoresis. Some these contain covalently bound carbohydrate groups; the major components of bovine - lactalbumin are devoid of carbohydrates others of minor components seem to have fewer amide groups than the major ones, and one minor - latalbumin containing three instead of four disulfide. In total the minor components donor account for more than 10% of the -lactalbumin. The amino acid sequence of -lactalbumin is similar to lysozymes. Indeed, bovine -lactalbumin B and chicken egg white lysozyme have identical amino acids residues at 49 positions, and the four disulfide groups are located identically; they are at 6-120, 28-111, 61-77 and 73-91 in -lactalbumin. It is considered that -lactalbumin arose in evolution by gene duplication of an ancestral gene coding for lysozyme. Although the conformation of -lactalbumin has not been defined completely by X-ray crystallography, preliminary indications are that its structure is similar to that of the well defined lysozyme. The two proteins must have very different active centers, however, since neither has the biological activity of the other; nor do they interfere with each others activity. The biological activity of -lactalbumin is interaction with galactosyl transferase to promote the transfer of galactosefrom UDP galactose to glucose to

form lactose. The nature of the effects of LA was used to develop a model for the arrangement of binding sites for acceptor substrates and LA on galactosyl transferase. Fig (7). The acceptor binding site of galactosyl transferase is viewed as containing at least two subsides that are capable of binding monosaccharide, one of which can bind free N-acetylglucosamine or glucose with low affinity substrate (S1) while the second (S2) binds an additional sugar in extended substrates. In the model, the binding of LA at this site in galactosyl transferase, adjacent to the monosaccharide substrate binding site, brings an eight boring site in LA close to S1 this site in LASLA, which is proposed to be region corresponding to part of the lysozyme active site cleft, forms favorable, stabilizing interactions with a monosaccharide bound to S1. Secondary structure including four  $\alpha$ -helices, several regions of 10 Helix and  $\beta$ -pleated sheet table (2)

## (2) $\alpha$ -Lactoglobulin:

### **Effect of pH on the structure of $\alpha$ -lactoglobulin**

The properties of  $\alpha$ -lactoglobulin have been examined since the 1930 by effectively every available technique (Sawyer & Kontopidis, 2000).  $\alpha$ -Lg from ruminant milk is polypeptide of 162 amino acids that exists as a dimer. The pH affects the structure through a series of reversible conformational changes leading to dissociation at both high and low pH. Four out of the five cysteine residues from two disulphide bridges, 66-160 and 106-119. Leaving cys 121 as the free thiol that appears to be responsible through disulphide interchange, for the formation of aggregates upon heating (Manderson, et al., 1999). Between pH 6 and 8 there is a significant change in the reactivity of the free thiol, the disinterment of a carboxyl, now known to be Glu 89 (Qin et al., 1998) and the opening up the central, legend  $\beta$ -binding site. The legends that bind  $\alpha$ -Lg tend to be hydrophobic and include fatty acids, cholesterol and more weakly, hydrocarbon molecules such as toluene and pentane.

### **Structural Variations of native bovine $\alpha$ -Lg:**

Both NMR and X-ray methods reveal the same general structure for bovine  $\alpha$ -Lg, even though NMR experiments were done at pH 2 where the protein exists in monomeric form, and the X-ray diffraction experiments span the range of pH 6-8.1, where in all cases, including those where  $\alpha$ -Lg is liganded to fatty acid. A common dimeric structure is observed. (Jameson et al., 2002)

### **Denaturation of bovine $\alpha$ -Lg:**

Various schemes of different degree of complexity have been proposed over the years for the thermal denaturation of bovine  $\alpha$ -Lg

Although most agree that the basic steps involve (1) .The dissociation of the dimer into monomers, (2). The loosening of the structure into a form that may be, or at least may be resemble, "molten globule state"(3) the molten state leads to the unfolding of the protein, (4). The formation of aggregates by both disulphides interchange and cross linking, and non covalent interaction (Qi et al., 1997). More recently it has been found that on early step gives rise to non-native monomer  $\alpha$ -Lg that contains disulphide bond in non-native configuration. The temperature, the ionic strength, the pH and the presence of legends and cations are some of the parameters determining this complex process. It is possible to use structure based approach to study a series of changes that lead to gelation or precipitation through the denatured and aggregated state, the structure must be perturbed in some way and the effects must be monitored by X-ray or crystallography or by NMR spectroscopy.

The methods of perturbing the structure are to use a denaturing agent such as urea, to raise the temperature, to modify the protein chemically or by adding legends that make the protein more or less resistant to denaturation, or to vary the pressure. All of this methods have been used,  $\alpha$ -Lg and there are a large number of reports published over the years describing the chemical and spectroscopic effects in solution (Sawyer & Kontopidis, 2000). However, the tertiary structural details resulting from the application of these methods, where they have been applied, is recent.

### **Solvent unfolding:**

Urea as a denaturing agent has been applied recently to study the refolding of  $\alpha$ -Lg by NMR. The protein is first denatured or at least substantially unfolded in urea. The urea is then abruptly diluted, perhaps 20-fold to a concentration well below that required to unfold the protein. As the protein refold, the peptide N-H protons of certain residues lose their ability to exchange with solvent. Those residues, whose amide proton exchangeability is lost rapidly are those that refolded first whereas those that continue to exchange all long times after dilution of the urea are on the surface or in solvent accessible regions of the structure. With  $\alpha$ -Lg, the protein core involving strands F, G and H and the helix form rapidly with the rest of the protein then folding around this central core. One further feature of this process is that there strong evidence of some transient structure (  $\alpha$ -helix) that is not observed in the final native structure. The evidence in this case is that (re-) folding of  $\alpha$ -Lg is a hierarchical process (Kuwata et al., 2001b).

### **Effect of temperature on the structure of $\alpha$ -Lacto globulin**

Monitoring the effects of temperature has been most efficiently carried out using spectroscopic techniques. For example, circular diachronic (CD) or Fourier transform infrared (FTIR) spectroscopy can conveniently be used to show the melting of the three turn -helix of -Lg at around 65 °C (Qi et al., 1997). Crystallized protein at pH 7.6 that had been heated to 60 °C, and the structure at 2.6 Å resolution was that of the native protein this resolution is substantially worse than that typically observed for -Lg, and is a warning that the material crystallized may not be representative of the entire sample, a small part of which may have become irreversibly denatured. Although studies in solution indicate that thermal effects are reversible up to 68 °C (Qi et al., 1997), the differential scanning calorimetric (DSC) technique used would not be sensitive to a significant fraction (up to 10 %) of the protein having become irreversibly denatured. However, many studies have been shown that -Lg at natural pH gradually denatures irreversibly if held at temperatures as low as 59 °C., As not earlier, high-quality protein crystals are best obtained from pure, homogeneous material uncontaminated by other proteins or by material in non native or denatured conformations. Crystal's isomorphism with the native protein crystals will certainly grow at 50° C.

NMR studies also using deuterium exchange techniques at several temperature and pH 2 (Belloque & Smith 1998) reveal that the denaturation occurs in stages at 55 °C strand E and the B loop unfold, and the A strand becomes flexible. At 75°C the A strand and the helix unfold, but the residues in the two sheets, BCD and FGH, are surprisingly resistant to amid H/D exchange. Prolonged heating leads to the formation of a transparent gel and this does not involve disulphide bond cross-linking (Schokker, et al, 2000). At pH 7.4, similar effects are noticed but their rate is faster. Similar, but not identical observation have been made by (Edwards et al, 2002) who used a slightly different denaturation protocol and pure -Lg B.

#### **Effect of pressures on the structure of -Lacto globulin**

Another means of perturbing the structure is to use hydrostatic pressure and there the unfolding of the protein has been monitored by NMR (Kuwata, et al., 2001a). This show that the two B-sheet surfaces appear to unfold independently in excellent agreement with the results referred to above using temperature. The crystal structure of -Lg at ambient pressure, after pressurization to 250 bars in a process that was not completely reversible, shows both reduced unit cell dimension and also a slight contraction of the structure, which was most marked around - strand A.

#### **Stabilization by bound Legends:**

Legend – binding studies of -Lg have been carried out in solution using several different spectroscopic techniques including NMR. (Muresan& de Wolf.2001). IT has emerged from their crystallographic studies that the majority of legends, e.g. palmitate, retinal and cholesterol bind within the central cavity of the protein (Sawyer & Kontopidis, 2000). The effect of this is to increase the stability of the protein to both urea and thermal denaturation in a manner that is expected when a solvent accessible internal cavity is filled (Jameson et al., 2002).

#### **Side-chain modification:**

It is clear that chemical modification of the amino side chains of -Lg leads to changes that often result in the protein becoming more susceptible to denaturation and aggregation. (Morgan etal., 1999) Modification of Cys 121 by most reagents significantly enhanced dimmer dissociation. Thus heavy metal ions, such as mercury or gold, lead to a significant change in both the unit cell dimensions and in the structure of the dimer interface the separation of the ant parallel -strands I and I increase by around 1A° as well as small but significant changes in the structure of the monomer subunits. It is not obvious, however why reaction of Cys 121 should affect the interface because the residue is on the underside of the helix distal to the dimer interface, and moreover, the helices themselves do not interact directly in any of the distinct crystal structure of bovine -Lg.

#### **Site-directed mutations:**

A method somewhat akin to chemical modification as a perturbing influence is that of protein engineering (Batt, 1997). Although significant studies have been made using the natural genetic variation present in the different alleles within one species, and the variation between species, modern molecular biological techniques make site-specific mutation convenient and efficient (Sambrook & Russell,2001). For example, the two "half mixed" forms (D33G with V118, and D33 with V118A) of the A and B genetic variants promise to provide to the reason behind the different stabilities of the two forms. Similarly, C12 1S will remove the ability of -Lg to aggregate by disulphide interchange mechanism. Both porcine -Lg and equine -Lg are monomers at pH 7 but the X-ray structure of the porcine (Hoedemaeker etal.,2002) and NMR structure of the equine (Kobayashi et al.,2000) protein, respectively, show a high degree of similarity to the bovine and ovine crystal and NMR structure. Structural studies combined with site directed mutagenesis will uncover many of the determinants of stability, or instability, of bovine -Lg of importance to milk technology, the physiological role of -Lg to both the lactating cow as donor and the calf as recipient

remains almost as mysterious now as it was more than 60 years ago. Molecular biological techniques have now provided methods to address explicitly these mysteries by examining the physiological effects on both mother and calf of cows that are unable to express -Lg.

#### **Immunoglobulin:**

Immunoglobulin is antibodies synthesized in the response to stimulation by macromolecular antigens foreign to the animal. They are polymers of two kinds of polypeptide chains, light (L) of MW 22,400 and heavy (H). The latter are of several types, including (MW 52,000), (MW 52,000-56,000), and (MW 69,000). Each of the L and H chains consists of a relatively constant and highly variable sequence and appears to be coded for by two genes. IgG1 and IgG2 are each polymers of two light chains and two heavy chains of the type (1 and 2). The chains are joined by disulfide linkages to form two antibody sites, each consisting of the variable portion of an H and L chain. IgG1 and IgG2 have about 2.9% bound carbohydrate and MW of about 150,000. They differ slightly in electrophoresis mobility. IgA and IgM immunoglobulin likewise have the basic structure of two H and two L chains joined by disulfide bridges. In IgA the H chains are of the type, and in IgM they are of the type. IgA is secreted as a dimer of two of the basic four-chain units joined by polypeptide of MW about 25,000,

called J-component, and associated with another called secretor component, Sc. This complex is called secretor IgA (SIgA) and has a MW of about 385,000. The secretor component is a protein of MW about 75,000, consisting of a single polypeptide chain with a number of internal disulfides. It has a relatively high content (10- 15 %) of bound carbohydrate consisting of N-acetylglucosamine, N-acetylgalactosamine, D-galactose, D-mannose L-fucose, and N-acetylneuraminic acid. In addition to the amount that is bound in SIgA, it occurs in the free state in milk in concentrations of 50-100 mg liter

IgM consists of a pentamer of the basic four-chain units joined by the J-component. It has 11-12% bound carbohydrate and MW of 900,000; its diameter is about 30nm. In all cases the sites that bind antibodies consists of the variable protein of an H and L chain in juxtaposition to each other. IgG has two such sites, SIgA has four, and IgM has ten. The immunoglobulin of an animal thus consists of numerous different proteins; each antigen encountered has caused the synthesis of Ig with a different variable portion.

The immunoglobulin in milk can exert and antimicrobial action, particularly IgM, which acts as agglutinin, for instant, against some streptococci. Moreover, cow smilk (but not that of buffalo, goat, or

sheep) contains a cry globulin (mainly consisting of IgM) that is involved in the cold agglutination of milk fat globules and in the attachment of bacteria to fat globules. The immunoglobulin is among the most heat sensitive of the whey proteins.

#### **Protease-Peptone Fraction:**

Fractions of the whey proteins amounting to about 1g per kilogram of milk are not rendered acid-insoluble by previously heating them milk. It has long been called protease-peptone. Four principal groups of components of protease-peptone are distinguishable electrophoretically. The first components, which is probably derived from a fat globule membrane constituent, and components 5,8 fast, and 8-slow, which are derived by proteolysis of casein.

#### **Other whey proteins:**

A group of acid glycoproteins is retained on DEAE cellulose when blood serum or whey is passed through it at pH 4.5. Fractional elution separates a number of components. The total amount that can be obtained from bovine blood serum, colostrums, and milk are respectively, about 2.0, 1.0 and 0.3 g-liter. One protein of this group is acid glycoprotein, formerly called orosomucoid. It has been isolated from human serum, colostrums, and milk, and from bovine serum and colostrums, but it has not been detected in bovine milk. It consists of a polypeptide chain of 181 residues to which five heteropolysaccharide groups are linked to asparagine residues. The carbohydrate constitutes about 45% of the total molecules. The function of this protein is not known. In any event, acid glycoprotein comprises only a small portion of the acid glycoproteins obtainable from fractionation of colostrums or milk on DEAE cellulose. Five other fractions have been obtained in varying states of homogeneity. All contain carbohydrate and phosphate and promote the growth of *Bifidobacterium bifidum* var. *pennsylvanicus* (formerly *Lactobacillus bifidus*). The possibility that some of these glycoproteins represent partial degradation products of caseins or membranematerials has not been elucidated.

A specific protein that binds folate (FBP) has been isolated from milk. Affinity chromatography on sepharose to which folate has been attached is especially effective in isolating this protein. Its concentration in normal milk is about 8mg/kg.

#### **Fat globule membrane protein:**

The fat globule membrane contains approximately 50% protein and accounts for about 15 of the total protein of the milk. Some of the protein constituents of the membrane are enzyme but it is not possible at present to estimate the ratio of enzymatic and non enzymatic components. The fat globule

membrane proteins are difficult to resolve analytically and to separate privately because they interact strongly with one another and with lipids.

### **2-Microglobulin:**

This protein consists of a single polypeptide chain of about 100 amino acids, residues and MW of 11800. It is present in several body fluids and in membranes of various type4s of cells. Its amino acid sequence indicates homology with the constant regions of immunoglobulin light and heavy chains.

A protein that had long previously been crystallized from bovine milk and designated lactollin called bovine  $\alpha$  microglobulin. It is a polypeptide of 98 residues, two of which are Cys.

Direct analysis for microglobulin concentration have not been made in bovine milk; the amounts of lactollin that have been isolated from colostrums and milk are about 6 and 2 mg liter respectively.

### **Lactoferrin and Transferrin**

Two iron-binding proteins are found in milk. One of them, transferrin (Tf), is a common blood plasma protein; the other , lactoferrin (Lf), is secreted not only by mammary glands but also by kidney and endometrial mucosa. Both Tf and Lf appear to be large single-chain polypeptides of 600-700 residues. Reported molecular weight differ somewhat; recent work favors 75000 to 77000 for Tf, but values for Lf are not so consistent either 700 or 93000 being reported, In both proteins about 4 mol% of the residues are Cys, and both have covalently linked carbohydrate consisting of N-acetylgucosamine, mannose, galactose, and N-acetylneuraminc acid. All transferrins and lactoferrins yet worked with appear to bind 2 mol of Fe + per mole. Tf and Lf differ markedly from each other on amino acid composition and in electrophoretic mobility. They can be detected readily in electrophoretic patterns by autoradiography with Fe. Electrophoreticx patterns of milk and boold preparations from individual animals reveal the occurrence of genetic variants of both proteins. No immunological cross-reaction between Tf and Lf has been demonstrated even when both are from a single species. Amino acid analysis and partial sequences of human Lf and Tf indicate some degree of homology between the two and some internal homologoyf peptide segments within each; sequencing is far from complete, however, Both Tf and Lf can be determined quantitatively in a biological fluid by immunodiffusion using a specific antiserum. The concentrations and ratios of Tf and Lf in milk vary greatly among species and with stage of lactation. The concentration of Lf in colostrums is about 1250 mg/kg <sup>1</sup>; in mid lactation the concentration falls to less than 100mg/kg <sup>1</sup>.

concentrations of Yf in milk have not been determined accurately but may be similar to those of Lf. Lactoferrin is an inhibitor of bacteria because it deprives them of iron. The concentration of Lf in bovine milk is so low, however, that it does not exert any significant antibacterial effect.

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6/1/2010

# The Application Of Micro-Relief Meter For Soil Tillage Studies

M.A.Ghazavi<sup>1</sup>, A.Yosufvand<sup>1</sup>, B.Hosseinzadeh<sup>1</sup>

<sup>1</sup> Department of mechanical farm machinery, University of Shahrekord, Shahrekord 115 Iran

[Bahram\\_hs@yahoo.com](mailto:Bahram_hs@yahoo.com)

**Abstract:** Measuring the physical properties of soil provides a good opportunity for careful study of processes such as evaporation from the soil surface, formation of water runoff, sediments and erosion. In this research, the change of some soil hydrological properties was studied in four kinds of primary soil cultivation activities by using a mechanical micro-relief meter. This study was conducted in a randomized complete block design. Data was collected in frames with  $9025 \text{ cm}^2$  area and 400 data height were collected for every frame. Measured soil properties were: Root Mean Square (RMS) of height data, superficial Profile Length Ratio (RZ) of soil roughness, Infiltration Recession Factor (RECS), plough depth, the volume of displaced soil, comparison of the area change in relation to superficial evaporation of soil. The RMS of height data was higher ( $p<0.05$ ) with moldboard plow and modified disk plow than chisel and traditional ploughshares. The analysis of height data collected from plots showed that surface evaporation of soil moisture didn't differ by plowing with moldboard plow or traditional ploughshares, compared with two other ploughshares. This statistic was less than the recorded value of developed dish-like ploughshare ( $p<0.01$ ). Also displaced soil mass by these two ploughshares was much more than chisel and traditional ones ( $p<0.01$ ). The developed ploughshare prevents more evaporation. Therefore, plowing surface with this instrument provides high pot-hole store and penetration coefficient compared with other ploughshares. [Journal of American Science. 2010;6(11):85-89]. (ISSN: 1545-1003).

**Keywords:** RMS of height data, RZ, plough, height data, surface evaporation, pot-hole store

## Introduction

The data derived from a micro-relief meter is used in graphic technology and models and geography information system. These data is used to specify channel networks and characterize the location of micro-relief by analysis.

To conserve soil moisture in semiarid areas, superficial evaporation of soil should be prevented. In different of soil cultivation operations, the amount of upper area of plough exposed to evaporation is different so superficial evaporation of soil changes in proportion to the type of soil cultivation. The surface exposed to soil evaporation is obtained by numeral data analysis of roughness. Elliot et al (1997) stated that photometric technology is a good method because of its vast data collection with regard to its collection of more data in every square meter, but it is better to use a brook meter with photometric method. Pini et al (1997) made a laser micro-relief meter which was used by Sevelbent in 2003 to detect spatial distribution plan of sedimentation and micro-morphology. Nicola et al (1999) showed that during heavy rainfall, the physical properties of soil change significantly. They measured change of soil surface roughness by using a laser micro-relief meter. Gazavi et al (1999) compared the operation of moldboard and disk plower. The measured changes consist of the analysis of soils' measure, resistance against penetration, apparent and specific mass, and

roughness of the land surface, existing humidity and the amount of mixing materials in soil. They found that developed dish-like ploughshare has better performance in higher speeds. Kamphorest et al (2000) compared the ability of roughness indexes to define maximum pothole store. Five indexes were computed using Digital Elevation Model (DEM) as following: random roughness (RR), tortuosity (T), limiting elevation difference (LD), slope (LS), and Mean Unslop Depression (MUD). Regression analysis on five indexes showed that RR is the best definition about pothole store. Oliver et al (2000) made an automatic micro-relief meter to measure elevation distribution in 5cm network in order to use distribution equation (effusion) of rain erosion. Also, he presented an electronic micro-relief meter with 5cm networked pattern in 2001 and an algorithm to compute the capacity of soil superficial store with millimeter unit in 2002. Michael et al (2003) indicated that using the technique of "acoustic backscatter" shows similar evaluation with roughness statistics of laser micro-relief meter. When roughness degree was sufficiently measured by "acoustic backscatter", the difference between laser micro-relief meter and "acoustic backscatter" technique to compute RMS was less than 9% and for correlation length was less than 13%.

The rainfall modeling, penetration, store and water flowing procedures have been performed by

Brasington and Smart (2003), Betts et al (2003), Darboux and Huang (2005). The geometric definition of soil surface is needed in all models to determine roughness quantity and maximum superficial store.

Carvajal et al (2006) studied the relationship between digital model of ups and downs and estimation accuracy of maximum pothole store. They achieved a prediction model to estimate the error of maximum pothole store. In their study, usable data were roughness of soil surface and analysis of digital model. Arvidsson et al (2006) computed ups and downs of soil surface using a micro-relief meter with 0.64m area to study moisture effects of soil during plough and showed that in moldboard plowing in low soil moisture conditions, soil softness is more than plowing with chisel. The change in soil surface elevation has been performed in primary soil cultivation by measurement. Their study investigated individually the effect of several kinds of soil cultivation on soil physical properties. Mechanical micro-relief meter was used to remove roughness data of the surface. Measured properties were RMS of height data, superficial RZ of soil roughness, RECS, plough depth, the volume of displaced soil, comparison the rate of soil porosity and area change in relation to surface evaporation of soil.

### Material and Methods

Measurements were performed in Khatoonabad research farm of Azad Islamic University, Khorasan branch, in July 2007. Khorasan is located in east of Esfahan and with 51° 46' longitude and 32° 38' latitude. The farm soil has heavy clay texture and the wheat residues of previous season were in farm. The study was done according to randomized complete blocks. Four kinds of soil tillage machines moldboard plow, including converter, used chisel plow, traditional plow and modified disk plow (Yule and Roddy 1994; Ghazavi 1997) were used on three adjacent blocks. The width and length of every plot were 3 and 25 meters, respectively and slope of all plots was (0.004) and vertical on them (0.002). After soil plowing on plots, height data was measured by mechanical micro-relief meter. Micro-relief meter consisted of one row of pins which could be used to measure one row of height data on graded sheet by contacting the tip of pins ground. The height data could be read by the position of pin top tip from the graded sheet. Finally one plot with 9025 cm<sup>2</sup> area and 5cm networking were measured, and 400 height data were obtained for every set of micro-relief meter. Micro-relief meter provide data by which the surface of different lands after plough with different tillage machines can be compared. The erosion is studied by the data of surface roughness before and after rainfall

in different times. Furthermore, soil pothole store can be measured and according to rainfall statistics, land's roughness can be modified by soil tillage machines to prevent water flowing. This provides a good practical method for preventing erosion in both agricultural and grasslands.

The measured data was rendered as matrixes with coordinates (x , y , z) and in the form of digital elevation model (DEM) data. Then data of numeral model was transferred to the computer and its relevant micro-topography with drawn by SURFER (V.8) software, then parameters of these micro-topography were computed. The statistical analysis of data was done by SAS (v.8) software. Two factors were used to determine roughness indexes with RMS and superficial RZ. RMS is height numeral data and superficial RZ is obtained as following:

$$\text{RZ} = \frac{\text{the upper area of frame unit}}{\text{the area of frame unit}}$$

The more area of roughness is the more soil surface in direct contact with air increases. So evaporation increases that this matter is effective in wasting of water in arid and semiarid areas. Therefore, the surface exposed to evaporation was computed in different tillage's and then compared with each other. Also, positive micro-topographic volume was computed in high level of plough depth and called soil displacement volume for every plow. EUROSEM (The European Soil Erosion Model) has been designed to simulate the erosion of water and sediment by using grooving procedures. In this model, application scale is farms and small areas. EUROSEM uses two parameters to determine soil roughness. These parameters are RFR (roughness measure) and RECS. RECS is the mean of elevation difference between highest and lowest of width section of soil. This study uses RECS to specify the tool of soil cultivation. Micro-relief meter was used to determine RECS.

### Results and Discussion

Figure 1 shows the micro-topographies of some plots. The results of variance analysis have been shown as tables. According to these tables, RMS of plowed soil with moldboard plow is more than RMS of chisel and traditional ( $p < 0.05$ ). RMS of modified disk plow did not have differ with moldboard plow. In result, these two machines have the most roughness and pot-hole store. Superficial RZ of plots showed the same RMS order of height data. Also, the area exposed to evaporation and superficial RZ show meaningful difference in 1% statistical probability level. The analysis of removed height data showed that superficial evaporation of soil moisture does not indicate significant difference between moldboard

plow and composite plows (the evaporation in modified disk plow is less than moldboard plow because of plant remains on land surface, while for two others is less ( $p<0.01$ ) than modified disk plow). Table 5 shows the values of RECS measured for

tools. The coefficient of variation for measurements of elevations RMS is 12.7%. Where RZ and S had values 4.9% and 3%, respectively, showing appropriate accuracy of measurements.

**Table (1): Analysis of variance for root mean square of elevation data**

Source	DF	Adj MS	F
R	2	0.3756	2.43 <sup>ns</sup>
T	3	1.4248	9.21*
Error	6	0.1574	

ns, non significant ( $p>0.05$ )

\* and \*\*, significant at 0.05 ( $p<0.05$ ) and 0.01 ( $p<0.01$ )

**Table (2): Analysis of variance for RZ**

Source	DF	Adj MS	F
R	2	0.00142	1.12 <sup>ns</sup>
T	3	0.019810	15.69**
Error	6	0.001263	

ns, non significant ( $p>0.05$ )

\* and \*\*, significant at 0.05 ( $p<0.05$ ) and 0.01 ( $p<0.01$ )

RZ, superficial Profile Length Ratio of soil roughness

**Table (3): Analysis of variance for S**

Source	DF	Adj MS	F
R	2	115756	1.13 <sup>ns</sup>
T	3	1614195	15.71**
Error	6	102749	

ns, non significant ( $p>0.05$ )

\* and \*\*, significant at 0.05 ( $p<0.05$ ) and 0.01 ( $p<0.01$ )

S, area change in relation to superficial evaporation of soil

**Table (4): Mean analysis**

Tillage type	RMS	RZ	S (cm <sup>2</sup> )	V (cm <sup>3</sup> )
Moldboard plow	3.969(a)	1.286(a)	11607(a)	105327
Modified disk plow	3.404(ab)	1.284(ab)	11320(ab)	107723
Chisel	2.831(bc)	1.168(bc)	10538(bc)	71752
Traditional plough	2.282(c)	1.108(c)	10000(c)	50368

RMS, root mean square of elevation data

RZ, superficial Profile Length Ratio of soil roughness

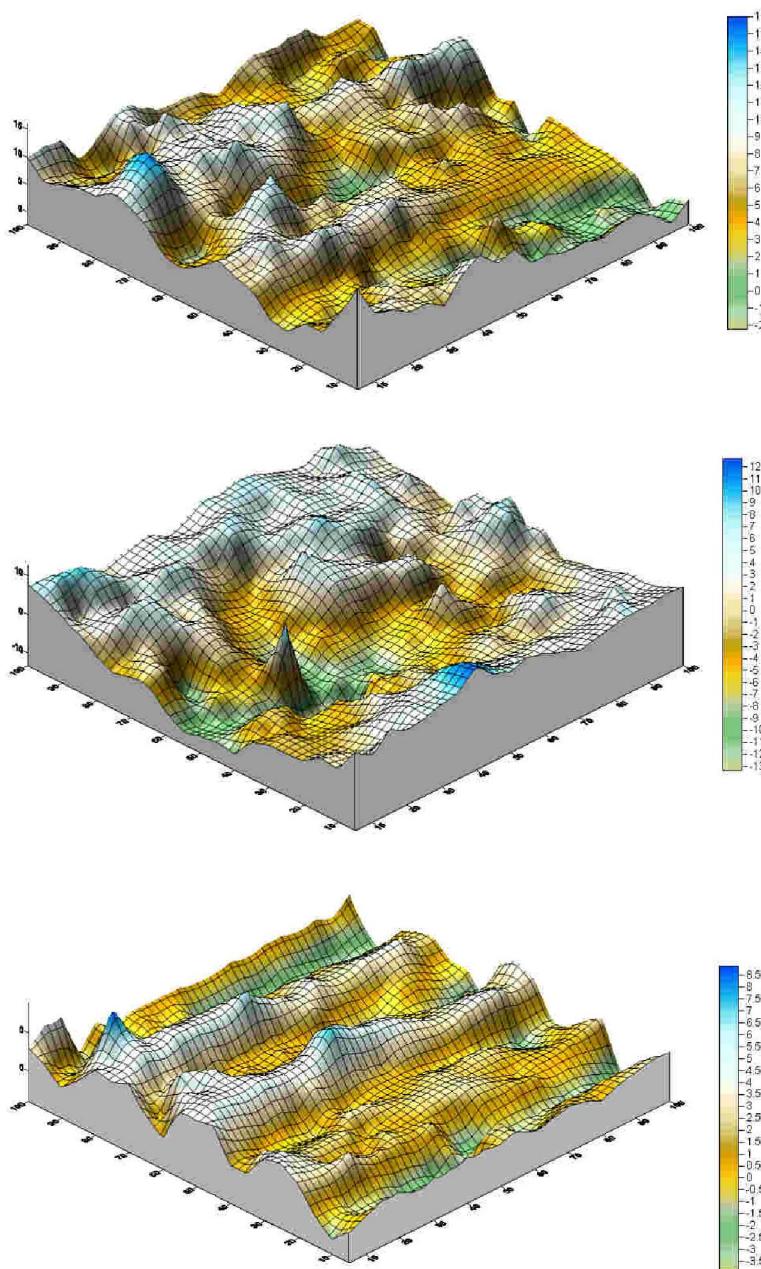
S, area change in relation to superficial evaporation of soil

V, volume of displaced soil

**Table (5): RECS measures for tillage machines**

	Moldboard plow	modified disk plow	Chisel	Traditional plough
RECS	17.33	15.42	10.21	8.35

RECS, infiltration recession factor

**Figure (1): The micro-topographies of some plow.**

### Conclusion

Displaced soil by modified disk and moldboard plows was much more than chisel and traditional plows. Because of high soil displacement and pothole store, the modified disk plow displaces soil with an angle less than moldboard plow. It can preserve soil moisture and prevents water evaporation. Therefore, plowed soil surface with this machine had high pot-hole store and penetration coefficient, reduces water run off, Prevents soil surface crusting and soil erosion in comparison with moldboard plows. In addition it would be possible to work at higher speed by using a more powerful tractor. Although, the moldboard plow is a standard tillage tool. The new one with above mentioned benefits can be used as an alternative machine for tillage operations and rainwater storage system.

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## Variations in some heavy metal concentrations in soil and *Manihot esculanta* tuber from the East and North eastern part of Nigeria

S. T. Garba,<sup>1\*</sup> J. T., Barminas,<sup>2</sup> and A. H. Santuraki,<sup>1</sup>

<sup>1</sup>Department of Chemistry, University of Maiduguri, Borno state. P. M. B. 1069. Nigeria.

<sup>2</sup>Department of Chemistry, Federal University of Technology Yola (FUTY), P. M. B. 2076. Adamawa state. Nigeria. [stelagarba@yahoo.com](mailto:stelagarba@yahoo.com)

**Abstract:** The levels of the heavy metals: Cr, Cd, Pb, Cu, Zn and Mn were determined in *Manihot esculanta* tubers and the soil used for its cultivation. This was done to asses the pollution level of the farmland and hence the safety status of *Manihot esculanta* tubers produced. Samples were collected from Konduga local government area of Borno state in the North East and from Umuahia local government area of Abia state in the East, all in Nigeria. These were treated and digested and the heavy metal concentrations were determined using Atomic Absorption Spectroscopy (AAS). The result obtained shows that, the samples from the North East had the highest levels of the elements Pb 30.14, Zn 88.65, Cd 3.15, Cu 16.00, Cr 6.74, and Mn 13.00 ppm in the soil sample while 5.47, 9.09, 5.05, 2.60, 3.37 ppm for the elements Pb, Zn, Cu, Cd, and Mn respectively were observed in *manihot esculanta* tubers sample. All these were found higher than what was observed in the soil and *manihot esculanta* samples collected from the East. Cr, was however, found below detection limits in *manihot esculanta* tuber samples from both the regions. [Journal of American Science. 2010;6(11):90-94]. (ISSN: 1545-1003).

**Keywords:** Health, Safety, Environment, Development, Borno State, Abia State, Nigeria.

### INTRODUCTION

Food safety is the major public concern world wide, the increasing demand for food has stimulated research regarding the risk associated with the consumption of food stuffs contamination by pesticide, heavy metals and/or toxins (D'Mello, 2003). Plant constitute essential component of our daily food, contributing protein, vitamins, iron, calcium and energy which are must needed by our system (Thompson and Kelly, 1990). However, these plants may contain both essential and toxic elements, such as heavy metals, at a wide range of concentrations (Bahemuka and Mubofu, 1999).

Heavy metal pollution of the environment even at low concentrations and their resulting long-term cumulative health effects are among the leading health problem all over the world (Oluyemi *et al.*, 2008). Metals such as Pb, Cr, Cd and Cu are cumulative poisons. These metals cause environmental hazards and are reported to be exceptionally toxic (Ellen *et al.*, 1990). Bioaccumulation of Pb for instance, interferes with the functioning of mitochondria, there by impairing respiration, and also causes constipation, swelling of the brain, paralysis and eventual death (Chang, 1992). Contamination of plants with heavy metals may be due to irrigation with contaminated water, the application of fertilizers and based pesticides, industrial emissions, transportation, the harvesting process, storage and/or at the point of sale (Maleki *et al.*, 2008). The use of dumpsite as farmlands is a common practice in urban centres in

Nigeria because of the fact that decayed and composted waste enhances soil fertility (Ogunyemi *et al.*, 2003). These metals often contains heavy metals in various forms and at different contamination levels. Some heavy metals like, As, Cd, Hg and Pb are particularly hazardous to plants, animals and humans (Alloway and Ayres, 1997).

Human beings are encouraged to consume more of edible parts of plants, vegetables and fruits, which are a good source of vitamins, minerals, fibre, energy and beneficial for health (Maleki *et al.*, 2008). Soil is a vital resource for sustaining two human needs of quality food supply and quality environment. Plants grown on a land polluted with municipal, domestic or industrial waste can absorb heavy metals inform of mobile ions present in the soil solution through their roots or through foliar absorption. These absorbed metals get accumulated in the roots, stems, fruits, grains and leaves of plants (Fatoki, 2000). Plants roots have an ability to take up significant quantities of lead while simultaneously greatly restricting its translocation to above ground (Lane and Martins, 1977).

The importance of these plants as food and energy not only to human but other domesticated ruminants further prompts the interest in wanting to know the level of these heavy metal pollutants in the plants and the soil where they grow. By accumulating metals in above-ground level tissues, plants can transfer heavy metal pollutants from soil into the food chain, and this accumulation is one of the most serious

environmental concerns of the present day not only because of the phytotoxicity of many of these metals to the plants themselves, but also because of the potentially harmful effects , toxic metals could have on animal and human health (Radojevic and Bashkin, 1999).The monitoring of heavy metals in crop plants and other foods is therefore of great importance in protecting the public from the hazards of toxic pollution.

Cassava is important, not only as a food crop but even more so as a major source of income for rural households. It is largely consumed in many processed forms in Nigeria. Its use in the industry and livestock feed, is well known, but is gradually increasing, especial import substitution becomes prominent in the industrial sector of the economy. As a cash crop, cassava generates cash income for the largest number of household in comparison with other stables and as a food crop, cassava has some inherent characteristics which make it attractive, especially to the small holder farmers in Nigeria. First, it is rich in carbohydrates especially starch and consequently has a multiplicity of end uses. Secondly it is available all year round, making it preferable to other, more seasonal crops such as grains, peas and beans and other crops for food security. Compared to grains, cassava is more tolerant of low soil fertility and more resistant to drought, pest and diseases. Furthermore, its roots are storable in the ground for months after they mature. This study therefore was carried out to asses the contamination level of the soils and the extent to which the cassava tubers grown on the soil were exposed to heavy metals, and hence the safety levels of the tubers for consumption.

## MATERIALS AND METHODS

### Sample and Sampling Area

Five spots at a distance of 50 metre from each other were mapped out for the sample collections. Samples were collected using clean stainless steel trowel from 0 - 10cm depth. Cassava (*Manihot esculanta*) tubers grown on the soil were randomly collected with stainless steel trowel and knife. In the North East samples were collected in Konduga local government area of Borno state while in the East samples were collected from Umuahia local government area of Abia state, Nigeria. The Cassava tuber samples were identified by a botanist in Biological science department, university of Maiduguri, Borno state.

### Sample Preparation and Analysis

The collected dried soil samples were thoroughly homogenized in a clean plastic bucket to

obtain a representative sample, crushed and sieved with 2 mm mesh and stored in a well - labelled polythene bags before analysis. The cassava tubers were washed with water, sliced and dried to a constant weight in an oven at 70°C. The dried samples were pulverized using a pestle and mortor, and kept in polyethylene bags for further analysis. The soil samples were digested in a crucible with a mixture of HNO<sub>3</sub>, HCl and HF acids. Similarly the dried powdered cassava tubers were digested with a mixture of 60% HClO<sub>4</sub>, concentrated HNO<sub>3</sub> and H<sub>2</sub>SO<sub>4</sub> acids. Blanks were also prepared to check for background contamination by the reagents used. The digested samples were analyzed for heavy metal concentrations using atomic absorption spectroscopy (AAS) model Unicamp 969 in Jos mining coporation, Plateau state, Nigeria.

## STATISTICAL ANALYSIS

Statistical analysis using Student's t-test showed that the soil texture, pH and organic matter content of the soils were not significantly different.

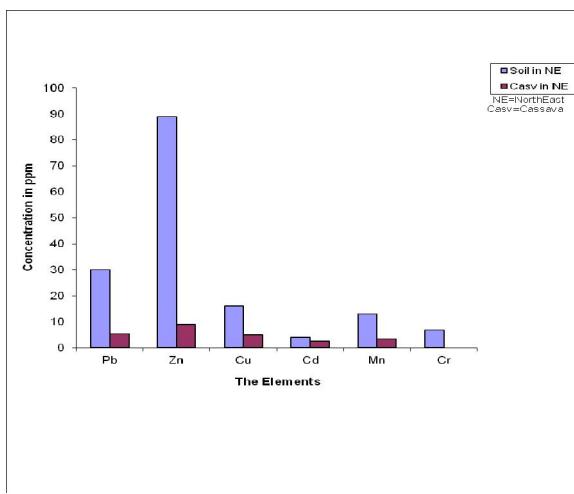
## RESULTS AND DISCUSSION

The soil properties obtained in this study are as shown in **table 1**.The percentage sand and clay were observed to be high in the soil samples from the east. This soil was found to be sandy clay loam in texture while, the soil samples from the North-east was found to be sandy clay in texture. The pH of the soil samples from the east were found to be slightly alkaline (7.4) with higher organic matter content while the soil from the North-east were found slightly acidic (6.65) with low organic matter content. The high pH level observed from the East may probably be due to the high organic matter content. This region also experienced high and frequent volume of rainfall which dilutes the soil solution leading to the high observed pH level.

The high organic matter content from this region may be due to the presence of to-much plant waste, frequent rainfall coupled with the high humidity level which catalyzed the decomposition of the plant waste. Organic matter plays an important role in soil structure, water retention, cation exchange and in the formation of complexes (Alloway and Ayres, 1997). In the North-east, the low pH level, may be attributed to the low and non-frequent rainfall, punctuated at times by dry air and high temperature hence low level of organic matter content was observed. At low pH level, metals are more soluble in the soil solution and therefore more available for plants use. Hence toxicity problems are more severe in acidic than in alkaline soils (Oluyemi *et al.*, 2008).

The heavy metal concentration in soil and cassava tubers from the East and the North-east are as

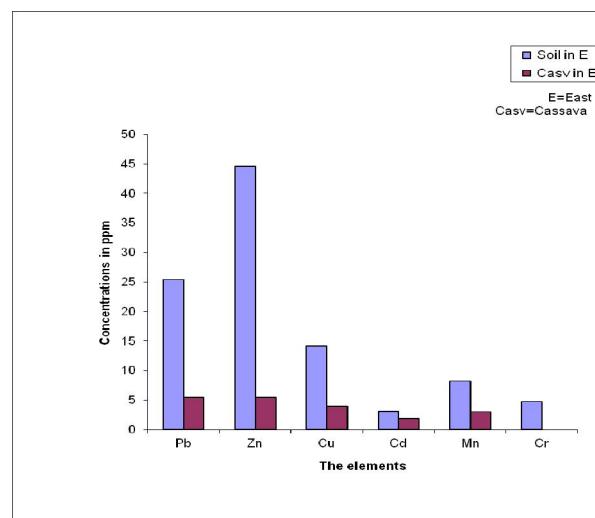
shown in **table 2**. The levels of these heavy metals in the samples from the North-east were found to be higher than what was observed in the samples from the East. This might be due to the dusty nature of the North-east region. Although samples were collected in the raining season, the rainfall was not frequent and heavy enough to wash or leached the deposited aerosol particulate matter. These aerosol particles originate mainly from the sahara desert from the neighbouring countries that share border with Nigeria in the North-east in the form of sandstorms. The sandstorm picks along with it a lot of aerosol particulate matter, loaded with heavy metals which get precipitated on the soil or plant by either rain or brought down by gravity under calm condition. Hence the high levels of these heavy metals in the soil and cassava tubers samples from the North-east than what was observed in the samples from the East. Figure 1 and 2.



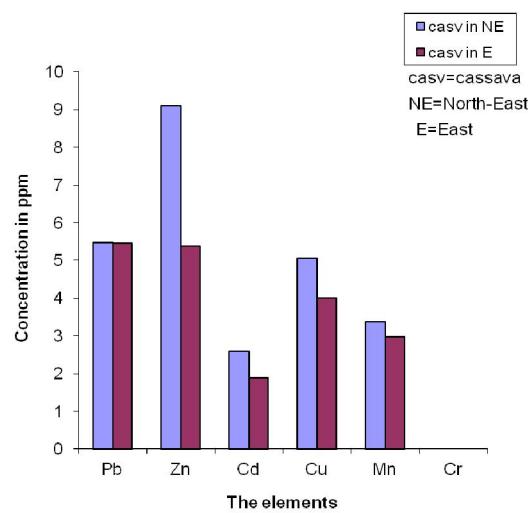
**Figure 1. Variation in the mean concentration of elements determined in Cassava and Soil From the North Eastern Part of Nigeria.**

As to the safety of the cassava tubers analyzed in this study regard to the levels of these heavy metal pollutants, it shows that, the level of contamination by Pb was 5.47 and 5.46 ppm in the samples from North - East and the East respectively. Zn had 9.09 and 5.34 ppm, Cd 2.60 and 1.88 ppm, Cu 5.05 and 4.00 ppm, Mn 3.37 and 2.98 ppm respectively. While Cr was found below the detection limits as shown in figure 3. It has been reported that, the normal non-toxic range of these metals in plants are: 0.2 - 20 mgkg<sup>-1</sup> for Pb, 1 - 400 mgkg<sup>-1</sup> for Zn, 0.2 - 2.4 mgkg<sup>-1</sup> for Cd, 5 - 20 mgkg<sup>-1</sup> for Cu and 20 - 1000 mgkg<sup>-1</sup> for Mn and the critical levels above which toxicity is likely are: 5 - 30 mgkg<sup>-1</sup> for Cd and Cr, 20 - 100 mgkg<sup>-1</sup> for Cu, 100 - 400 mgkg<sup>-1</sup> for Zn, 30 - 300 mgkg<sup>-1</sup> for Pb and 300 -

500 mgkg<sup>-1</sup> for Mn. Although the levels of metals observed in this study were slightly high, with the exception of Cr which was not detected, the levels are found within the range of the normal, non-toxic levels reported by Alloway, (1995).



**Figure 2. Variation in the mean concentration of elements determined in Soil and Cassava from the Eastern part of Nigeria**



**Figure 3. Variation in the levels of the heavy metals determined in cassava tubers from the East and the North-Eastern part of Nigeria**

**Table 1:** Soil properties

Soil	Depth (cm)	% Sand	% Clay	% Silt	pH	% Organic matter Content
Soil from Konduga, Borno State in the North-east	0 – 10	55.96	28.14	15.90	6.65	6.64
Soil from Umuahia, Abia state in the east.	0 – 10	58.54	22.23	19.23	7.4	7.54

**Table 2:** The mean  $\pm$  SD of heavy metal concentration in soil and cassava tubers from the East and North - eastern part of Nigeria.

Elements / Sampling Area	Zn	Cu	Cd	Pb	Mn	Cr
Soil from Konduga, the North-East	88.95 $\pm$ 0.02	16.00 $\pm$ 0.02	4.00 $\pm$ 0.03	30.14 $\pm$ 0.03	13.00 $\pm$ 0.03	6.74 $\pm$ 0.03
Soil from Umuahia the East	44.65 $\pm$ 0.03	14.10 $\pm$ 0.02	3.15 $\pm$ 0.03	25.44 $\pm$ 0.03	8.18 $\pm$ 0.03	4.73 $\pm$ 0.08
Cassava tubers from Konduga the North-East	9.09 $\pm$ 0.03	5.05 $\pm$ 0.03	2.60 $\pm$ 0.41	5.47 $\pm$ 0.02	3.37 $\pm$ 0.03	ND
Cassava tubers from Umuahia the East	5.38 $\pm$ 0.03	4.00 $\pm$ 0.03	1.88 $\pm$ 0.16	5.46 $\pm$ 0.02	2.98 $\pm$ 0.01	ND

Key ND = Not detected; SD = Standard deviation

### Conclusion

Food safety is now a global issue. Therefore monitoring the levels of contaminants in food items for proper health management has become imperative and should be encouraged. The consumption of tuber crops nowadays to supplement the energy provided by other food items is becoming higher. Because of this, tuber crops are gaining more financial support from the government of Nigeria to boost production for subsequent exportation. It is therefore imperative to certify the safety in its consumption. The levels of the pollutant observed in this study were found to be within the safe limits of consumption.

### Corresponding author \*:

Shuaibu T. Garba  
Department of Chemistry,  
University of Maiduguri, Borno State. P. M. B. 1069.  
Nigeria.  
Email address: [stelagarba@yahoo.com](mailto:stelagarba@yahoo.com)

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Sumission Date: June 2010

# Exploitation of palms wine in the municipality of Ze (Benin): socio-economic and physical impacts

Ade Romaric Herman<sup>1\*</sup>, Bio Bigou Bani Leon<sup>2</sup>, Luo Zhaohui<sup>3</sup>

1. School of Environmental Studies, China University of Geosciences, Hubei province, 388 lomo Road, 430074 Wuhan, P.R China, , 008613797056028
2. University of Benin, (Abomey-Calavi), General Secretary of university Box: 526- Abomey- Calavi-Benin
3. China University of Geosciences, Department of Environmental Sciences, 388 Lumo Road, Wuhan City, Hubei province, 430074, P.R. China

**Abstract:** The exploitation of palm wine is one of activities which many people from southern Benin particularly the municipality of Ze have engaged. This study aims to analyze the socio-economic and environmental impacts of the exploitation of palm wine in the municipality. The methodology consisted of collecting demographic, agricultural, socio economic data, processing and analysis of data collected in real area based on components of the Leopold matrix. The exploitation of palm wine has changed the agricultural landscape of the municipality of Ze. The soil is becoming unproductive for food crops after a long fallow palm wine causing impoverished land due to overuse. The vegetation is endangered because of the rarity of certain plant species like *Acacia* sp (acacia), *Antiaris africana*, *sapadi Blighia* (bligia tasty), *Chlorophora excelsa*, *Cola nitida*, etc. But, in other aspect this activity is contributing to improve the living conditions of farmers and reduce the production of oil palm in this area. The production of alcohol provides employment to about 42% of the active population in the municipality of Ze, (municipality of Ze, 2007). At various levels of production that is from felling to alcohol distillation there are large numbers of people being employed. [Journal of American Science. 2010;6(11):95-102]. (ISSN: 1545-1003).

**Key words:** palm wine; sodabi; socio- economic and physical impact; municipality of Ze.

## 1- Introduction

Benin is one of the developing countries in Western Africa. Its size is just over 110.000 km<sup>2</sup> with a population of almost 8.500.000. Agricultural is a backbone of Benin in which about 70% of the active population is involved. It represents 90% of exports and contributes more than 40% to Gross Domestic Product (INSAE, 2008). Exploitation of Palm wine for local alcohol production is an activity in which farmers of southern Benin especially the people from the municipality of Ze have been involved. There are different types of palm trees which are palm wine, palm date and palm toddy (Brand and Durosset, 1995). However, this study was focused only on palm oil which has been grown in the municipality.

Previous studies on palm oil have not identified the impacts of exploitation of palm wine on the economic environment of Ze. Most of studies carried out in Benin focused on the palms of the plateau of Adja and Oueme without providing both the main socio-economic and environmental impacts of palm wine exploitation. The goal of this study is to identify and analyze the socio-economical and physical impacts of palm wine exploitation. This analysis is important to the authorities to have a view for mitigation of impacts through: the reduction strategy for felling of palm wine for alcohol production, improved processing techniques,

improved living conditions of farmers and their role. The production of alcohol is specific and important to local inhabitants of southern Benin. Historically, The “sodabi”, drinking palm wine and homemade, was prepared for the first time by the brothers Gbèhalaton Bonou and Sodabi Kiti in the village Sèdjè Houègoudo Allada in the municipality of Ze in Benin. However, constantly growing of the population and the number of agricultural active has promoted the increment production of alcohol. After one decade, production of palm schemes is constantly decreasing in the locality due to periodic and selective felling of palms for the production of alcohol added to climate change.

## 2-Methodology

### 2-1 Area of study

The municipality of Ze is one of the 77 municipalities of Benin located between 6° 34' and 6° 37' North latitude and 2° 10' and 2° 13' longitude in the southern Benin. It covers an area of 543 km<sup>2</sup>. The municipality of Ze housed a population of 58185 inhabitants in 1992. In 2002, the population rose to 68315 inhabitants, an increase of 10130 people in ten years (INSAE). This evolution reduces the arable land. Also, People attach themselves most importantly to the transformation of derivatives of palm oil especially palm wine. Land is moderately favorable

for the cultivation of perennial plants. The choice of this area is also justified by the fact that forest resources are threatened (OVIGEPAF, 2005) and

there are poorer soils as a result of the abuse of palm wine.

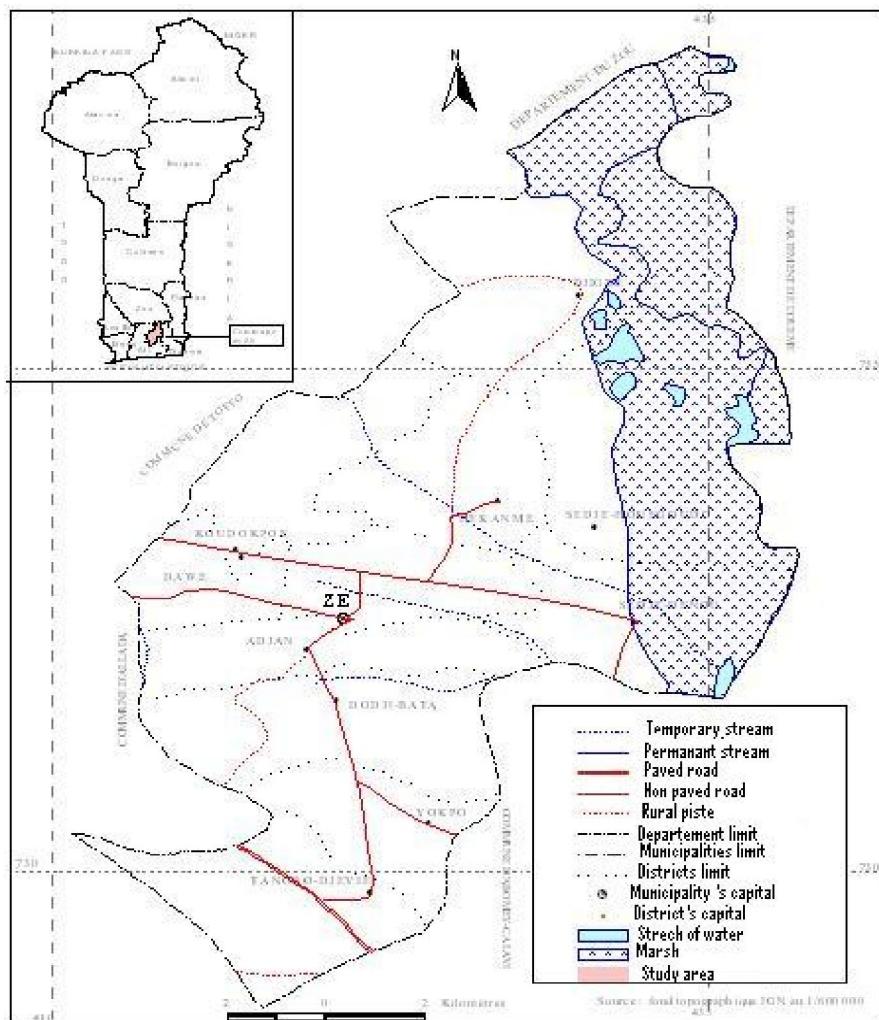


Figure 1: Geographic localization of area of study (Ze)

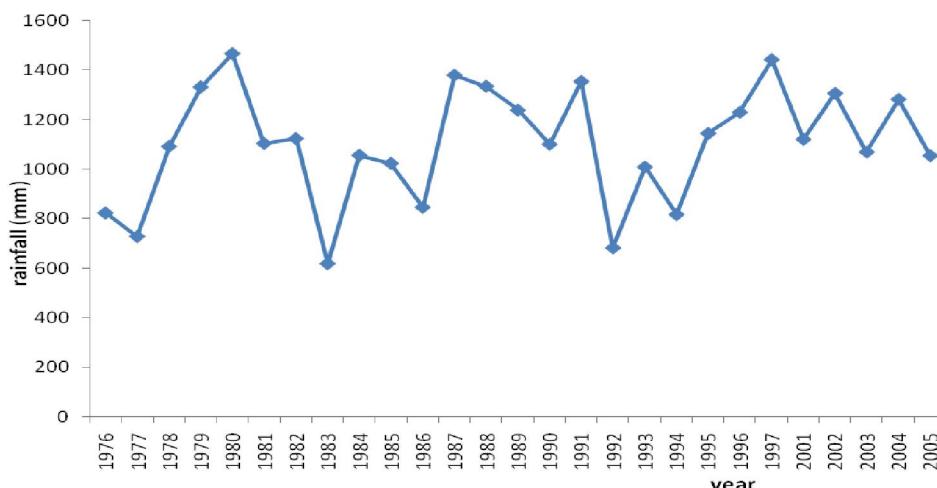
## 2-1-1 Climate

As we know, wine palm is a perennial plant that needs a minimum of 1800 mm of rain per year to grow up. A high and constant temperature with maximum average of about 29 ° and 33 ° and the mean minimum of about 22° to 24° is necessary for the palm wine. Also, they need constant sunshine of at least five to seven hours per day. However, the

municipality is under influence of climate subequatorial whose features are moderately favorable to the exploitation of the palm wine.

## 2-1-2 Precipitation

The average annual rainfall is around 1365.91 mm in this municipality. Figure 2 reflects the rainfall in the municipality of Ze from 1976 to 2005.



**Figure 2:** evolution of precipitation in Niaouilli (1976-2005)

Source: ASECNA, 2005.

The rainfall is generally unimodal, one year in five. The observation of Figure 2 identifies three levels. Of the 30 years the maximum rainfall 1478 mm was in 1987, 1310.7 mm in 1998 and 1395.2 mm in 2001. This situation has not been very favorable for the cultivation of palm trees, since the minimum required for its Growth is 1800 mm / year. This regime has encouraged the development of natural plant species which are threatened daily by the strong human influence.

### 2-1-3 Vegetation and soil

It is an area mostly covered with a large industrial plantation of palm trees organized into five cooperatives with an average of 600 hectares each, and private plantations of natural selected palm trees. The municipality of Ze is characterized by a sedimentary cover moderately favorable for the cultivation of palm trees. There are three types of soil, illustrated in Figure 3 (CeCPA, Ze).

- Ü The land bar or Continental Terminal, which covers more than half of the locality. It is favorable for growing food and palm wine;
- Ü Hydro-morphic soils in the valley of Oxeye at Djigbé, Sèdjè-Denou and Houégoudo. They are not favorable for the cultivation of palm wine;
- Ü Lateritic soils which are also favorable to palm wine.

Overall, the Continental Terminal is mostly favorable to the cultivation of palm wine than other types of soil.

### 2-2 Data analysis

The methodological approach used here was the collection, processing and analysis of data, and interpretation of results. The data collected here was both qualitative (access to land, access to credit, farming techniques, labor, etc.) and quantitative (the profitability of farming, the agricultural workforce, etc.) which allows the determination of parameters to identify the impacts associated with the exploitation of palm wine. There was much focus on those relating to socio-economic, agronomic and biophysical indicators and in addition, the collection of these data required an appropriate method in collecting literature.

#### 2-2-1 Data collection

Firstly, a literature review was conducted which provided guidance in collecting information that was relevant and gave orientation on the choice of the title to this paper. Information related to statistical data on palm wine, the evolution of vegetation cover and the felling of palms, the agricultural economy and living conditions of farmers were collected from different institutions namely: INSAE, INRAB, MAEP, OVIGEPAF, CeRPA and MEPN in Benin.

The second phase was for the field survey. It took place in villages of six districts of the municipality where the wine activity is very intense (Ze centre, Koundokpoe, Djigbé, Adjan, Sèdjè Houegoudo, and Hekanmè). Research units selected were the operators of palm wine or liquor producers as well as the various technical institutions responsible and Elders of the locality.

The choice of sampling was based on three basic criterions:

- Ø Farmer or producer of palm wine in the resort;

- Ø Processor of palm wine from the resort;
- Ø Occupation of the land under palm trees.

These investigations were made on the basis of self-administered questionnaires sent to farmers and processors derivatives of palm wine. The semi-direct have been made for the simple reason that over 50% of people surveyed were illiterate and were unable to complete questionnaires. The interviews were conducted, which helped to confront and complement the information gathered for using questionnaires and focus groups. This technique enhanced community participation at each level of the research.

Finally, observations were made on field to understand types of plantations, the palm operating systems, the state of vegetation cover, the chain of production of alcohol, systems of land tenure, etc.

## 2-2-2 Analytical method

Identification of potential impacts of the exploitation of palm wine on each element of the environment and the socio-economic framework was made by using Leopold Matrix method. It is a two dimensional array used to identify interactions between activities, appearing in rows, and environmental components which appears in the columns. It allowed us to establish the link between wine activity and its impacts on the natural environment. Impacts of the exploitation of palm wine identified were characterized by their nature (direct or indirect), their importance and scope. The overall impact was evaluated according to the magnitude of the impact and value of the element affected.

		susceptible activities and actions cause impacts								
		trunks of palm trees abandoned in fields	investment related to the preparation of alcohol	Sales and marketing channel	distillation of alcohol	extraction of wine	pruning		Remove of waste water	
<b>legend</b>										
1.Minor negative impact										
2. Minor positive impact										
3. Major negative impact										
4. Major positive impact										
0. impact not significant										
Or Impact not determined										
<b>Environmental component</b>										
<b>biophysical area</b>	<b>Soil and geology</b>	1	3	0	0	1	0	0	3	3
	<b>Vegetation</b>	2	3	0	0	0	0	1	0	1
	<b>Wildlife</b>	1	1	0	0	0	0	0	0	0
	<b>Air quality</b>	0	0	0	1	1	0	0	0	3
<b>Socio-economic area</b>	<b>Population</b>	4	4	4	4	4	2	4	0	1
	<b>Health risks</b>	0	0	0	0	1	0	0	0	1
	<b>Profitability</b>	4	2	0	0	4	4	4	0	0

**Table:** Leopold matrix method adapted to the exploitation of palm wine in the municipality of ZE.

In order to understand the magnitude of impacts in the municipality using the Leopold matrix method, two indicators were used which were, the degree of impact on the environment element (dI) and the frequency of impact fI which could allow to establish the nature of the impact. Two formulae have been used:

∨

$$dI = \sum_{i=1}^n \frac{xij}{n}$$

with

- xij is the positive or negative matrix of the environment component which appear frequently
- n= 9

Analysis of degree of impact is based on following criteria: when  $dI < 0.5$  is considered that the element is partially affected,  $0.5 < dI < 1.5$ , the element is said affected and  $dI > 1.5$ , the element is said severely affected.

$$\checkmark \quad fI = \frac{e}{n} * 100 \text{ with}$$

- e is number of positive impact (minor and major) or negative impact(minor and major) on the element
- n=9

### **3-Results and discussion**

Two types of exploitation were observed in the municipality of Ze; the first one is traditional exploitation which is an artisanal process of local alcohol which needs to meet certain conditions and processing techniques before the actual production. This kind of exploitation occurs to both natural and private palm grove users. Second one is selective exploitation which relates to the palm industry and is organized by the authorities of the CAR and UR-CAR in the municipality. Palms which are felled are sold to local alcohol producers. For these two types of exploitation, they both have an implication on socio-economic and environmental impacts.

#### **3-1 Socio-economic impact of exploitation of Palm wine**

Local alcohol production is providing employment to population of the municipality of Ze. These jobs are at different levels in the chain production. The degree of impact  $dI= 3 > 1.5$  with  $nI= 88.88\%$  for the population and  $dI= 2.11 > 1.5$  with  $nI= 46.6\%$  for the profitability. Analysis shows that more than 33.33% of palm wine producers surveyed employ between 3-12 workers per hectare against 71.41% of wine processors that employ between 5-20 workers per day and per hectare for work on the felling, pruning, and the extraction of wine; in other way how the transformation of derivatives of palm wine is labor-intensive. The manpower recruited running a seasonal periods due to unfavorable wine activities that occur during the year. Also, according to surveys, 80% of producers of palm wine put their legacy parcels in palm grove planting. Thus, the retail foot palm allowed them to meet food needs and fulfill the obligations of customary order. In fact, from 300 feet of natural palm wine felled per hectare, on average 4800 liters of alcohol were produced locally by the processor in the interval of three months. After sales, the gross income per season from the operator was approximately \$969 per month an estimated profit of more than \$80 if the activity covered all year. It means that their income can contribute up to 50% for the family. Furthermore, in

the case of processors who sorely depend on this business, the production exceeds 4800 liters per farmer.

In the districts where investigations were conducted, the production of alcohol occurs only in dry season which is the peak period; the rainy season was often reserved for fieldwork. Figure 2 shows information on the proportion of each activity by peasant farmers from the total income.

The production of alcohol remains the main financial source. The income from this production is used for household, health care,etc. The evolution of alcohol production, reduced production of palm oil in the municipality of Ze has led to the scarcity of palm schemes which are also linked to climatic conditions, the pruning of young seedlings, especially the felling of palms. The low productivity of palm oil is also due to high costs and number of cheap laborers employed throughout the transformation process scale. According to the surveys, 80% of palm oil producers used an average 15 workers per day against an average of 8 workers in the alcohol producers. Figure 3 shows the frequency of each product from the processing of derivatives in the municipality of Ze.

#### **3-2 Environmental impact**

The exploitation of palm wine affects two major components of the environment. From the analysis, it has been shown that  $dI$  of soil was inferior to 1.5 and  $nI= 33.33\%$ . That mean the soil is the most negatively affected. Impoverished land due to overuse (cropping systems consist of a virtually continuous food crop palm wines plantations) has completely changed the agricultural landscape in the district of Adjan, Koundokpoé, Djigbé, Ze -center and Hékanmè (CeCPA-Ze, 2007). The soils become unproductive for food crops after a long fallow palm wine. Nutrients in vegetation and soils were slow to be recovered, therefore, causing a decrease in soil fertility (INRAB). This work gives an understanding as to why the municipality of Ze still imports food despite its climate favorable conditions to agriculture. However, palm improves soil fertility which enhances growth for certain food crops such as maize and cassava. It is also advantageous since people are compelled not to make fires. Ultimately, the vegetation that grows eventually decomposes thus increasing the organic matter in the soil there by returning the biological life.

In the district of koundokpoe, the field observation and analysis of data collected shows that after a fallow, there arises a problem of overuse of land, which does not favor good growth of some crops such as beans and peanut. Figure 4 illustrates the evolution of the felling of palms for the production of alcohol from 1997 to 2007 in the municipality of Ze. From 1997 to 2000, felling private Palms as well as industrial plantations had increased. This is due to the

increment of demand for alcohol and increased number of the alcohol producers. From 2000 to 2005, that amount had fallen due to the scarcity of natural palm trees and also the culling campaign co-organized by Rural Development of the municipality. In 2006, this amount had increased due to the regeneration of palms and also the lack of culling campaign by cooperatives.

On the other hand, the felling Palms' site has significantly better physical and chemical fertility characteristics within the radius of one meter and increases even more above 2m. All this is explained as the radius of one meter is rich and mostly because of inflorescences, fruits and almonds which fall on the ground and eventually decompose. Within the radius of 2 meters that is enriched by palm, there are about 100 plants per hectare. The fertilizing effect begins in the 2nd year. During, the first year after the felling of palms, there is decomposition of roots and plant debris in soil, immobilizing some of the nutrients such as nitrate (N), phosphate (P), potassium (K), calcium (Ca), magnesium (Mg) and nitrogen (Na). After plant decomposition, soils become richer and nutrients are available for crops.

In regards to the vegetation, the degree of impact dI was equal to  $0.77 > 0.5$  and nI equal to 55.55%. That means the vegetation was also mostly negatively affected. The cultivation of the palm wine and its exploitation for the production of alcohol requires destruction of natural vegetation formations. The use of plant species for the production of alcohol and the felling of regular palm is an alarming call to reduce deterioration of the physical environment of Ze. This was confirmed by comparative analysis of land use maps of 1994 and 2006 which show the evolution of vegetation cover. Figure 4 shows the decline of plant species, which is linked to human activities including palms exploitation for the production of alcohol.

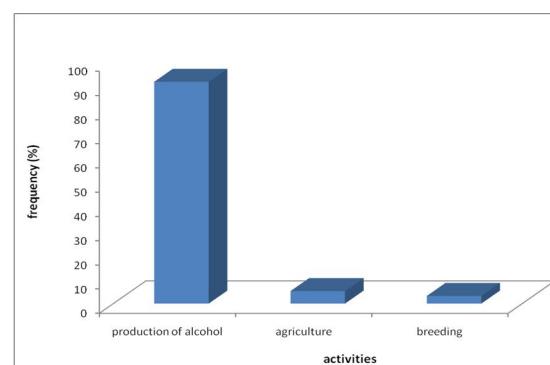
Figure 6 shows the rate of decline and increasing environmental components in the municipality of Ze. Training swamps, savannas and shrub lands as the relict forests have declined significantly between 1994 and 2006. The cultivation of over 40,000 of palm trees had lead to the destruction of vegetation cover in about 50 hectares in the northwestern part of the town. (CeCPA, Ze). The vegetation such as savannas, patches of dense forests (forest of worship "ORO"), swamp forests and forest reserves on Djigbé were threatened. The energy source used in the preparation of alcohol is the wood energy provided by different forest types of riverside villages. The lack of arable land due to the increment of population numbers is leading to pressure on forest products despite the prohibition laws. This has led to the loss of over a tenth of the total forest area of

3647ha per year. Plant species such as *Acacia sp* (acacia), *Antiaris Africana*, *Blighia sapadi*, *Chloroophora excelsa*, *Cola nitida* etc are gradually disappearing at an expense of agricultural crops in the municipality.

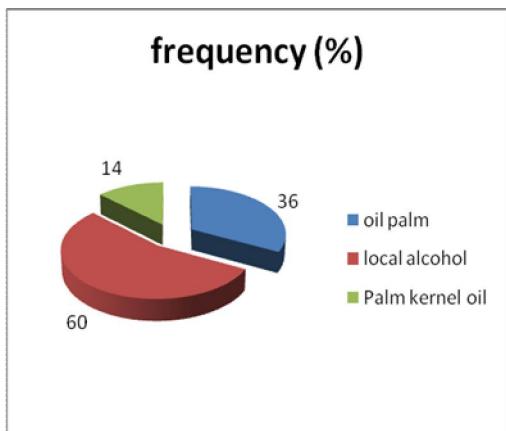
#### 4-Conclusion

The palm wine, particular oil palm is a multidimensional plant due to its multifaceted and varied character. The exploitation is one of the main activities of people in the municipality of Ze. From the production, processing and marketing of raw materials high numbers of labor are mobilized. It is emerging as a real source of income for the population and has been contributing for the improvement of living standards of farmers. In environmental terms, the exploitation of palm wine contributes to the degradation of the environment, including soil and vegetation. It is therefore necessary to have mitigation measures related to reduction strategies and improvement of impacts associated with the exploitation of palm wine.

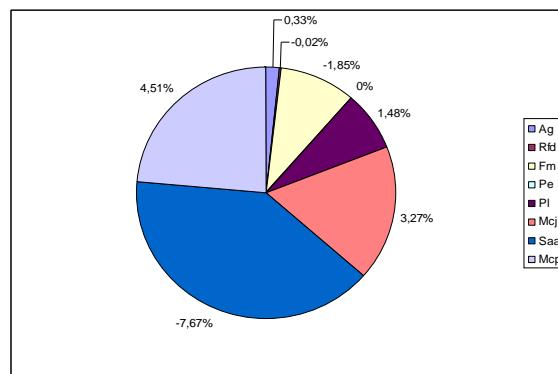
Amongst these measures, some of them are; the strategy of reducing felling of palm wine for the production of alcohol; adoption of the technique known as "tapping", which can improve processing techniques, soil fertility and living conditions of farmers by diversifying to other agriculture activities such as food crops and livestock farming. Diversification has a positive effect on the well-being of farmers in the sense that total revenue is high and the quality of the alcohol from palms is also improved since the volume of the alcohol produced is not much which brings in much focus during the production. In the long run, the beverage can also be enjoyed in all corners of Benin.



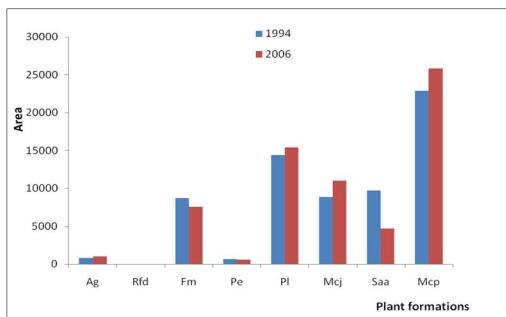
**Figure 3:** Comparison of annual activities for alcohol producers, farmers and ranchers. **Source:** Survey Results, January 2009



**Figure 4:** Frequency of production of palm wine processing. **Source:** Survey Results, January 2007



**Figure 7:** Evolution of environmental components. **Source:** CENATEL, May 2008



**Figure 5:** Evolution of the felling of palm wine in the municipality of ZE. **Source:** OVIGEPAF, January 2007

#### Legend (Figure 5 and 6)

Ag=built-up area;

Rfd= Relics of dense forest;

Fm= Swamp training;

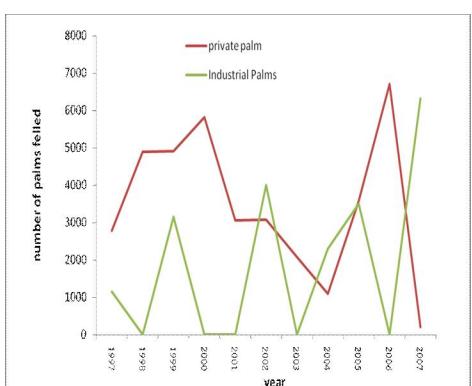
Pe= Water;

PI=Plantations;

Mjc= Mosaic of cultures and fallow land;

SAA= Tree and shrub savanna;

Mcp= Mosaic of cultures palm



**Figure 6:** areas of environmental components. **Source:** CENATEL, May 2008

#### List of Abbreviations

ASECNA- Agence pour la Sécurité de la Navigation Aérienne en Afrique et à Madagascar

CAR- Coopérative d'Aménagement Rural

CeCPA - Centre Communal de la Production Agricole

CeRPA - Centre Régional de la Production Agricole INRAB - Institut National des Recherches Agricoles du Bénin

INSAE - Institut National de la Statistique et de l'Analyse Economique

MAEP - Ministère de l'Agriculture, de l'Elevage et de la Pêche

MEPN - Ministère de l'Environnement et de la Protection de la Nature

OVIGEPAF - Organisation Villageoise de la Gestion Participative de la Forêt

UR-CAR - Union Régionale des Coopératives d'Aménagement Rural

#### Acknowledgement

I would like to thank the following Professors; BIO BIGOU Leon Bani, AKPAKI Joseph and CeCPA-101- ZE for their technical assistance.

\*For correspondence: [hermanade@yahoo.fr](mailto:hermanade@yahoo.fr)

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04/06/2010

## Sorption characteristics of copper in some calcareous soils of western Iran

A. R. Hosseinpur<sup>1</sup> and F. Dandanmozd<sup>2</sup>

<sup>1</sup>.Soil Sci. Dep. Shahrekord Univ. Shahrekord, Iran

<sup>2</sup>.Soil Sci. Dep. Bu-Ali Sina Univ. Hamadan, Iran.

E-mail: [hosseinpur-a@agr.sku.ac.ir](mailto:hosseinpur-a@agr.sku.ac.ir)

**Abstract:** The environmental impact of metal additions to soil depends on its sorption ability. To evaluate the sorption of copper (Cu) on to some soils an experiment was conducted with ten calcareous soils of Hamadan province in the west of Iran. Half g soil samples were equilibrated at 25±1 and 45±1°C with 25 ml of 0.01 M CaCl<sub>2</sub> containing 0 to 30 mgL<sup>-1</sup> Cu as CuSO<sub>4</sub>. Suspensions were centrifuged, filtered and concentration of Cu in the clear extract solution was calculated. The thermodynamic parameters viz. K, G, H and S were determined by using sorption data and concentration of Cu in equilibrium solution at two different temperature. Thermodynamic parameters revealed that Cu sorption increased as the values of K and G increased with rise in temperature from 25 to 45 °C. The G° values at 25 and 45°C were negative and ranged from -18.39 to -24.10 and -21.167 to -26.267 kJ mol<sup>-1</sup> respectively. The values of enthalpy (H°) and entropy (S) were positive and ranged from 8.184 to 42.852 kJ mol<sup>-1</sup> and 102.457 to 206.184 J mol<sup>-1</sup> K<sup>-1</sup>. The results showed that Cu sorption is a spontaneous process and endothermic reaction. The results also showed that calcareous soils can sorb high amounts of Cu and that thermodynamic parameters are useful in describing Cu sorption. [Journal of American Science. 2010;6(11):103-108]. (ISSN: 1545-1003).

**Keywords:** Sorption isotherm; Calcareous soils; Thermodynamic parameter; Copper;

### 1. Introduction

Adsorption is one of the most important chemical processes in soils. It determines the quantity of plant nutrients, metals, radionuclides, pesticides and other organic chemicals that are retained on soil surfaces and, therefore, is one of the primary processes that affect transport of nutrients and contaminants in soils. Sorption also affects the electrostatic properties of suspended particles and colloids. The electrostatic properties affect coagulation and settling (Sparks 1995). Sorption reactions on soil mineral surfaces potentially attenuate toxic soil solution (Goldberg 2002). Sorption isotherm analysis is a useful technique to study the retention of metals in soils. Sorption isotherms provide useful information about the soil retention capacity and the strength by which the sorbate is held on to the soil.

Sorption isotherm batch experiments are very important in soil science research. Theoretically, maximum monolayer sorption, empirical adsorption constants, and other important adsorption parameters can be determined by this method.. Selective adsorption of Cu and Zn has been related to their susceptibility to hydration, their charge / radius ratio, their electronegativity and their Misono softness (McBride 1989). Study of Zn and Cu adsorption onto soils of various kinds and onto certain individual soils fractions and other substrates have shown that the main physical and chemical factors governing these processes are pH and presence of organic and/or inorganic colloids (Lopez et al. 1998; Kardivelu et al. 2001; McBride et al. 1997). Copper is more soluble in acid soils

than in calcareous soils and their marked sorption hysteresis is most marked in the case of sorption by organic soil components (McLaren et al. 1983; Barow et al. 1989; Wu et al. 1999). In the absence of other metals, copper is generally considered to be adsorbed in greater quantities than other metals (Elliott et al. 1986), although they behave more similarly in soils with low organic matter content (Karathanasis 1999).

Adsorption increase with increasing temperature, due to the increase in number of active sites (Yavuz et al. 2003; Bouberka et al. 2005). To gain further insight into sorption process and its mechanism, thermodynamic approach can predict the final state of metal in the soil system from an initial nonequilibrium state (Sposito 1984). A thermodynamic approach was used to study the effect of temperature on the adsorption processes of lindane on a number of adsorbents (Mills and Bigger 1969a, b, c). Evaluation of the free energy change corresponding to the transfer of element from bulk solution into the appropriate site of the double layer or clay mineral lattice are helpful to express the sorption process. Similarly, an understanding of change in enthalpy and entropy helps in determining the free energy change and disorders occurring during sorption process. The high values of G° both for Pb and Cd indicated that both the reactions are spontaneous (Adhikari and Singh 2003). The values of H° were found to be negative for Cd and positive for Pb concluded that Cd sorption reaction was exothermic while Pb sorption was found to be an endothermic reaction in all the soils. (Adhikari and Singh 2003). The values of S were found to be positive due to the

exchange of the metal ions with more mobile ions present on the exchanger, which would cause increase in the entropy, during the adsorption process (Unlu and Ersoz, 2006). Information about Cu sorption characteristics and sorption thermodynamic parameters (STP) were limited in soils of Iran. The objectives of this research were to study the Cu sorption characteristics and determination of STP in some calcareous soils of Hamadan province in western Iran.

## 2.Materials and Methods

We collected bulk samples of the top soils (0-30cm depth) from ten calcareous soils from Hamadan province in the west of Iran. All samples were air dried, crushed and sieved through a 2-mm prior to soil analysis and sorption studies. Characteristics of the soils such as, particle size distribution, pH, EC, CEC, organic C, and calcium carbonate equivalent were determined using standard analytical methods (Gee and Bauder 1986; Rhodes 1996; Summer and miller 1996; Nelson and Sommers 1996 and Nelson 1982). Concentration of available Cu in soil samples was determined using DTPA method (Lindsay and Norvell 1978).

To study the sorption of Cu by different soils, 0.5 g soil sample was placed in 100 ml plastic bottles and equilibrated with 25 ml of 0.01 M  $\text{CaCl}_2$  solution containing graded levels of Cu, i.e. 0, 2, 5, 10, 15, 30  $\text{mg l}^{-1}$  Cu as  $\text{CuSO}_4$  solution. Solutions were prepared in 0.01 M  $\text{CaCl}_2$  to keep the ionic strength almost constant. Each sorption set, for Cu, was replicated thrice. The soil suspension shaken for 30 min and was equilibrated for 24 h at  $25\pm 1$  and  $45\pm 1^\circ\text{C}$  on an incubator. Based on preliminary studies, an equilibrium period of 24 h and soil/solution ratio of 1:50 were found optimum beyond which no significant change in metal content of equilibrium solution was recorded. After equilibration time, the suspension was filtered, and concentration of Cu in the clear extract solution was determined using Varian Atomic Absorption Spectrophotometry. Amount of Cu sorbed by soils was calculated from the difference between the initial and final concentration of Cu in the equilibrium solution. For studying the sorption relationship, the data were fitted to the following equations:

$$\text{C}_e/q = 1/K_b + 1/b\text{C}_e \quad \text{Conventional Langmuir}$$

$$\log q = \log K_f + n \log \text{C}_e \quad \text{Freundlich equation}$$

Where  $\text{C}_e$  is the Cu concentration in the equilibrium solution ( $\text{mg/l}$ ),  $q$  is the amount of Cu sorbed by the soil ( $\text{mg kg}^{-1}$ ),  $b$  is the sorption maxima ( $\text{mg kg}^{-1}$ ) and  $K_b$  is the bonding energy coefficient ( $\text{l mg}^{-1}$ ). The  $K_f$  is the Freundlich distribution coefficient and  $n$  is an empirical constant.

Langmuir and Freundlich isotherms don't give any idea about sorption mechanism, but Dubinin-Radushkevich (D-R) isotherm describes sorption on a single type of uniform pores. In this

respect the D-R isotherm is an analogue of Langmuir type but it is more general, because it does not assume a homogeneous surface or constant sorption potential (Unlu and Ersoz 2006). In order to understand the adsorption type, D-R isotherms were obtained. The D-R isotherm has the form:

$$\ln q = \ln q_m - k^2$$

and

$$= [RT \ln (1 + (1/C_e))]$$

Where  $\ln q$  is Polanyi potential,  $q$  is the amount of Cu sorbed by the soil ( $\text{mol g}^{-1}$ ),  $k$  is a constant related to the adsorption energy ( $\text{mol}^2 \text{kJ}^{-2}$ ) and  $q_m$  is the adsorption capacity ( $\text{mol g}^{-1}$ ). The mean free energy of adsorption ( $E$ ) was calculated from the  $k$  values using the equation:

$$E = (-2k)^{-0.5}$$

The magnitude of  $E$  is useful for estimating the type of adsorption process. If this value is between 8 and  $16 \text{ kJ mol}^{-1}$ , adsorption process can be explained by ion exchange (Unlu and Ersoz 2006).

Thermodynamic parameters were calculated from the variation of the thermodynamic equilibrium constant,  $K^\circ$ , computed by following the procedure outlined by Biggar and Cheung (1973). The value of  $K^\circ$  for adsorption reaction can be defined as:

$$K^\circ = a_s/a_e = sC_s/eC_e$$

where  $a_s$  denotes activity of adsorbed metals,  $a_e$  to activity of metals in equilibrium solution,  $C_s$  to milligrams of metals adsorbed per litre of solution in contact with the adsorbent surface,  $C_e$  to milligrams of solute per litre of solution in equilibrium solution,  $s$  is the activity coefficient of the sorbed metals and  $e$  represents the activity coefficient of metals in equilibrium solution. Since at lower concentration, activity coefficient approaches unity, above Eq. was reduced to:

$$K^\circ = C_s/C_e$$

The values of  $K^\circ$  were obtained by plotting  $\ln (C_s/C_e)$  vs.  $C_s$  and extrapolating to zero  $C_s$ . The standard free energy ( $G^\circ$ ) was calculated as follows:

$$G^\circ = -RT \ln K^\circ$$

The standard enthalpy ( $H^\circ$ ) was obtained from integrated form of the Vant Hoff equation:

$$\ln K_2^\circ/K_1^\circ = -H^\circ/R[1/T_2 - 1/T_1]$$

The standard entropy ( $S^\circ$ ) was calculated as

$$S^\circ = (H^\circ - G^\circ)/T$$

## 3.Results and Discussion

Selected chemical and physical characteristics of the soils are presented in Table 1. The texture of the soil samples are clay loam to sandy clay loam. The calcium carbonate equivalent ranged from 5 to 53.8%. Organic matter content ranged from 0.68% to 2.40%. The pH of the soils ranged from 7.44 to 8.20 which indicated that all of the soils are alkaline. The CEC ranged from 10 to 25.1  $\text{Cmol}_{(+)} \text{Kg}^{-1}$ . The DTPA extractable Cu ranged from 0.49 to  $4.15 \text{ mg kg}^{-1}$ .

For proper evaluation of the environmental threat posed by Cu or of its availability, it is necessary to supplement the individual sorption characteristics. Sorption isotherm for these soils exhibited difference in the amounts retained. Cu sorption didn't described by Langmuir equation ( $R^2=0.11-0.60$ ). Freundlich and D-R equation were described Cu sorption (Table 2). Constants of these equations were shown in Table 2. Freundlich distribution coefficient ( $K_f$ ) ranged from 798.18 to 73063.42 mg kg<sup>-1</sup>. Distribution coefficient represents the sorption affinity of the metal cations in solution for the soil solid phase and can be used to characterize the mobility and retention of Cu in a soil system. A distribution coefficient can be related to both plant uptake and environmental pollution. Low distribution coefficients indicate that most of the metals present in the system remain in the solution and are available for transport, chemical processes and plant uptake (Jalali and Moharrami 2007). Whereas higher values indicate lower

mobility and higher retention of metal in the soil. Soil no 8 had the highest  $K_f$ , its high CEC and clay content. Soil no 10 had the lowest  $K_f$ , its low CEC and equivalent calcium carbonate. So that Cu in soil no 8 had the lowest mobility and Cu in soil no 10 had the highest mobility. Freundlich distribution coefficient order was:

soil no 8>soil no 5>soil no 7> soil no 9>soil no 2>soil no 6>soil no 4>soil no 3>soil no 1> soil no 10

Freundlich constant n which indicates adsorption intensity (Jalali and Moharrami, 2007) ranged from 0.86 to 1.75. Soil no 3 and 10 had highest and lowest n respectively. Elzinga et al. (1999) also evaluated the batch sorption data and established general purpose, Freundlich isotherms for different heavy metals in soils. Mafton et al. (2000) reported that Zn adsorption in eight calcareous soils of Iran followed a Freundlich adsorption isotherm.

Table 1. Physicochemical properties of the experimental soils

Soil No.	pH (1:2.5)	CEC cmol(+) <sup>1</sup> kg <sup>-1</sup>	EC ds.m <sup>-1</sup>	O.M	Clay %	Silt	CaCO <sub>3</sub>	Zn- DTPA (mg kg <sup>-1</sup> )
1	7.80	10.9	0.23	0.90	27.0	7.5	11.0	1.09
2	8.10	11.5	0.30	1.03	33.2	15.0	14.1	4.15
3	7.96	14.2	0.19	0.83	34.2	19.1	33.5	1.49
4	7.45	23.5	0.13	0.68	38.3	10.3	5.0	0.66
5	8.20	16.0	0.34	2.40	22.0	15.0	53.8	0.49
6	8.00	15.5	0.20	0.80	20.8	25.3	17.1	0.56
7	7.44	20.9	0.19	1.06	27.0	27.8	22.1	0.63
8	7.90	25.1	0.20	1.10	36.4	25.9	27.4	0.65
9	7.90	14.5	0.26	0.80	38.1	17.6	39.4	1.15
10	7.85	10.0	0.30	1.40	27.6	9.5	6.1	2.56

Table 2. Freundlich and D-R equation constants of soils

Soil no	Freundlich constants			D-R constants			
	$k_f$ $1 \text{ kg}^{-1}$	n	$R^2$	K Mol <sup>2</sup> kJ <sup>-2</sup>	$q_m$ $\text{mmol g}^{-1}$	E $\text{kJ mol}^{-1}$	$R^2$
1	1498.99	1.02	0.92**	-0.0043	2.086	10.783	0.99**
2	4652.65	1.31	0.74*	-0.0082	15.182	7.8087	0.79*
3	2310.47	0.86	0.99**	-0.0044	1.313	10.660	0.99**
4	4152.41	0.99	0.93**	-0.0055	2.434	9.535	0.96**
5	30881.61	1.09	0.98**	-0.0054	4.192	9.623	0.97**
6	4296.35	1.12	0.98**	-0.0062	3.560	8.980	0.99**
7	27567.67	1.57	0.95**	-0.0088	56.591	7.538	0.97**
8	73063.42	1.75	0.95**	-0.0094	223.347	7.293	0.97**
9	25852.35	1.51	0.94**	-0.0084	42.712	7.715	0.95**
10	798.18	0.89	0.87**	-0.0036	2.513	11.785	0.96**

\* Significant at the 0.05 probability level

\*\* Significant at the 0.01 probability level

Distribution coefficient significantly correlated with CEC(Table 3). Reyhanitarabar et al. (2007) studying Zn retention of 20 calcareous soils

of central Iran, reported a significant relationship between Freundlich  $K_f$  with percentage of clay, CEC and CCE. Karimian and Moafpourian (1999)

reported that in calcareous soils of the southern part of Iran, Freundlich  $K_f$  showed a highly significant relationship with soil pH and Clay, but it didn't significantly correlate with CEC, CCE and OM.

Freundlich n didn't significantly correlate with soil properties. Reyhanitabar et al. (2007) reported a significant relationship between Freundlich n with percentage of clay. Elrashidi and O'Connor (1982) reported a significant relationship

between Freundlich coefficients and percentage of clay, CEC and pH, but not OM.

The values of  $q_m$  constant (D-R isotherm) which is the adsorption capacity ranged from 1.313 to 223.347 ( $\text{mol g}^{-1}$ ) (Table 2). Soil no 8 had the highest  $q_m$ , its high CEC and clay content and soil no 9 had the lowest  $q_m$ , its low OM and CEC. This parameter significantly correlated with percentage of clay and CEC (Table 3).

Table 3- Correlation between soil properties and adsorption isotherm parameters

Soil Properties	Freundlich constants		D-R constants		
	n	$k_f$	K	$q_m$	E
CCE	-0.25	0.23	-0.11	0.29	0.09
Clay	-0.19	0.33	-0.63*	0.69*	-0.62*
Silt	-0.61*	0.53	-0.32	0.32	-0.27
O.M	0.045	0.04	0.11	-0.17	0.07
EC	0.002	-0.16	-0.01	-0.05	-0.05
CEC	-0.47	0.66*	-0.44	0.61*	-0.42
pH	0.07	-0.01	0.1	-0.08	0.09

\* Significant at the 0.05 probability level

The magnitude of E which is calculated from D-R isotherm is useful for estimating the type of adsorption process. In this study, E values for Cu ranged from 7.29 to 10.78  $\text{kJ mol}^{-1}$  (Table 2) that are between the values of ion exchange. Therefore it is possible to say that adsorption mechanism of Cu ions on soils can be explained with an ion-exchange process.

The values of k (D-R isotherm) which is constant related to the adsorption energy ranged from -0.0036 to -0.0094  $\text{mol}^2 \text{kJ}^{-2}$ . Soil no 8 had the highest k, its high CEC and Clay. Soil no 10 had the lowest k, its low CEC and CCE.

Evaluation of thermodynamic parameters viz.  $K^\circ$ ,  $G^\circ$ ,  $H^\circ$ ,  $S^\circ$  provide an insight into mechanism of Cu sorption in the soils. The data in Tables 4 indicates that value of  $K^\circ$  increased with rise in temperature from 25 to 45 °C in all the soils. The  $G^\circ$  values for Cu were negative in all the soils (Tables 4). These negative values indicate that the sorption process is spontaneous. The  $G^\circ$  at 25 °C ranged from -18.391 to -24.101  $\text{kJ mol}^{-1}$ . The  $G^\circ$  at 45°C ranged from -21.167 to -26.267  $\text{kJ mol}^{-1}$ . In all the soils, the free energy ( $G^\circ$ ) of the Cu sorption was more negative at higher temperature, which suggested that the spontaneity of the process increased with rise in temperature.

Table 4. Thermodynamic parameters of Cu sorption

Soil no	K		$G^\circ (\text{kJ mol}^{-1})$		$H^\circ (\text{kJ mol}^{-1})$	$S^\circ (\text{J mol}^{-1}\text{K}^{-1})$	
	25°C	45°C	25°C	25°C		25°C	45°C
1	1814.56	5385.15	-18.591	-22.714	42.853	206.184	206.184
2	1862.36	3409.43	-18.655	-21.506	23.821	142.538	142.538
3	4517.89	7700.96	-20.851	-23.660	21.001	140.467	140.467
4	2861.21	5447.98	-19.719	-22.745	25.369	151.303	151.303
5	7396.78	11883.43	-22.072	-24.807	18.676	136.740	136.740
6	5604.92	7069.89	-21.385	-23.434	9.147	102.457	102.457
7	4539.63	6555.11	-20.863	-23.234	14.473	118.576	118.576
8	16772.57	20645.53	-24.101	-26.267	8.184	108.338	108.338
9	4065.39	6481.45	-20.589	-23.204	18.374	130.751	130.751
10	1674.21	2998.90	-18.391	-21.167	22.962	138.771	138.771

Soil no 8 had the highest  $G^\circ$  at 25 and 45 °C, its high CEC and calcium carbonate equivalent. Soil no 10 had the lowest  $G^\circ$  at 25 and 45°C, its low CEC and calcium carbonate equivalent.

The values of isoteric heat (enthalpy) of Cu sorption ( $H^\circ$ ) were positive and ranged from 8.184 to 42.853  $\text{kJ mol}^{-1}$  (Table 4). This provides an indication that sorption reaction was endothermic for Cu. Similar findings were also observed by

Bigger and Chung (1973), Adhikari and Singh (2003), Dali-yousef et al. (2006) and Unlu and Ersoz (2006).

Although there are no certain criteria related to the  $H^\circ$  values that define the adsorption type, the heat of adsorption values between 5.0 and 100  $\text{kcal mol}^{-1}$  (20.9-418.4  $\text{kJ mol}^{-1}$ ), which are heats of chemical reactions, are frequently assumed as the comparable values for the chemical adsorption

processes (Unlu and Ersoz, 2006). Soil no 1 had the higher H°, and soil no 8 had the lowest H°.

The values of H° were calculated from plots of ln K° versus 1/T. The linear nature of the plot indicates that the mechanism of adsorption is not changed as temperature changed. But the amount of adsorption is changed because the supply of thermal energy is different. The endothermic nature of the adsorption processes shows that these processes don't energetically stable (Bigger and Cheung 1973).

If the values of H° for heavy metals had been within the range of the enthalpy change of adsorption for ion exchange (8.4-12.6 kJ mol<sup>-1</sup>) suggesting that the adsorption process of those ions is of ion exchange in nature (Helfferich 1962). The values of H° for Cu in this study were greater than 12.6 kJ mol<sup>-1</sup> which indicated that the presence of other mechanism for the adsorption of these ions occurs on soil beside ion exchange mechanism.

Table 5. Correlation coefficients between soil properties and thermodynamic parameters

Soil properties	K° (25)	K° (45)	G° (25)	G° (25)	H° (25)	S° (25)	S° (45)
CCE	0.74*	0.71*	-0.72*	-0.68*	-0.23	-0.17	-0.17
Clay	0.41	0.33	-0.53	-0.42	-0.25	-0.22	-0.22
Silt	0.08	0.37	-0.20	-0.30	0.05	0.08	0.08
O.M	0.54	-0.10	-0.33	0.12	-0.59	-0.59	-0.59
EC	0.12	0.02	0.07	0.13	-0.05	-0.06	-0.06
CEC	0.63*	0.14	-0.71*	-0.29	-0.65*	-0.62*	-0.62*
pH	0.21	0.34	-0.10	-0.30	0.19	0.21	0.21

\* Significant at the 0.05 probability level

The values of S° for Cu sorption were positive and ranged from 102.457 to 206.184 J mol<sup>-1</sup> K<sup>-1</sup>. The positive values of S° indicates that the increased randomness at solid-solution interface during the adsorption of this cation on sorbent, while the negative value of S° indicates the decrease of the degree of randomness at the surface of the sorbent during the adsorption process ions. This decreasing in the randomness lead to an increase in the adsorption capacity of the ion on sorbent (Abou-Mesalam 2003).

The thermodynamic equilibrium constant (K°) at 25°C significantly correlated with calcium carbonate equivalent and CEC, while at 45°C it only correlated with CCE. Standard free energy (G°) at 25°C significantly (p<0.05) correlated with CCE and CEC, but at 45°C it highly correlated (p<0.05) with CCE. Standard enthalpy (H°) and standard entropy (S°) were significantly correlated with CEC (Table 5).

### Conclusion

The results of this research showed that calcareous soils have a high capacity for Cu sorption. The experimental data were better fitted by D-R than Freundlich equation. The soil properties such as clay and CEC play an important role in Cu sorption.

Thermodynamic parameters revealed that Cu sorption reaction in soils studied were spontaneous and endothermic. These suggested that the sorption capacity of these soils enhanced with increased in temperature. Thus, for developing suitable strategies for proper management of heavy metal pollution, moreover soil properties and nature of pollutant, soil condition, particularly temperature

needs to be considered. Thermodynamic parameters significantly correlated with CCE and CEC.

Correspondence to:

A.R. Hosseinpur. Sil Sci. Dep.  
Shahrood, Univ. Shahrood, Iran,  
E-mail: hosseinpur-a@agr.sku.ac.ir

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5/2/2010

## Association between Single nucleotide polymorphisms in Gallinacin genes and resistance to Marek's disease in White Leghorn chicken

Yacoub, H. A<sup>\*1</sup>, Galal, A<sup>2</sup>, El Fiky, S.A<sup>1</sup> and Fathi, M. M<sup>2</sup>,

<sup>1</sup>Cell Biology Department, National Research Center, Giza, Egypt

<sup>2</sup>Poultry Production Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt

<sup>\*</sup>Haitham\_yakoub@yahoo.com

**Abstract:** Gallinacins are antimicrobial peptides that play a significant role in innate immunity in chicken. The aim of this study was to determine the relationship between candidate genes of innate immunity and resistance to Marek's disease and to predict whether the amino acids substitutions lead to produce new phenotypes. We used in current study two inbred lines of White Leghorn chickens, line 6 which selected for resistant to Marek's disease and line 7 which selected to susceptible to Marek's disease from ADOL, ARS, USDA. We examined Gal-1 and Gal-2 in current study by sequenced a 1.38 kb in two directions from two inbred lines (6 and 7). A total of 6 SNPs were identified within the sequenced regions. This equates to an SNP rate of 4.34 SNPs/kb, nearly to the previously reported 5 SNPs/kb across the entire chicken genome. The current study showed that the gallinacin genes are polymorphic because there are many single nucleotide polymorphisms (SNPs) in both inbred lines of White Leghorn chickens and some of these SNPs are nonsynonymous and others are synonymous and some of them are located in intronic region and the rest are in exonic region. All identified SNPs were intronic; except for Gal-1 was exonic resulting in amino acids changes which have a non-synonymous SNP resulting in amino acids alterations of asparagine to serine, histidine to tyrosine and tyrosine to serine, respectively. From SIFT (Sorting Intolerant from tolerant) program which used to predict whether an amino acids substitutions can affect protein function resulting in phenotypic effect , that is may be made the inbred line 7 of White Leghorn chickens are susceptible to Marek's disease rather than line 6. We are concluded that a new chromosomal region with effects on the response to Marek's disease in chickens was characterized in this study. Within this region, the SNPs in the gallinacin candidate genes could potentially be used in a marker assisted selection program to enhance the response to Marek's disease. Analysis of the gallinacin genes in the protective pathways of disease resistance has also opened the possibilities for therapeutic strategies using endogenous antimicrobial peptides. [Journal of American Science. 2010;6(11):109-114]. (ISSN: 1545-1003).

**Keywords:** single nucleotide polymorphisms, Gallinacin, genes, Marek's disease, resistance

### 1. Introduction

Global production of chickens has experienced massive change and growth over the past 50 years. The commercial broiler and layer markets produce more than 50 billion birds annually to meet current worldwide consumer demands of more than 74 metric tons of meat and more than 66 million metric tons of eggs (Muir *et al.*, 2008).

In fact, poultry has become the leading meat consumed in the United States and most other countries and is the most dynamic animal commodity in the world; production has increased by 436% since 1970, more than 2.3 times and 7.5 times the corresponding growth in swine and beef, respectively (<http://faostat.fao.org>). Unfortunately, the poultry industry continues to be confronted with new and emerging infectious diseases such as Newcastle disease, avian leucosis, avian influenza and Marek's disease that can led to significant economic losses.

Marek's disease (MD) is a lymphoproliferative disease, caused by a member of

the herpesvirus family, that is estimated to cost the poultry industry nearly \$1 billion annually (Purchase, 1985). Diseased chickens infected by the Marek's disease virus (MDV), the causative pathogen, commonly exhibit paralysis, blindness, and visible lymphoid tumors that result in condemnation of the birds. Although vaccination programs have effectively reduced the incidence of MD, there is evidence that current vaccines do not protect well against some highly pathogenic MDV strains that have emerged in recent years (Witter and Hunt, 1993). Also, MD vaccines control rather than eliminate losses from MD because they do not block MDV infection, thus as a result, MDV is ubiquitous on poultry farms, and all chickens are exposed to the pathogenic agent at 1 day of age (Vallejo *et al.*, 1997).

All these factors point to the need to complement vaccinal protection with alternative methods such as genetic resistance (Spencer *et al.* 1974; Gavora and Spencer, 1979). And even if a

specific disease has been controlled through vaccination, genetic resistance is of value because it represents a safeguard against heavy losses in the case of disease outbreaks (Vallejo *et al.*, 1997).

Genetic resistance to MD has been known for more than 60 years (Calnek, 1985). Genetic resistance is a complex trait controlled by many genes though genetic selection for high levels of resistance can be obtained within relatively few generations (Cole, 1968), this is because of selection for certain MHC haplotypes, something that would not be done now to maintain biodiversity. The development of effective vaccines in the late 1960s, however, greatly reduced interest in the genetic control of MD. Ironically, genetically resistant lines were shown to have greater vaccinal immunity and higher egg production than susceptible lines (Von Krosigk *et al.*, 1972; Spencer *et al.* 1974; Gavora and Spencer, 1979).

One such class of genes that may play a role in resistance to Marek's disease are gallinacin genes, one family of antimicrobial peptides (AMP). Antimicrobial peptides (AMP) are relatively small molecules that are less than 100 amino acids in length and have a broad spectrum of antimicrobial activity (Ma *et al.*, 2007). Defensins are a type of AMP characterized by the presence of a conserved cysteine (Cys)-rich defensin motif. The three defensin subfamilies (α, β, and γ-defensins) found in humans and mammals, only α-defensins have been found in birds (Sugiarto and Yu, 2004; Satchell *et al.*, 2003; Bensch *et al.*, 1995 and Higgs *et al.*, 2005).

These Gals are widely expressed across most tissues, including those of the digestive system, respiratory system, genitourinary system, and several other anatomical areas in the chicken (Ma *et al.*, 2007). Further, different Gals are expressed in different tissues (Higgs *et al.*, 2005; Harwing *et al.*, 1994 and Lynn *et al.*, 2004).

The main objectives of this study is

1. To identify and analyze new candidate genes for their association with resistance to Marek's disease in the inbred White Leghorn Lines 6 subline 3 (6<sub>3</sub>) and 7 subline 2 (7<sub>2</sub>).
2. To predict whether an amino acid substitution in a protein will have a phenotypic effect.

## 2. Material and Methods

This study was carried out, at the Avian Disease and Oncology laboratory (ADOL),

Agricultural Research Service (ARS), United States Department of Agriculture (USDA), USA and Cell Biology Department, National Research Center of Egypt. The inbred White Leghorn Lines 6 subline 3 (6<sub>3</sub>) and 7 subline 2 (7<sub>2</sub>) had been taken to be used in current study, differ greatly in MD susceptibility (6<sub>3</sub> is resistant and 7<sub>2</sub> is highly susceptible; Crittenden, 1975; Pazderka *et al.*, 1975).

### 1. DNA isolation, PCR

Genomic DNA was prepared from chicken erythrocytes by using QIAgen DNA purification kit. To characterize the 3'-untranslated region of each gene, a pair of primers (**Table 1**) was developed using FastPCR, based on the published chicken genome assembly. PCRs were performed using 25-μl reaction mixture volumes that contain 25 ng of chicken genomic DNA, 0.8 μM of each primer, 200 μM of each deoxynucleoside triphosphate, 1 unit of Taq DNA polymerase, 2.5 μl of 10x PCR buffer, and 1.5 mM MgCl<sub>2</sub>.

The following cycling conditions were used:

1. An initial denaturation step at 94 °C for 3 min, followed by 35 cycles at
2. 94 °C for 1 min,
3. at the optimal annealing temperature for 1 min, and at
4. 72 °C for 1 min and
5. Final extension at 72 °C for 5 min. The PCR products were separated by electrophoresis through 1.5% gel.

### 2. The sequencing

The PCR products were purified using Sephadex-G, An ABI3100 DNA analyzer (Applied Biosystems, Foster City, CA) was used for direct sequence using nucleotide dye terminators. PCR products were sequenced at Avian Disease and Oncology Laboratory (ADOL), ARS, USDA.

### 3. Sequencing analysis

Sequencing alignment was achieved using Nucleotide-nucleotide BLAST (blastn) software in <http://www.ncbi.nlm.nih.gov/blast/> and CLASTALW 2.0.12. To detect the SNPs in inbred White Leghorn lines using Sequencher program version 4.8, also, to predict whether an amino acid substitution in a protein will have a phenotypic effect using Sorting Intolerant from Tolerant (SIFT) program [http://sift.jcvi.org/www/SIFT\\_aligned\\_seqs\\_submit.html](http://sift.jcvi.org/www/SIFT_aligned_seqs_submit.html).

## 3. Results

### 1. Sequence variation

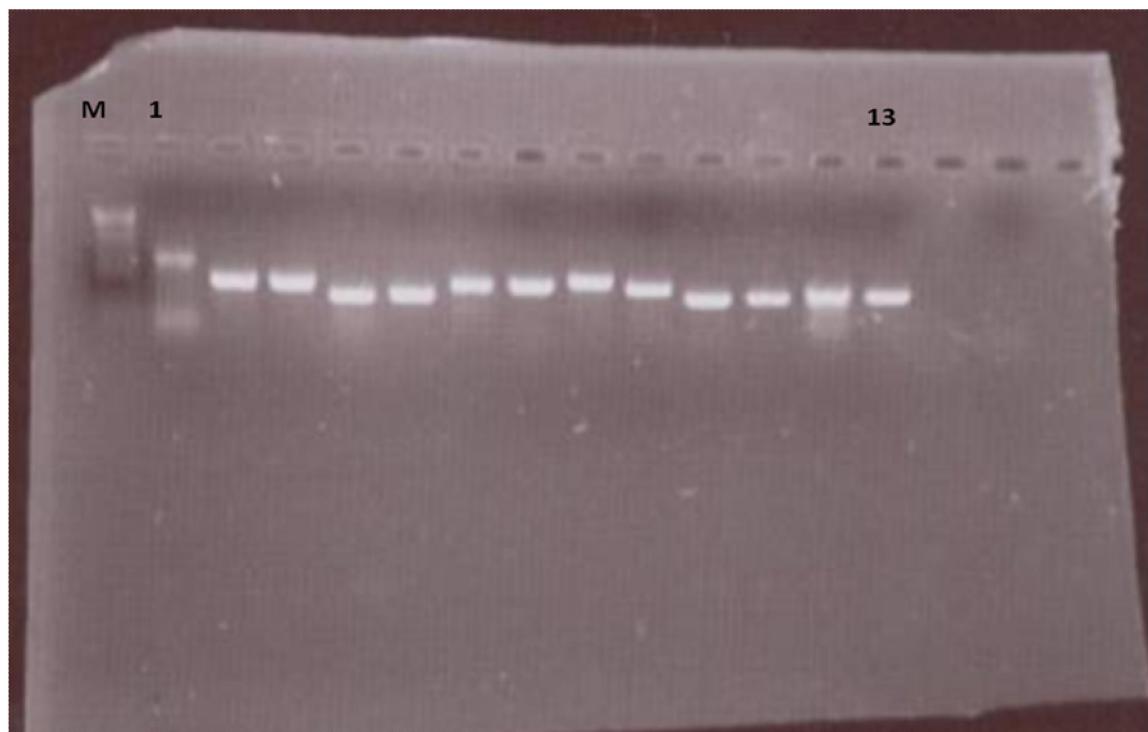
#### 1.1. Gallinacin-1

Single nucleotide polymorphism in gallinacin 1 in line 6 (resistant to Marek's disease) and line 7 (susceptible to Marek's disease) is showed in Fig (3). Primers designed from gallinacin 1 genomic DNA amplified a 872 bp fragment of the gene with SNPs of A-to-G, C-to-T and A-to-C within the exonic sequence in line 6 (resistant to MD) and line 7 (susceptible to MD), respectively. At positions 110,260,751, 110,260,716 and 110,260,781 of the chicken genome assembly the nucleotides are G, T and C, respectively. These nonsynonymous SNPs produced an amino acid change from asparagine to serine, histidine to tyrosine and tyrosine to serine, respectively.

## 1.2. Gallinacin-2

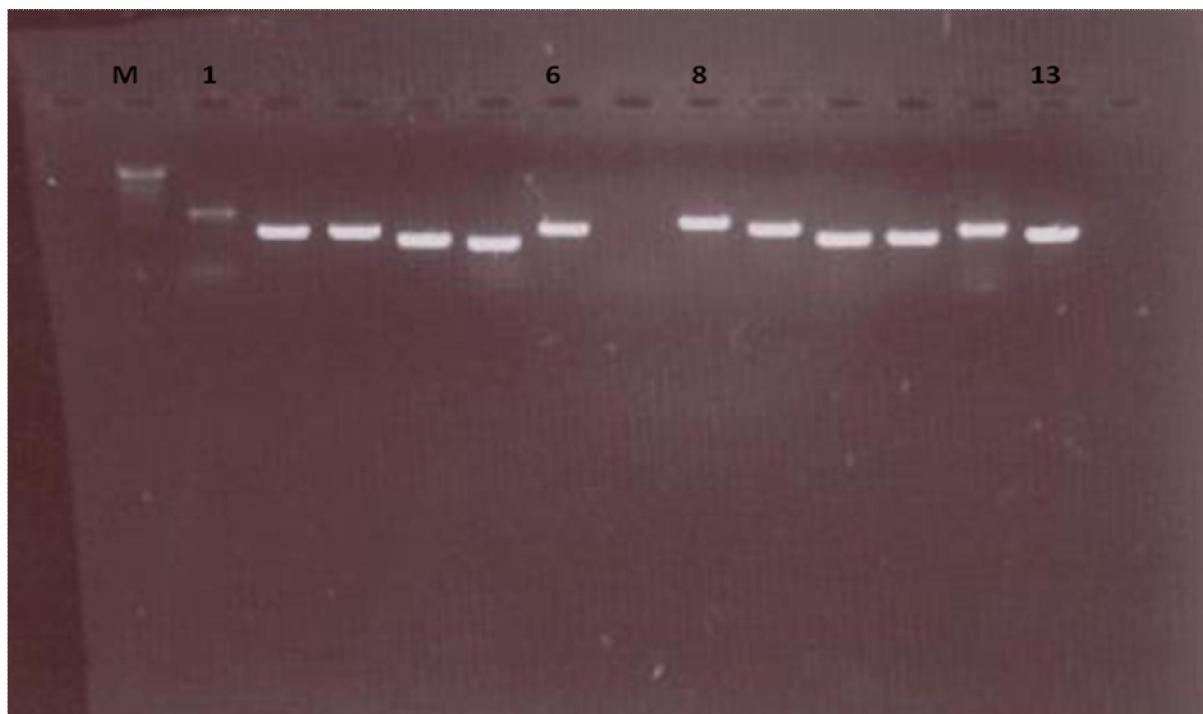
**Table. 1. Primer sequence of Gal-1 and Gal-2 .**

Gene	Primer sequence (Forward/reverse)	PCR product size bp	Annealing temperature	Accession Number
<b>Gal -1</b>	<b>5'-ACTGCAGGCCCATGGTGGATGTC-3'</b>	827	<b>58</b>	<b>HM136609</b>
	<b>5'-TGTTAGACTGAGATCCATGGGAC-3'</b>			<b>HM136612</b>
<b>Gal-2</b>	<b>5'-GCTGCTGAGGCCTTGCTGTAGC-3'</b>	553	<b>58</b>	<b>HM136610</b>
	<b>5'-ATGGCCATAGATGCCAGCAC-3'</b>			<b>HM136611</b>



**Fig.1. Amplified fragment of gallinacin genes (1-13) in inbred White Leghorn line 6 sub line 3. Lane M, DNA molecular weight marker. Lane 1-13, Gal-1- Gal-13.**

Single nucleotide polymorphism in gallinacin 2 in line 6 (resistant to Marek's disease) and line 7 (susceptible to Marek's disease) is noticed in Fig (4). For gallinacin 2 , a 553 bp product that contain two substitutions in an intron A- to G, A-to-G and A-to-G in line 6 (resistant to MD) and line 7 (susceptible to MD), respectively. At position 110,258,387,110,258,196 and 110,258, 137 of the chicken genome assembly the nucleotides are A, A and G respectively. This SNP is a synonymous SNP and it doesn't change amino acid.



**Fig.2.** Amplified fragment of gallinacin genes (1-6)(8-13) in inbred White Leghorn line 6 sub line 3. Lane M, DNA molecular weight marker. Lane 1-6 , Gal-1 –Gal-6. Lane 8-13, Gal-8 – Gal-13.

#### 4. Discussion

##### SNP detection and its rate

In total, 1.38 kb was sequenced in two directions from two inbred lines (6 and 7). A total of 6 SNPs were identified within the sequenced regions. This equates to an SNP rate of 4.34 SNPs/kb, nearly to the previously reported 5 SNPs/kb across the entire chicken genome (Wong *et al.*, 2004).

All identified SNPs were intronic, except for Gal-1 was exonic resulting in an amino acids changes which have a non-synonymous SNP resulting in amino acids changes of asparagine to serine, histidine to tyrosine and tyrosine to serine, respectively.

Non-synonymous SNP are of interest due to their potential effect on protein expression and, ultimately have minimal effects on genes expression (exceptions might be those nucleotides that are important in DNA–protein interactions in the promoter and the genomic regions or those nucleotides that are involved in RNA stability) and both synonymous and non-synonymous SNP are excellent genetic markers for mapping studies (Emara and Kim, 2003).

##### SNPs location

The current study showed that the gallinacin genes are polymorphic because there are many single nucleotide polymorphisms (SNPs) in both inbred lines of White Leghorn chickens and some of these SNPs are nonsynonymous and others are synonymous and some of them are located in intronic region and the rest are in exonic region.

##### Intronic SNPs

There were many intronic SNPs are located in non-coding region in gallinacin genes specifically for gal-2, 3 SNPs. Intronic SNPs, while not the causal mutations, can provide excellent markers for genetic selection for an increased immune response to Marek's disease.

##### Exonic SNPs

In a gallinacin 1 there were three nonsynonymous substitutions A-to-G, C-to-T and A-to-C within the exonic sequence in line 6 (resistant to MD) and line 7 (susceptible to MD), respectively. And these alterations lead to protein modification through changes of asparagine to serine, histidine to tyrosine and tyrosine to serine in lines 6 and 7, respectively.

From SIFT (Sorting Intolerant from tolerant) program which used to predict whether an amino acids substitutions can affect protein function resulting in phenotypic effect , that is may be made the inbred line 7 of White Leghorn chickens are susceptible to Marek's disease rather than line 6.

Most genetic variation is considered neutral but single base changes in and around a gene can affect its expression or the function of its protein products (Collins *et al.*, 1997 and Risch and Merikanges, 1996). A nonsynonymous or missense variant is a single base change in a coding region that causes an amino acid change in the corresponding protein.

If a nonsynonymous variant alters protein function, the change can have drastic phenotypic consequences. Most alterations are deleterious and so are eventually eliminated through purified selection. However, beneficial mutations can sweep through the population and become fixed, thus contributing to species differentiation.

It was observed that disease-causing Amino Acid Substitutions (AASs) had common structural features that distinguished them from neutral substitutions, suggesting that structure could also be used for prediction (Sunyaev *et al.*, 2000 and Wang and Moult, 2001).

The gallinacin genes are clustered within an 86-kb distance on the 3q3.5-q3.7 chromosome (Xiao *et al.*, 2004). The location of molecular markers within this cluster could be useful for marker assisted genetic selection and positional cloning works (Hasenstein *et al.*, 2006).

Bar-Shira *et al.* (2006) hypothesized that innate effector mechanisms such as gallinacin enable immune protection during the first week after hatching until functional maturation of the adaptive immune system occurs. They showed that mRNA levels of *Gal1* and *Gal2* decreased relative to the day of hatching throughout the first week of life and then increased again during the second week.

## 5. Conclusion:

We concluded that a new chromosomal region with effects on the response to *Marek's disease* in chickens was characterized in this study. Within this region, the SNPs in the gallinacin candidate genes could potentially be used in a marker assisted selection program to enhance the response to Marek's disease. Analysis of the gallinacin genes in the protective pathways of disease resistance has also opened the possibilities for therapeutic strategies using endogenous antimicrobial peptides.

## Corresponding author

Yacoub, H. A

Cell Biology Department, National Research Center, Giza, Egypt.  
Haitham\_yakoub@yahoo.com

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6/1/2010

# Endometrial Cytology and Bacteriological Isolates From Buffaloes With Retained Fetal Membranes and Their Effects on the Reproductive Efficiency

Amer H. A.<sup>1\*</sup>, AbouZeid N. Z.<sup>2</sup> and Barakat T. M.<sup>1</sup>

Department of Theriogenology<sup>1</sup> and Infectious Diseases<sup>2</sup>, Faculty of Veterinary Medicine, Zagazig University, Egypt

\*Corresponding author: [samarmed84@yahoo.com](mailto:samarmed84@yahoo.com)

**Abstract:** This study aimed to determine if the buffaloes with retained fetal membranes (RFM) and without systemic involvement had an effect on the subsequent reproductive efficiency. One hundred buffaloes with or without placental retention were allocated into 4 groups, including 25 buffaloes at day 15 post-calving had RFM (1<sup>st</sup> group), 25 buffaloes at 45 days post-calving had RFM (2<sup>nd</sup> group), 25 buffaloes without RFM at day 15 post-calving as control (3<sup>rd</sup> group) and 25 buffaloes without RFM at day 45 post-calving as control (4<sup>th</sup> group). The intrauterine perfusion fluid (10ml) was collected and examined bacteriologically and cytologically to evaluate the intrauterine environment. The reproductive parameters were determined in both buffaloes with or without retained fetal membranes. The detection rate of bacterial spp. was significantly ( $P<0.05$ ) higher in buffaloes with RFM collected at day 15 after parturition than those in other groups. All 25 buffaloes with RFM at 15 days post-partum (100.0 %) showed positive results. From 22 of them (88.0%), more than one bacterial species was isolated. An *Archonobacterium pyogenes* (A. pyogenes) was isolated from 56.0% of buffaloes with RFM after 15 days post-calving. On the other hand, 5 (20.0%) out of 25 buffaloes with RFM at 45 days post-partum showed positive results. Nine out of 25 (36.0%) buffaloes without RFM at 15 days post-partum showed positive results. Moreover, 4 out of 25 (16.0%) control buffaloes at 45 days post-partum showed positive results. The bacterial species most frequently isolated was *Lactobacillus* spp. The number of buffaloes with  $\geq 70\%$  PMNs or  $\leq 40\%$  lymphocytes cells was higher (24/25, 96%) in the 1<sup>st</sup> group (RFM) at 15 days than those in 2<sup>nd</sup> group (RFM) at 45 days post-calving. The number of buffaloes with  $\geq 70\%$  PMNs or  $\leq 40\%$  lymphocytes cells was also significantly ( $P<0.01$ ) higher in control group (17/25, 65%) at 15 days than those in control group (6/25, 24%) at 45 days. There were no significant variations among the groups of the buffaloes with retained placenta and the control groups at 15 and 45 days post-calving in postpartum uterine involution, the number of days from parturition to initial insemination, the number of days to conception and the number of services per conception. The overall conception rate was 15(60%) and 16(64%) in the RFM group, meanwhile, it was 19(76%) and 20(80%) in the control groups. It could be concluded that, in most buffaloes, the retained fetal membranes without systemic involvement had no major effect on the postpartum reproductive performance. [Journal of American Science. 2010;6(11):115-121]. (ISSN: 1545-1003).

**Keywords:** Buffaloes, Bacteriologically, Cytologically, Insemination, Conception.

## 1. Introduction

Risk factors for acute metritis were categorized by Sheldon and Dobson (2004) as intrauterine damages (Stillbirth, dystocia, twins, caesarean section, retained placenta, delayed uterine involution), metabolic disorders (milk fever, ketosis, left displaced abomasums) and the balance between pathogenicity and immunity (disruption of neutrophil function, type of bacterial flora, progesterone and glucocorticoids administration, early formation of corpus luteum, level of hygiene). The infection and to some extent the inflammation of the uterine wall during and after parturition must be accepted as a physiological process (Lewis, 1997; Hertl et al., 2010). Pathogenic species for metritis isolated from the uterine cavity are *Escherichia coli*, *A. pyogenes* and obligate anaerobic species *Fusobacterium (F) necrophorum* and *Prevotella* spp. (Lewis, 1997;

Sheldon et al., 2004; Bicalho et al., 2010). Besides the quantity and quality of bacteria in the uterus, the efficiency of uterine defense mechanisms determines the severity of metritis. The uterine defense mechanisms consist of anatomical and physical barriers i.e. the vulvar and cervical closure as well as the cell mediated and humeral immune systems. The initial cellular response to an infection of the uterine wall is an influx of PMNs and macrophages. Immunoglobulins and opsonins are released from the endometrium (Bondurant, 1999; Dhaliwal et al., 2001; Földi et al., 2006). Knowledge and characteristics of the intrauterine environment following placental retention is needed to establish effective measures for an improving the reproductive efficiency in cattle with retained placenta (Joosten et al., 1988; Salama et al., 1993). This study aimed to determine if the buffaloes with retained fetal

membranes (RFM) and without systemic involvement had an effect on the subsequent reproductive efficiency based on the bacteriological and cytological examinations of intrauterine perfusion fluid.

## 2. Material and Methods

### 2.1. Animals

This study was conducted "between" November, 2008 to October, 2009 using 800 Egyptian buffaloes housed in barn stalls belonging to private farms related to Balkas, Dakahlia Province. The age of the animals ranged between 3-8 years. The animals stall fed and had unrestricted access to hay and 8-10 kg concentrate feed for each. Fifty buffaloes had not expelled the placenta after 24 hours post calving were assigned to the experimental group (RFM), while the other fifty had expelled the placenta without manual interference were used as a control group (C). No treatment was administered to the buffaloes with placental retention (expect those animals showing systemic involvement associated with fever, were both systemically and locally treated and excluded from the study). One hundred buffaloes with or without placental retention were allocated into 4 groups, including 25 buffaloes at day 15 post-calving had RFM (1<sup>st</sup> group), 25 buffaloes at 45 days post-calving had RFM (2<sup>nd</sup> group), 25 buffaloes without RFM at day 15 post-calving as control (3<sup>rd</sup> group) and 25 buffaloes without RFM at day 45 post-calving as control (4<sup>th</sup> group).

### 2.2. Collection of intrauterine perfusion fluid

The intrauterine perfusion fluid was collected once only from four groups by the method performed by Kaneko et al. (1996). A vaginal speculum was inserted into the vagina after cleaning of the vulva with disinfection (Betadine). The tip of a balloon catheter (Terumo Inc. Tokyo, Japan Fr. 22) was inserted into the cervix as deep as possible without touching the vaginal wall. The vaginal speculum was removed, and then the balloon catheter was advanced into the uterus using the recto-vaginal method. The balloon was inflated with air. Sterile physiological saline (100 ml) was infused into the uterus through a balloon catheter and recovered by gentle massaging of the uterus.

### 2.3. Bacteriological examination of the intrauterine perfusion fluid

The perfusion fluid (10 ml) was centrifuged at 1000 rpm for 10 minutes and after removal of the supernatant, the sediment was resuspended in 1 ml of physiological saline. An allocate of the resuspended sediment (100 µl) was applied to soy agar with 5% sheep blood and incubated for 2-7 days at 37°C in both aerobic and anaerobic atmospheres. Using the

criteria of Kaneko et al. (1996), samples showing growth of more than 50 identical colonies were defined as positive control for bacteria and were considered to indicate bacteriological deterioration of the intrauterine environment. Gram-negative, a typical, pine leaf-like rods, which showed hemolytic reaction on sheep blood containing agar medium were negative to the catalase test, were judged to the *A. pyogenes*. Samples showing the growth of a more than one *A. pyogenes* colony were defined as positive for *A. pyogenes*. All bacterial isolates were identified according to Bergey's manual of a systemic bacteriology (Holt et al., 1994).

### 2.4. Cytological examination of the intrauterine perfusion fluid

The perfusion fluid (10 ml) was centrifuged as described above and the sediment was smeared on a glass slide, dried in air, fixed for 3 minutes with methyl alcohol and then the sediment was stained with Giemsa. A total of 200 cells was counted at x1000 in each specimen and classified into PMNs, eosinophils, lymphocytes and macrophages like cells. The percentage of PMNs and lymphocytes were calculated and recorded. Specimens with a PMNs ratio exceeding 70% or a lymphocytes ratio below 40% were considered to indicate a poor cytologically intrauterine environment (Kaneko et al., 1996).

### 2.5. Investigation of the postpartum reproductive performance

The postpartum uterine involution, number of days from parturition to initial insemination, number of days until conception and number of inseminations required to achieve conception, as well as the overall conception rate, were determined in both buffaloes with or without placental retention.

### 2.6. Statistical analysis

The positive rates for bacteria and for *A. pyogenes* were compared between groups by using Chi-square test. The PMNs ratio and the lymphocytes ratio in sediment, inflammatory cells and the reproductive performance were compared by a student's T-test according to Snedecor & Cochran (1982).

## 3. Results

The detection rate of *Streptococcus spp.*, *Bacteroids melaninogenias*, *Fusebacterium necrophorum*, *Escherichia coli*, *Pasteurella multocida*, *Proteus vulgaris*, *Lactobacillus spp.*, *Staphylococcus aureus*, *Enterococcus spp.* and *A. pyogenes* was significantly ( $P<0.05$ ) higher in buffaloes with RFM collected at day 15 after parturition than that in other groups (Table 1). All 25

RFM buffaloes at 15 days post-partum (100.0 %) showed positive results. From 22 of them (88.0%), more than one bacterial species was isolated. A mixed culture of *E. coli*, *Streptococcus spp.*, *Pasteurella multocida*, *A. pyogenes* and *Fusebacterium necrophorum* were most common. The bacterial species most frequently isolated was *E. coli*, (25 isolates, (100.0%), followed by *Streptococcus spp.*, (22 isolates, 88.0 %), *Pasteurella multocida* (15 isolates, 60.0%) and *A. pyogenes*, (14 isolates, 56.0%). Other bacteria as Bacteroids melaninogenias and *Fusebacterium necrophorum* were found at lower frequencies (10 isolates for each one) as presented in Table 1. On the other hand 5 (20.0%) out of 25RFM buffaloes at 45 days post-partum showed positive results. The bacterial species

most frequently isolated was *Staphylococcus spp.*, *Pasteurella multocida* and *Proteus vulgaris*.

Whereas 9 (36.0%) out of 25 control buffaloes at 15 days post-partum, showed positive results. From 4 of them (16.0%), more than one bacterial species was isolated. A mixed culture of *E. coli*, *Streptococcus spp.*, *Lactobacillus spp.* and *Enterococcus spp.* were most common. The bacterial species most frequently isolated was *E. coli* and *Lactobacillus spp.* (4 isolates for each, 16.0%), followed by *Streptococcus spp.* and *Enterococcus spp.* (3 isolates for each, 12.0 %) and *Fusebacterium necrophorum* (2 isolates, 8.0%). Moreover, 4 out of 25 (16.0%) control buffaloes at 45 days post-partum showed positive results. The bacterial species most frequently isolated was *Lactobacillus spp.* (Table 1).

**Table 1. Proportion of buffaloes with or without placental retained showing positive cultures of aerobic and anaerobic bacteria in the intrauterine perfusion fluid collected at 15 and 45 days after parturition**

Group	Bacterial species	No./Frequency of isolation (%)
<b>Buffaloes with retained fetal membranes</b>		
<b>at 15 days post-partum</b>	<i>Streptococcus spp.</i>	22/25 (88.0)
	<i>Bacteroids melaninogenias</i>	10/25 (40.0)
	<i>Fusebacterium necrophorum</i>	10/25 (40.0)
	<i>Escherichia coli</i>	25/25 (100.0)
	<i>Pasteurella multocida</i>	15/25 (60.0)
	<i>A. pyogenes</i>	14/25 (56.0)
<b>Total positive</b>		25/25 (100.0) <sup>a</sup>
<b>at 45 days post-partum</b>	<i>Streptococcus spp.</i>	2/25
	<i>Staphylococcus aureus</i>	1/25
	<i>Pasteurella multocida</i>	2/25
	<i>Proteus vulgaris</i>	2/25
<b>Total positive</b>		5/25 (20.0) <sup>c</sup>
<b>Control groups</b>		
<b>at 15 days post-partum</b>	<i>Escherichia coli</i>	4/25 (16.0)
	<i>Streptococcus spp.</i>	3/25 (12.0)
	<i>Fusebacterium necrophorum</i>	2/25 (8.0)
	<i>Lactobacillus spp.</i>	3/25 (12.0)
	<i>Enterococcus spp.</i>	3/25 (12.0)
<b>Total positive</b>		9/25 (36.0) <sup>b</sup>
<b>at 45 days post-partum</b>	<i>Streptococcus spp.</i>	2/25 (8.0)
	<i>Lactobacillus spp.</i>	4/25 (16.0)
<b>Total positive</b>		4/25 (16.0) <sup>c</sup>

Different superscripts in the same column (Total positive) mean significant difference P<0.05

The main number of PMNs in the 1<sup>st</sup> group (RFM) at 15days post-calving was higher (P<0.01) than any those in other groups. There was also significant different in the percentage of PMNs (P<0.01) between control groups at 15 and 45 days. The mean number of a lymphocytes was significantly lower (P<0.01) in 1<sup>st</sup> group (RFMs) at 15 days than those in other groups. Also, there was a significant

differences (P<0.01) between control groups at 15 and 45 days. The number of buffaloes with ≥70% PMNs or ≤ 40% lymphocytes cells was higher (24/25, 96%) in the 1<sup>st</sup> group (RFM) at 15 days than those in 3<sup>rd</sup> group (RFM) at 45 days post-calving. The number of buffaloes with ≥70% PMNs or ≤40% lymphocytes cells was also significantly (P<0.01) higher in control group (17/25, 65%) at 15 days than

those in control group (6/25, 24%) at 45 days (Table 2).

**Table 2. The mean value of PMNs and lymphocytes (mean  $\pm$  SD) in the intrauterine perfusion fluid of the buffaloes with and without RFM, collected 15 and 45 days after parturition**

Groups	No.	PMNs Mean $\pm$ SD	Lymphocytes Mean $\pm$ SD	No. (%)
<b>Buffaloes with retained fetal membranes</b>				
at 15 days post-partum	25	83.5 $\pm$ 17.7 <sup>a</sup>	14.4 $\pm$ 16.0 <sup>d</sup>	25/25 (100.0) <sup>a</sup>
at 45 days post-partum	25	52.4 $\pm$ 29.5 <sup>c</sup>	41.7 $\pm$ 27.4 <sup>b</sup>	5/25 (20.0) <sup>c</sup>
<b>Control groups</b>				
at 15 days post-partum	25	63.9 $\pm$ 26.0 <sup>b</sup>	31.02 $\pm$ 3.3 <sup>c</sup>	9/25 (36.0) <sup>b</sup>
at 45 days post-partum	25	36.9 $\pm$ 26.3 <sup>d</sup>	55.4 $\pm$ 27.1 <sup>a</sup>	4/25 (16.0) <sup>c</sup>

Different superscripts in the same column (Total positive) mean significant difference P<0.05

There were no significant variations among the groups of the buffaloes with retained placenta and the control groups at 15 and 45 days post-calving in postpartum uterine involution (29.30 $\pm$ 1.21 and 28.47 $\pm$ 1.38 vs. 28.41 $\pm$ 1.04 and 27.95 $\pm$ 1.60), the number of days from parturition to initial insemination (90.0 $\pm$ 28.5 and 83.0 $\pm$ 3.22 vs. 84.0 $\pm$ 20.6 and 79.2 $\pm$ 28.7), the number of days to conception

(124.7 $\pm$ 56.4 and 131.2 $\pm$ 66.0 vs. 114.11 $\pm$ 19.46 and 116.0 $\pm$ 31.51), and the number of services per conception (1.78 $\pm$ 1.03 and 1.97 $\pm$ 1.06 vs. 1.62 $\pm$ 1.02 and 1.53 $\pm$ 1.03), respectively. The overall conception rate was 15(60%) and 16(64%) in the RFM group, meanwhile, it was 19(76%) and 20(80%) in the control groups (Table 3).

**Table 3. Postpartum reproductive performance in buffaloes with or without retained fetal membranes**

Groups	Buffaloes number	Uterine involution	No. of days to initial insemination	No. of days to conception	No. of services per conception	Overall conception rate
<b>Buffaloes with retained fetal membranes</b>						
RFM15	25	29.30 $\pm$ 1.21 <sup>a</sup>	90.0 $\pm$ 28.5 <sup>b</sup>	124.7 $\pm$ 56.4 <sup>c</sup>	1.78 $\pm$ 1.03 <sup>d</sup>	15(60%)
RFM45	25	28.47 $\pm$ 1.38 <sup>a</sup>	83.0 $\pm$ 3.22 <sup>b</sup>	131.2 $\pm$ 66.0 <sup>c</sup>	1.97 $\pm$ 1.06 <sup>d</sup>	16(64%)
<b>Control buffaloes</b>						
C15	25	28.41 $\pm$ 1.04 <sup>a</sup>	84.0 $\pm$ 20.6 <sup>b</sup>	114.11 $\pm$ 19.46 <sup>c</sup>	1.62 $\pm$ 1.02 <sup>d</sup>	19(76%)
C45	25	27.95 $\pm$ 1.60 <sup>a</sup>	79.2 $\pm$ 28.7 <sup>b</sup>	116.0 $\pm$ 31.51 <sup>c</sup>	1.53 $\pm$ 1.03 <sup>d</sup>	20(80%)

The same superscripts within the same column means non-significant difference P<0.05

RFM: Retained Fetal Membrane C: Control

#### 4. Discussion

The period immediately after calving is very important in the reproductive life cycle of buffalo because of the vast influence on reproductive efficiency. A normal uterine involution and the re-establishment of the ovarian function postpartum are crucial to obtain short calving to conception interval that is required to optimize milk and calf production. Retention of foetal membranes with the dominance of *E. coli* in the uterine lumen might favor the colonization of other bacteria including facultative anaerobic and strictly anaerobes in the uterine wall of buffaloes (Paisley et al. 1986; Hussain 1989). High prevalence of bacterial isolation from buffaloes after

15 days of calving in both buffaloes with or without RFM revealed mainly *E. coli*. This suggests that the bacterial contamination which is mainly *E. coli* present in the uterus shortly after parturition. This observation is in agreement with Dohmen and Sheldon (Dohmen et al. 2000; Sheldon et al. 2006) in cattle. This suggests that the bacterial contamination which is mainly *E. coli* present in the uterus after parturition might favor the development of uterine infection by other highly pathogenic organisms. While other facultative anaerobic bacteria and strictly anaerobic bacteria, which lack the ability to invade intact epithelium, are usually considered facultative pathogens (Dohmen et al. 1995; Sheldon et al. 2004).

The development of uterine disease depends on the immune response of the cattle, as well as the species and number (load and challenge) of bacteria (Sheldon et al. 2006). Therefore, damage to epithelium is usually required to establish infection (Cohen et al. 1995; Sheldon and Dobson 2004; Sheldon et al. 2004) either by *E. coli* infection or damaged epithelium resulting from obstetrical manipulation (Paisley et al. 1986; Hussain 1989). *A. pyogenes* and strictly anaerobic bacteria were never isolated from buffaloes with normal parturition after 15 days of parturition. *A. pyogenes* was isolated from 56.0% of buffaloes with RFM after 15 days post-calving. *A. pyogenes* induces metritis by synergism with gram-negative bacilli such as *F. necrophorum* and *prevotella* spp., where *F. necrophorum* is known to produce a potent leukocidal endotoxin, these toxins facilitate tissue invasion by *A. pyogenes* which in turn produces growth stimulating factor for the species of bacteriodes which seen to have unusual potent lipopolysaccharide molecules (Ruder et al., 1981; Kaneko et al., 1997; Lewis, 1997; Sheldon et al., 2004).

Bacterial isolation from RFM buffaloes after 15 days of calving included non-specific bacteria mainly *E. coli*, *A. pyogenes*, *Fusebacterium necrophorum*, *Bacterioids melaninogenias*, *Streptococcus* spp., and *Pasteurella multocida*. These results were concordant with those reported by Azawi and Taha (2002); Jadon et al. (2005); Azawi (2006). Retained foetal membranes diminish uterine ability to eliminate contaminated organisms. The exact causes of uterine infections during the postpartum period remain unknown (Lewis 1997 and Azawi, 2008.). The detection rate of bacteria and *A. pyogenes* were decreased at day 45 after parturition and were no longer significantly different from those of the control animals. From the previous results, it is clear that infection and to some extent the inflammation of the uterine wall during and after parturition must be accepted as a physiological process (Lewis, 1997; Stephen et al., 2008). *Lactobacillus* spp. was isolated only from the uterus of buffaloes after 15 and 45 days of calving in buffaloes without RFM (control groups). These bacteria were never isolated from buffaloes with RFM. This result suggests that the presence of *Lactobacillus* sp. in the uterus indicated a healthy uterus (Bondurant 1999).

In the cytological examination, the number of buffaloes with  $\geq 70\%$  PMNs or  $\leq 40\%$  lymphocytes cells was higher (24/25, 96%) in the 1<sup>st</sup> group (RFM) at 15 days than those in 2<sup>nd</sup> group (RFM) at 45 days post-calving. The number of buffaloes with  $\geq 70\%$  PMNs or  $\leq 40\%$  lymphocytes cells was also significantly ( $P<0.01$ ) higher in control group (17/25, 65%) at 15 days than those in control group (6/25,

24%) at 45 days (Table 3). The high percent of PMN in the uterus of RFM buffaloes suggests both direct and indirect effects of bacterial toxins to attract or stimulate PMN infiltration in the uterus. These observations were in agreement with the earlier observations of Zerbe et al. (2001). Others provided an evidence that placenta attracts PMN to uterine lumen (Hoedemaker et al. 1992). Moreover chemo-attractive properties of uterine fluid have been described in vitro and the uterus response quickly to an antigen with release of PMN-chemotactic mediators, which results in a rapid migration of PMNs into the uterine lumen (Pycock, 1994 and Watson et al., 1987). It could be suggested that the combined effect of RFM and bacterial infection and their toxins in the uterus attracts high number of PMN in uterine discharge of RP buffaloes. It appears that retained placenta does adversely affect the postpartum intrauterine environment but the injury is repaired at day 45 after parturition. Mechanical aspects of the uterine defense system are currently believed to be a major contributor in uterine clearance of a bacteria and inflammatory products (Troedsson & Liu, 1991; Troedsson et al., 1993; Stephen, 2008).

Retained placenta has been reported as risk factors to induce metritis and thereby to reduce subsequently fertility (Coleman et al., 1985; Dohoo & Martin, 1984; Erb et al., 1981; Halpern et al., 1985; Heinonen & Heinonen, 1989; Sheldon & Dobson, 2004). In this study, non significant difference in reproductive performance was found between the buffaloes with or without RFM. It has been reported that fertility was not affected in the buffaloes with retained placenta if metritis was not induced after placental retention or if the cattle had recovered from metritis by the time of an insemination (Stevenson & Call, 1988; and Werven et al., 1992 and Claire & Chery, 2007). *In conclusion*, as any system in the body, bacteria are regularly present in the genital tract of normal buffaloes during the different reproductive stages. These bacterial flora play an important role in genital tract protection against infection. Moreover, in most buffaloes, the retained fetal membranes without systemic involvement had no major effect on the postpartum reproductive performance.

#### Corresponding author:

H. Amer,  
Department of Theriogenology, Faculty of Veterinary Medicine, Zagazig University, El-Zeraa str. 114; 44511-Zagazig; Egypt  
Tel.: +2(055)2367711; Fax: +2(055)2283683.  
[samamed84@yahoo.com](mailto:samarmed84@yahoo.com)

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6/1/2010

## Toxoplasmosis in Naturally and Experimentally Infected Goats

AbouZeid N.Z.<sup>1</sup>, Amer H.A.<sup>2\*</sup>, T.M. Barakat<sup>2</sup>, Selim A.M.<sup>1</sup> and El-Balkemey F.A.<sup>1</sup>

Department of Infectious Diseases<sup>1</sup>, Theriogenology<sup>2</sup>, Faculty of Veterinary Medicine, Zagazig University, Egypt

\*Corresponding author: [samamed84@yahoo.com](mailto:samarmed84@yahoo.com)

**Abstract:** One hundred slaughtered goats (2-3 years old) were used for diagnosis of toxoplasmosis in naturally infected goats, and 12 healthy pregnant and nonpregnant goats were used to study the pattern of toxoplasmosis as experimental study. Prevalence of toxoplasmosis in 100 slaughtered goats revealed that 29 (29%) and 27 (27%) were seropositive by LAT and IHA tests, respectively. There was agreement between LAT and IHA 97.3% in seronegative and 93.1% in seropositive sera in goats. There was complete concordance between LAT and bioassay in cats and mice. While the agreement between IHA result and bioassay in cat and mice was 93.1% in goats. Clinical examination of experimentally infected goats revealed that all goats had slight rise of body temperature; depression, anorexia, cough, muscular hyperesthesia and diarrhea by day 5 and returned to normal by day 11. The age of fetus at the time of *T. gondii* infection is one of the known causes for the variability in clinical response. As infection of goats in early stage of pregnancy result in fetal reabsorption, while infection in mid pregnancy lead to abortion in one goat at 28 days post-infection and the other was aborted at 40 days post-infection. Moreover infections in late pregnancy resulted in delivery of viable kids. On the other hand controls goats were clinically normal and pregnant does were birth viable kids. LAT showed rapid response after 14 days post-infection, while IHA detected antibodies after 3 weeks post-infection. The antibody titers of both tests remained high until the end of experiment (48 weeks), while the titers were decreased around abortion or parturition and increased again after one week. Both LAT and IHA tests were insensitive in the pre-suckling kids from infected goats, whereas PCR gave positive results. In conclusion, PCR considered the most reliable tool for diagnosis of prenatal infection of toxoplasmosis, while LAT and IHA were considered unreliable tools for diagnosis of toxoplasmosis if they applied one week before or after kidding. [Journal of American Science. 2010;6(11):122-129]. (ISSN: 1545-1003).

**Keywords:** Prevalence, Toxoplasmosis, Goats, Abortion, Parturition.

### 1. Introduction

Improvement of goats breeding can be done through elimination of destructive factors affecting their productive potential. Toxoplasmosis is one of the most common zoonotic protozoal diseases caused by an obligatory intracellular apico-complexan protozoan. The disease is widely distributed affecting people worldwide. *Toxoplasma gondii* was firstly discovered by Nicolle & Manceaux (1908) in North Africa. *T. gondii* may be transmitted vertically by Tachyzoites to the fetus via placenta or horizontally through ingesting sporulated oocytes or tissue cysts of infected animals (Tenter et al., 2001). The infection of goats by *T. gondii* occurred through contaminated food and water by sporulated oocytes from infected cat feces (Dubey & Beattie, 1988). Therefore, toxoplasmosis in these animals was significantly associated with the presence of cats roaming in the farms. If the parasite is encountered during pregnancy, fetal infection, abortion and neonatal loss can occur, thus toxoplasma infection in goats has a major economic impact upon their farming (Dubey & Towle, 1986). The latent infected goats constitute potential source of human toxoplasmosis whose acquired infection mainly through ingesting of tissue cysts in undercooked meat

(Dubey & Beattie, 1988; El-On & Peiser, 2003). Several techniques employed for diagnosis of toxoplasmosis including coprological (feces), histological (tissues), bioassay (inoculation of cat and mice) and serological tests including dye test, IHA, LAT, ELISA and PCR (Hurtado et al., 2001; Pereira-Bueno et al., 2002; Pierglili-Fioretti, 2004). In view of the above argument, this work was planned to investigate the seroprevalence and pattern of toxoplasmosis in naturally infected and experimentally challenged goats with *T. gondii*.

### 2. Material and Methods

#### 2.1. Animals

2.1.1. Goats: One hundred goats (2-3 years old) that slaughtered at Cairo and Zagazig abattoirs were used for diagnosis of toxoplasmosis in naturally infected goats. Moreover, 12 healthy pregnant and nonpregnant goats (2-3 years old) were used to study the pattern of toxoplasmosis as experimental study. The experimental animals were proved to be free from parasitic infestation after clinical and parasitological examination (Radostits, 2007).

2.1.2. Cats: seventy four apparent normal cats 2-3 months age were used in this study at different

intervals. After parasitological examination, they proved free from; Nematodes, Cestodes, Isospora, Eimeria and *T. gondii* oocysts and serologically negative against *T. gondii* infection. They fed *ad libitum* diet according to Charles (1979). Cats were used for tissues bioassay.

**2.1.3. Mice:** one hundred and twenty three white Swiss laboratory mice, about 25 gm body weights were obtained from Unit of Laboratory Animals, Faculty of Veterinary Medicine, Zagazig University. They proved to be free from toxoplasmosis. They were used for blood bioassay.

## 2.2. Samples

**2.2.1. Blood Samples:** Ten ml of blood were collected in clean sterile dry screw capped bottle from examined goats and cats. The collected blood were left to clot at room temperature for one hour and centrifuged at 3000 rpm for 15 minutes. Sera were aspirated by Pasteur pipette in other clean dry crocked bottle which labeled in a serial number and stored at -20°C until used. Moreover, 10 ml of blood collected from goats one week post infection were put in a tube containing EDTA (ethylene diamine tetra acetic acid) and subsequently inoculated subcutaneous (S/C) into mice and also used for detection of *T. gondii* antigen by PCR.

**2.2.2. Faecal Samples:** The entire faeces from each cat were examined for oocysts as described by Dubey (2001). Moreover fecal samples from goats that used for experimental studies were examined before experiment according to Soulsby (1986).

**2.2.3. Tissue Samples:** Tissue specimens were obtained from skeletal muscles (diaphragm) of slaughtered goats; in addition to placenta and tissues of aborted fetuses. The tissue specimens were used for bioassay in mice and cats according to Dubey (2001).

## 2.3. Seroprevalence of toxoplasmosis in slaughtered and experimental goats

Serum samples were collected from slaughtered goats as well as from experimentally infected and control goats weekly interval. The serum samples were tested serologically by LAT and IHA according to (Jacob, 1973) and Camargo & Leser (1976), respectively. Blood samples on EDTA were taken one week post infection and tissue specimens from each aborted fetus and placenta examined by PCR according to Esteban-Redondo et al. (1999) and bioassay in mice according to Dubey et al. (1997).

**2.4. Polymerase chain reaction (PCR)<sup>1</sup>** according to Esteban-Redondo et al., (1999): Primer 1 corresponds to B1 gene nucleotides 694 to 714 (5' - GGAAGTGCATCCGTTCATGAG) and primer 2 is of the opposite sense and corresponds to nucleotides 887 to 868 (5' -TCTTTAAAGCGTCGTGGTC) on the antisense strand.

## 2.5. Diagnosis of toxoplasmosis in serologically positive slaughtered goats through bioassay:

Fifty gm of fresh meat (from diaphragm) were collected from serologically positive slaughtered goats (29) against toxoplasmosis. Twenty-five grams from each animal were taken and used for determination of infection by bioassay in mice through digestion technique (3 mice for each sample). Another 25gm were used for determination of infection by bioassay in cats (2 cats for each sample).

### 2.5.1. Determination of infection by digestion technique

Twenty five grams from each carcass were minced and digested in 250 ml of Pepsin hydrochloric acid; after one hour at 37°C, the suspension was filtrated via gauze and the filtrate was centrifuged for 15 min. at 2000 rpm. The supernatant was removed and the sediment was washed with sterile saline then centrifugation was repeated. To the final precipitate, 10ml of saline containing 500 units of penicillin and 0.5 mg of Streptomycin were added. Three ml of suspension were inoculated I/P into 3 mice. The mice were killed two weeks post-inoculation and their peritoneal exudates were examined microscopically for *T. gondii* tachyzoites (Dubey et al., 1997).

### 2.5.2. Determination of infection by bioassay in kittens

Twenty-five grams of diaphragms from each animal were minced and fed to 2 kittens. Entire feces of each cat were collected daily post infection and examined microscopically for the presence of *T. gondii* oocysts according to Dubey (2001). The sporulation and testing the infectivity of isolated oocysts were done according to Dubey & Beatlie (1988). These sporulated oocysts were used for experimental study. Before inoculation of infective sporulated oocysts in goats, H<sub>2</sub>So<sub>4</sub> was neutralized by 3.3% NaOH.

<sup>1</sup> Biotechnological Unit of Al Borg Lab, Elmouhandsein, 55 Abd Elmoneim Read St. Cairo

## 2.6. Ultrasonic pregnancy detection of goats

A real-time ultrasound scanner equipped with transrectal and transabdominal transducers (Pie-medical, Genus 240, Japan) was used for this study, and a well lubricated 6 MHz transducer with Carboxymethyl-cellulose conducted gel was introduced as described by Haibel (1990); Kaehn (1994) and Hesselink & Taverne (1994). Two methods of ultrasonographical examination (trans-rectal and trans-abdominal) were used to check pregnant goats

## 2.6. Disease pattern in experimental goats

Twelve goats were divided into 5 groups. Each animal (in group 1-4) was inoculated with 60,000 sporulated oocysts:

- Group-1 included 2 goats at early gestation (1-2 month).
- Group-2 included 2 goats at mid gestation (3 months).
- Group-3 included 2 goats at late gestation ( $\geq 4$  months).
- Group-4 included 2 goats (nonpregnant).
- Group-5 (control) included 4 goats (2 pregnant and 2 nonpregnant).

Daily observation of goats for any clinical manifestation was applied according to Radostits et al. (2007). Serum samples were collected from each animal in the experimental and control groups weekly (up to 48 weeks) for estimation of infection by toxoplasmosis by LAT and IHA, and another blood samples were collected on EDTA (Ethylene Diamine Tetra-acetic Acid) to detect infection by PCR and bioassay in mice.

## 2.7. Statistical analysis

The obtained data were analysed for significance using T-test (Selvin, 1996), and the variabilities were done by variant NOVA (SAS, 1996).

## 3. Results

### 3.1. Seroprevalence results

Out of 100 goats sera samples that collected from Cairo and Zagazig abattoir 29(29%) and 27 (27%) of goats were seropositive against toxoplasmosis by LAT and IHA tests. The seroprevalence was higher at Zagazig abattoir (28%) than that at Cairo abattoir (24.2%) (Table 1). The titer of LAT were ranged from 1/16 to 1/512, with the most frequency 1/32 to 1/256, while the titer of IHA were ranged from 1/80 to 1/1280 with the most frequently 1/160 (Table 2).

### 3.2. Disease pattern in goats

Daily investigation of all infected goats revealed slight rise of body temperature. The temperature started to rise at day 5, reached a peak of

41°C at day 6 and 7 and return to normal at day 11. All goats showed depression, anorexia, cough, muscular hyperesthesia, and diarrhea. Experimentally infections of goats in early stage of gestation (Group I) result in fetal reabsorption and were negative by ultrasonographical examination one-month post-infection (Image1a,b). While infection of 2 mid pregnant does (group II) result in one of these does was aborted at 28 days post-infection, whereas the other was aborted at 40 days post-infection. Moreover infection of 2 late pregnant does (Group III) were birth 4 normal viable kids their weight were 1.9 - 2 kg. The main gross lesions in the placenta were multiple focal areas of necrosis and calcification which were grayish white in color and firm in consistency (Image 2). On the other hand control does (Group V) were clinically normal and pregnant does were birth normal viable kids their weight ranged from 2 - 2.2 kg.

**Table 1: Seroprevalence of toxoplasmosis in slaughtered goats.**

Locality	Total No.	Goats			
		LAT +ve	%	IHAT +ve	%
Zagazig Abattoir	67	20	29.9	19	28.4
Cairo Abattoir	33	9	27.3	8	24.2
<b>Total</b>	<b>100</b>	<b>29</b>	<b>29</b>	<b>27</b>	<b>27</b>

LAT: Latex Agglutination Test; IHAT: Indirect Haemagglutination Test.

**Table 2: Titers of LAT and IHAT during seroprevalence of toxoplasmosis in slaughtered goats.**

Titre	No.	Goats					
		LAT -ve	IHAT 1/80	IHAT 1/160	IHAT 1/320	IHAT 1/640	IHAT 1/1280
-ve	71	71	-	-	-	-	-
1/16	5	2	1	2	-	-	-
1/32	8	-	1	6	1	-	-
1/64	7	-	-	4	2	1	-
1/128	3	-	-	-	2	1	-
1/256	4	-	-	-	1	2	1
1/512	2	-	-	-	-	1	1
<b>Total</b>	<b>100</b>	<b>73</b>	<b>2</b>	<b>12</b>	<b>6</b>	<b>5</b>	<b>2</b>

LAT: Latex Agglutination Test; IHAT: Indirect Haemagglutination Test.

Tachyzoites of *T. gondii* were recovered from the blood of 5 out of 8 experimentally infected goats one week P.I. through blood bioassay in mice. Only 5 out of 24 mice injected with blood became infected (Image 3). *T. gondii* were isolated from all placenta and aborted fetuses of infected does using tissue bioassays in mice. There were 6 out of 12 mice

and 5 out of 6 mice injected with tissue of placenta and aborted fetuses became infected. Tissue bioassay in cats revealed *T. gondii* from all placenta and aborted fetuses of experimentally infected does. There were 7 out of 8 and 4 out of 4 cats became

infected and shed oocysts after feeding tissues of placenta and aborted fetuses of experimentally infected does (Image 3 and Image 4). Blood and tissues bioassay of non-infected control goats revealed no tachyzoites or tissue cysts (Table 3).

**Table 3. Bioassay results in goats infected with sporulated oocysts.**

Sporulated oocysts	Infected stage	Groups	No. of goats	Stages of gestation	Does for Each group	Bioassay in mice			Bioassay in cats		
						No. of mice +ve /no. of inoculated	No. of mice +ve /no. of inoculated	No. of mice +ve /no. of inoculated	No. of cats +ve /no. of inoculated	No. of cats +ve /no. of inoculated	
Control	1	Early	60,000 sporulated oocysts	1	0/3	-	-	-	-	-	
	2	Early			1/3	-	-	-	-	-	
	3	Mid			1/3	1/3	2/3	2/2	2/2	2/2	
	4	Mid			1/3	2/3	3/3	2/2	2/2	2/2	
	5	Late			0/3	1/3	-	1/2	-	-	
	6	Late			1/3	2/3	-	2/2	-	-	
	7	Non			0/3	-	-	-	-	-	
	8	Non			1/3	-	-	-	-	-	
	9	Mid			0/3	0/3	-	0/2	-	-	
	10	Mid			0/3	0/3	-	0/2	-	-	
	11	Non			0/3	-	-	-	-	-	
	12	Non			0/3	-	-	-	-	-	
Total		12			5/24	6/12	5/6	7/8	4/4		

Serum samples collected from infected goats as well as control revealed that all goats were seronegative at the time of inoculation. LAT showed rapid response as antibody titer first appear after 10 days in one goat, while all infected does developed antibody titer of 1/32 or more after 12 days P.I. *T. gondii* serum antibodies peaked usually to a plateau level at 8 to 22 weeks after inoculation of the does and showing slight fluctuations until the end of experiment (48 weeks). Abortion or kidding coincided with or preceded these peak levels and antibodies titers of aborted does ranged from 1/64 – 1/128 (Table 4). Whereas IHA detected antibodies against *T. gondii* in some goats (2 does) on day 14 P.I. and all infected goats were seropositive after 3 weeks P.I. *T. gondii* serum antibodies peaked usually

to a plateau level at 9 to 26 weeks after inoculation of the does and showing slight fluctuations until the end of the experiment (48 weeks). Abortion or kidding preceded these peak levels and antibodies titers of aborted does ranged from 1/80-1/160 (Table 4). The results also revealed that antibody titer of either LAT or IHA were decreased around abortion or parturition and increase again after one week (Table 4). Both LAT and IHA were negative and insensitive in detection of congenital infection in pre-suckling kids. Whereas PCR gave positive results in the blood of all infected does one week P.I., but gave negative results in control group. Moreover, PCR gave positive results in the placenta and tissues of all aborted kids from infected does as well as in the blood of life congenitally infected kids (Table 4 and Image 5).

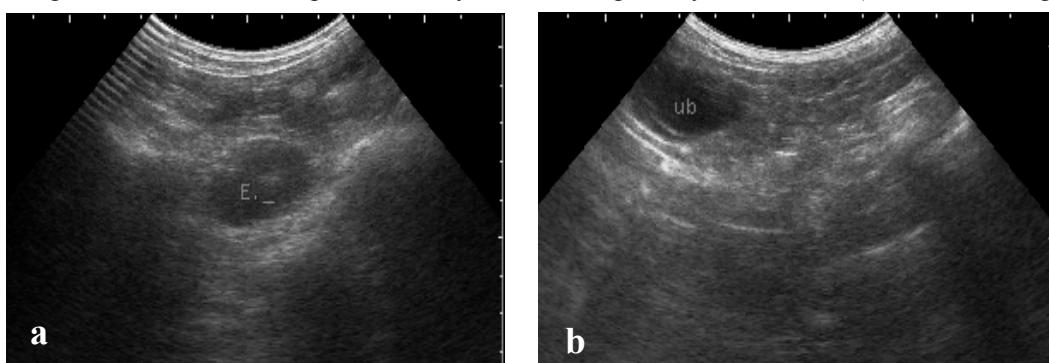


Image 1: Pregnant goat at 2<sup>nd</sup> month (a), and foetal resorption after one month post infection (b)



Image 2: A gross lesions in the placenta, where multiple focal areas of necrosis and calcification which were grayish white in

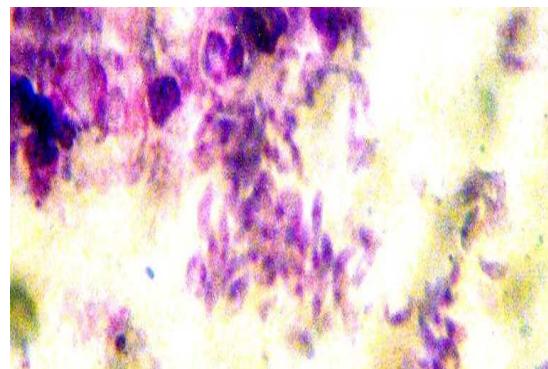


Image 3: Tachyzoites of toxoplasma gondii from peritoneal fluid of mice experimentally infected with blood of infected goats.



Image 4: Oocyst of *T. gondii* from cat faeces.

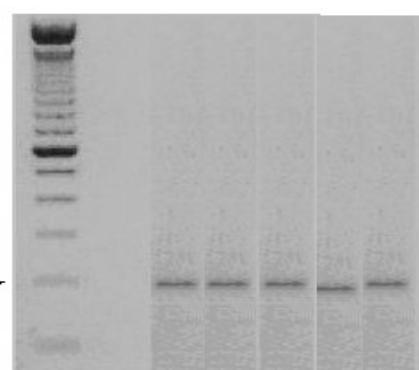


Image 5: Positive PCR results in the placenta and tissues of aborted kids from infected does as well as in the blood of life congenitally infected kid.

**Table 4. Outcomes of pregnancy in experimentally infected goats with *T. gondii* oocysts**

		Animals		Early pregnant		Mid pregnant		Late pregnant		Non-pregnant	
Does	Serological results	No		1	2	3	4	5	6	7	8
		LAT		Initial	<16	<16	<16	<16	<16	<16	<16
		IHA		Peak/plateau	32768 (70)	16384 (63)	65536 (70)	32768 (70)	16384 (56)	16384 (63)	16384 (84)
		At abortion Or kidding		-	-	128	128	64	64	-	-
		IHA		Initial	-ve	-ve	-ve	-ve	-ve	-ve	-ve
		Peak/plateau		1/10240 (84)	1/10240 (70)	1/20480 (70)	1/10240 (84)	1/20480 (63)	1/10240 (70)	1/5120 (84)	1/1024 0 (84)
		At abortion Or kidding		-	-	1/160	1/160	1/80	1/160	-	-
		PCR on blood		+	+	+	+	+	+	+	+
		At abortion or kidding days after inoculation		Fetal resorption	Fetal resorption	28	50	25	30	-	-
Kids	Condition of kid		-	-	Fresh	Fresh	Viable	Viable	-	-	
	LAT		-	-	<16	<16	<16	<16	-	-	
	IHA				<1/80	<1/80	<1/80	<1/80			
	PCR				+ve	+ve	+ve	+ve			
	T.gondii isolation		Mice	Cat	+ve	+ve	+ve	+ve	-	-	

LAT: Latex Agglutination Test; IHA: Indirect Haemagglutination Test, PCR: Polymerase Chain Reaction.

#### 4. Discussion

The sole source of infection of herbivore especially goats by *T.gondii* is sporulated oocysts in contaminated food and water from infected cat feces. Toxoplasmosis has a potential risk to human, in addition to its economic losses in goats. If the parasite is encountered during pregnancy, fetal infection; abortion and neonatal loss can occur (Buxton 1998; Bisson et al., 2000). Clinical symptoms of toxoplasmosis in goats are not specific especially in the early stage of infection. Therefore, the detection of specific toxoplasma antibodies appears to be an important tool for diagnosis of toxoplasmosis in ovine species.

Evaluation of serological tests becomes important in order to use sensitive and specific tests in serological surveys (Moreno et al., 1991). In the present study, out of 100 goats' sera slaughtered at Cairo and Zagazig abattoir, 29 (29%) and 27 (27%) of goats were seropositive against toxoplasmosis by LAT and IHA tests respectively. This percentage of infection among goats seems to be nearly similar to that reported by Pita-Gondim et al. (1999); Sharma et al. (2003) and Jittapalapong et al. (2005) using the same tests. Our result was different than those reported by Matsuo & Husin, (1996); Da Silva & Langoni (2001) and Mainardi et al. (2003). In the present study it was noticed that the percentage of infection varied from region to region. This variation may be attributed to the difference of the environmental and ecological condition, which affect the biology of the parasite or the system of breeding and hygienic measures inside farms.

Regarding the quantitative agreement in antibody titers obtained by IHA and LAT in 100 caprine serum samples examined for toxoplasmosis, it showed that out of 5 LAT positive sera at titers 1/16, 2 sera were IHA negative and all LAT result >1/32 was positive by IHA. This was in agreement with Chhabra et al. (1981), Dubey et al. (1987) and Tress et al. (1988) who found 100% correlation between LAT, IHA and dye test. They concluded that LAT is sensitive, reliable, rapidly responsive serological test and is efficient for screening purposes. Regarding to the comparison between serological results and the results of bioassay in mice and cats, the results revealed that there were complete concordance between LAT and bioassay in cats and mice, as all LAT positive sera samples of goats were positive by bioassay in cats and mice. While the agreement between IHA and bioassay in cat and mice were 93.1% in goats. These results reflect that LAT is more sensitive than IHA. This result agreed with that reported by Dubey et al. (1987); Tress et al. (1988); Figueiredo et al. (2001) and Lhafi et al. (2004) and Érica et al. (2010).

The results of clinical examination of experimentally infected goats revealed that all infected goats had slight rise of body temperature (41°C), depression, anorexia, cough, muscular hyperesthesia, and diarrhea by day 5 and returned to normal by day 11. These results were in agreement with Dubey et al. (1980) and Nishi et al. (2001). Goats in group (I), which infected at early stage of gestation revealed fetal reabsorption and negative ultrasonographical examination, one month post-infection. This result was in agreement with that recorded by Dubey (1981). Infection of 2 mid pregnant does (group II), result in abortion of one at 28 days post-infection, whereas the other was aborted at 40 days post-infection. This result was concordant with that of Buxton (1998). Moreover infections of 2 late pregnant does (Group III) were birth normal viable kids their weight were 1.9-2 kg. This result coincided with that reported by Dubey & Beattie (1988). The previous results indicate that age of fetus at the time of *T. gondii* infection in the goats is one of the known causes for this variability in clinical response. This concordant with that reported by Blewett and Watson (1983).

Blood bioassay in mice revealed *T. gondii* tachyzoites from the blood of 4 out of 8 experimentally infected goats one week P.I. only 4 out of 24 mice injected with blood became infected. These results were in agreement with that recorded by Dubey et al. (1980) and Freyre et al. (2008) who detected parasitaemia in 7 out of 7 goats that lasted 3 to 10 days, and Nishi et al. (2001) who detected parasitaemia in 50% of infected goats by mice bioassay from 7 to 14 days P.I. and isolated viable *T. gondii* from all infected goats which killed after 8 weeks P.I. Tissue bioassays in mice and cats revealed *T. gondii* from all placenta and aborted fetuses of infected does. This result was in agreement with that recorded by Dubey (1981) who isolated *T. gondii* from the fetal placenta of 6 out of 7 goats as early as 10 and as late as 15 days after inoculation. Regarding PCR results, *T. gondii* was detected from blood of life fetuses, all placenta and aborted fetuses of infected does. This result was coincided with that reported by Sreekumar et al. (2004) who found that lung, muscles and mesenteric lymph node aspirates of the doe and lung tissue of the aborted fetus were PCR positive.

With regard to the results of serological tests on experimentally infected goats LAT showed rapid response as all infected does developed antibody titer of 1/32 or more after 14 days P.I., while IHA detected antibodies after 3 weeks P.I. These results were in agreement with that reported by Vitor et al. (1999) who found that antibodies against *T. gondii* in

the sera of experimentally infected goats were ranged from 1:256 and 1:32000.

Our results revealed that both LAT and IHA tests were insensitive in detection of *T. gondii* antibodies in the post-suckling lambs from infected does. These results coincided with that reported by Dubey et al. (1987). The antibodies of LAT and IHA remained high until the end of experiment (at 48 weeks P.I.), this clarified that the high antibody titers are not necessarily diagnostic of recent infection. This result consensus with that recorded by Dubey (1985) who reported that the concentration of antibody may remain high, even into the next breeding season.

It could be concluded that, clinical symptoms of toxoplasmosis in goats are not specific; therefore serological tests appeared to be an important tool for diagnosis. Moreover, PCR considered the most reliable tool for diagnosis of prenatal infection of toxoplasmosis, while LAT and IHA were considered unreliable tools for diagnosis of toxoplasmosis if they applied one week before or after kidding.

#### **Corresponding author**

H. Amer,  
Department of Theriogenology, Faculty of Veterinary Medicine, Zagazig University, El-Zeraa str. 114; 44511-Zagazig; Egypt  
Tel.: +2(055)2367711; Fax: +2(055)2283683.  
[samarmed84@yahoo.com](mailto:samarmed84@yahoo.com)

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6/2/2010

# Photocatalytic Degradation of Monoazo and Diazo Dyes in Wastewater on Nanometer-Sized TiO<sub>2</sub>

S.A. Abo-Farha

Chemistry Department, Faculty of Science, Al-Azhar University (Girls), Naser City, Cairo, Egypt  
samiaelhosieny@yahoo.com

**Abstract :** Advanced oxidation processes (AOPs) have proved very effective in treatment of the various hazardous organic pollutants in water. The photocatalytic degradation of two azo dyes, monoazo dye Acid Orange 10(AO10) and diazo dye Acid Red114(AR114) present in wastewater were studied. Homogeneous photocatalytic degradation of the two azo dyes with UV/Visible/H<sub>2</sub>O<sub>2</sub> process was investigated. The rates of disappearance of the two azo dyes were monitored spectrophotometrically at the visible maximum absorption wavelengths. It was found that the rate of decolorization rises by increasing the initial dosage of H<sub>2</sub>O<sub>2</sub> up to a “critical” value at which it is maximum and beyond which it is inhibited. The rates of reactions follow pseudo-first-order kinetics. Also heterogeneous photocatalytic degradation of the two azo dyes with UV/Visible/TiO<sub>2</sub> (titanium dioxide) interface was investigated. The photocatalytic degradation rate depends on dye structure, dye concentration, TiO<sub>2</sub> loading and pH of the medium. The mechanism of the photodegradation process under UV-visible light illumination involves an electron excitation into the conduction band of the TiO<sub>2</sub> semiconductor leading to the generation of very active oxygenated species that attack the dye molecules leading to photodegradation .

Photocatalytic activity of TiO<sub>2</sub> was examined by focusing on its enhancement by electron scavengers in the photocatalytic decomposition of the two azo dyes. The electron scavenger employed was inorganic oxidant such as H<sub>2</sub>O<sub>2</sub>, adequate dose of H<sub>2</sub>O<sub>2</sub> led to a faster degradation of the two azo dyes in the TiO<sub>2</sub> photocatalytic system. The fast decolorization of monoazo dye (AO10) than diazo dye (AR114) is an indication that, the number of azo and sulphonate groups in the dye molecule may be a determining factor for increasing the degradation rates. TiO<sub>2</sub> can be recycled at least twice without significant change in its efficiency. The photodegradation rates of the two recycled catalysts RC-1 and RC-2 were examined. [ Journal of American Science. 2010; 6(11): 130-142]. (ISSN: : 1545-1003).

**Keywords:** Azo dyes; UV/H<sub>2</sub>O<sub>2</sub> oxidation, Titanium dioxide; Photodegradation; Semiconductor.

## 1. Introduction

Environmental pollution on a global scale, as well as the lack of sufficient clean energy sources, have drawn much attention to the need for developing ecologically clean chemical technology, materials, and process [1-4]. Azo dyes, being the largest group of synthetic dyes, constitute up to 70% of all the known commercial dyes produced. Highly substituted aromatic rings joined by one or more azo groups characterize their chemical structures. These substituted ring structures make the molecules recalcitrant which the conventional wastewater treatment processes do not degrade. Being released into the environment, these dyes not only impart colors to water sources but also damage living organisms by stopping the reoxygenation capacity of water, blocking sunlight, and therefore disturbing the natural growth activity of aquatic life [5,6]. Thus, the color removal of textile wastewater is a major environmental concern [7].

In recent years, research in new non-biological methods has led to processes which

actually destroy these pollutants in stead of simply extracting them from water (e.g., adsorption by active carbon, air stripping, etc.). It has been shown that the use of TiO<sub>2</sub>, O<sub>3</sub>, H<sub>2</sub>O<sub>2</sub>, and Fenton (a mixture of ferrous ion with H<sub>2</sub>O<sub>2</sub>) are more efficient in the photodegradation of organic pollutants in comparison to that of direct photolysis [8-10]. Among them, one of the common observations is that the enhancement of organic decomposition is due to the generation of powerful non-selective hydroxyl radical ( $\cdot$ OH) produced in the process of photodegradation.

The efficiency of advanced oxidation processes for the degradation of recalcitrant compounds has been extensively studied [11-16]. Photocatalytic process, which utilizes TiO<sub>2</sub> semiconductor photocatalyst, has received increasing attention because of its low cost, non-toxicity, relatively high chemical stability of the catalyst, and the possibility of using sunlight as a source of irradiation [5,8,11,14]. However, it has a limitation that the quantity of  $\cdot$ OH radicals cannot be increased infinitely because overdosing of TiO<sub>2</sub> scatters the light in the solution [8,17]. Therefore, new

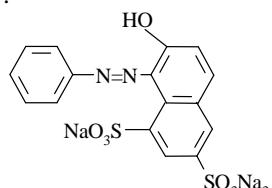
developments of these technologies have focused on searching for better oxidants to increase the generation of radicals or to optimize the photodegradation process.

It was reported that the use of inorganic oxidants, such as  $\text{H}_2\text{O}_2$ ,  $\text{ClO}_3^-$ ,  $\text{BrO}_3^-$ , and  $\text{S}_2\text{O}_8^{2-}$ , in  $\text{TiO}_2$  system increased the quantum efficiencies either by inhibiting electron-hole pair recombination through scavenging conduction band electrons at the surface of  $\text{TiO}_2$  or by offering additional oxygen atom as an electron acceptor to from the superoxide radical ion ( $\text{O}_2^\cdot$ ) [8,18]. According to the investigation on  $\text{H}_2\text{O}_2$ , adequate dose of  $\text{H}_2\text{O}_2$  led to a faster degradation of organic compounds in the  $\text{TiO}_2$  photocatalytic system [8,19]. However, the degradation was suppressed if excess  $\text{H}_2\text{O}_2$  was used. This is due to the undesirable consumption of  $\cdot\text{OH}$  radical that was previously formed in the solution by  $\text{H}_2\text{O}_2$ , leading to generation of less-reactive  $\text{HO}_2^\cdot$  radicals [8, 20]. Enhancement of  $\text{TiO}_2$ -catalyzed photodegradation of organic compounds by several inorganic oxidants was mainly attributed to the

## 2. Materials and Methods

### 2.1. Materials

Two azo dyes, monoazo dye Acid Orange 10 (AO10) and diazo dye Acid Red 114(AR114) were obtained from Lingxian Shine Coating and



C.I. Acid Orange 10

(Mol. wt. 452.376 g and  $\lambda_{\max}$  478 nm)

Hydrogen peroxide (30% w/w) was obtained from Merck. Titanium dioxide P-25 from Degussa Corporation (70% anatase, 99.8% purity, average particle size 30 nm and specific surface of 50  $\text{m}^2/\text{g}$ ). It was dissolved in deionised water using (New water purification system, Human RO 180. RO, product).

### 2.2. Physical measurements

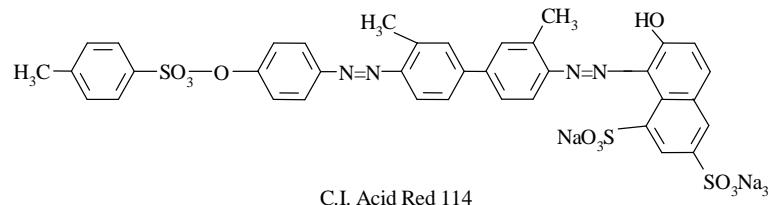
For the UV/Visible/ $\text{H}_2\text{O}_2$  and UV/Visible/ $\text{TiO}_2$  processes, irradiations were performed in a batch photoreactor. All experiments were conducted in a batch microsol light tester equipped with a pre-settable timer and water-cooling jacket (BS 1006 UK-TN) fitted with 400 W MB/U lamp show in Fig. (1) (made in England).

increased electron scavenging from the extra oxidant sources [8,21].

Several studies of photocatalytic degradation of dyes have been reported [8,22,23]. Factors influencing the photodegradation rate of aqueous system have been studied in the subjects such as the initial concentration of dyes, the effect of pH values, dissolved oxygen contents and amounts of photocatalyst added to the aqueous solution [24,25].

This paper describes the kinetics of the color removal for two dyes, monoazo dye Acid Orange 10(AO10) and diazo dye Acid Red 114 (AR114) by homogeneous photocatalytic degradation in presence of (UV/Visible/ $\text{H}_2\text{O}_2$ ), which is a 'friendly' oxidant and by heterogeneous photocatalytic degradation in presence of (UV/Visible/ $\text{TiO}_2$ ) and enhancing the photocatalytic activity of  $\text{TiO}_2$  by employing electron scavenger such as  $\text{H}_2\text{O}_2$ . Variable factors such as the initial dyes concentration,  $\text{H}_2\text{O}_2$  does,  $\text{TiO}_2$  loading and pH values have been studied. Moreover the efficiency of the recycled  $\text{TiO}_2$  was examined

auxiliaries Co. LTD. were used without further purification. Their structure are drawn.



C.I. Acid Red 114

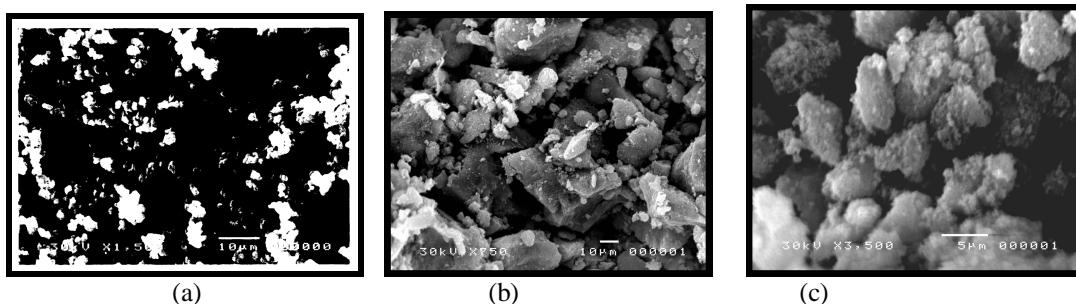
(Mol. wt. 830.8308g and  $\lambda_{\max}$  514 nm)



**Fig. (1):** Batch microsol photoreactor light tester

The pH values of the solution were adjusted, using microcomputer pH-vision DATALOGGER 6209; JENCO ELECTRONICS-LTD (made in U.S.A.). PH adjusted using dilute hydrochloric acid and sodium hydroxide solutions. Hydrochloric acid was chosen because its effected on the adsorption surface properties of the  $\text{TiO}_2$  is negligible [26]. The absorption spectra were recorded with JENWAY-6300 UV-Visible spectrophotometer. The absorbance of solutions measured using a 1cm quartz cell (made in U.K.).

### 2.3. SEM analysis



**Fig. (2):** SEM micrograph of (a)  $\text{TiO}_2$  and (b) (AO10) adsorbed on  $\text{TiO}_2$  (c) (AR114) adsorbed on  $\text{TiO}_2$

The SEM picture of pure  $\text{TiO}_2$  and (AO10) and (AR114) adsorbed on  $\text{TiO}_2$  are shown in Fig. (2). The SEM picture of pure  $\text{TiO}_2$  Fig. 2a shows that, the size of titanium dioxide particles are uniform and needle-like particles [9]. In case of (AO10) and (AR114) agglomeration (particle-particle interactions) is observed. The distribution of dye on the surface of  $\text{TiO}_2$  is not uniform and SEM pictures Fig. 2b,c show that, dyes contain irregular shaped particles which are the aggregation of tiny crystals. However, it cannot be ruled out, that some dye particles are too small to be observed at the resolution of the used microscope [4,18]. The image from Fig. 2b, c reveals that, the presence of great agglomerates with particle size of monoazo dye (AO10) than diazo dye (AR114). From this result, it is clear that the morphology has been strongly influenced by the type of acid dye [27].

### 2.4. Photocatalytic degradation experiments

#### 2.4.1. Homogeneous photocatalytic degradation

The experiments are carried out in a batch-type photoreactor.  $\text{H}_2\text{O}_2$  is acts as photocatalyst and UV/Visible/light as illuminating light source. Reaction system is setup by adding the photocatalysts into 250 ml dye solutions prepared in appropriate concentrations using deionized water. The pH is adjusted to the desired values with HCl and NaOH.

Centrifug model 800 of a maximum speed 4000r/min is used for complete separation for the semiconductor particles used ( $\text{TiO}_2$ ) from the sample solution. Scanning electron microscope (SEM) analysis is performed to identify the catalyst surface morphology using a JEOL-JSM-5400S scanning electron microscope (made in Japan). The SEM is measured in National Center for Radiation Research and Technology.

The dye solutions are stirred and divided to 5 ml samples and illuminated in a batch-type photoreactor. At regular time intervals the dye concentrations are measured spectrophotometrically.

#### 2.4.2. Heterogeneous photocatalytic degradation

Also the experiments are carried out in the same batch photoreactor. Pure  $\text{TiO}_2$  powder adding into 250 ml dye solutions prepared in appropriate concentrations using deionized water. The pH also is adjusted to the desired values. The dye solutions are stirred and divided to 5 ml samples and illuminated in batch photoreactor. At regular time intervals the samples centrifuged and dye concentrations are measured spectrophotometrically.

### 3. Results and Discussion

#### 3.1. Homogeneous photocatalytic degradation with $\text{H}_2\text{O}_2$

##### 3.1.1. Effect of initial dye concentration.

Initial dye concentrations  $C_0$  were set in the range  $1.0 \times 10^{-5}$  to  $1.0 \times 10^{-4}$  M for both two azo dyes Acid Orange 10 and Acid Red 114. Photocatalysis were occurred in presence of 5mM  $\text{H}_2\text{O}_2$  for both two azo dyes and pH 3.0. Initially, a large degree of removal is observed. This is due to fast decomposition of  $\text{H}_2\text{O}_2$  producing the hydroxyl radicals.

Degradation of the dye is due to the hydroxyl radicals generated upon photolysis of hydrogen peroxide [28-30], following the reaction:



This radical is a very powerful oxidizer, able to react with inorganic as well with aliphatic or aromatic organic compounds [31,32].

According to the results of Shu *et al.*, [28] the photooxidation reaction is pseudo-first-order with respect to azo dye concentration. This frequently occurs when the contaminant is very dilute in solutions. The kinetic constant can also be linked to the dye absorbance by Eq. (2).

$$\ln \frac{A}{A_0} = -kt \quad (2)$$

Where :  $A_0$  and  $A$  are the initial and final absorbance values of solution before and after irradiation [33].

Moreover, decolorization of dye is mainly due to hydroxyl radicals generated. Azo bonds are more active: AO10 contain one azo bond and AR114 contain two azo bonds and degradation of this dyes are due to the initial electrophilic cleavage of its chromophoric azo ( $-\text{N}=\text{N}-$ ) bond attached to naphthalene ring [34].

The values of photodegradation pseudo first order rate constants for different concentrations of dyes calculated from the linear plots of  $\ln A/A_0$  against irradiation time are given in Figs. (3,4). Taking into account that, the life-time of hydroxyl radical is very short (only few nanoseconds), they can only react where they are formed [35]. Increasing the concentrations of AO10 and AR114 lead to decrease in the degradation rates see Fig. 4 and Table 1. However, the molar extinction coefficient of the two dyes are high ( $\epsilon=16.9 \times 10^3$  and  $18.3 \times 10^3$  liter mole $^{-1}$  cm $^{-1}$  for AO10 and AR114 respectively), so that a rise in its concentration induce an inner filter effect, i.e., incident light would largely be wasted for dye excitation rather than for the hydroxyl radical precursor excitation. Consequently, the solution becomes more and more impermeable to UV radiation. As the rate of hydrogen peroxide photolysis directly depends on the fraction of incident light absorbed by  $\text{H}_2\text{O}_2$  molecules, the degradation rate slows down. The effect of direct UV/Visible irradiation was insignificant. The observed first order rate constants were  $5.2 \times 10^{-3}$  and  $4.7 \times 10^{-3}$  (min $^{-1}$ ) for AO10 and AR114 respectively. For reaction combined UV/Visible irradiation and hydrogen peroxide addition, the decolorization was extremely powerful than that of direct photolysis.

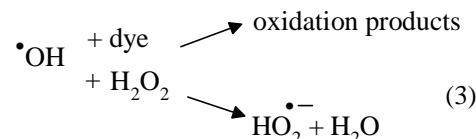
### 3.1.2. Effect of initial $\text{H}_2\text{O}_2$ concentration

The effect of varying the initial  $\text{H}_2\text{O}_2$  concentration increase from 5mM to 100 mM for dye

concentration  $1.0 \times 10^{-5}\text{M}$  at pH 3.0 for both the two azo dyes AO10 and AR114. A very large excess of  $\text{H}_2\text{O}_2$  in comparison to the dye was introduced in the solutions. Fig. 5, shows that, the initial hydrogen peroxide concentration strongly modifies the rates of degradation of the two azo dyes, Acid Orange 10 and Acid Red 114 in the UV/Visible/ $\text{H}_2\text{O}_2$  processes [19,29].

At low hydrogen peroxide concentrations, formation of  $\cdot\text{OH}$  is the kinetic determining step.  $\text{H}_2\text{O}_2$  cannot generate enough hydroxyl radicals and the oxidation rate is logically slow. Further, most of free radicals are directly consumed by the dye.

An increase of the hydrogen peroxide concentration up to 50 mM leads to an important rise in the solution discolouration rate (see Table (1)). On the other hand, further increase in the  $\text{H}_2\text{O}_2$  concentration partly inhibits the oxidation rate. This behaviour is proof of the existence of an optimal dosage in  $\text{H}_2\text{O}_2$ . We must underline the fact that hydroxyl radicals produced upon photolysis of hydrogen peroxide can react with dye molecules, but also with an excess of  $\text{H}_2\text{O}_2$ .



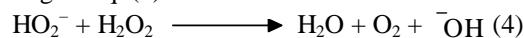
In the presence of high concentration of peroxide ( $>50$  mM), we could expect that more  $\cdot\text{OH}$  radicals would be produced. However these radicals preferentially react with the excess of  $\text{H}_2\text{O}_2$ . This undesirable reaction competes with the destruction of the dye chromophore [36,37].

### 3.1.3. Effect of initial pH

To study the effect of pH on photodegradation, experiments are conducted at  $1.0 \times 10^{-5}\text{M}$  dye concentration for the two azo dyes Acid Orange 10 and Acid Red 114 in presence of 50mM  $\text{H}_2\text{O}_2$  dose at different initial pH values ranges from 1.0 to 11.0, the calculated pseudo first-order rate constants (Fig. 6) are given in Table (1). The results show that, high degradation rate constant values are observed at pH 3.0 for both two azo dyes and decrease significantly in alkaline media. Similar results have already been reported for azo dyes [35,36].

The high rate constant value observed at lower pH can be explained by the change in the molecule structure. The presence of labile H atom makes the molecule of dye especially vulnerable toward attack of  $\cdot\text{OH}$  radicals [36].

In alkaline medium, hydrogen peroxide undergoes decomposition leading to dioxgen and water rather than producing hydroxyl radicals under UV/Visible/irradiation [38]. Therefore the instantaneous concentration in  $\cdot\text{OH}$  is lower than expected. The base-catalyzed decomposition involves the  $\text{HO}_2^-$  anion: the conjugated base of  $\text{H}_2\text{O}_2$  reacts with non-dissociated molecule of  $\text{H}_2\text{O}_2$  according to Eq. (4).



Furthermore, the deactivation of  $\cdot\text{OH}$  is greater when the pH of the solution is high (the reaction of  $\cdot\text{OH}$  with  $\text{HO}_2^-$  being approximately 100 times faster than its reaction with  $\text{H}_2\text{O}_2$  [30,38].



The reactivity of  $\text{HO}_2^-$  and its basic form  $\text{O}_2^-$  with organic compounds is very weak. They preferentially disproportionate producing some hydrogen peroxide and oxygen, according to the Eq. (7).



### 3.2. Heterogeneous photocatalytic degradation

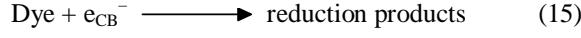
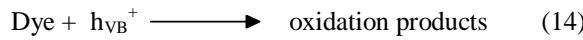
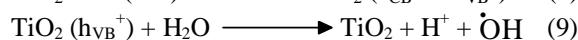
#### 3.2.1. Photocatalysis of $\text{TiO}_2$ suspension containing azo dyes

##### 3.2.1.1 Photodegradability of the dyes

Initial control experiments are carried out in order to evaluate the photocatalysis viability in the degradation of the azo dyes AO10 and AR114 under the following conditions : (i) self photolysis of dye solution with UV/Visible/light and (ii) under irradiation of UV/Visible/light with photocatalyst. Fig. (7) shows the change in absorption intensity on irradiation of an aqueous solutions of AO10 and AR114, in the presence and absence of titanium dioxide.

From the obtained results it is clear that the dyes are small decolorization to (i) direct photolysis of UV/Visible/light. Simultaneous irradiation and aeration in the presence of  $\text{TiO}_2$  caused excellent decolorisation of the dyes [39,40]. This suggested that the photocatlytic activity of  $\text{TiO}_2$  degussa P-25 is remarkable, and the photocatalytic degradation of these dyes under UV/Visible/light is significant [41-43]. This is due to the fact that when  $\text{TiO}_2$  is illuminated with the light of  $\lambda < 390\text{nm}$ , conduction band electrons ( $e^-$ ) and valence band holes ( $h^+$ ) are generated.  $\text{TiO}_2$  suspension is irradiated with light energy greater than its band gap energy (Eg, 3.2 eV).

The photogenerated electrons could reduce the dyes or react with electron acceptors such as  $\text{O}_2$  adsorbed on the Ti(III) surface or dissolved in water, reducing it to superoxide radical anion  $\text{O}_2^-$ . The photo-generated holes can oxidize the organic molecule to form  $\text{R}^+$ , or react with  $\text{OH}^-$  or  $\text{H}_2\text{O}$  oxidizing them into  $\cdot\text{OH}$  radicals. Together with other highly oxidant species (peroxide radicals) they are reported to be responsible for the heterogeneous  $\text{TiO}_2$  photodecomposition of organic substrates as dyes. According to this, the relevant reactions at the semiconductor surface causing the degradation of dyes can be expressed as follows:



The resulting  $\cdot\text{OH}$  radicals, being a very strong oxidizing agent can oxidize most of azo dyes to the mineral end-products. Substrates not reactive toward hydroxyl radicals are degraded employing  $\text{TiO}_2$  photocatalysis with rates of decay highly influenced by the semiconductor valence band edge position [44]. The role of reduced pathway (Eq. (15)) in heterogeneous photocatalysis has been investigated also in the degradation of several dyes but in a minor extent than oxidation [43,45].

##### 3.2.1.2. Factors influencing the photocatalytic degradation

###### 3.2.1.2.1 Effect of initial dye concentration

The effect of initial concentration of the dyes on the rate of degradation was performed by varying the initial dye concentration from  $1.0 \times 10^{-5}$  to  $1.0 \times 10^{-4}\text{M}$  at  $\text{pH} = 3.0$  with constant loading of  $\text{TiO}_2(0.2\text{ g/l})$  for both two azo dyes AO10 and AR114.

The values of the first order rate constants calculated according to Eq. 2. are shown in Fig. (8) [15,43,45]. It is generally noted that the degradation rate reach to maximum level [ $1.0 \times 10^{-5}\text{M}$ ] for both two azo dyes and a further increase in dyes concentrations lead to decrease the degradation rate of the dyes [46,47]. The rate of degradation relates to the probability of  $\cdot\text{OH}$  radicals formation on the

catalyst surface and to the probability of  $\cdot\text{OH}$  radicals reacting with dye molecules. The degradation efficiency of the dye decreases as the dye concentration increases further. The presumed reason is that at high dye concentrations the generation of  $\cdot\text{OH}$  radicals on the surface of catalyst is reduced since the active sites are covered by dye ions. Another possible cause for such results is the UV/Visible screening effect of the dye itself. At a high dye concentration, a significant amount of UV/Visible may be absorbed by the dye molecules rather than the  $\text{TiO}_2$  particles and that reduce the efficiency of the catalytic reaction because the concentrations of  $\cdot\text{OH}$  and  $\text{O}_2^{\cdot-}$  decrease [40,48].

Moreover the major portion of degradation occurs in the region near to the irradiated side (termed as reaction zone) where the irradiation intensity is much higher than in the other side [49]. Thus at higher dye concentration, degradation decreases at sufficiently long distances from the light source or the reaction zone due to the retardation in the penetration of light.

Hence, it is concluded that as initial concentration of the dye increases, the requirement of catalyst surface needed for the degradation also increases [50].

### 3.2.1.2.2. Effect of $\text{TiO}_2$ loading

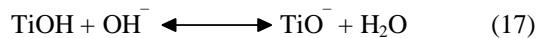
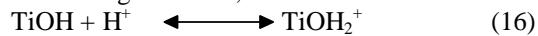
$\text{TiO}_2$  dosage is an important parameter that can affect the degradation rate. The initial reaction rates were found to be directly proportional to catalyst concentration indicating the heterogeneous regime. However, it was observed that, above a certain level of concentration the reaction rate decreases and becomes independent on the catalyst concentration.

In this work, the effect of  $\text{TiO}_2$  loading on the photodegradation rate of the two azo dyes AO10 and AR114 has been examined by varying its amount from 0.05 to 0.2 g/l ( $[\text{AO10}]=[\text{AR114}]=1.0 \times 10^{-5}\text{M}$ , pH = 3.0 for both two azo dyes). The linear plots of  $\ln A/A_0$  against irradiation time (Fig. 9) and photodegradation rate constants for both two azo dyes are given in Fig. (10). Fig. (10) shows that, the rate of photodegradation increase with catalyst loading up to 0.2 g/l. Above this loading, increase in turbidity of the solution reduces the light transmission through the solution, so this work stopped at this level. While below this level, it is assumed that the catalyst surface and absorption of light by the catalyst are the limiting factors. Many authors have investigated the reaction rate as a function of catalyst loading under different experimental conditions [51]. The enhancement of

the removal rate is due to (i) the increase in the amount of catalyst weight which increases the number of dye molecules adsorbed (ii) the increase in the density of particles in the area of illumination [34]. Thus higher amount of the catalyst may not be useful both in view of possible aggregation as well as reduced irradiation field due to increase in light scattering [52].

#### 3.2.1.2.3. Effect of pH

The interpretation of pH effects on the efficiency of dye photodegradation process is a very difficult task because of its multiple roles. First, is related to the ionization state of the surface according to the following reactions,



as well as to that of reactant dyes and products such as acids and amines. pH changes can be thus influence the adsorption of dye molecules onto the  $\text{TiO}_2$  surfaces, an important step for the photocatalytic oxidation to take place [53]. Bahnemann *et al.* [54] have already reviewed that acid-base properties of the metal oxide surfaces can have considerable implications upon their photocatalytic activity. The point of zero charge (PZC) of the  $\text{TiO}_2$  (Degussa P-25) is 6.8 [55]. Thus, the  $\text{TiO}_2$  surface is positively charged in acidic media ( $\text{pH} < 6.8$ ), whereas it is negatively charged under alkaline conditions ( $\text{pH} > 6.8$ ).

Second, hydroxyl radicals can be formed by the reaction between hydroxide ions and positive holes. The positive holes are considered as the major oxidation species at low pH whereas hydroxyl radicals are considered as the predominant species at neutral or high pH levels [48]. It was stated that in alkaline solution  $\cdot\text{OH}$  are easier to be generated by oxidizing more hydroxyl ions available on  $\text{TiO}_2$  surface, thus the efficiency of the process is logically enhanced [42]. Similar results are reported in the photocatalysed degradation of acidic azo dyes and triazine containing azo dyes [50], although it should be noted that in alkaline solution there is a coulombic repulsion between the negative charged surface of photocatalyst and the hydroxide anions. This fact could prevent the formation of  $\cdot\text{OH}$  and thus decrease the photoxidation. Very high pHs have been found favorable even when anionic azo dyes should hamper adsorption on the negatively charged surface [56]. At low pH, reduction by electrons in conduction band may play a very important role in the degradation of dyes due to the reductive cleavage of azo bonds.

Third the  $\text{TiO}_2$  particles tend to agglomerate under acidic condition and the surface area available

for dye adsorption and photon absorption would be reduced [53]. Hence, pH plays an important role both in the characteristics of textile waters and in the reaction mechanisms that can contribute to dye degradation, namely, hydroxyl radical attack, direct oxidation by the positive hole and direct reduction by the electron in the conducting band.

The wastewater from textile industries usually has a wide range of pH values. Generally, pH plays an important role both in the characteristics of textile wastes and generation of hydroxyl radicals [50]. Hence, attempts have been made to study the influence of pH in the degradation of the two azo dyes AO10 and AR114. The photocatalytic reactions are conducted at different pH values ranges from 1.0 to 11.0 and two dyes concentration [ $1.0 \times 10^{-5}$  M] at catalyst loading of 0.2 g/l. All reactions followed an apparent first-order kinetics confirmed by the linear transform of  $\ln(A/A_0) = f(t)$ . The rate constants of disappearance k in  $\text{min}^{-1}$  for two azo dyes AO10 and AR114 are illustrated in Fig. (11).

The results obtained in Fig. (11) indicated that, at  $\text{pH} < 6$  a strong adsorption of the two azo dyes on the  $\text{TiO}_2$  particles is observed as a result of the electrostatic attraction of the positively charged  $\text{TiO}_2$  with the dyes. At  $\text{pH} > 6.8$  as two azo dyes molecules are negatively charged in alkaline media, their adsorption is also expected to be affected by an increase in the density of  $\text{TiO}^-$  groups on the semiconductor surface. Thus, due to coulombic repulsion the dyes are scarcely adsorbed [42]. For the above reasons the photocatalytic activity of anionic dyes (mainly sulphonated dyes) reached a maximum in acidic conditions followed by a decrease in the pH range 7-11 [40,43,47].



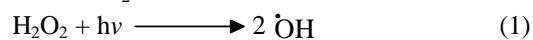
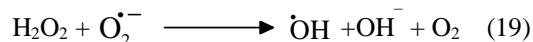
Moreover, the higher degradation rate at acid pH is seen also for UV/Visible/ $\text{TiO}_2$  experiments due to the efficient electron-transfer process due to strong surface complex bond formation. This effect is less marked in neutral/basic pH solutions [57]. The inhibitory effect seems to be more pronounced in the alkaline range ( $\text{pH}=11-13$ ). At high pH values the hydroxyl radicals are rapidly scavenged and they do not have the opportunity to react with dyes [58].

Since the influence of the pH is dependent on dye type and on properties of  $\text{TiO}_2$  surface. This effect on the photocatalytic efficiency must be accurately checked before any application.

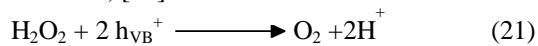
### 3.2.2. Photocatalysis of $\text{TiO}_2$ suspension with $\text{H}_2\text{O}_2$ containing azo dyes

It was observed that  $\text{H}_2\text{O}_2$  addition was beneficial for the photooxidation of the two azo dyes AO10 and AR114 [46]. The reactive radical

intermediate  $\cdot\text{OH}$  formed from  $\text{H}_2\text{O}_2$  by reactions with the photogenerated electrons can exert a dual function : as strong oxidant themselves and as electron scavengers, thus inhibiting the electron-hole recombination of the semiconductor surface [59] according to the following equations :



Moreover, the solution phase may at times be oxygen starved, because of either oxygen consumption or slow oxygen mass transfer. Peroxide addition thereby increases the rate towards what it would have been an adequate oxygen supply. However,  $\text{H}_2\text{O}_2$  can also become a scavenger of valence band holes and  $\cdot\text{OH}$ , when present at high concentration, [40].



As both  $\text{h}_{\text{VB}}^+$  and  $\cdot\text{OH}$  are strong oxidants for dyes, the photocatalytic oxidation will be inhibited when  $\text{H}_2\text{O}_2$  level gets too high. Furthermore, can be adsorbed onto  $\text{TiO}_2$  particles to modify their surfaces and subsequently decrease its catalytic activity.

In this work, the effect of varying dose of  $\text{H}_2\text{O}_2$  [5mM-100mM] in presence of constant weight of  $\text{TiO}_2$  (0.05 g/l) was also studied in the presence of the two azo dyes ([AO10] and [AR114] =  $1.0 \times 10^{-5}$  M) at constant  $\text{pH}=3.0$  Fig. (12). The results demonstrate that, the rate of photodegradation enhanced in presence of  $\text{H}_2\text{O}_2$  with  $\text{TiO}_2$  from 50mM  $\text{H}_2\text{O}_2$  without  $\text{TiO}_2$  (Fig. 5) to 10mM  $\text{H}_2\text{O}_2$  in presence of 0.05 g/l  $\text{TiO}_2$  for AO10 (Fig. 13), while the rate of photodegradation remain constant in both two cases at 50 mM  $\text{H}_2\text{O}_2$ , for AR 114 without  $\text{TiO}_2$  (Fig. 5) and in presence of 0.15 g/l  $\text{TiO}_2$  Fig. (13).

On the other hand, the effect of  $\text{TiO}_2$  loading on the photodegradation rates of AO10 and AR114 has been examined by varying its amount from 0.05 to 0.20 g/l, ([AO10] = [AR114] =  $1.0 \times 10^{-5}$  M),  $\text{pH}=3.0$ ,  $[\text{H}_2\text{O}_2]=10\text{mM}$ , 50mM for AO10 and AR114 respectively. The calculated photodegradation rate constants are given in Fig. (14). The results, demonstrate that the rate of photodegradation reached to maximum value, when loading by ([0.05 g/l  $\text{TiO}_2$ ] and in presence of 10 mM  $\text{H}_2\text{O}_2$  for AO10) compared to [0.20 g/l  $\text{TiO}_2$ ] without  $\text{H}_2\text{O}_2$  for the same dye Fig. (10). Also the rate of photodegradation of AR114 enhances in presence of  $\text{H}_2\text{O}_2$  with  $\text{TiO}_2$  from 0.20 g/l

TiO<sub>2</sub> without H<sub>2</sub>O<sub>2</sub> Fig. (10) to 0.15 g/l TiO<sub>2</sub> in presence of 50 mM H<sub>2</sub>O<sub>2</sub> Fig. (14).

Also the concentrations of the two azo dyes were increased in presence of H<sub>2</sub>O<sub>2</sub> with TiO<sub>2</sub> from [1.0 x 10<sup>-5</sup> M] to [5.0 x 10<sup>-5</sup> M] for (AO10) and [1.0 x 10<sup>-4</sup> M] for (AR114) in presence (0.05 g/l TiO<sub>2</sub> with 10 mM H<sub>2</sub>O<sub>2</sub> for (AO10) and 0.15 g/l TiO<sub>2</sub> with 50 mM H<sub>2</sub>O<sub>2</sub> for AR114) at pH 3.0. The photodegradation rates reached to maximum at 75.4x10<sup>-3</sup> (min<sup>-1</sup>) for (AO10) and 42.9x10<sup>-3</sup> (min<sup>-1</sup>) for (AR114) Fig. (15). The effect of use TiO<sub>2</sub> with H<sub>2</sub>O<sub>2</sub> on the pH values was fixed at pH 3.0 for both two azo dyes [1.0 x 10<sup>-5</sup> M].

From the above results it was found that, there were optimum conditions for each process to obtain excellent degradation for each dye. The optimum conditions in presence of UV/H<sub>2</sub>O<sub>2</sub> or UV/TiO<sub>2</sub> alone was 50 mM H<sub>2</sub>O<sub>2</sub> or 0.20 g/l TiO<sub>2</sub> for both two azo dyes. These conditions were reduced in presence of UV/H<sub>2</sub>O<sub>2</sub>/TiO<sub>2</sub> to (10mM H<sub>2</sub>O<sub>2</sub> and 0.05 g/l TiO<sub>2</sub> for AO10) and (50 mM H<sub>2</sub>O<sub>2</sub> and 0.15 g/l TiO<sub>2</sub> for AR114) Fig. (16).

### 3.2.3 Efficiency of the recycled TiO<sub>2</sub>

Photocatalysis is a clean technology, which normally dose not involve any waste disposal

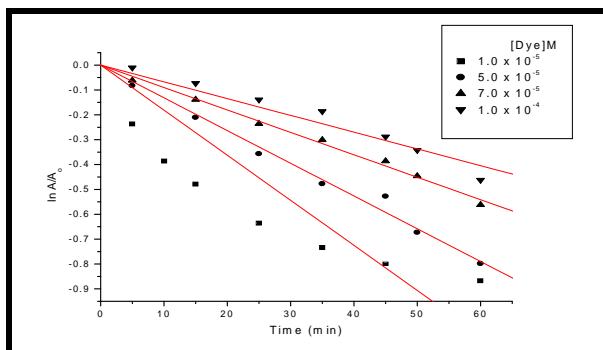
problem. The catalysts can be recycled. TiO<sub>2</sub> can be used at least twice without significant change in the efficiency [60]. The economy of the photocatalytic process depends upon how many times a catalyst can be reused without sacrificing its efficiency and the type of regeneration it requires.

The photodegradation rates of the two recycled catalysts RC-1 and RC-2 were examined . RC-1 achieved 50.4 x 10<sup>-3</sup> (min<sup>-1</sup>) for (AO10) and RC-2 achieved 43.8 x 10<sup>-3</sup> (min)<sup>-1</sup> for (AR114) compared to 73.2 x 10<sup>-3</sup> and 42.9 x 10<sup>-3</sup> (min)<sup>-1</sup> for (AO10) and (AR114) respectively obtained with the fresh catalyst under the same experimental conditions. The used catalyst was regenerated to get RC-1 first by treating with boiling distilled water till a colorless wash liquid was obtained and then by drying it in a hot air oven at a temperature of 90 to 100°C. RC-1 was heated in a muffle furnace at about 600°C to yield RC-2.

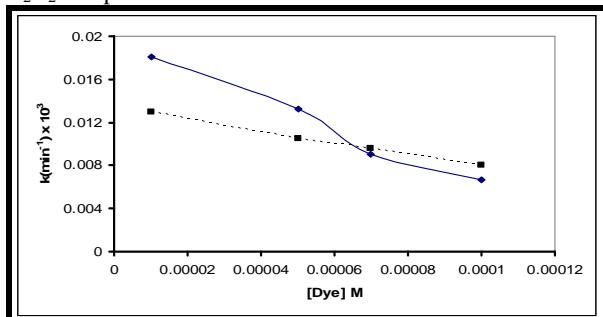
The decrease in the efficiency of the recycled catalyst may be attributed to the deposition of photoinsensitive hydroxides (Fouling) on the photocatalysts surface blocking its active sites.

**Table (1):** Photodegradation rates of (AO10) and (AR114) in presence of different parameters.

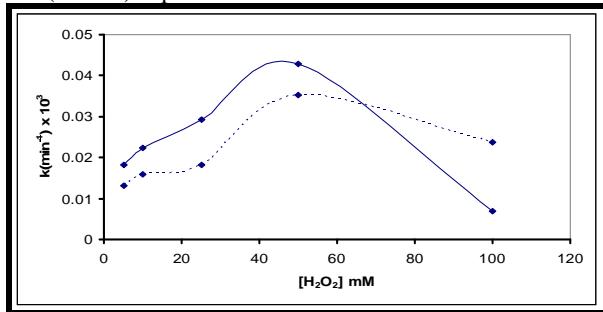
Parameters	k(min <sup>-1</sup> ) x 10 <sup>3</sup>	
	AO10	AR114
[Dye] M		
1.0 x 10 <sup>-5</sup>	18.1	13.0
5.0 x 10 <sup>-5</sup>	13.2	10.5
7.0 x 10 <sup>-5</sup>	9.0	9.6
1.0 x 10 <sup>-4</sup>	6.7	8.0
[H <sub>2</sub> O <sub>2</sub> ]mM		
5	18.1	13.2
10	22.4	15.8
25	29.3	18.3
50	42.9	35.2
100	6.8	23.8
pH		
1	20.5	15.3
3	42.9	35.2
5	15.0	10.8
7	10.0	9.0
9	9.3	8.3
11	5.7	2.0



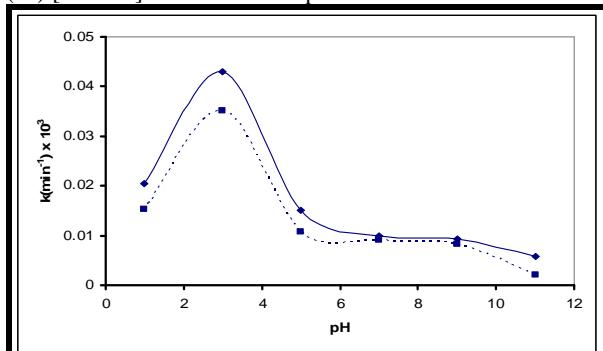
**Fig. (3):** Kinetics of photodegradation of (AO10) at different initial dye concentrations in presence of 5mM  $H_2O_2$  and pH 3.0.



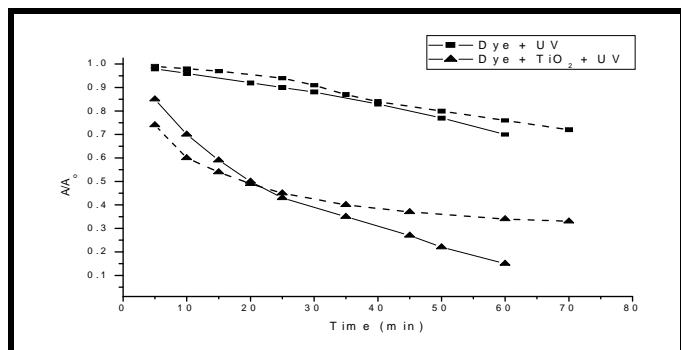
**Fig. (4):** Plot of  $k$  ( $\text{min}^{-1}$ ) vs. [dye] for (—) [AO10] and (---) [AR 114] in presence of [5mM  $H_2O_2$ ] for both (AO10) and (AR114) at pH 3.0.



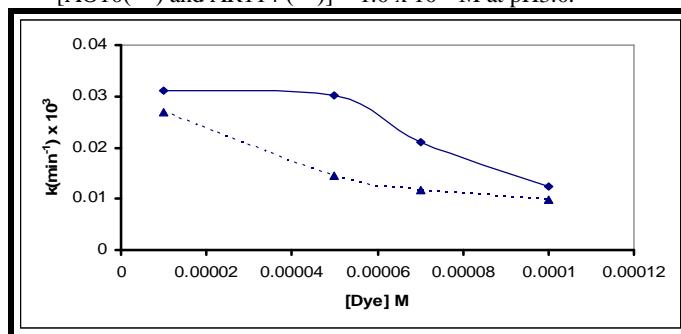
**Fig. (5):** Plot of  $k$  ( $\text{min}^{-1}$ ) vs.  $[H_2O_2]$  mM for (—) [AO10] and (---) [AR 114] =  $1.0 \times 10^{-5}$  M at pH3.0.



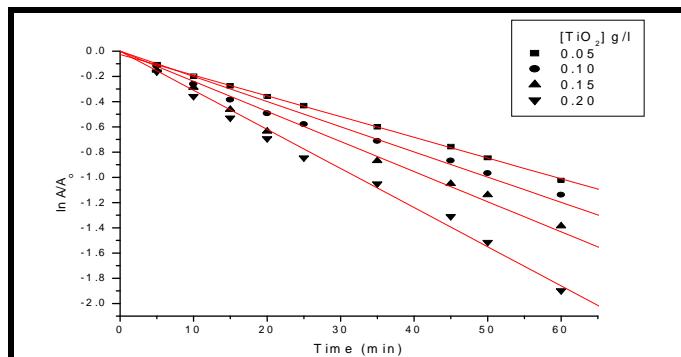
**Fig. (6):** Plot of  $k$  ( $\text{min}^{-1}$ ) vs. pH for (—) [AO10] and (---) [AR 114] =  $1.0 \times 10^{-5}$  M in presence of 50mM  $H_2O_2$  for both (AO10) and (AR114).



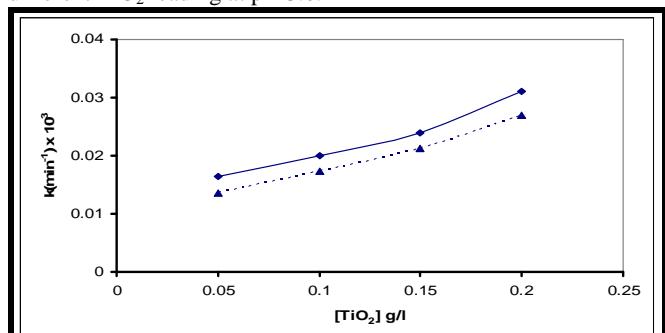
**Fig. (7) :** Plot of absorbance at  $\lambda_{\text{max}}$  (478, 514 nm) vs. time for [AO10](—) and AR 114 (---) =  $1.0 \times 10^{-5}$  M at pH3.0.



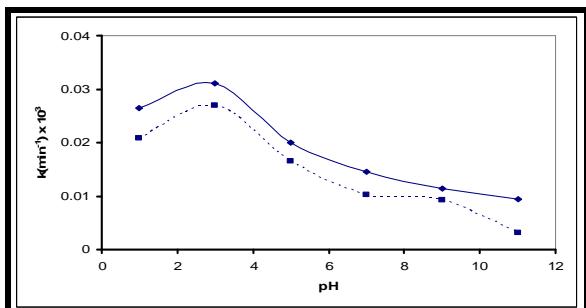
**Fig. (8):** Plot of  $k$  ( $\text{min}^{-1}$ ) vs. [dye] for (—) [AO10] and (---) [AR 114] in presence of 0.2 g/l  $TiO_2$  for both two azo dyes at pH3.0.



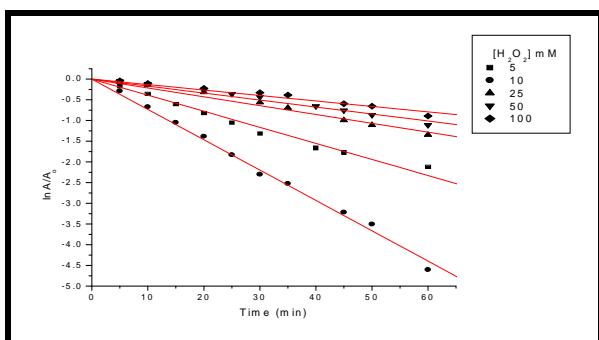
**Fig. (9):** Kinetics of photodegradation of [AO10] =  $0 \times 10^{-5}$  M at different  $TiO_2$  loading at pH 3.0.



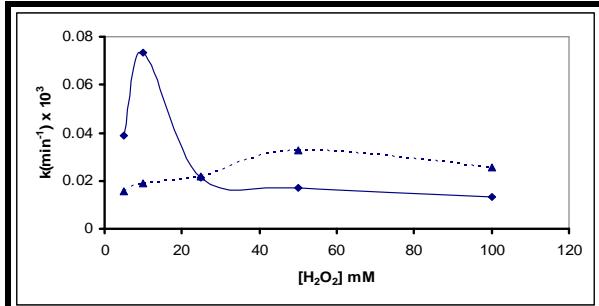
**Fig. (10):** Plot of  $k$  ( $\text{min}^{-1}$ ) vs.  $[TiO_2]$  for (—) [AO10] and (---) [AR 114] =  $1.0 \times 10^{-5}$  M at pH3.0.



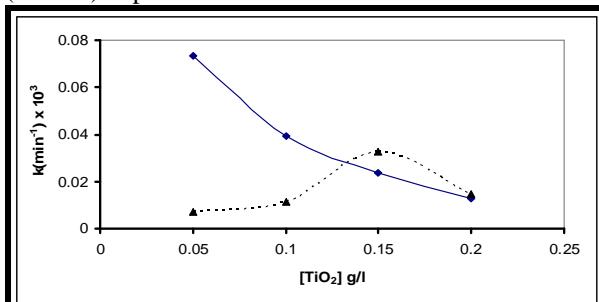
**Fig. (11):** Plot of  $k$  ( $\text{min}^{-1}$ ) vs. pH for (—) [AO10] and (---) [AR 114] =  $1.0 \times 10^{-5}$  M in presence of 0.2 g/l  $\text{TiO}_2$  for both two azo dyes.



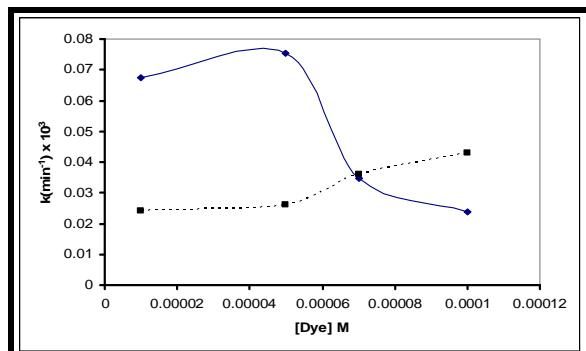
**Fig. (12):** Kinetics of photodegradation of [AO10] =  $1.0 \times 10^{-5}$  M at different  $\text{H}_2\text{O}_2$  concentrations in presence of 0.05 g/l  $\text{TiO}_2$  at pH 3.0.



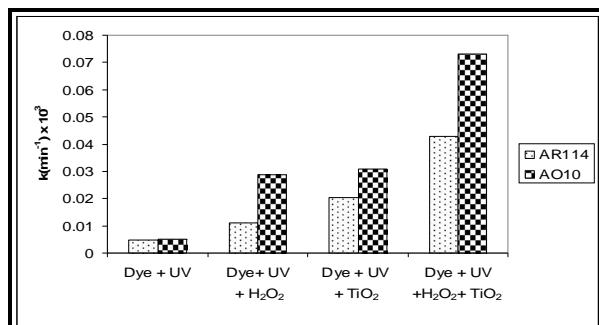
**Fig. (13):** Plot of  $k$  ( $\text{min}^{-1}$ ) vs.  $[\text{H}_2\text{O}_2]$  for (—) [AO10] and (---) [AR 114] =  $1.0 \times 10^{-5}$  M in presence of 0.05 g/l  $\text{TiO}_2$  for (AO10) and 0.15 g/l  $\text{TiO}_2$  for (AR114) at pH3.0.



**Fig. (14):** Plot of  $k$  ( $\text{min}^{-1}$ ) vs.  $[\text{TiO}_2]$  for (—) AO10 and (---) [AR 114] =  $1.0 \times 10^{-5}$  M in presence of 10 mM  $\text{H}_2\text{O}_2$  for (AO10) and 50mM for (AR114) at pH3.0.



**Fig. (15):** Plot of  $k$  ( $\text{min}^{-1}$ ) vs. [dye] for (—) [AO10] and (---) [AR 114] in presence of 0.05 g/l  $\text{TiO}_2$  and 10mM  $\text{H}_2\text{O}_2$  for (AO10) and 0.15 g/l  $\text{TiO}_2$  and 50mM  $\text{H}_2\text{O}_2$  for (AR114) at pH3.0.



**Fig. (16):** Plot of  $k(\text{min}^{-1})$  vs(AO10) and (AR114) at the best conditions for each process to obtain degradation at pH 3.0

#### 4. Conclusion

The impact of  $\text{H}_2\text{O}_2$  initial concentration in color removal kinetics under UV/ $\text{H}_2\text{O}_2$  process for two azo dyes (monoazo dye (AO10) and diazo dye (AR114)) was investigated. The results prove that the pseudo-first order kinetic model is in good agreement with the experimental data. A set of parameters for the model depicting this degradation were determined. These parameters, will naturally vary with the operating conditions, such as (dye concentration,  $\text{H}_2\text{O}_2$  dose, pH values). An increase in  $\text{H}_2\text{O}_2$  dose leads to a faster degradation up to a critical value; at a higher ratio the degradation process become slower.

The photodegradation of the two azo dyes catalyzed by UV/ $\text{TiO}_2$  was carried out with good results. Various parameters such as (dye concentration,  $\text{TiO}_2$  loading, pH values) were tested. The photocatalytic degradation rates for the two dyes were enhanced in presence of UV/ $\text{H}_2\text{O}_2/\text{TiO}_2$  at optimum conditions for each process and dye. The monoazo dye (AO10) was degraded faster than the diazo dye (AR114).

It may be postulated that, the dye color removal and degradation rates are proportional to the number of azo and sulphonic groups present in their molecules.

In summary, this work demonstrates that photocatalysis is a very effective technology for degrading Acid dyes with azo and sulphonic groups. Moreover, this technology can be utilized directly in dye baths before they are mixed with other textile effluents, which make their treatment difficult and costly due to dilution.

#### **Corresponding author**

S.A. Abo-Farha

Chemistry Department, Faculty of Science, Al-Azhar University (Girls), Naser City, Cairo, Egypt ,  
E-mail: [samiaelhosieny@yahoo.com](mailto:samiaelhosieny@yahoo.com)

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6/1/2010

# Phenotypic and genetic trends for milk production in Egyptian buffaloes

Fooda, T. A.; Kawthar A. Mourad and Gebreel, I. A

Animal Production Research Institute-Buffalo Breeding Research Department- Dooki- Giza - Egypt

**Abstract:** A total of 3495 records collected from 904 buffalo cows progeny of 174 sires and 470 dams through period from 1990 to 2008 in all Stations belonging to Animal Production Research Institute, Ministry of Agriculture were used to estimate the phenotypic and genetic trends for total milk yield. LSM for total milk at different year of calving ranged between 1334 kg and 1692 kg, 1028 kg and 1561 kg, 1209 kg and 1633 kg, 1355 kg and 1415 kg and 1137 kg and 1355 kg for El-Nattafe El-Gidid (NG), El-Nattafe El-Kadim (NK), Mahalet Mousa (MM), Gemiza (G) and Sids (S) stations, respectively. Estimates of the positive breeding value (BV, %) at different year of calving ranged between 40 % and 52 %, 31 % and 52 %, 40 % and 56 %, 37 % and 55 % and 45 % and 59 % for NG, NK, MM, G and S stations, respectively. Annual phenotypic trend for milk production ranged between -11.7 kg and +36.7 kg for S and NK stations, respectively. While, the annual genetic trend ranged between -0.16 kg and +0.6 kg for G and NG stations, respectively. The results of the present study showed that there are increased of improvement of phenotypic and genetic trend in all MM farms from 1990 until now. [Journal of American Science. 2010;6(11):143-147]. (ISSN: 1545-1003).

**Keywords:** buffalo, phenotypic trend, genetic trend, breeding value and milk production

## 1. Introduction

The Egyptian buffaloes occupies an important role among the domestic animals as a provider of dairy produce beef. It contribution about 70 % of total country milk production, although the population of dairy animals (8.5 million) is almost equally divided between cows and buffaloes. Milk yield in buffaloes is affected by several genetic and non-genetic factors, that modulate the expression of the genetic merit (Khattab and Mourad, 1992 and Mourad *et al.*, 2005).

Since the productivity of the Egyptian buffaloes in nearly similar to that of other buffalo breeds, the introduction of foreign breed of buffalo will not contribute significantly in improving the genetic make up of the Egyptian buffaloes as in case of the native cattle. Therefore, improving the productivity of the buffalo done through selection. Open nucleus herd system (Steane, 1990) were applied from 1997 until now and established in El-Nattafe El-Gidid. Objectives of the present study were to estimate genetic and phenotypic trends in a five herds over 18 years and genetic evaluation of nucleus scheme.

## 2. Material and Methods

The data were collected from Stations : El-Nattafe El-Gidid, El-Nattafe El-Kadim, Mahalet Mousa (Kafr El-Sheikh Governorate), Sids (Bani Sewafe Governorate) and Gemiza (Gharbia Governorate) belonging to Animal Production Research Institute, Ministry of Agriculture. A total of 3495 records collected from 904 buffalo cows'

progeny of 174 sires and 470 dams and their sires having 5 progeny or more through period from 1990 to 2008 were used to estimate the phenotypic and genetic trend for total milk yield.

Buffaloes were kept in open sheds and grazed on berseem from December to May. They were hand-milked twice daily. Heifers were served for the first time when they reach 330 kg and / or 24 mo.. The cows should be dried off two months before the calving date, and they served not before two months after calving.

The Animal model (derivative - free restricted maximum likelihood: DFREML, Meyer, 1997) used to prediction of buffaloes breeding value for total milk yield according to the following model:

$$\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{Z}_a \mathbf{a} + \mathbf{Z}_c \mathbf{c} + \mathbf{e}$$

where:  $\mathbf{y}$  = Vector of observations,  $\mathbf{X}$  = Incidence matrix relating fixed effects to  $\mathbf{y}$ ,  $\mathbf{b}$  = Vector of an overall mean and fixed effects (parity, year, season of calving and lactation period as a covariate),  $\mathbf{Z}_a$  = Incidence matrix relating direct additive genetic effects to  $\mathbf{y}$ ,  $\mathbf{a}$  = Vector of random effect (direct additive genetic associated with the incidence matrix  $\mathbf{Z}_a$ ,  $\mathbf{Z}_c$  = Incidence matrix for permanent environmental effect,  $\mathbf{c}$  = Vector of permanent environmental effect associated with the incidence matrix  $\mathbf{Z}_c$  and  $\mathbf{e}$  = Vector of random residual effects  $N(0, I\sigma^2_e)$ ;  $I$  is an identity matrix. The variance-covariance of the random effects was as follows

$$\text{Var} \begin{bmatrix} a \\ c \\ e \end{bmatrix} = \begin{bmatrix} A\sigma^2_a & 0 & 0 \\ 0 & I_c\sigma^2_c & 0 \\ 0 & 0 & I_n\sigma^2_e \end{bmatrix}$$

Where: A = Numerator relationship matrix,  $I_c$ ,  $I_n$  = Identity matrix with order equal to number of animals and number of records, respectively.

The phenotypic trends were measured as the regression of least squares means on calving years. Also, the genetic trends were measured as the regression of breeding value on calving years.

$$Y = a + Xb$$

Where: Y = total milk yield or breeding value, A = Intercept, X = Calving Year and b = the regression coefficient for Y on X.

### 3. Results and Discussion

Least square means for TMY, LP and BV (TMY) (mean, minimum, maximum and percentage of positive) are presented in Table 1. The present estimate of TMY is lower than those reported by Soliman *et al.* (1985) and Badran *et al.* (1991) (2159 and 2241 kg, respectively) on Egyptian buffaloes. But similar to those reported by Khattab *et al.* (1985) 1456 kg, otby *et al.* (1989) 1292 kg, Ashmawy (1991) 1564 kg, Khalil *et al.* (1992) 1249 kg, Khattab and Mourad (1992) 1309 kg, Khalil (1993) 1249 kg, Mansour *et al.* (1993) 1363 kg, Abd El-Raoof (1995) 1505 kg and Mourad *et al.* (2005) 1581 kg, on Egyptian buffaloes.

The differences between the estimates in present study and other studies may be attributed to the herds size, climatic and managerial conditions and / or different genetically make up. The percentage of positive for BV (TMY) ranged from 40 to 52 %, from 31 to 52 %, from 40 to 56 %, from 40 to 50 %, from 37 to 55 %, from 45 to 59 % and from 41 to 50 % in NG, NK, MM, All MM, G, S and All stations, respectively (Table 1).

Table (2) shows the intercept (a), regression coefficient (b) and accuracy ( $R^2$ ) for TMY and BV (TMY) on calving years. The accuracy ranged from

55 to 79 % for TMY and from 51 to 72 % for BV (TMY).

Table (3) showed the amount of phenotypic and genetic change for TMY. The annual phenotypic change ranged from -11.7 to +36.7 kg in S and NK farms, respectively. While, the annual genetic change ranged from -0.16 to +0.6 kg in G and NG farms, respectively. Also NK farm have a good annual phenotypic and genetic change (+36.7 and +0.57 kg, respectively). The experimental stations in all MM were best annual phenotypic change (+26.0 kg). But, different in Sids and Gemiza farms (-11.7 and +2.3 kg, respectively). While, the experimental stations in all MM and Sids farms were best annual genetic change (+0.58 and +0.54 kg, respectively). But, different in Gemiza farm (-0.16 kg). The differences between the experimental stations may be attributed to different nutritional level, climatic conditions and management practices in different herds.

Very limited literature on phenotypic and genetic trend for TMY in Egyptian buffaloes. Khattab and Mourad (1992) reported that the phenotypic and genetic trends for TMY in all MM farms from 1966 to 1987 were +16.2 and -1.6 kg, respectively. These results were lower than the estimates in this study. The results of the present study showed that there are increased of improvement of phenotypic and genetic trend in these farms from 1987 until now.

Fig. (1). showed the phenotypic and genetic trend for TMY in all farms. Increases of both trends for all farms except in Sids and Gemiza farms for phenotypic and genetic trend, respectively. This results explain clearly, the importance of genetic – environmentally interaction effect on milk yield traits.

From the present study we recommended that, construct selection indexes to increase genetic and phenotypic improvement of milk yield traits in Egyptian buffaloes. The importance of genetic – environmentally interaction effect in all milk production traits.

**Table (1). LSM $\pm$ SE for total milk yield (TMY), lactation period (LP) and mean, minimum, maximum and % of positive for breeding value (BV, TMY) at different birth year.**

Farm	TMY (kg)	LP (Day)	BV (TMY, kg)			
			LSM $\pm$ SE	LSM $\pm$ SE	Mean	Min.
<b>NG : (955)*</b>						
<b>1993</b>	1368 $\pm$ 76	220 $\pm$ 11.2	-0.28	-99.61	189.85	44
<b>1996</b>	1334 $\pm$ 42	216 $\pm$ 6.00	-3.66	-187.65	232.78	40
<b>1999</b>	1606 $\pm$ 46	217 $\pm$ 5.00	-1.30	-222.65	220.11	47
<b>2002</b>	1580 $\pm$ 45	209 $\pm$ 4.80	5.73	-260.72	328.53	47
<b>2005</b>	1692 $\pm$ 37	208 $\pm$ 4.20	4.26	-279.10	339.51	50
<b>2008</b>	1667 $\pm$ 31	209 $\pm$ 3.70	6.12	-268.23	326.63	52
<b>NK : (721)*</b>						

<b>1993</b>	1028±112	182±18.6	0.06	-57.24	176.73	31
<b>1996</b>	1147±61	192±9.20	-2.38	-100.12	194.24	48
<b>1999</b>	1521±43	206±5.90	-4.62	-152.61	258.97	48
<b>2002</b>	1492±42	204±4.50	3.53	-243.64	226.16	52
<b>2005</b>	1561±41	204±4.40	4.05	-248.44	238.38	49
<b>2008</b>	1555±41	204±4.40	6.30	-352.31	243.11	51
<b>MM : (830)*</b>						
<b>1993</b>	1209±103	209±15.0	-1.17	-111.27	115.62	40
<b>1996</b>	1300±68	214±9.90	-0.79	-197.20	182.56	56
<b>1999</b>	1628±58	232±7.00	-1.76	-265.14	362.82	41
<b>2002</b>	1633±45	222±5.10	5.79	-280.98	394.36	47
<b>2005</b>	1619±41	217±4.50	5.51	-289.44	523.18	47
<b>2008</b>	1546±41	211±4.30	5.49	-289.58	556.45	47
<b>All MM : (2506)*</b>						
<b>1993</b>	1251±56	210±12.0	-0.48	-111.27	189.85	40
<b>1996</b>	1282±32	212±11.5	-2.64	-197.20	232.78	46
<b>1999</b>	1585±28	220±10.0	-2.49	-265.14	362.82	45
<b>2002</b>	1568±25	216±9.00	5.00	-280.98	394.36	49
<b>2005</b>	1626±23	222±9.80	4.66	-289.44	523.18	48
<b>2008</b>	1594±22	220±10.5	5.94	-352.31	556.45	50
<b>G : (580)*</b>						
<b>1993</b>	1366±76	284±11.6	-2.37	-85.84	101.10	37
<b>1996</b>	1355±44	264±7.40	2.49	-159.04	260.83	55
<b>1999</b>	1410±45	249±7.80	-3.97	-252.06	241.54	44
<b>2002</b>	1415±46	243±6.60	-3.99	-210.88	368.68	41
<b>2005</b>	1407±38	246±14.4	1.98	-335.97	552.10	48
<b>2008</b>	1381±33	241±4.00	-5.49	-279.21	601.08	46
<b>S : (409)*</b>						
<b>1993</b>	1355±82	278±16.0	2.12	-56.37	75.68	56
<b>1996</b>	1242±89	273±11.7	-1.99	-245.47	178.89	59
<b>1999</b>	1318±70	271±8.80	-4.33	-203.57	139.21	48
<b>2002</b>	1206±48	249±7.60	1.68	-253.59	148.61	57
<b>2005</b>	1229±29	248±6.40	7.56	-243	185.24	45
<b>2008</b>	1137±34	240±5.60	6.58	-241.69	176.47	53
<b>All : (3495)*</b>						
<b>1993</b>	1284±43	205±14.0	-0.62	-111.27	189.85	41
<b>1996</b>	1292±26	210±12.5	-1.65	-245.47	260.83	48
<b>1999</b>	1534±24	215±11.8	-2.89	-265.14	362.82	46
<b>2002</b>	1506±21	210±9.90	3.31	-280.98	394.36	48
<b>2005</b>	1513±18	215±12.0	4.89	-335.97	552.09	48
<b>2008</b>	1493±18	202±11.5	3.97	-352.31	601.08	50

NG: El-Nattafe El-Gidid

NK: El-Nattafe El-Kadim

MM: Mahalet Mousa

All MM: NG, NK and MM

G: Gemiza S: Sids

All: all farms

\*: Number of records.

**Table (2). Intercept (a), Regression coefficient (b) and accuracy ( $R^2$ ) for Total milk yield (TMY) and breeding value (BV, TMY) on calving years.**

Farm	TMY			BV (TMY)		
	A	b	$R^2$	a	b	$R^2$
<b>NG</b>	-46909	24.22	79	-1194.49	0.60	67
<b>NK</b>	-71930	36.65	77	-1116.07	0.56	56
<b>MM</b>	-48942	25.21	57	-1136.20	0.57	72
<b>All MM</b>	-50529	26.00	74	-1169.87	0.59	69
<b>G</b>	-3107	2.25	55	324.86	-0.16	51
<b>S</b>	24892	-11.82	71	-1083.29	0.54	53
<b>All</b>	-30571	16.00	60	-928.02	0.46	63

NG: El-Nattafe El-Gidid

NK :El-Nattafe El-Kadim

MM: Mahalet Mousa

All MM: NG, NK and MM

G: Gemiza

S: Sids

All: all farms

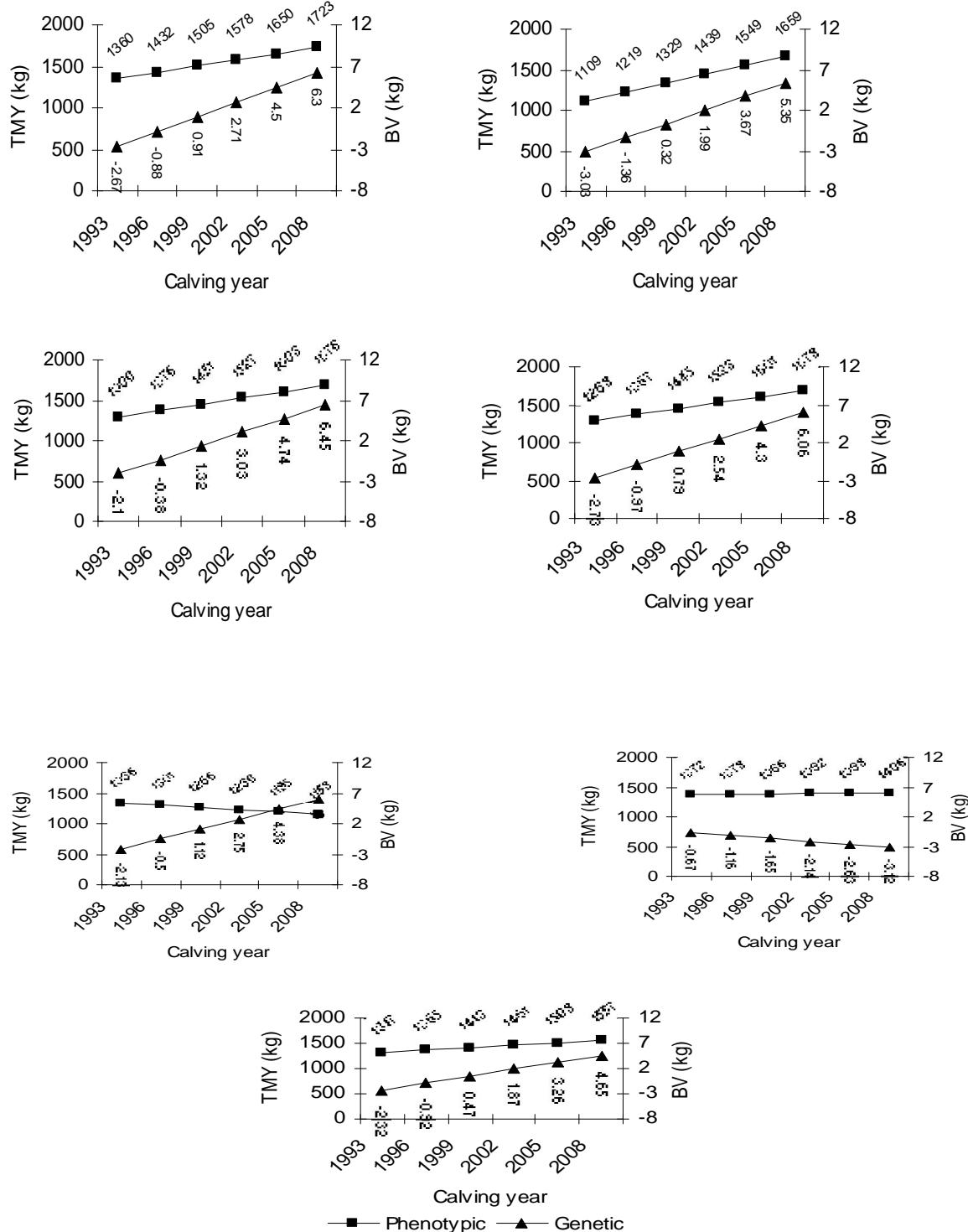


Fig. (1) Phenotypic and genetic trend for total milk yield in all farms

NG: El-Nattafe El-Gidid

NK: El-Nattafe El-Kadim

MM: Mahalet Mousa

All MM: NG, NK and MM

G: Gemiza

S: Sids

All: all farms

**Table (3). Amount of phenotypic and genetic change (kg) for total milk yield**

Farm	Phenotypic		Genetic	
	Change*	Annual	Change*	Annual
NG	+73	+24.3	+1.80	+0.60
NK	+110	+36.7	+1.70	+0.57
MM	+76	+25.3	+1.70	+0.57
All MM	+78	+26.0	+1.75	+0.58
G	+7	+2.30	-0.49	-0.16
S	-35	-11.7	+1.63	+0.54
All	+48	+16.0	+1.40	+0.47

NG: El-Nattafe El-Gidid

All MM: NG, NK and MM

NK: El-Nattafe El-Kadim

G: Gemiza

MM: Mahalet Mousa

All: all farms

\* Every 3 years

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5/20/2010

# Computer Aided Design, Synthesis and Biological Evaluation of Novel Acridine Derivatives a Topoisomerase I Inhibitors

Gamil Mahmoud El Taliawi<sup>1</sup>, Enayat Ibrahim Ali<sup>1</sup>, Gehan Hegazy Hegazy<sup>\*1</sup>, Nasser S. M. Ismail<sup>2</sup>  
and Walaa Ramadan<sup>1</sup>

<sup>1</sup>Department of Pharmaceutical Chemistry, Faculty of Pharmacy Cairo University, <sup>2</sup> Pharmaceutical Chemistry Department, Faculty of Pharmacy Ain Shams University, Egypt.

<sup>\*</sup>[gehan\\_hegazy@yahoo.com](mailto:gehan_hegazy@yahoo.com)

**Abstract:** A series of novel 9-anilinoacridines was designed and their molecular docking studies into the active site were examined as topoisomerase I inhibitor. Several compounds showed significant high simulation docking score. The designed compounds were synthesized and biologically evaluated against mammary carcinoma cell line (MCF-7), where compounds 8,11e,11f,13b,14b,14e and 14f showed significant inhibitory activity at a concentration 10 $\mu$ g/mL). It appears that the *in vitro* activity of compounds 8,11e,11f,13b,14b,14e and 14f were consistent with their molecular modeling results, and compound 14b showed the highest activity with IC<sub>50</sub> value of 7.8  $\mu$ g. [Journal of American Science. 2010;6(11):148-158]. (ISSN: 1545-1003).

**Keywords:** Molecular docking, Acridine derivatives, Antitumor

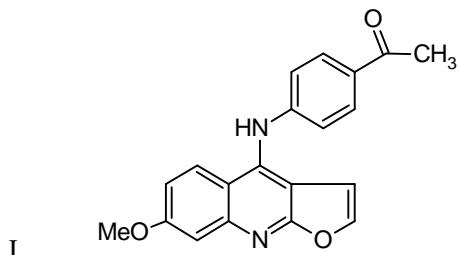
## 1. Introduction

Topoisomerase-targeting agents that stabilize the cleavable complex formed between the enzyme and DNA have proved to be effective in the treatment of cancer [1]. Topoisomerases are nuclear enzymes; there are two major subtypes, topoisomerase I (TOP1) and topoisomerase II (TOP2) based upon differences in their initial mechanisms wherein a single- or double-stranded DNA break is implicated [2-4]. Topoisomerase I participates in the control of the topological state of DNA, and as such this enzyme is essential for DNA transcription and replication as well as other vital processes including chromosome condensation/opening and mitosis [5, 6]. All topoisomerases act through a conserved active-site tyrosine residue to cleave the phosphodiester backbone and form a covalent phosphotyrosine intermediate with the DNA [7]. Human topoisomerase I (Top1) cleaves a single DNA strand through transesterification of Tyr723 and forms a 3'-phosphotyrosine linkage to the DNA. After cleavage, the broken (scissile) DNA strand can rotate around the unbroken (nonscissile) strand and remove DNA supercoils [8]. The enzyme allows both the rewinding of underwound negatively supercoiled DNA and the unwinding of overwound positively supercoiled DNA [9]. The DNA phosphodiester backbone is restored in a second transesterification reaction when the 5'-OH of the broken DNA strand attacks the 3'-phosphotyrosine bond. This religation reaction therefore liberates top1 for subsequent

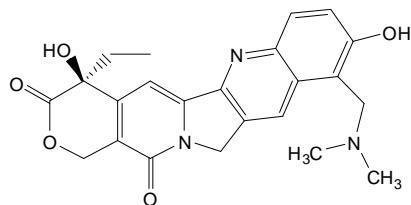
cleavage/unwinding reactions. Human topoisomerase I (TOP1) is the molecular target of a diverse set of anticancer compounds, including the camptothecins,

indolocarbazoles, indenoisoquinolines and 9-anilinoacridines [10]. Camptothecin was the first agent identified as a TOP1-targeting agent [11]. Irinotecan and topotecan are the only current Top1 inhibitors approved by the Food and Drug Administration (FDA) for the treatment of cancer, and they validate Top1 as a therapeutic target for anticancer drug development. However, these camptothecin derivatives are not ideal drug molecules. These compounds bind to a transient TOP1-DNA covalent complex and inhibit the resealing of a single-strand nick that the enzyme creates to relieve super helical tension in duplex DNA[12]. On the other hand, acridines are known to possess antitumor activity. They exert their antitumor activity through DNA intercalation, [13] inhibition of topoisomerase enzymes [14,15] or inhibition of telomerase[16]. 9-Anilinoacridines is an important class which attracted considerable attention as DNA intercalators[17]. Also, some acridines as the acridine derivative 3-(9-acridinylamino)-5-(hydroxymethyl) aniline (AHMA) were proved to be potent topoisomerase inhibitor[18]. Moreover, some methoxy derivatives of 4-anilinofuro[2,3-b]quinoline I (Fig.1), a bioisotere of 9-anilinoacridines, have been shown to exhibit excellent cytotoxicity against cancer cells [19]. Also, methoxy 2-phenylquinoline derivatives, another bioisosters of the acridine ring, as compound II (Fig.1) were found to be active against the growth of certain solid cancer such as NCI-H226 non small cell lung cancer, MDA-MB-231/ATCC breast cancer and Sf-295 CNS cancer [20]. Concerning the antitumor activity

many pyridine-2-one and 3-cyano-2-imino pyridine derivatives exhibit antiproliferative activity [21, 22]. Based on the pre-mentioned review and the urgent need to develop new potential antitumor agents, our current investigation is based on optimization of lead compound by molecular docking studies, using the enzyme bound crystal structure of the Top1 inhibitor topotecan (III)(Fig.1). This involves the synthesis of new substituted acridines such as 9-anilinoacridine;

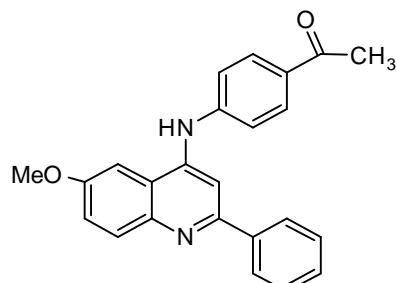


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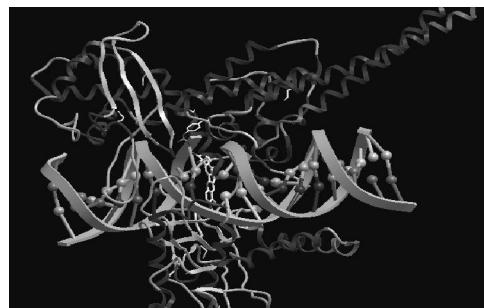


III (Topotecan)

9-anilinonitroacridines or 9-anilinomethoxy acridines combined to 4-aryl-3-cyanopyridine-6-yl-2-one or 4-aryl-3-cyanopyridine-2-imino-6-yl in order to combine the antitumor activity of both moieties aiming to increase the potency of the resulting hybrid compounds.



II



**Figure 1. TopI inhibitors and the crystal structure of compound III with the TopI complex**

In this work, molecular modeling simulation studies were performed in order to predict the biological activity of the proposed compounds. Docking Study using Molsoft ICM software was performed [23]. The crystal structure of Topotecan/topoisomerase I (Fig. 1) was obtained from protein data bank website (pdb). This regularized protein complex structure was used in determination of the active site that is mentioned in the literature. Docking process was carried out for the test set of compounds (10a-f -12a-f and 13a-f – 15a-f) using the enzyme-ligand interaction energy as scoring function [24].

## 2. Materials and Methods

All melting points are uncorrected and determined by the open capillary method using Gallenkamp melting point apparatus (MFB-595-010M; Weiss-Gallenkamp,London,UK). IR spectra were recorded on a Shimadzu 435 Spectrometer(IR-435;Shimadzu,Japan) using KBr disks. <sup>1</sup>H NMR spectra were recorded on a Perkin-Elmer NMR FXQ-

200 MHZ Spectrometer (Tokyo, Japan), using TMS as internal standard. Mass spectra were recorded on a GCMS-QP 1000 EX, Mass Spectrometer. Elemental analyses for C, H, and N were within  $\pm 0.4\%$  of the theoretical values and were performed at the Microanalytical Center, Cairo University, and they were of the theoretical values. Progress of the reactions was monitored by TLC using precoated aluminum sheets silica gel MERCK 60 F 254(Merck,Germany) and was visualized by UV lamp.

### 2.1. Chemistry

Diphenylamine-2-carboxylic acid 1 and 5-Nitro-diphenylamine-2-carboxylic acid 2 [25].

In this work, compounds 1 and 2 were prepared using "Ullmann reaction" via reaction of o-chlorobenzoic acid or its derivatives and aniline according to the reported method.

4'-Methoxydiphenylamine-2-carboxylic acid 3 [25].

It was prepared according to "Ullmann reaction" using o-chlorobenzoic acid and p-anisidine as reported.

9-Chloroacridine 4, 9-Chloro-3-nitroacridine 5 and 9-Chloro-2-methoxyacridine 6 [26].

They were prepared by reaction of compounds 1-3 with phosphorus oxychloride according to the reported method.

9-(4-Acetylaniino) acridine 7, 9-(4-Acetylaniino)-3-nitroacridine 8 and 9-(4-Acetylaniino)-2-methoxyacridine 9.

A mixture of 4, 5, or 6 (0.04 mol) and p-aminoacetophenone (5.40g, 0.04 mol) was dissolved in DMF (9 mL) and piperidine (2 drops). The mixture was refluxed for 3 hours. The formed precipitate was filtered and crystallized from ethanol. compound 8 Yield 75%; m.p. >300 °C. MS: m/z (%): 357 [M+](0.5) Anal. Calcd. for C<sub>21</sub>H<sub>15</sub>N<sub>3</sub>O<sub>3</sub>(357): C 70.58, H 4.20, N 11.76 Found C 70.34, H 4.13, N 11.76.

General procedure for preparation of 9-[p-(4-Aryl-3-cyano-2(1H)-oxopyridin-6-yl) anilino] acridine derivatives 10a-f, 11a-f and 12a-f.

A mixture of 7, 8 or 9 (0.01 mol), ethylcyanoacetate(1.20 g, 0.01mol ), the appropriate aldehyde (0.01mol) and ammonium acetate (6.19g,0.08mol) in n-butanol (30mL) was refluxed for 7 hours. The solid separated on cooling was filtered, dried and crystallized from dimethylformamide and water to provide desired compounds. 10a-f, 11a-f and 12a-f.

9-[p-(4-Phenyl-3-cyano-2(1H)-oxopyridin-6-yl) anilino] acridine 10a

Yield:54%;mp:195-197°C. IR(cm<sup>-1</sup>):3330 (NHs), 2215(CN), 1660 (CO). <sup>1</sup>H-NMR(DMSO-d<sub>6</sub>) ppm: 7.20-8.40(m,18,ArH), 12.20(s, 2H, 2NH) exchanged with D<sub>2</sub>O. Anal. Calcd. for C<sub>31</sub>H<sub>20</sub>N<sub>4</sub>O(464): C,80.17;H, 4.31; N, 12.06. Found:C,80.20;H,4.40;N,12.65.

9-[p-(4-p-Fluorophenyl-3-cyano-2(1H)-oxopyridin-6-yl) anilino] acridine 10b

Yield:65%;mp:222-224°C. IR(cm<sup>-1</sup>):3300 (NHs), 2210 (CN), 1665 (CO). <sup>1</sup>H-NMR(DMSO-d<sub>6</sub>) ppm: 7.20-8.40 (m, 17H, ArH), 11.80(s, 2H, 2NH) exchanged with D<sub>2</sub>O. MS: m/z (%)483 [M<sup>+</sup>] (62.0). Anal.Calcd.for C<sub>31</sub>H<sub>19</sub>FN<sub>4</sub>O (482) :C,77.17 ;H,3.94 ;N,11.62 .Found:C,77.20;H, 4.40 ;N,11.55.

9-[p-(4-p-Chlorophenyl-3-cyano-2(1H)-oxopyridin-6-yl) anilino] acridine 10c

Yield:63%;mp:215-217°C. IR(cm<sup>-1</sup>):3310 (NHs), 2220 (CN), 1670 (CO). <sup>1</sup>H-NMR(DMSO-d<sub>6</sub>) ppm:7.20-8.30 (m,17H,ArH),11.60(s,2H,2NH) exchanged with D<sub>2</sub>O. Anal.Calcd.for C<sub>31</sub>H<sub>19</sub>ClN<sub>4</sub>O(498.5): C,74.62 ;H,3.81;N,11.23 .Found: C,74.50;H,4.00;N,10.95.

9-[p-(4-p-Hydroxyphenyl-3-cyano-2(1H)-oxopyridin-6-yl)anilino] acridine 10d

Yield:68%;mp193-196°C. IR(cm<sup>-1</sup>):3409(OH), 3332-3239 (NHs), 2200 (CN), 1650 (CO). <sup>1</sup>H-NMR(DMSO-d<sub>6</sub>) ppm: 6.80-8.60(m, 17H, ArH), 11.50(s, 2H, 2NH) exchanged with D<sub>2</sub>O, 12.21(s, 1H, OH ) exchanged with D<sub>2</sub>O. MS: m/z(%) 480 [M<sup>+</sup>] (1.70). Anal.Calcd.for C<sub>31</sub>H<sub>20</sub>N<sub>4</sub>O<sub>2</sub>(480) :C , 77.50 ;H, 4.16 ;N,11.66 .Found:C,77.34;H,4.05;N,11.55.

9-[p-(4-p-Methoxyphenyl-3-cyano-2(1H)-oxopyridin-6-yl) anilino] acridine 10e

Yield:70%;mp:257-260°C. IR(cm<sup>-1</sup>):3420-3233 (NHs), 2214 (CN), 1716 (CO). <sup>1</sup>H-NMR(DMSO-d<sub>6</sub>) ppm: 3.90 (s,3H,OCH<sub>3</sub>),7.20-8.60 (m,17H,ArH),11.8(s,2H,2NH) exchanged with D<sub>2</sub>O. Anal.Calcd.for C<sub>32</sub>H<sub>22</sub>N<sub>4</sub>O<sub>2</sub> (494):C, 77.73;H,4.45;N,11.33.Found:C,77.53;H,4.40;N,1 1.38.

9-[p-(4-p-Dimethylaminophenyl-3-cyano-2(1H)-oxopyridin-6-yl)anilino] acridine 10f

Yield:68%;mp:268-270°C. IR (cm<sup>-1</sup>):3335-3201(NHs), 2192(CN), 1659(CO). <sup>1</sup>H-NMR(DMSO-d<sub>6</sub>) ppm: 3.70 (s,6H,2CH<sub>3</sub>), 7.40-8.60 (m,17H,ArH) ,11.00 (s,2H,2NH) exchanged with D<sub>2</sub>O. MS:m/z(%) 508 [M<sup>+</sup>] (50.0). Anal.Calcd.for C<sub>33</sub>H<sub>25</sub>N<sub>5</sub>O (507):C,78.10 ;H,4.93 ;N,13.80 .Found:C,78.10 ;H,5.00;N,13.65.

3-Nitro-9-[p-(4-phenyl-3-cyano-2(1H)-oxopyridin-6-yl) anilino] acridine 11a

Yield:60%;mp:>300°C. IR(cm<sup>-1</sup>): 3447-3231(NHs), 2218(CN), 1731(CO). <sup>1</sup>H-NMR(DMSO-d<sub>6</sub>) ppm: 7.64-8.65 (m,17H,ArH ),12.21 (s,2H,2NH) exchanged with D<sub>2</sub>O. MS:m/z (%)508 [M<sup>+</sup>] (4.15). Anal. Calcd. for C<sub>31</sub>H<sub>19</sub>N<sub>5</sub>O<sub>3</sub>(509):C,73.08;H,3.73;N,13.75.Found: C,73.00;H,3.90;N,13.54.

9-[p-(4-p-Fluorophenyl-3-cyano-2(1H)-oxopyridin-6-yl)anilino] -3- nitro acridine 11b

Yield:68%;mp: >300°C. IR(cm<sup>-1</sup>): 3300 (NHs), 2200 (CN), 1660 (CO). <sup>1</sup>H-NMR(DMSO-d<sub>6</sub>) ppm: 7.42-8.62 (m,16H,ArH ), 12.00 (s,2H,2NH ) exchanged with D<sub>2</sub>O. Anal.Calcd.for C<sub>31</sub>H<sub>18</sub>FN<sub>5</sub>O<sub>3</sub> (527):C,70.59

;H,3.41 ;N,13.28 .Found:C,70.40;H,3.50;N,13.35.

9-[p-(4-p-Chlorophenyl-3-cyano-2(1H)-oxopyridin-6-yl)anilino]-3-nitro acridine 11c

Yield:75%;mp: >300<sup>0</sup>C. IR(cm<sup>-1</sup>):3404-3330(NHs), 2213(CN), 1650(CO). <sup>1</sup>H-NMR(DMSO-d<sub>6</sub>) ppm: 7.20-8.60 (m, 16H, ArH), 12.20(s, 2H, 2NH) exchanged with D<sub>2</sub>O. MS: m/z (%)543.5[M<sup>+</sup>] (1.34). Anal.Calcd.for C<sub>31</sub>H<sub>18</sub>ClN<sub>5</sub>O<sub>3</sub> (543.5): C,68.44; H,3.31; N,12.88. Found: C,68.16; H,3.72; N,13.01.

9-[p-(4-p-Hydroxyphenyl-3-cyano-2(1H)-oxopyridin-6-yl)anilino]-3-nitroacridine 11d

Yield:69%;mp: >300<sup>0</sup>C. IR(cm<sup>-1</sup>): 3401(OH), 3334-3245(NHs), 2220(CN), 1650(CO). <sup>1</sup>H-NMR(DMSO-d<sub>6</sub>) ppm: 6.80-8.60(m, 16H, ArH), 11.60(s, 1H, OH ) exchanged with D<sub>2</sub>O. 12.20(s, 2H, 2NH) exchanged with D<sub>2</sub>O. Anal.Calcd.for C<sub>31</sub>H<sub>19</sub>N<sub>5</sub>O<sub>4</sub> (525):C,70.86;H,3.62;N,13.33. Found: C,70.50; H,3.70;N,13.35.

9-[p-(4-p-Methoxyphenyl-3-cyano-2(1H)-oxopyridin-6-yl)anilino]-3-nitro acridine 11e

Yield:70%;mp: >300<sup>0</sup>C. IR(cm<sup>-1</sup>): 3320 (NHs), 2220(CN), 1710 (CO). <sup>1</sup>H-NMR(DMSO-d<sub>6</sub>) ppm:4.00 (s, 3H, OCH<sub>3</sub>), 7.20-8.60 (m, 16H, ArH), 12.20(s, 2H, 2NH) exchanged with D<sub>2</sub>O. MS: m/z(%) 538[M<sup>+</sup>] (1.34). Anal.Calcd.for C<sub>32</sub>H<sub>21</sub>N<sub>5</sub>O<sub>4</sub> (539):C,71.24;H,3.90;N,12.99. Found: C,70.99 ;H,4.02;N,12.82.

9-[p-(4-p-Dimethylaminophenyl-3-cyano-2(1H)-oxopyridin-6-yl)anilino]-3-nitroacridine 11f

Yield:65%;mp: >300<sup>0</sup>C. IR(cm<sup>-1</sup>): 3330 (NHs), 2200 (CN), 1660 (CO). <sup>1</sup>H-NMR(DMSO-d<sub>6</sub>) ppm:3.40 (s,6H,2CH<sub>3</sub>), 7.82-8.40 (m,16H,ArH), 12.00 (s,2H,2NH) exchanged with D<sub>2</sub>O. MS:m/z (%)551 [M<sup>+</sup>] (0.53). Anal.Calcd.for C<sub>33</sub>H<sub>24</sub>N<sub>6</sub>O<sub>3</sub> (552): C,71.74;H,4.35;N,15.22. Found:C,71.55 ; H,4.40 ;N,15.19.

2-Methoxy-9-[p-(4-phenyl-3-cyano-2(1H)-oxopyridin-6-yl) anilino] acridine 12a

Yield:65%;mp:195-197<sup>0</sup>C. IR(cm<sup>-1</sup>):3331(NHs), 2225(CN), 1718(CO). <sup>1</sup>H-NMR(DMSO-d<sub>6</sub>) ppm :3.80 (s, 3H, OCH<sub>3</sub>), 7.00-8.10 (m, 17H, ArH), 12.22(s, 2H, 2NH) exchanged with D<sub>2</sub>O. MS: m/z (%)494 [M<sup>+</sup>] (0.26). Anal.Calcd.forC<sub>32</sub>H<sub>22</sub>N<sub>4</sub>O<sub>2</sub>(494): C,77.73; H,4.45;N, 11.34 .Found: C,77.69 ;H,4.40;N,11.42.

9-[p-(4-p-Fluorophenyl-3-cyano-2(1H)-oxopyridin-6-yl) anilino]-2-methoxy acridine 12b

Yield:69%;mp:218-220<sup>0</sup>C. IR(cm<sup>-1</sup>):3320 (NHs), 2215 (CN), 1665(CO). <sup>1</sup>H-NMR(DMSO-d<sub>6</sub>)

ppm: 3.80 (s, 3H, OCH<sub>3</sub>), 7.00-8.40 (m, 16H, ArH), 12.00(s, 2H, 2NH) exchanged with D<sub>2</sub>O. Anal.Calcd.for C<sub>32</sub>H<sub>21</sub>FN<sub>4</sub>O<sub>2</sub>(512) :C,75.00; H,4.10;N,10.99. Found:C,75.10;H,4.00;N,11.40.

9-[p-(4-p-Chlorophenyl-3-cyano-2(1H)-oxopyridin-6-yl) anilino]-2-methoxy acridine 12c

Yield:70%;mp:183-185<sup>0</sup>C. IR(cm<sup>-1</sup>): 3350 (NHs), 2210 (CN), 1650 (CO). <sup>1</sup>H-NMR(DMSO-d<sub>6</sub>) ppm: 3.61 (s,3H, OCH<sub>3</sub>), 7.21-8.42 (m,16H,ArH),12 (s,2H,2NH) exchanged with D<sub>2</sub>O. Anal. Calcd.for C<sub>32</sub>H<sub>21</sub>ClN<sub>4</sub>O<sub>2</sub> (528.5) :C,72.65 ;H,3.97; N,10.60. Found:C,72.70; H,4.00;N,10.24.

9-[p-(4-p-Hydroxyphenyl-3-cyano-2(1H)-oxopyridin-6-yl)anilino]-2-methoxy acridine 12d

Yield:62%;mp:160-162<sup>0</sup>C. IR(cm<sup>-1</sup>):3408(OH), 3334-3246(NHs), 2215(CN), 1651(CO). <sup>1</sup>H-NMR(DMSO-d<sub>6</sub>) ppm: 3.41 (s,3H,OCH<sub>3</sub> ),6.91-8.42 (m,16H,ArH ),11.44 (s,1H,OH) exchanged with D<sub>2</sub>O, 12.10 (s,2H,2NH) exchanged with D<sub>2</sub>O. Anal. Calcd.for C<sub>32</sub>H<sub>22</sub>N<sub>4</sub>O<sub>3</sub>(510) :C,75.29 ;H,4.31 ;N,10.98 .Found:C,75.30;H,4.30;N,10.98.

2-Methoxy-9-[p-(4-p-methoxyphenyl-3-cyano-2(1H)-oxopyridin-6-yl) anilino] acridine 12e

Yield:70%;mp:240-242<sup>0</sup>C. IR(cm<sup>-1</sup>):3400-3232(NHs),2200(CN), 1722(CO). <sup>1</sup>H-NMR(DMSO-d<sub>6</sub>) ppm: 3.70 (s, 3H, OCH<sub>3</sub>), 4.10(s, 3H, OCH<sub>3</sub>), 7.20-8.40 (m,16H, ArH), 11.8(s, 2H, 2NH) exchanged with D<sub>2</sub>O. MS: m/z (%)524 [M<sup>+</sup>] (0.28). Anal.Calcd.for C<sub>33</sub>H<sub>24</sub>N<sub>4</sub>O<sub>3</sub> (524): C,75.57;H,4.58;N,10.69 .Found: C,75.40 ;H,4.60;N,11.10.

9-[p-(4-p-Dimethylaminophenyl-3-cyano-2(1H)-oxopyridin-6-yl)anilino]-2-methoxyacridine 12f

Yield: 68%; mp:218-220<sup>0</sup>C. IR(cm<sup>-1</sup>): 3340-3400(NHs), 2200(CN),1665(CO). <sup>1</sup>H-NMR(DMSO-d<sub>6</sub>) ppm: 3.86 (s, 6H, 2CH<sub>3</sub>), 4.12(s, 3H, OCH<sub>3</sub>), 7.54-8.65 (m,16H, ArH), 11.8(s, 2H, 2NH) exchanged with D<sub>2</sub>O. MS: m/z (%)537 [M<sup>+</sup>] (0.13). Anal.Calcd.for C<sub>34</sub>H<sub>27</sub>N<sub>5</sub>O<sub>2</sub> (537): C,75.98; H,5.03; N,13.04. Found: C,75.59; H,5.00; N,13.03.

9-[p-(4-Aryl -3- cyano-2(1H)-iminopyridin-6-yl) anilino] acridine derivatives 13a-f, 14a-f and 15a-f.

A mixture of 7, 8 or 9 (0.01 mol), malononitrile (0.65g, 0.01mol), the appropriate aldehyde (0.01mol) and ammonium acetate (6.19g, 0.08mol) in n-butanol (30mL) was refluxed for 7 hours. The solid separated on

cooling was filtered, dried and crystallized from dimethylformamide and water.

**9-[p-(4-Phenyl-3-cyano-2(1H)-iminopyridin-6-yl) anilino] acridine 13a.**

Yield:66%;mp:270-272 0C. IR(cm<sup>-1</sup>): 3400-3300(NHs),2215(CN). 1H-NMR(DMSO-d<sub>6</sub>) ppm: 7.21-8.42 (m,18H,ArH), 11.80 (s, 3H, 3NH) exchanged with D<sub>2</sub>O. Anal.Calcd. for C<sub>31</sub>H<sub>21</sub>N<sub>5</sub>(463): C,80.35; H,4.54; N,15.12. Found: C,80.10; H,4.60; N,15.72.

**9-[p-(4-p-Fluorophenyl-3-cyano-2(1H)-iminopyridin-6-yl) anilino] acridine 13b.**

Yield: 68%; mp: 219-220 0C. IR(cm<sup>-1</sup>): 3450-3220 (NHs), 2210 (CN). 1H-NMR(DMSO-d<sub>6</sub>) ppm: 7.22-8.41 (m, 17H, ArH), 11.75 (s, 3H, 3NH) exchanged with D<sub>2</sub>O. Anal.Calcd. for C<sub>31</sub>H<sub>20</sub>FN<sub>5</sub> (481): C,77.34; H,4.15; N,14.55. Found: C,77.38; H,4.33; N,14.44.

**9-[p-(4-p-Chlorophenyl-3-cyano-2(1H)-iminopyridin-6-yl) anilino] acridine 13c.**

Yield:64%;mp:278-2800C. IR(cm<sup>-1</sup>): 3500-3215(NHs),2225(CN). 1H-NMR(DMSO-d<sub>6</sub>) ppm: 7.43-8.54(m,17H,ArH),11.50(s,3H,3NH) exchanged with D<sub>2</sub>O. MS: m/z (%)497[M<sup>+</sup>] (0.1). Anal.Calcd.for C<sub>31</sub>H<sub>20</sub>CIN<sub>5</sub> (497.5) :C,74.77; H,4.02; N,14.07. Found:C,74.80;H,4.10;N,14.15.

**9-[p-(4-p-Hydroxyphenyl -3- cyano- 2 (1H) -iminopyridin -6 -yl) anilino] acridine 13d.**

Yield:70%;mp:248-250 °C. IR(cm<sup>-1</sup>): 3455(OH), 3300-3224 (NHs), 2209 (CN). <sup>1</sup>H-NMR(DMSO-d<sub>6</sub>) ppm: 7.44-8.22(m,17H,ArH),9.50 (s,1H,OH) exchanged with D<sub>2</sub>O , 11.82(s,3H,3NH) exchanged with D<sub>2</sub>O. MS: m/z (%)480[M<sup>+</sup>] (0.27). Anal.Calcd.for C<sub>31</sub>H<sub>21</sub>N<sub>5</sub>O(479) : C,77.66 ;H,4.38 ;N,14.61. Found:C,77.38;H,4.62;N,14.19.

**9-[p-(4-p-Methoxyphenyl-3-cyano-2(1H)-iminopyridin-6-yl) anilino] acridine 13e.**

Yield:65%;mp:235-238 °C. IR(cm<sup>-1</sup>): 3421-3200 (NHs), 2220 (CN). <sup>1</sup>H-NMR(DMSO-d<sub>6</sub>) ppm: 3.41(s,3H,OCH<sub>3</sub>) , 7.43-8.51(m,17H,ArH),12.00 (s,3H,3NH) exchanged with D<sub>2</sub>O. MS: m/z (%) 493[M<sup>+</sup>] (32.61). Anal.Calcd.for C<sub>32</sub>H<sub>23</sub>N<sub>5</sub>O(493): C,77.89 ;H,4.66 ;N,14.20. Found: C,77.90; H,4.60; N,14.20.

**9-[p-(4-p-Dimethylaminophenyl-3-cyano-2(1H)-iminopyridin-6-yl) anilino] acridine 13f.**

Yield:72%;mp:271-273°C. IR(cm<sup>-1</sup>): 3500-3231(NHs), 2216(CN). <sup>1</sup>H-NMR(DMSO-d<sub>6</sub>) ppm: 3.3 (s, 6H, 2CH<sub>3</sub>), 6.8-8.6(m, 17H, ArH), 12.2(s, 3H, 3NH) exchanged with D<sub>2</sub>O. Anal.Calcd.for C<sub>33</sub>H<sub>26</sub>N<sub>6</sub>

(506) : C,78.26; H,5.14 ; N,16.60. Found: C,78.10; H,4.90; N,16.45.

**3-Nitro-9-[p-(4-phenyl-3-cyano-2(1H)-iminopyridin-6-yl) anilino] acridine 14a.**

Yield: 65%; mp:>300°C. IR(cm<sup>-1</sup>): 3500-3225(NHs), 2201(CN). <sup>1</sup>H-NMR(DMSO-d<sub>6</sub>) ppm: 7.20-8.40 (m, 17H, ArH), 12.22(s, 3H, 3NH) exchanged with D<sub>2</sub>O. MS: m/z (%) 507 [M<sup>+</sup>] (0.03). Anal.Calcd. for C<sub>31</sub>H<sub>20</sub>N<sub>6</sub>O<sub>2</sub> (508) : C,73.23; H,3.94; N,16.53. Found:C,73.00; H,4.00; N,16.22.

**9-[p-(4-p-Fluorophenyl-3-cyano-2(1H)-iminopyridin-6-yl)anilino]3-nitro acridine 14b.**

Yield:62%;mp: >300°C. IR(cm<sup>-1</sup>): 3225(NHs), 2220(CN). <sup>1</sup>H-NMR(DMSO-d<sub>6</sub>) ppm: 7.20-8.40(m, 16H, ArH), 12.14(s, 3H, 3NH) exchanged with D<sub>2</sub>O. MS: m/z (%) 527[M<sup>+</sup>] (0.38). Anal.Calcd.for C<sub>31</sub>H<sub>19</sub>FN<sub>6</sub>O<sub>2</sub> (526): C,70.72 ; H,3.61; N,15.97. Found:C,70.80; H,3.70; N,15.76.

**9-[p-(4-p-Chlorophenyl-3-cyano-2(1H)-iminopyridin-6-yl)anilino]-3-nitro acridine 14c.**

Yield:68%; mp: >300°C. IR(cm<sup>-1</sup>): 3450-3211(NHs), 2209(CN). <sup>1</sup>H-NMR(DMSO-d<sub>6</sub>) ppm: 6.81-8.62 (m, 16H, ArH), 12.35(s, 3H, 3NH) exchanged with D<sub>2</sub>O. Anal.Calcd. for C<sub>31</sub>H<sub>19</sub>CIN<sub>6</sub>O<sub>2</sub> (542.5): C,68.57 ; H,3.50 ; N,15.48. Found: C,68.89; H,3.40; N,15.31.

**9-[p-(4-p-Hydroxyphenyl-3-cyano-2(1H)-iminopyridin-6-yl)anilino]-3-nitro acridine 14d.**

Yield:73%;mp: >300°C. IR(cm<sup>-1</sup>): 3450(OH),3320-3220(NHs),2220(CN). <sup>1</sup>H-NMR(DMSO-d<sub>6</sub>) ppm: 6.93-8.44 (m,16H,ArH), 9.91(s,1H,OH) exchanged with D<sub>2</sub>O 12.20 (s,3H,3 NH) exchanged with D<sub>2</sub>O. Anal.Calcd. for C<sub>31</sub>H<sub>20</sub>N<sub>6</sub>O<sub>3</sub> (524): C,70.99; H,3.82; N,16.03. Found:C,71.35; H,3.89; N,16.03.

**9-[p-(4-p-Methoxyphenyl-3-cyano-2(1H)-iminopyridin-6-yl)anilino]-3-nitro acridine 14e.**

Yield:70%;mp:>300 °C. IR(cm<sup>-1</sup>): 3400-3300 (NHs), 2200 (CN). <sup>1</sup>H-NMR(DMSO-d<sub>6</sub>) ppm: 3.60 (s,3H,OCH<sub>3</sub>), 7.22-8.42 (m,16H,ArH), 12.11 (s,3H,3NH) exchanged with D<sub>2</sub>O. MS: m/z (%) 537 [M<sup>+</sup>] (1.92). Anal.Calcd. for C<sub>32</sub>H<sub>22</sub>N<sub>6</sub>O<sub>3</sub> (538): C,71.37; H,4.09; N,15.61. Found: C,71.30; H,4.26; N,15.31.

**9-[p-(4-p-Dimethylaminophenyl -3- cyano- 2 (1H) -iminopyridin -6 -yl) anilino]-3-nitroacridine 14f.**

Yield: 68%;mp: >300 °C. IR(cm<sup>-1</sup>): 3450-3300 (NHs), 2210 (CN). <sup>1</sup>H-NMR(DMSO-d<sub>6</sub>) ppm: 3.11 (s, 6H, 2CH<sub>3</sub>), 7.21-8.00 (m, 16H, ArH), 12.14(s, 3H, 3NH) exchanged with D<sub>2</sub>O. Anal.Calcd. for C<sub>33</sub>H<sub>25</sub>N<sub>7</sub>O<sub>2</sub> (551) : C,71.87; H,4.53; N,17.78. Found:C,71.90; H,4.60; N,17.60.

2-Methoxy-9-[p-(4-phenyl-3-cyano-2(1H)-iminopyridin-6-yl)anilino] acridine 15a.

Yield:75%;mp: 228-230°C. IR(cm<sup>-1</sup>): 3450-3300 (NHs),2220 (CN). <sup>1</sup>H-NMR(DMSO-d<sub>6</sub>) ppm: 3.75(s,3H,OCH<sub>3</sub>), 7.11-8.22 (m,17H,ArH),12.00 (s,3H,3NH) exchanged with D<sub>2</sub>O. Anal.Calcd. for C<sub>32</sub>H<sub>23</sub>N<sub>5</sub>O (493) : C,77.89 ; H,4.66 ; N,14.20. Found: C,77.80; H,4.64; N,14.35.

9-[p-(4-p-Fluorophenyl-3-cyano-2(1H)-iminopyridin-6-yl)anilino]-2-methoxyacridine 15b.

Yield:68%;mp:258-260°C. IR(cm<sup>-1</sup>): 3300 (NHs), 2210 (CN). <sup>1</sup>H-NMR(DMSO-d<sub>6</sub>) ppm: 3.65(s,3H,OCH<sub>3</sub>), 7.32-8.52 (m,16H,ArH),11.95 (s,3H,3NH) exchanged with D<sub>2</sub>O. Anal.Calcd. for C<sub>32</sub>H<sub>22</sub>FN<sub>5</sub>O (511) : C,75.15 ;H,4.30 ;N,13.70 . Found:C,74.95;H,4.45;N,13.91.

9-[p-(4-p-Chlorophenyl-3-cyano-2(1H)-iminopyridin-6-yl)anilino]-2-methoxy acridine 15c.

Yield:72%; mp:230-232°C. IR(cm<sup>-1</sup>): 3400-3201(NHs), 2225(CN). <sup>1</sup>H-NMR(DMSO-d<sub>6</sub>) ppm: 3.82 (s, 3H, OCH<sub>3</sub>), 7.22-8.42 (m, 16H, ArH), 12.35(s, 3H, 3NH) exchanged with D<sub>2</sub>O. Anal.Calcd. for C<sub>32</sub>H<sub>22</sub>CIN<sub>5</sub>O (527.5): C,72.80; H,4.17; N,13.27. Found:C,72.56; H,4.18; N,13.14.

9-[p-(4-p-Hydroxyphenyl-3-cyano-2(1H)-iminopyridin-6-yl)anilino]-2-methoxyacridine 15d.

Yield:70%; mp: 222-225°C. IR(cm<sup>-1</sup>): 3450 (OH), 3320(NHs), 2210(CN). <sup>1</sup>H-NMR(DMSO-d<sub>6</sub>) ppm: 4.00 (s, 3H, OCH<sub>3</sub>), 6.8-8.6 (m, 16H, ArH), 9.6(s, 1H, OH) exchanged with D<sub>2</sub>O, 11.73 (s, 3H, 3NH) exchanged with D<sub>2</sub>O. MS: m/z (%) 508 [M<sup>+</sup>] (3.11). Anal.Calcd. for C<sub>32</sub>H<sub>23</sub>N<sub>5</sub>O<sub>2</sub> (509): C,75.44; H,4.52; N,13.75. Found: C,74.21; H,4.39; N,14.00.

2-Methoxy-9-[p-(4-p-methoxyphenyl-3-cyano-2(1H)-iminopyridin-6-yl) anilino] acridine 15e.

Yield:65%;mp:165-167 °C. IR(cm<sup>-1</sup>): 3334-3246(NHs), 2199(CN). <sup>1</sup>H-NMR(DMSO-d<sub>6</sub>) ppm: 3.22(s,3H,OCH<sub>3</sub>), 3.92(s,3H,OCH<sub>3</sub>), 6.91-8.43 (m,16H,ArH), 11.95 (s, 3H,3 NH) exchanged with D<sub>2</sub>O. MS: m/z (%)523 [M<sup>+</sup>] (1.75). Anal.Calcd. for C<sub>33</sub>H<sub>25</sub>N<sub>5</sub>O<sub>2</sub> (523): C,75.72; H,4.78; N,13.38. Found:C,75.40; H,4.50; N,13.42.

9-[p-(4-p-Dimethylaminophenyl-3-cyano-2(1H)-iminopyridin-6-yl)anilino]-2-methoxyacridine 15f.

Yield:67%;mp:180-182°C. IR(cm<sup>-1</sup>): 3400-3300(NHs),2206(CN). <sup>1</sup>H-NMR(DMSO-d<sub>6</sub>) ppm: 3.00 (s, 6H, 2CH<sub>3</sub>), 3.9(s, 3H, OCH<sub>3</sub>), 6.8-8.6 (m, 16H, ArH), 11.8 (s, 3H, 3NH) exchanged with D<sub>2</sub>O. Anal.Calcd. for C<sub>34</sub>H<sub>28</sub>N<sub>6</sub>O (536) : C,76.12; H,5.22; N,15.67. Found:C,76.20; H,5.30; N,15.53.

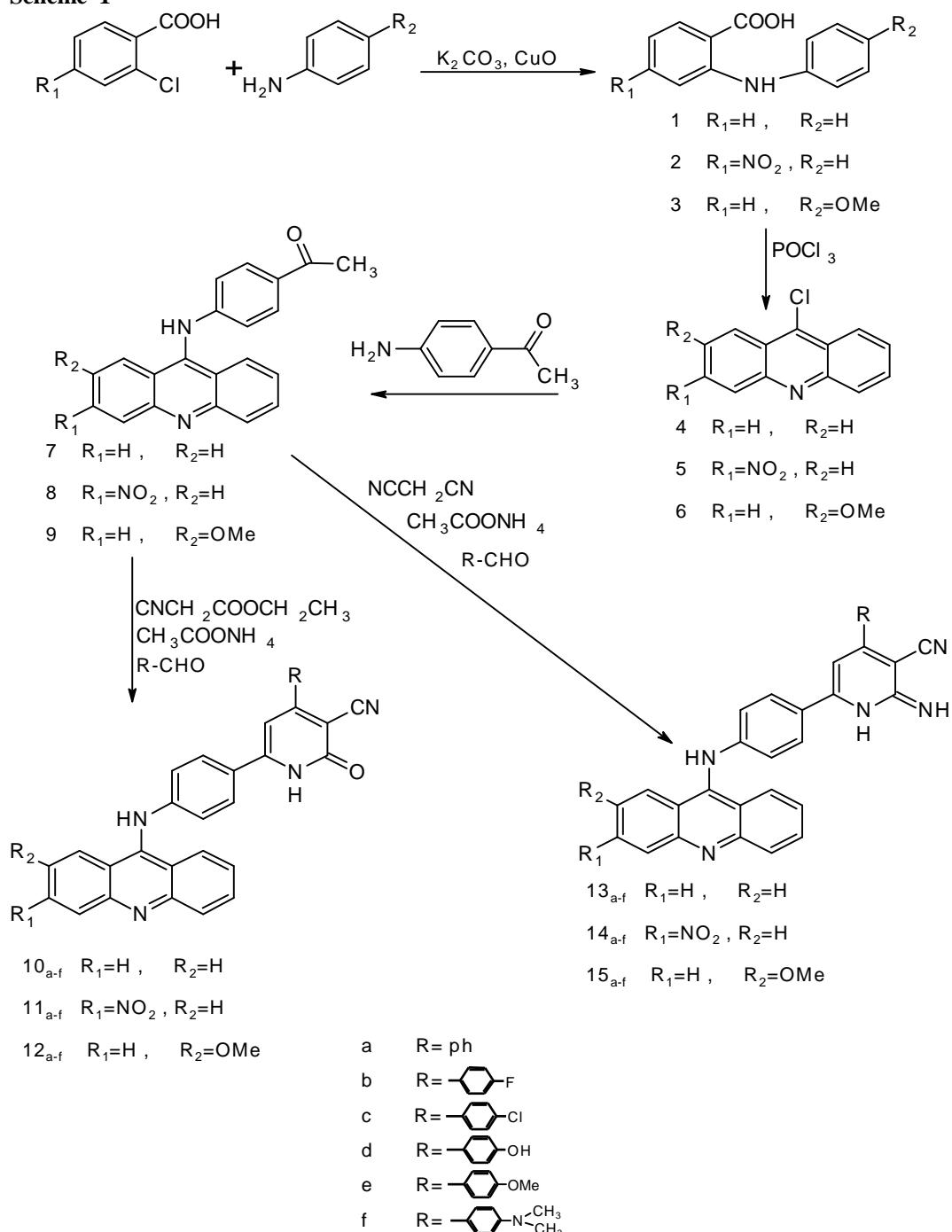
## 2.2. Biological screening

MCF-7 breast cancer cells were plated in 96 multiwell plates (104 cells/well) for 24 hours before treatment with the compounds to allow attachment of cells to the wall of the plate. Different concentrations of the compound under test (0, 1, 2.5, 5 and 10 µg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 hours at 37 °C and in atmosphere of 5% CO<sub>2</sub>. After 48 hours, cells were fixed, washed and stained with Sulforhodamie B stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader.

## 3. Results and Discussion

### 3.1. Synthesis.

For the synthesis of the target compounds 10a-f - 15a-f the following straightforward pathway was pursued. Compounds 1-3 were prepared using Ullmann reaction according to the reported method [25]. Compounds 1-3 were used to prepare the intermediate compounds 4-6 respectively according to the reported method [26]. Compounds 7-9 were prepared from compounds 4-6, respectively [27]. The infrared spectrum of the compounds showed reappearance of NH and C=O groups. The final compounds were obtained as shown in scheme 1 using a combinatory chemistry model using multicomponent reaction (MCRs) [28, 29]. This type of reaction is preferred since it is easier to perform, gives higher yield and is less time consuming [30]. The time needed for completing the reaction was monitored by TLC using chloroform: methanol 9.5:0.5. The final compounds were prepared by refluxing an equimolar amount of compounds 7-9 and the appropriate aldehyde in the presence of excess ethylcyanoacetate or malononitrile to afford the corresponding compounds 10a-f -12a-f or compounds 13a-f -15a-f, respectively.

**Scheme 1**

### 3.2. Molecular docking studies of the new compounds with topoisomerase I

This technique is considered direct molecular modeling where the 3D structure of the enzyme is known and is used to know the detailed intermolecular interactions between the ligand and

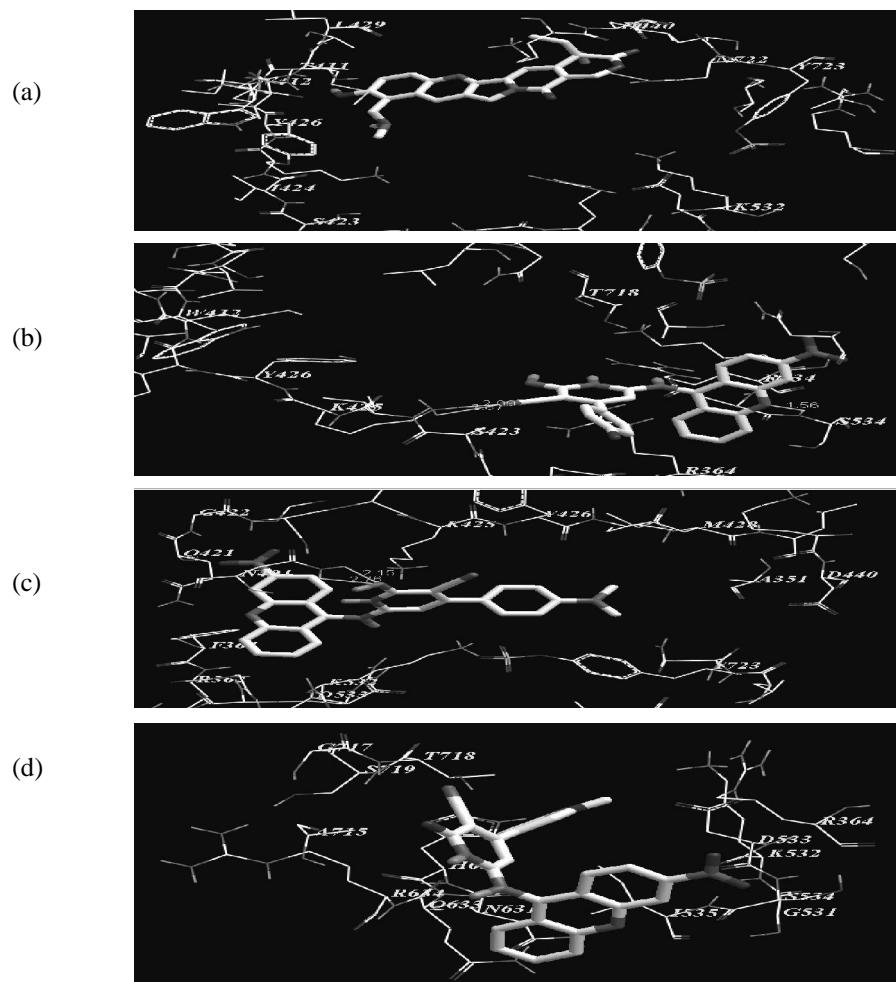
the target protein. An automated docking study was carried out using the crystal structure of inhibitor Topotecan/topoisomerase I complex obtained from protein data bank website (pdb) entry 1SC7; having resolution of 2.0 Å°. This regularized protein complex structure was used in determination of the enzyme active site that is mentioned in the literature. The performance of

the docking method on topoisomerase I inhibitors was evaluated and validated by re-docking the crystal ligand topotecan where RMSD value obtained was 0.00421. Docking process was carried out for the test set of compounds (10a-f -12a-f and 13a-f – 15a-f).

In the flexible-ligand-rigid enzyme docking, the enzyme was represented by six potential energy maps, namely, electrostatic, hydrogen bond, hydrophobic, and three van der Waals. Interactive docking using Mol table ligand was carried out for all the conformers of each compound of the test set to the selected active site of topoisomerase I. Each docked compound was assigned a score according to its fit in the ligand binding pocket (LBP).

The predicted binding energies of the new compounds are listed in Tables 1&2. The docking poses of Topotecan III and the three compounds possessing the lowest binding energies, 14b, 14e and 11e, into the active site of TopI is shown in Fig. 2.

Docking results provided useful information in understanding the structural features of the target and the necessary chemical features of the ligands. This was extended to the successful designing of our acridine derivatives where most analogs were highly active analogs against Top I.



**Figure 2;** (a), (b), (c) and (d) the proposed binding mode of Topotecan III, compound 14b, 14e and 11e inside the active site of TopI resulting from docking, respectively. The most important amino acids are shown together with their respective numbers. Compound 14b form two hydrogen bonds with Ser423.

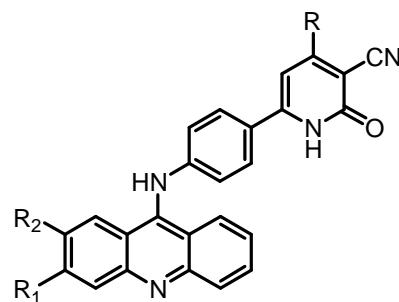
### 3.3. Anticancer screening

Measurement of potential cytotoxicity of the new compounds against breast cancer MCF-7 cell line by sulforhodamine B (SRB) assay: Potential cytotoxicity of the compounds against MCF-7 breast cancer cell line was tested using the method of skehan co-worker [31]. The relation between surviving fraction and drug concentration was plotted to get the survival curve for each compound. Also, IC<sub>50</sub> for each derivative was determined which is the dose of the compound reduces survival to 50%. Results are shown in Table 3. It can be seen from the data obtained that compounds 8, 11e, 11f, 13b, 14b,

14e and 14f showed activity at a concentration 10 µg or less. The most active one is 14b. Regarding the structure of the active compounds we can conclude that acridine derivatives substituted with nitro group showed higher activity compared to the unsubstituted or methoxy congeners as shown by 11e and 14b.

The p-substituted-3-cyano-2-iminopyridine derivatives exhibited higher antitumor activity than their 2-oxocongeners.

**Table 1. Best docking conformer for each compound in the test set (10a-f -12a-f) docked into the active site of Top1.**



R	R <sub>1</sub>	R <sub>2</sub>	Cpd No.	Docking value (kcal/mol)	R	R <sub>1</sub>	R <sub>2</sub>	Cpd No.	Docking value (kcal/mol)
Ph	H	H	<b>10a</b>	-66.1	p-OH-C <sub>6</sub> H <sub>5</sub>	NO <sub>2</sub>	H	<b>11d</b>	-65.3
p-F-C <sub>6</sub> H <sub>5</sub>	H	H	<b>10b</b>	-79.1	p-MeO-C <sub>6</sub> H <sub>5</sub>	NO <sub>2</sub>	H	<b>11e</b>	-79.9
p-Cl-C <sub>6</sub> H <sub>5</sub>	H	H	<b>10c</b>	-69.9	p-N(CH <sub>3</sub> ) <sub>2</sub> -C <sub>6</sub> H <sub>5</sub>	NO <sub>2</sub>	H	<b>11f</b>	-72.7
p-OH-C <sub>6</sub> H <sub>5</sub>	H	H	<b>10d</b>	-63.4	Ph	H	MeO	<b>12a</b>	-66.4
p-MeO-C <sub>6</sub> H <sub>5</sub>	H	H	<b>10e</b>	-71.3	p-F-C <sub>6</sub> H <sub>5</sub>	H	MeO	<b>12b</b>	-78.4
p-N(CH <sub>3</sub> ) <sub>2</sub> -C <sub>6</sub> H <sub>5</sub>	H	H	<b>10f</b>	-70.6	p-Cl-C <sub>6</sub> H <sub>5</sub>	H	MeO	<b>12c</b>	-72.7
Ph	NO <sub>2</sub>	H	<b>11a</b>	-66.9	p-OH-C <sub>6</sub> H <sub>5</sub>	H	MeO	<b>12d</b>	-70.4
p-F-C <sub>6</sub> H <sub>5</sub>	NO <sub>2</sub>	H	<b>11b</b>	-70.1	p-MeO-C <sub>6</sub> H <sub>5</sub>	H	MeO	<b>12e</b>	-73.1
p-Cl-C <sub>6</sub> H <sub>5</sub>	NO <sub>2</sub>	H	<b>11c</b>	-64.1	p-N(CH <sub>3</sub> ) <sub>2</sub> -C <sub>6</sub> H <sub>5</sub>	H	MeO	<b>12f</b>	-75.8

### 4. Conclusion,

Compounds 8, 11e, 11f, 13b, 14b, 14e and 14f showed activity at a concentration 10 µg or less. The most active one is 14b. Regarding the structure of the active compounds we can conclude that acridine derivatives substituted with nitro group showed higher activity compared to the unsubstituted or methoxy congeners as shown by 11e and 14b. The p-substituted-3-cyano-2-iminopyridine derivatives exhibited higher antitumor activity than their 2-oxocongeners.

### Acknowledgements

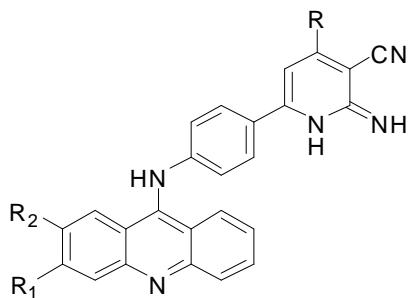
Author thank all the member of pharmacology unit at the National Cancer institute ,Cairo university for preparing the cytotoxicity testing.

### Corresponding author

Gehan Hegazy Hegazy

<sup>1</sup>Department of Pharmaceutical Chemistry, Faculty of Pharmacy Cairo University, Cairo, Egypt.

[gehan\\_hegazy@yahoo.com](mailto:gehan_hegazy@yahoo.com)

**Table 2.** Best docking conformer for each compound in the test set (13a-f-15a-f) docked into the active site of Top1.

R	R <sub>1</sub>	R <sub>2</sub>	Cpd No.	Docking value (kcal/mol)	R	R <sub>1</sub>	R <sub>2</sub>	Cpd No.	Docking value (kcal/mol)
Ph	H	H	<b>13a</b>	-63.1	p-OH-C <sub>6</sub> H <sub>5</sub>	NO <sub>2</sub>	H	<b>14d</b>	-67.3
p-F-C <sub>6</sub> H <sub>5</sub>	H	H	<b>13b</b>	-77.4	p-MeO-C <sub>6</sub> H <sub>5</sub>	NO <sub>2</sub>	H	<b>14e</b>	-82.4
p-Cl-C <sub>6</sub> H <sub>5</sub>	H	H	<b>13c</b>	-70.3	p-N(CH <sub>3</sub> ) <sub>2</sub> -C <sub>6</sub> H <sub>5</sub>	NO <sub>2</sub>	H	<b>14f</b>	-75.7
p-OH-C <sub>6</sub> H <sub>5</sub>	H	H	<b>13d</b>	-66.6	Ph	H	MeO	<b>15a</b>	-67.3
p-MeO-C <sub>6</sub> H <sub>5</sub>	H	H	<b>13e</b>	-73.7	p-F-C <sub>6</sub> H <sub>5</sub>	H	MeO	<b>15b</b>	-78.4
p-N(CH <sub>3</sub> ) <sub>2</sub> -C <sub>6</sub> H <sub>5</sub>	H	H	<b>13f</b>	-72.6	p-Cl-C <sub>6</sub> H <sub>5</sub>	H	MeO	<b>15c</b>	-69.9
Ph	NO <sub>2</sub>	H	<b>14a</b>	-68.3	p-OH-C <sub>6</sub> H <sub>5</sub>	H	MeO	<b>15d</b>	-71.2
p-F-C <sub>6</sub> H <sub>5</sub>	NO <sub>2</sub>	H	<b>14b</b>	-86.8	p-MeO-C <sub>6</sub> H <sub>5</sub>	H	MeO	<b>15e</b>	-74.1
p-Cl-C <sub>6</sub> H <sub>5</sub>	NO <sub>2</sub>	H	<b>14c</b>	-73.1	p-N(CH <sub>3</sub> ) <sub>2</sub> -C <sub>6</sub> H <sub>5</sub>	H	MeO	<b>15f</b>	-73.5

**Table 3:** IC<sub>50</sub> values of the most active compounds against MCF-7 breast cancer cell line.

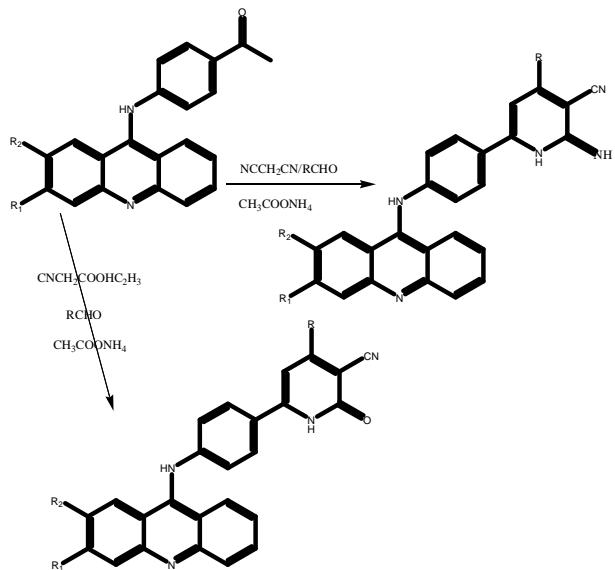
Compound	IC <sub>50</sub> in $\mu\text{g}/\text{mL}$
<b>8</b>	9.23
<b>11e</b>	8.86
<b>11f</b>	10
<b>13b</b>	9.93
<b>14b</b>	7.80
<b>14e</b>	9.73
<b>14f</b>	9.41

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## Graphical abstract



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6/1/2010

## Effect of Probiotic (*Saccharomyces cerevisiae*) Adding to Diets on Intestinal Microflora and Performance of Hy-Line Layers Hens

Saadia M. Hassanein<sup>1</sup> and Nagla K. Soliman<sup>2</sup>

<sup>1</sup>Microbiology Dept, Faculty of Science, <sup>2</sup>Poultry Production Dept., Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

**Abstract:** An experiment was conducted to evaluate the effect of adding various levels of a live yeast to laying hen diets on their laying and feeding performance, egg shell, egg components and some blood constituents, as well as the intestinal microflora make-up. This was studied to validate the mode of a live yeast action in improving laying hens performance. For this purpose 75 Hy line (W-36) white layers were reared from 70 to 79 weeks of age in individual cages and randomly distributed into five experimental groups of 15 layers each. The individual hen was represented as an experimental unit. The five experimental groups were fed on five graded levels of a live yeast as 0.0% (control), 0.4%, 0.8%, 1.2% and 1.6%. The main results indicated an increase in egg production percentage of layers fed with 0.4% and 0.8% a live yeast which recorded 83.4% and 80.6% respectively compared with 74% of control which was similar to the groups of layers fed 1.2% (74.9%) and 1.6% (74.6%). Average egg weight was not influenced by adding yeast into diets. Egg mass results were parallel to these of egg production where the values of 46.7, 51.0, 50.2, 48.3 and 46.1 g egg/hen/day were recorded for the group of birds fed with 0%, 0.4%, 0.8%, 1.2% and 1.6% a live yeast respectively. Egg albumen and egg yolk were affected significantly. There was a slight improvement in egg shell thickness and percentage. Feed intake values were approximately similar within the different treatments. Feed conversion ratios (g feed/g egg) of layers fed yeast levels of 0.4% (2.08) and 0.8% (2.07) were better than the control group (2.27). Blood total protein levels of birds fed 0.4% (3.82), 0.8% (3.65) and 1.2% (3.97) yeast were lower than the control (4.16), while the value of 1.6% yeast (4.16) was slightly higher than control. Blood albumen levels were parallel to those of blood protein while blood globulin values were not affected. Blood cholesterol levels of layers fed yeast-supplemented diets were lower than the control. Blood total lipids were not affected by treatments. Ileal content pH of layers fed 0.8% and 1.2% yeast levels was lower than the control. Microbiological examination of ileal content indicated an obvious reduction in bacterial total count. While Lactobacilli bacterial count was increased. There were reductions in bacterial strains of *Escherichia coli* (*E.coli*), *Klebsiella sp.*, *Staphylococcus sp.*, *Micrococcus sp.*, *Campylobacter sp.*, and *Clostridium perfringens* of layers fed various yeast levels. The results of this study suggest adding live yeast at 0.4% or 0.8% into laying hen diets can enhance the productive performance and nutrients utilization via the inhibitory effect of yeast against pathogenic bacteria. [Journal of American Science. 2010;6(11):159-169]. (ISSN: 1545-1003).

**Keywords:** yeast level, laying hen, egg production, ileal microflora, blood constituents.

### 1. Introduction

Microorganisms used as probiotics in animal nutrition: Probiotics are live microorganisms that, when administered through the digestive tract, have a positive impact on the host's health. Microorganisms used in animal feed are mainly bacterial strains belonging to different genera, e.g. *Lactobacillus*, *Enterococcus*, *Pediococcus* and *Bacillus*. Other probiotics are microscopic fungi, including *Saccharomyces* yeasts. Some probiotic microorganisms are normal residents in the digestive tract, while others are not (Guillot, 2009). Different mechanisms of probiotic action have been suggested,

but most are only hypothetical. The positive effect can result either from a direct nutritional effect of the probiotic, or a "health" effect, with probiotics acting as

bioregulators of the intestinal microflora and reinforcing the host's natural defences (Fuller, 1977; Fuller, 2001).

Kabir (2004) indicated that the gut microflora forms with its host animal a complex ecosystem and microbial interactions ensure the stability of the ecosystem and the health of the host. In some cases the gut microflora is unbalanced and the biological defences against pathogenic agents less effective. The positive effect observed can be the result of either a direct nutritional effect, similar to the effect obtained with antibiotics, or a "health" or sanitary effect, where the probiotic acts as a bioregulator of the gut microflora and reinforces the natural defences.

The different mechanisms of action suggested are: (i) nutritional effect include: (1) Reduction of metabolic

reactions that produces toxic substances (2) Stimulation of indigenous enzymes (3) Production of vitamins or antimicrobial substances.

(ii) Sanitary effect include (1) Increase in colonization resistance. (2) Stimulation of the immune response. Some experiments have demonstrated in vitro the effects of strains of *Saccharomyces cerevisiae* on the activity of anaerobic rumen microorganisms. The addition of *S. cerevisiae* live cells to cultures of some cellulolytic fungal species stimulated zoospores germination and cellulose degradation. The addition of yeasts stimulates also the growth of some anaerobic bacteria, including the cellulolytic and the lactic acid utilising bacteria (Chaucheyras et al., 1995; Yoon and Stern, 1996).

Kizerwetter and Binek, (2009) reported that probiotics have reduced the incidence and duration of diseases. Probiotic strains have been shown to inhibit pathogenic bacteria both in vitro and in vivo through several different mechanisms. The mode of action of probiotics in poultry includes: (i) maintaining normal intestinal microflora by competitive exclusion and antagonism (ii) altering metabolism by increasing digestive enzyme activity and decreasing bacterial enzyme activity and ammonia production (iii) improving feed intake and digestion iv) stimulating the immune system (Apata 2008; Kabir, 2009).

Kabir et al. (2005) attempted to evaluate the effect of probiotics with regard to clearing bacterial infections and regulating intestinal flora by determining the total viable count (TVC) and total *lactobacillus* count (TLC) of the crop and cecum samples of probiotics and conventional fed groups at the 2<sup>nd</sup>, 4th and 6th week of age. Their result revealed competitive antagonism. The result of their study also evidenced that probiotic organisms inhibited some nonbeneficial pathogens by occupying intestinal wall space. They also demonstrated that broilers fed with probiotics had a tendency to display pronounced intestinal histological changes such as active impetus in cell mitosis and increased nuclear size of cells, than the controls. Recently, Mountzouris et al. (2007) demonstrated that probiotic species belonging to *lactobacillus*, *Streptococcus*, *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Aspergillus*, *Candida*, and *Saccharomyces* have a potential effect on modulation of intestinal microflora and pathogen inhibition.

A few years ago active living yeast, has been documented as probiotic feed additive for poultry, due to its improvement effect on performance characteristics. Including a live yeast into laying hen diets improved egg production percentage (Kim et al., 2002 and Shivani et al., 2003), and egg weight (Han et

al., 1999; Park et al., 2001 and Park et al., 2002). Dumanovski (2000); Sharma et al. (2001); Kim et al. (2002) and Kabir (2009) reported that, adding a live yeast into laying hens diet improved feed intake and feed conversion ratio.

Inclusions of yeast into laying hen diets enhanced egg shell breaking strength (Park et al., 2002), and reduced soft or broken eggs (Park et al., 2001).

In Egypt, a very few studies have been conducted to investigate the effect of feeding yeast on performance of laying hens. Soliman (2003) studied the effect of supplementing a constant level of live yeast into laying hens diets, he observed an improvement in average egg weight, feed conversion values and nutrients utilization. The mode of beneficial action of yeast can be attributed to its antagonistic bacteria and altering gut microflora make up Line et al., 1998; Wakwak et al. (2003) and Kabir 2009) observed a sharp reduction in bacterial total count of ileum content, due to supplementing yeast into Japanese quail diets. In contrast ileal content of lactobacilli bacteria increased significantly due to adding yeast into laying hen diets (Kim et al., 2002; Hossain et al., 2005). Adding yeast to poultry diets leads to reduced bacterial counts of *E.coli* and *Clostridium perfringers* (Park et al., 2002; Nava et al., 2005), *Salmonella* and *Campylobacter* (Line et al., 1998). In this concern the research is still lacking under Egyptian conditions.

the objective of this study aimed to investigate the effect of enriching Hy line (W-36) laying hen diets with various levels of active a live yeast on their laying and feeding performance, egg shell, egg components and some blood constituents. As well as ileal bacterial make-up will be studied to validate the mode of yeast action in improving performance of laying hens.

This study provides a summary of the use of probiotic (*Saccharomyces cerevisiae*) for prevention of bacterial diseases in poultry as well as demonstrating the potential role of probiotics in the growth performance and immune response of poultry.

## 2. Materials and Methods

This study was carried out at (Layer Nutrition Research Unit), Faculty of Agriculture, Ain Shams University.

It was conducted using 75 Hy-Line (W-36) white layers which were randomly sited from 70 to 79 week of age in individual battery cages located in open sided laying house. The hens were randomly

distributed into five treatment groups of 15 layers each. The individual hen was represented as experimental unit. For nine weeks experimental period the hens were fed on a basal diet supplemented with five graded levels of active live yeast *Saccharomyces cerevisiae* (produced by Starch, Yeast and Clean Co., Alex.) as 0.0% (control) 0.4%, 0.8%, 1.2% and 1.6%.

The basal diet was formulated (Table 1) to meet all nutrient requirements of laying hens according to (Hy-Line 2000) management guide. Feed was provided ad lib in an individual feeders and water was supplied through automatic nipples. Lighting hours were 17 hours per day. Egg weight in grams was recorded daily for each hen throughout the experimental period. Average egg weight, egg production percentage and average egg mass (g/hen/day) were calculated for each

hen and treatment group. Feed consumption in grams per hen was recorded weekly and average feed consumption per treatment group was calculated. Feed conversion ratio was calculated as gram feed consumed per gram egg produce (g. feed/ g. egg). Body weight gain was calculated for each hen and treatment group by subtracting individual body weight of hen at 70 weeks from that at 79 weeks of age. Egg component percentages were assessed by using 12 eggs per treatment represent 6 hens as two consecutive eggs per hen. For this purpose, egg was individually weighted, broken, yolk and albumin was separated weighed and related as percentage to whole egg weight. Egg shell with membrane were cleaned, dried, weighed and related as percentage e to the whole egg.

**Table (1): Composition and calculated analysis of experimental diet.**

Feed Ingredient	Percentage (%)
Yellow corn	59.93
Soybean meal (48%)	24.23
Corn gluten meal	2.0
Calcium carbonate	9.16
di-calcium phosphate	1.84
Oil	2.0
Common salt	0.364
Methionine	0.076
Premix*	0.4
<b>Total</b>	<b>100</b>
<i>Calculated analysis:</i>	
ME (kcal/kg)	2806
Protein (%)	17.39
Calcium (%)	3.97
Av. Phosphorus (%)	0.465
Meth. + Cyst (%)	0.66
Lysine (%)	0.86

\*: Vitamins and minerals Premix: each 1 kg supplied the following per kilogram of diet; vit. A: 12000 lu, vit. D3: 3000 lu, vit. E.: 12 mg.  
vit. B12 0.02 mg, vit. B1 1 mg, Choline chloride 0.16 mg, Copper 3 mg, Iron 30 mg.  
Manganese 40 mg, Zinc 45 mg and Selenium 3 mg according to NRC (1994).

Egg shell thickness (millimeter) was determined using a micrometer. Initial and final body weights of layers were recorded and average body weight gain was calculated.

#### Blood Analysis and Microbiological Examination:

At the end of the experiment five hens per experimental group were slaughtered, blood samples

were collected and centrifuged for 15 minutes. Plasma total protein was determined according to Biuret method (Henery, 1964), albumin according to Doumas et al. (1971). Plasma globulin was calculated by subtracting albumin from total protein. Then albumin to globulin ratio was calculated. Plasma total lipid was determined according to Knight et al. (1972) and total cholesterol according to Watson (1960).

For microbial experimentation, ileal content samples were collected by pressing the outer wall of cut ileal to push its content into clean, sterile glass bottle. The pH value of ileum content were determined using pH meter. Microbiological experimentation procedure was done as follows: One gram of ileal content was adjustely weighed and transferred into test tube containing 9 ml of 0.1 sterile peptone the samples were mixed well and serial dilutions were prepared.

#### Cultivation and Enumeration of Bacteria:

Bacterial total count was examined with nutrient agar medium composed of (per liter) yeast extract 2.5 g tryptone 5 g, glucose 1 g, agar 15 g and distilled water up to one liter (Swanson et al., 1992).

*Lactobacilli* bacteria was counted with M.R.S. agar medium which is composed of casein peptone 10 g meat extract 10 g, yeast extract 5 g, glucose 20 g, tween 80 1 g, K<sub>2</sub>hpo<sub>4</sub> 2 g, sodium acetate 5 g, diammonium citrate 2 g, Mnso<sub>4</sub> 0.2 g and distilled water up to 1 liter (Laner and Kandier, 1980)..

Coliforms bacteria were counted by using MacConkey agar medium that is composed as pancreatic digest of gelatin 17 g, pancreatic digest of casein 1.5 g, peptic of animal tissue 1.5 g, lactose 10 g, bile salts 1.5 g, sodium chloride 5 g, neutral red 0.03 g, crystal violet 0.001 g, agar 3.5 g, and distilled water up to 1 liter (Oxoid, 1992).

*Campylobacter* strains were grown in stationary cultures in 5 ml of Rosef broth without antibiotics for 48 hours in a microaerobic atmosphere created by using BBL gas pak plus anaerobic system envelopes without the palladium catalyst. Rosef broth contains (per liter) peptone 10g, lablemco (oxid) 8 g, yeast extract 1 g, Nacl 5 g, rezasurin solution (0.025% wt/vol) 1.6 g (Ryan and Ray, 2004).

*Colstridium perfringers* were grown in a stationary culture in an anaerobic atmosphere and subsequently diluted in sterile Rosef broth or sterile saline to concentrations of 10<sup>6</sup> to 10<sup>8</sup> CFU per ml, then PCR procedure was used for examination (Baumgart et al., 2007).

*Klebsiella* and *Proteus* gram negative *Enterobacteria* were grown in MacConkey agar medium and eosin/methylene blue agar medium composed (per liter) of peptone 10 g, lactose 5 g, dipotassium phosphate 2 g, eosin Y 0.4 g, methylene blue 0.065 g, and agar 13.5 g (Oxoid, 1992).

*Staphylococcus* sp. and *Micrococcus* sp. gram positive bacteria was grown in nutrient agar medium, MacConekay agar medium and *Staphylococcus*

medium (No. 110) that composed (per liter) yeast extract 2.5 g, tryptone 10 g, glateene 30 g, lactose 2 g, D/manitol 10 g, Nacl 75 g, dipotassium phosphate 5 g, agar 15 g, pH 7 ± 0.02 (Mathews et al., 1997).

#### Statistical Analysis:

Statistical analysis was carried out using statistical program SAS (1988). Dukan's multiple tests was used to separate means.

### 3. Results and Discussion

Shareef and Dabbagh (2009) reported that *Saccharomyces cerevisiae* supplementation of broilers, to the level of 1, 1.5 and 2%, were significantly, increase the body weight gain, feed consumption and feed conversion efficiency. The beneficial effect of *Saccharomyces cerevisiae* is attributed to the fact that it is a naturally rich source of proteins, minerals and B-complex vitamins.

It is well known that yeast culture, and its cell wall extract containing 1,3-1,6 D-glucan and Mannan oligosaccharide are the important natural growth promoters for modern livestock and poultry production (Van Leeuwen et al., 2005a). The advantages of these promoters over the traditional antibiotic growth promoters are 1) no withdrawal time, 2) no residual effect, and 3) no causes of microbial mutation (Gibson and Roberfroid, 2008). *Saccharomyces cerevisiae* is considered as one of the live microorganisms probiotic that, when administered through the digestive tract, have a positive impact on the hosts health through its direct nutritional effect. Field reports (Banday and Risam, 2002) have suggested that probiotic supplementation improved performance of broilers. The different mechanisms of probiotic action suggested are; nutritional effect by regulation of metabolic reactions that produces toxic substances; stimulation of endogenous enzymes and by production of vitamins or antimicrobial substances. Moreover, *Saccharomyces cerevisiae* could act as bioregulator of the intestinal micro flora and reinforcing the host natural defenses, through the sanitary effect by increasing the colonization resistance and stimulation of the immune response (Line et al., 1998). These effects were largely reflected by using mannan Oligosaccharide, the naturally derived extract from the cell wall of *Saccharomyces cerevisiae*. This oligosaccharide content is approxi-mately50% of the carbohydrate fraction and improved body weight gain in broiler chickens and that this effect can be attributed to the trophic effect of this product on the intestinal mucosa, because it increases villus height, particularly during the first 7 days of the chickens life (Santin et al., 2001).

Oligosaccharides used to control pathogenic scours of all kinds in livestock caused by *Salmonella*, and *E.coli* etc (Laegreid and Bauer, 2004). Mannan-oligosaccharides are thought- to block the attachment of pathogenic bacteria to the animal's intestine and colonization that may result in disease, while acting as a nutrient to other beneficial bacteria. It is also thought to stimulate the animal's immune system, thereby further reducing the risk of disease (Firon and Ofek, 1983). Oyofo et al. (1989) observed that the adherence of *Salmonella typhimurium* to enterocytes of the small intestine of chicks, in vitro, was inhibited in the presence of mannose. Later, they found that inclusion of mannose in the drinking water of chicks reduced *S. typhimurium* colonization of the cecum.

*Saccharomyces cerevisiae* Probiotic supplementation has been shown to reduce the

cholesterol concentration were reported in egg yolk by (Abdulrahim et al., 1996) and serum in chicken (Mohan et al., 1996). Recent report suggested that feeding of chicory beta fructans an oligosaccharide, a prebiotic, reduced the serum cholesterol and abdominal fat of broiler chicken (Yusrizal, 2003). Gilliland et al. (1985) suggested that the Prebiotic supplementation could have enhanced the lactobacilli count. Similar results have been reported by others (Mohan, 1996).

#### Laying Performance:

Egg production percentage of laying hens fed 0.4% (83.4%) and 0.8% (80.6%) live yeast was higher than the control value (74%) which was approximately similar to those fed with 1.2% (74.9%), 1.6% (74.6%) yeast in their diets. The differences between egg production percentages lacked significance (Table 2).

**Table (2): Effect of feeding different yeast levels on laying performance and egg components.**

Item	Yeast Level				
	0.0%	0.4%	0.8%	1.2%	1.6%
Egg production	74.0	83.4	80.6	74.9	74.6
Av. Egg weight (g)	63.1	61.2	62.7	64.5	61.8
Egg mass (g egg/hen/day)	46.7	51.0	50.2	48.3	46.1
<b>Egg component</b>					
Egg yolk (%)	27.3	28.1	28.8	27.6	27.7
Egg albumin (%)	63.7	62.6	61.7	63.1	62.9
Egg shell (%)	9.00	9.33	9.45	9.39	9.39
Egg shell thickness (mm)	0.396 <sup>b</sup>	0.425 <sup>ab</sup>	0.426 <sup>a</sup>	0.416 <sup>ab</sup>	0.420 <sup>ab</sup>

a, b: Means with different superscripts are significantly different (P<0.05).

The improvement in egg production due to low level of yeast inclusion is in agreement with the result of Kim et al., (2002); Shivani et al. (2003); Shareef and Al-Dabbagh (2009) who observed higher percentage of egg production for hens fed yeast-supplemented diets than the control hens.

Average egg weight was not influenced significantly by adding yeast into diets. Nursoy et al.

(2004) stated that, egg weight was not affected by adding yeast into diet. The improvement in egg production reflected on egg mass (g egg/hen/day) values which increased from 46.7 (control) to 51.0 and 50.2 by adding 0.4% and 0.8% yeast level respectively, while the high levels of yeast (1.2% and 1.6%) declined egg mass value to be 48.3 and 46.1 respectively.

The increment in egg production and egg mass with 0.4% and 0.8% yeast level may be attributed to the antagonistic effect of yeast against harmful enteric microflora which may cause mal-absorption of

nutrients. So that, adding yeast may enhance digestion, absorption and saving more nutrients for egg formation. Soliman (2003) attributed the best hen day egg production of hens fed dietary yeast to the decrease proliferation of pathogenic bacteria. The high inclusion of yeast level has an adverse effect on nutrient digestibility (Romashko, 1999). Thereby, laying performance was not improved due to adding of 1.2% or 1.6% live yeast into diet.

#### Feeding Performance and Body Weight Gain:

Feed intake values of different treated groups were approximately similar and lacked significance. Kim et al. (2002) stated that, feed intake values were not statistically different among yeast feeding groups and control.

Feed conversion ratios (g feed/g egg) of birds fed with 0.4% (2.08) and 0.8% (2.07) dietary yeast were better than that of control (2.22), while 1.2% (2.24) and 1.6% (2.25) yeast levels did not show any improvement compared to the control. Park et al. (2002); Soliman (2003) and Zhang et al., (2005)

observed an improvement in feed conversion ratio of laying hens fed yeast supplemented diets.

The slight improvement in feed conversion inherent with low inclusion levels of yeast (0.4% or 0.8%) may be attributed to the improvement in nutrients absorption and utilization associated with adding yeast which reduces the proliferation of enteric harmful bacteria that responsible of mal-absorption (Table 3). Bradle and Savag (1995) observed an improvement in energy utilization due to feeding

dietary yeast. Soliman (2003) reported that, supplementation of yeast into laying hen diets significantly improved digestion coefficient of crude protein.

Body weight gain values of layers fed different yeast levels were not significantly higher than control (Table 3). Sharma et al. (2001) stated that, the weight gain of egg type chicken fed yeast supplemented diet was higher than those fed control diet.

**Table (3): Effect of feeding various live yeast levels on feeding performance and body weight gain.**

Item	Yeast Level				
	0.0%	0.4%	0.8%	1.2%	1.6%
Feed intake (g/hen/day)	104.00	105.7	105.00	108.3	103.6
Feed conversion (g feed/g egg)	2.22	2.08	2.07	2.24	2.25
Initial body weight (g)	1475	1444	1480	1478	1481
Final body-weight (g)	1497	1494	1540	1555	1552
Body weight gain (g)	22	50	60	76.8	71.6

Non-significant differences.

#### Egg Component:

Incorporating of live yeast into laying hen diets did not influence egg albumin or egg yolk percentages and the difference; among treatments lacked significance (Table 2). Nursoy et al. (2004) did not find any affect on egg albumin or egg yolk of laying hens fed yeast-supplemented diet.

However, egg shell percentage and egg shell thickness values were improved due to feeding various yeast levels, especially at 0.8%, when compared to the control group (Table 2).

The improvement in egg shell percentage and egg shell thickness may be attributed to the enhancement of calcium absorption and retention associated with adding yeast into the diet (Bradly and Savage, 1995). Park et al. (2001) reported that, hens fed diets with yeast produced less soft shell and broken egg than control.

#### Blood Constituents:

Blood total protein values of birds fed on 0.4% (3.82), 0.8% (3.65), and 1.2% yeast (3.97) were lower than the control (4.16) (Table 4). However, the level of 1.6% yeast (4.33) was slightly higher than control. Similar results were recorded for blood albumin. There

was no effect on blood globulins due to adding yeast to the diet.

The results of blood protein did not agree with those obtained by Wakwak et al. (2003), who did not find any effect on blood protein or albumin due to adding yeast into growing quail diets.

The lower values of blood proteins of birds fed on 0.4%, 0.8% and 1.2% yeast than the control may be attributed to the inhibitory effect of yeast against harmful intestinal microflora because harmful enteric bacteria secretes inflammatory agents lead to increase protein synthesis in liver and accordingly increased blood content of protein. Klasing and Austic (1984) observed an increase in protein synthesis in liver of chickens infected with *Escherichia coli* bacteria. Similar explanation can be introduced for the higher blood protein value of layers fed 1.6% dietary yeast, that the high inclusion of active live yeast may induce an inflammation in the small intestine wall causing increase in blood protein level.

Blood cholesterol levels of layers fed yeast supplemented diets were lower than the control (Table 4). Victor et al. (1993) and Endo et al., (1999) found that cholesterol content was lower with inclusion of yeast into broiler chicks' diets. Blood total lipid was not affected by adding yeast into diets.

**Table (4): Effect of feeding various live yeast levels on blood constituents.**

Item	Yeast Level				
	0.0%	0.4%	0.8%	1.2%	1.6%

<b>Total protein (g/dL)</b>	4.16 <sup>a</sup>	3.82 <sup>ab</sup>	3.65 <sup>b</sup>	3.97 <sup>ab</sup>	4.33 <sup>a</sup>
<b>Albumin (g/dL)</b>	2.23 <sup>a</sup>	1.83 <sup>b</sup>	1.80 <sup>b</sup>	2.08 <sup>ab</sup>	2.36 <sup>a</sup>
<b>Globulin (g/dL)</b>	1.93	1.99	1.87	1.89	1.97
<b>Alb./Glob.</b>	1.16	0.92	0.97	0.91	0.84
<b>Cholesterol (g/dL)</b>	161.5 <sup>a</sup>	149 <sup>ab</sup>	133.7 <sup>b</sup>	158.2 <sup>ab</sup>	149 <sup>ab</sup>
<b>Total lipid (mg/dL)</b>	418.0	395.0	396.2	437.7	423.0

a, b: Means with different superscripts are significantly different ( $P<0.05$ ).

#### Ileal pH and Intestinal Bacteria:

Ileal content pH was not affected by adding active yeast into laying hens diets (Table 5). However, there were a reduction in digesta pH of layers fed yeast level of 0.8% and 1.2% which recorded 6.00 and 6.31 respectively against 6.58 for control. Dawson et al. (1990) and Gibson and

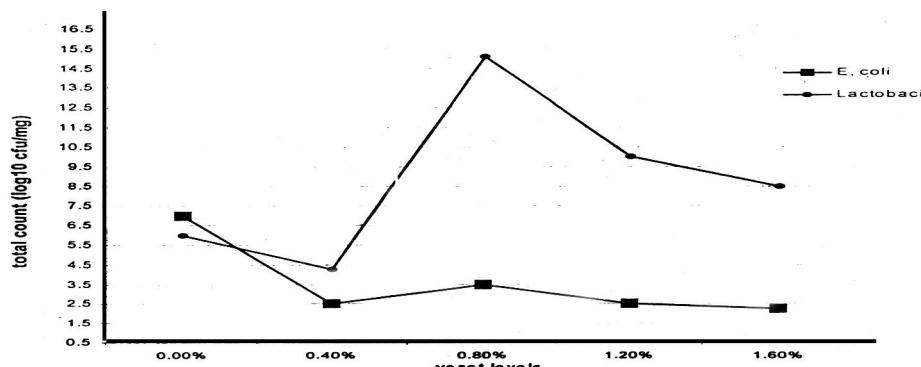
Roberfroid, (2008) observed a reduction in ruminal pH value of steers fed active yeast.

There was an effect yeast on bacterial total count which was sharply reduced when supplemented yeast level increased. The most reduction was recorded for the birds fed 1.6% live yeast (Table 5 and Fig. 1).

**Table (5): Effect of feeding active yeast levels on pH value of ileal content and intestinal bacteria make-up.**

<b>Microbial Strains</b>	<b>Yeast Level</b>				
	<b>0.0%</b>	<b>0.4%</b>	<b>0.8%</b>	<b>1.2%</b>	<b>1.6%</b>
Ileal content pH	6.58	6.88	6.00	6.31	6.58
<b>Log 10 cfu./mg</b>					
Bacterial total count	15	12.5	12.7	10.1	5.4
<i>Escherichia coli</i>	7.0	2.5	3.5	2.5	2.25
<i>Lactobacilli sp.</i>	6.0	4.25	15.1	10.0	8.5
<i>Klebsiella sp.</i>	1	1	N.d	1	1
<i>Staphylococcus sp.</i>	3	1	2	1	1
<i>Proteus sp.</i>	2	1	2	1	1
<i>Micrococcus sp.</i>	2	N.d	3	N.d	N.d
<i>Combylobacter sp.</i>	4	N.d	3	2	N.d
<i>Closteridium perfringers</i>	3	N.d	2	1	N.d

N.d: Non-dectable.



**Figure (1): Effect of yeast level on total count of *E.coli* and *lactobacilli sp.*.**

The inhibitory effect of yeast on intestinal microflora had been established by Line et al. (1998); Wakwak et al. (2003) and Nava et al., (2005), who reported that, yeast has a reduction effect against pathogenic gut microflora.

Count of *Lactobacilli* bacteria increased due to adding active live yeast at 0.8%, 1.2% and 1.6% into laying hens diets. This result confirms those of Kim et al. (2002), who added *Pichia farinose* yeast strain into laying hens' diets and Park et al. (2002) and Kabir (2009), who included *Saccharomyces cervisiae* into broiler diets. Their results indicated an increase in viable count of ileal lactobacilli's due to adding live yeast.

The viable counts of Lactobacilli are inversely related to the pH value of ileal digesta (Table 5), where the reduction in pH values is associated with increasing Lactobacilli count. This may confirm that Lactobacilli bacterial grow well in slightly acidic media (Fuller, 2001).

*Lactobacilli* bacteria secrete lactic acid which reduces digesta pH so the reduction in pH value may be due to direct action of intestinal bacilli bacteria or to indirect effect of yeast on increasing intestinal bacilli bacteria. Live yeast enriched diet led to a sharply reduction in pathogenic bacterial strains of *E.coli* and *Campylobacter sp.* These strains usually cause mild to moderate gastroenteritis, diarrhea and mal-absorption of nutrients in chickens.

The current results are in agreement with those of Park et al. (2002), who stated that the counts of *Clostridium perfringer* and *E.coli* bacteria were lower

due to adding *Saccharomyces cervisiae* yeast into broiler chicks' diets. The antagonistic effect of live yeast against intestinal microflora was elucidated by Line et al. (1998) and Laegreid and Bauery (2004) who stated that, several harmful pathogenic bacteria have been shown to exhibit a binding specific for the sugar mannose. A live yeast cells contain mannose in their wall. This mannose in the cell wall may cause the yeast to act as a decoy for the attachment of pathogens. Because yeast has been demonstrated not to permanently colonize animals, the yeast and any yeast-bound pathogens pass out in the bird excretion and bacterial colonization is diminished.

Kabir et al., (2004) reported that probiotic microorganisms, once established in the gut, may produce substances with bactericidal or bacteriostatic properties (bacteriocins) such as lactoferrin, lysozyme, hydrogen peroxide as well as several organic acids. These substances have a detrimental

impact on harmful bacteria, which is primarily due to a lowering of the gut pH. A decrease in PH may partially offset the low secretion of hydrochloric acid in the stomach. In addition, competition for energy and nutrients between probiotic and other bacteria may result in a suppression of pathogenic species. Numerous factors such as animal to animal variation, strain of yeast, and experimental procedures have contributed to the variation in results of yeast culture studies. However, the digestive advantages of enhanced nutrient digestibility, cecal fermentation and subsequent production parameters provide justification for nutritionists to continue to research yeast culture supplementation.

#### 4. Conclusion:

It can be concluded that adding live yeast *Saccharomyces cervisiae* can enhance the productive performance of laying hens and nutrients utilization via the inhibitory effect of yeast against pathogenic bacteria which may cause mild enteritis and mal-absorption of nutrients.

Probiotics constitutes now an important aspect of applied biotechnological research and therefore as opposed to antibiotics and chemotherapeutic agents can be employed for growth promotion in poultry. Scientists now are triggering effort to establish the delicate symbiotic relationship of poultry with their bacteria, especially in the digestive tract, where they are very important to the well being of man and poultry (Kabir, 2009). Since probiotics do not result in the development and spread of microbial resistance, they offer immense potential to become an alternative to antibiotics. The present study reveals that probiotics could be successfully used as nutritional tools in poultry feeds for promotion of growth, modulation of intestinal microflora and pathogen inhibition, immunomodulation and promoting meat quality of poultry.

#### Acknowledgment:

Great thanks to Dr. Hussein A. El-Alaily, Professor of Poultry Nutrition, Ain Shams University and the staff of Misr Hatchery Company for providing all facilities needed for this study.

**Corresponding author**

Saadia M. Hassanein  
 Microbiology Dept, Faculty of Science, Faculty of Agriculture, Ain Shams University, Cairo, Egypt

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6/2/2010

# Properties of enterotoxigenic *S. aureus* Isolated from mastitic cattle and buffaloes in Egypt

Jakeen Kamal Abdel Haleem El-Jakee<sup>1</sup>, Emad Rizkalla Zaki<sup>2</sup>, Randa Samy Farag<sup>2</sup>

1-Microbiology Department Faculty of Vet. Medicine Cairo University

2-Buffaloes Diseases Department, Animal Health Research Institute, Dokki, Giza.

[jeljakee@yahoo.com](mailto:jeljakee@yahoo.com)

**Abstract:** Enterotoxigenic *S. aureus* in milk posses a potential health hazard to consumers. In this paper 106 *S. aureus* isolated from cow and buffalo milk samples were investigated for production of enterotoxins. RPLA results showed high incidence of type C enterotoxin followed by type A and type B with incidence of 34 (32.1%), 19 (17.9%) and 15 (14.2%) respectively. Toxigenic *S. aureus* isolates produced golden yellow, creamy and white colonies on agar in percent of 69.11%, 27.94% and 2.94% respectively. Regarding to hemolytic activity on sheep blood agar, 92.65% of toxigenic *S. aureus* isolated from bovine milk samples were hemolytic. A correlation exists between toxigenic isolates and coagulase and DNase production. On crystal violet agar medium, 23.53% of the *S. aureus* isolates yielded yellow colonies, 64.71% yielded violet colonies, while 11.76% yielded white colonies from the toxigenic *S. aureus* isolates. It is clear that most of bovine isolates yielded violet colonies on the medium. Out of 68 isolates of toxigenic *S. aureus* isolates 51 (75%) showed SpA by agglutination test positive. Results obtained showed 100% agreement between RPLA and PCR techniques. [Journal of American Science. 2010;6(11):170-178]. (ISSN: 1545-1003).

**Keywords:** *S. aureus*, mastitis, enterotoxins, RPLA, PCR.

## 1. Introduction

Milk and its products can harbor a variety of microorganisms and can be important sources of food-borne pathogens. Livestock-associated *S. aureus* to be an underappreciated source of pathogenic strains (Bystron et al., 2010). Enterotoxigenic *S. aureus* in raw milk posses a potential health hazard to consumers, the identification of such strains should be used as part of a risk analysis of milk and milk products (Zouharova and Rysanek, 2008). Staphylococcal food poisoning is considered one of the leading food-borne illnesses in human worldwide and is associated with contaminated food of animal origin such as milk and dairy products (Tsegmed et al., 2007). *S. aureus* is a major causative agent of mastitis which is the most economically important diseases for the dairy industry so more effective therapeutic treatment and prophylactic approaches are surely needed (Chiang et al., 2007; Oviedo-Boyo et al., 2008).

Regarding the public health, *S. aureus* is a commensal organism and versatile pathogen in animals and human. It produces a broad spectrum of surface components (proteins and capsular polysaccharides) and exotoxins. Staphylococcal enterotoxins (SEs) are serologically grouped into five major classical types which are SEA, SEB, SEC, SED and SEE. Also new SEs such as SEG through SEM has recently been identified and characterized (Chiang et al., 2006). In addition to toxic shock syndrome toxin (TSST-1) which is the causative agent in toxic shock syndrome in human (Kenny et al., 1993). The direct detection of the

pathogen in the raw milk and dairy products by PCR technique can provide rapid results and highlight the presence of loads of *S. aureus* potentially representing the risk of intoxication (Ercolini et al., 2004). The analysis of the results obtained by SET-RPLA method for the productivity of classical enterotoxins A-D and the results obtained by PCR for the presence of *sea-sed* genes revealed the correlation between each other (Lawrynowicz-Paciorek et al., 2007). The present work aimed to determine the role of *Staphylococcus* species in bovine mastitis and study the most virulence factors associated with isolated strains using recent techniques for the detection of gene sequence concerned with toxin production as RPLA and PCR techniques.

## 2. Material and Methods

Milk samples:

A total Of 203 animals including 149 cows and 54 buffaloes from different farms in Egypt were examined for mastitis according to clinical observation (Schalm et al., 1971). A total of 554 individual quarter milk samples were collected from 406 quarters of lactating cows and 148 quarters of lactating buffaloes, distributed as shown in Table (1). The examined udders were thoroughly washed, dried with a clean towel and the teats were sprayed with 70% ethanol. After that the first few jets of milk were discarded and 10 ml of milk samples from each quarter were collected in a sterile McCartney bottle. All samples were kept at 4°C and transported immediately to the laboratory.

**Table (1):** Number of examined animals and quarters

Infected quarters	Cows		Buffaloes	
	No. of animals	No. of quarters	No. of animals	No. of quarters
One quarter	25	25	9	9
Two quarter	29	58	10	20
Three quarter	57	171	21	63
Four quarter	38	152	14	56
Total	149	406	54	148

#### Bacteriological examination:

The milk samples were activated by incubation for 18-24 hours at 37°C then the cream and supernatant fluids were discarded then milk samples were centrifuged at 3000 rpm for 20 minutes before bacteriological cultivation. The sediment was streaked on to the surface of nutrient agar (Difco) and Mannitol salt agar medium (Oxoid), then the inoculated plates were incubated for 24-48 hours at 37 °C after which they were examined for colony characters, cellular morphology and the purity of the culture. The suspected colonies were picked up and propagated on Baird-Parker agar (Oxoid) for further examination.

#### Identification and characterization of staphylococci isolates:

Pure cultures of the isolates were identified and characterized according to Cruickshank *et al.* (1975) and Mackie and MacCarteny (1996).-

Characteristics of coagulase positive staphylococci were identified according to Quinn *et al.* (2002) by: coagulase test using dry spot kit (staphy tect plus), acetoin production, pigment production on nutrient agar "Difco", hemolysis activity on blood agar base (Oxoid) plus 5% sheep blood, deoxyribonuclease activity using DNase agar (Oxoid), growth on Baird-

Parker medium (Oxoid) and crystal violet agar growth type (Rodgers *et al.*, 1999). Also SpA was detected using agglutination kits (welcome diagnostics) and latex slide agglutination test: Dry spot kit (staphy tect plus) (Oxoid, DR100M) was used for the identification of staphylococci which possess clumping factor.

#### Detection of staphylococcal enterotoxins by SET RPLA kit (Oxoid):

Using reversed passive latex agglutination (RPLA) the *S. aureus* isolates were examined for production of enterotoxins A, B, C and D.

#### Detection of enterotoxin by PCR

The DNA was extracted from *S. aureus* isolates using enzymatic method and the PCR products were visualized according to Sambrook *et al.* (1989) using primers synthesized by Metabion Company, Germany as described in Table (2). DNA molecular weight marker was supplied by Amers Co. Cleveland, Ohio, USA and standard *S. aureus* and *S. epidermidis* donated from Department of bacteriology, Navy American research Unit (NAMRU 3).

### 3. Results

Tables (3) demonstrate the distribution of affected quarters among mastitic cows and buffaloes. It is clear that affection in 3 quarters is higher than the others quarters affection (42.12 - 42.57%), followed by affection in 4 quarters (37.43 - 37.84%), then in 2 quarters (14.29 – 13.51%) and in one quarter (6.16 – 6.08%) respectively.

The distribution of staphylococcal species among the examined mastitic quarters was 23.29% as shown in Table (4). It is clear that 106 isolates were identified as *S. aureus* with an incidence of 19.13%, followed by 16 isolates (2.89%) identified as *S. intermedius* and 7 isolates (1.26%) were identified as *S. hyicus*.

Results obtained in Table (5) showed that 68 out of 106 *S. aureus* isolates were found to be toxicogenic with an incidence of 64.2% and distributed as follow: enterotoxin C were detected in 34 samples with an incidence of 32.1% , followed by enterotoxin A were isolated from 19 samples with an incidence of 17.9% and enterotoxin B were isolated from 15 samples with an incidence of 14.2%. It is clear from previous results that the enterotoxin C is the most predominant enterotoxin type than the others types.

*S. aureus* coagulase positive isolates produced endopigments when cultivated on nutrient agar. As shown in Table (6) toxicogenic *S. aureus* isolates produced golden yellow, creamy and white colonies on agar in percent of 69.1%, 27.94% and 2.94% respectively. Non toxicogenic *S. aureus* isolates produced golden yellow and creamy colonies on agar in percent

of 71.05% and 28.95% respectively. It is clear that golden yellow colony was the most predominant pigment among bovine *S. aureus* isolates.

In the present investigation sheep blood agar was used to determine types of hemolysis among the *S. aureus* isolates and the results were illustrated in Table (7). It is clear that 92.65% of toxigenic *S. aureus* isolates were hemolytic and 92.1% of non toxigenic *S. aureus* isolates were hemolytic.

Out of 68 toxigenic *S. aureus* isolates 46 (67.65%) were DNase positive as shown in Table (8). While out of 38 non toxigenic *S. aureus* isolates 26 (68.42%) were DNase positive.

As shown in Table (9), out of 68 toxigenic *S. aureus* isolates 66 were positive for tellurite reduction with an incidence of 97.06%, while all the 38 non toxigenic *S. aureus* isolates (100%) were positive.

Crystal violet agar medium was used as a selective medium for characterization of *S. aureus*. 3

characteristic appearances were recorded as shown in Table (10). Among toxigenic *S. aureus* isolates type A growth (yellow colonies) was detected in 23.53% of the isolates, and type C growth (violet colonies) was detected in 64.71% of the isolates, while type E (white colonies) was detected only in 11.76%. In non toxigenic *S. aureus* isolates Type A growth (yellow colonies) was detected in 23.68% of the isolates, and type C growth (violet colonies) was detected in 65.79% of the isolates, while type E (white colonies) was detected only in 10.53%. It is clear that most of bovine isolates had violet colonies on the medium.

Out of 68 isolates of toxigenic *S. aureus* isolates, 51 (75%) showed SpA agglutination test positive as shown in Table (11). Also out of 38 isolates of non toxigenic *S. aureus* isolates 27 (71.05%) were SpA positive.

**Table (2):** shows the primers used for PCR

Genes	Primer sequence (5'- 3')	PCR Program*			Size (bp)	Reference	
		No. of cycles	Temperature(°C) / time(minutes) of				
			Denaturation	Annealing	Extension		
16 S rRNA F	GTAGGTGGCAAGCGTTATCC	35	92 °C / 1 min	52°C / 1 min	72°C / 1 min	228	<b>Løvseth et al. (2004)</b>
16 S rRNA R	CGCACATCAGCGTCAG						
sea F	CCTTTGAAACGGTTAAAACG	35	92 °C / 1 min	58°C / 1 min	72°C / 1 min	127	<b>Becker et al. (1998)</b>
sea R	TCTGAACCTTCCCATAAAAAC						
seb F	TCGCATCAAACGTACAAACG					477	
seb R	GCAGGTACTCTATAAGTGCC						

Photo (1) showed that the *S. aureus* isolates previously proved to be toxigenic strains by using RPLA were confirmed to be toxigenic by using PCR. Results obtained showed that 100% agreement between RPLA & PCR.

**Table (3):** Distribution of quarters showing clinical signs of mastitis in 149 cows and 54 buffaloes.

Quarter	Cows		Buffaloes	
	No.	%	No.	%
1 Quarter	25	6.16	9	6.08
2 Quarter	58	14.29	20	13.51
3 Quarter	171	42.12	63	42.57
4 Quarter	152	37.43	56	37.84
Total	406	100	148	100

No. Positive number. % was calculated according to the total number of quarters.

**Table (4):** Distribution of *Staphylococcus* species isolated from the examined milk samples.

Sources of the isolates	No. of examined milk samples	Staphylococcus species						Total No. of isolates	%		
		<i>S. aureus</i>		<i>S. intermedius</i>		<i>S. hyicus</i>					
		No.	%	No.	%	No.	%				
Cows	406	85	20.94	11	2.71	5	1.23	101	24.88		
Buffaloes	148	21	14.19	5	3.38	2	1.35	28	18.92		
Total	554	106	19.13	16	2.89	7	1.26	129	23.29		

**Table (5):** Prevalence of toxigenic *S. aureus* isolates using RPLA test.

	No. of <i>S. aureus</i> isolates	Toxigenic isolates		Types of toxins					
				A		B		C	
		No.	%	No.	%	No.	%	No.	%
Cows	85	56	65.9	16	18.8	12	14.1	28	32.9
Buffaloes	21	12	57.1	3	14.3	3	14.3	6	28.6
Total	106	68	64.2	19	17.9	15	14.2	34	32.1

**Table (6) :** Percentage of pigment production among *S. aureus* isolates.

Sources of the isolates	Toxigenic isolates						Non toxigenic isolates *						Total isolates								
	No. of examined <i>S. aureus</i>	Golden yellow		Creamy		White		No. of examined <i>S. aureus</i>	Golden yellow		Creamy		White		No. of examined <i>S. aureus</i>	Golden yellow		Creamy		White	
		No.	%	No.	%	No.	%		No.	%	No.	%	No.	%		No.	%	No.	%	No.	%
Cows	56	38	67.9	16	28.6	2	3.6	29	20	68.97	9	31.03	-	0	85	58	68.2	25	29.4	2	2.4
Buffaloes	12	9	75	3	25	-	0	9	7	77.8	2	22.2	-	0	21	16	76.2	5	23.8	-	0
Total	68	47	69.1	19	27.94	2	2.94	38	27	71.05	11	28.95	-	0	106	74	69.81	30	28.3	2	1.89

**Table (7):** Percentage of hemolytic activity of *S. aureus* isolates on sheep blood agar.

Sources of the isolates	Toxigenic isolates				Non Toxigenic isolates *				Total isolates			
	No. of examined <i>S. aureus</i>	Hemolytic activity			No. of examined <i>S. aureus</i>	Hemolytic activity			No. of examined <i>S. aureus</i>	Hemolytic activity		
		No.	%	No.		No.	%	No.		%		
Cows	56	52	92.86	29	27	93.1	-	85	79	92.94	-	-
Buffaloes	12	11	91.67	9	8	88.89	-	21	19	90.48	-	-
Total	68	63	92.65	38	35	92.1	-	106	98	92.45	-	-

\*non toxigenic *S. aureus* using RPLA

**Table (8) :** Percentage of deoxyribonuclease activity of *S. aureus* isolates.

Sources of isolates	Toxigenic isolates				Non Toxigenic isolates *				Total isolates			
	No. of examined <i>S. aureus</i>	DNase activity		No. of examined <i>S. aureus</i>	DNase activity		No. of examined <i>S. aureus</i>	DNase activity		No. of examined <i>S. aureus</i>	DNase activity	
		No.	%		No.	%		No.	%		No.	%
Cows	56	38	67.86	29	21	72.41	85	59	69.41			
Buffaloes	12	8	66.67	9	5	55.56	21	13	61.9			
Total	68	46	67.65	38	26	68.42	106	72	67.92			

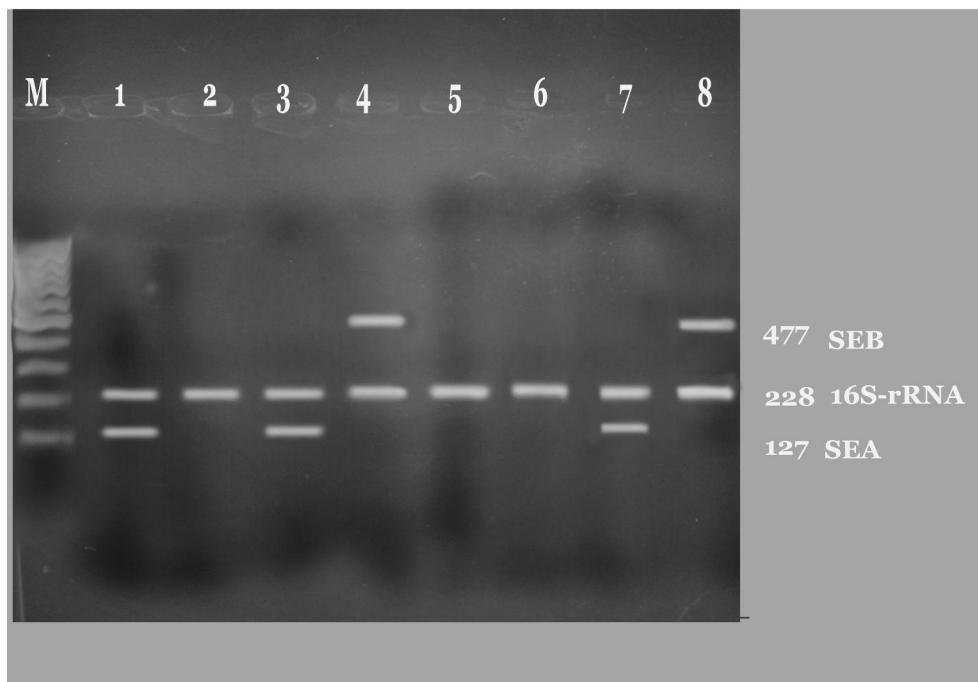
\*non toxigenic *S. aureus* using RPLA**Table (10):** Percentage of growth types of *S. aureus* isolates on crystal violet agar medium.

Sources of the isolates	Toxigenic isolates						Non toxigenic isolates *						Total isolates								
	No. of examined <i>S. aureus</i>	violet		yellow		White		No. of examined <i>S. aureus</i>	violet		yellow		White		No. of examined <i>S. aureus</i>	violet		yellow		White	
		No.	%	No.	%	No.	%		No.	%	No.	%	No.	%		No.	%	No.	%	No.	%
Cows	56	36	64.29	13	23.21	7	12.5	29	19	65.52	7	24.14	3	10.34	85	55	64.71	20	23.53	10	11.76
Buffaloes	12	8	66.67	3	25	1	8.33	9	6	66.67	2	22.22	1	11.11	21	14	66.67	5	23.81	2	9.52
Total	68	44	64.71	16	23.53	8	11.76	38	25	65.79	9	23.68	4	10.53	106	69	65.1	25	23.58	12	11.32

\*non toxigenic *S. aureus* using RPLA**Table (11):** Incidence of protein A in *S. aureus* isolates using agglutination test.

Sources of isolates	Toxigenic isolates				Non Toxigenic isolates *				Total isolates			
	No. of examined <i>S. aureus</i>	Staphylococcal protein A (SpA)		No. of examined <i>S. aureus</i>	Staphylococcal protein A (SpA)		No. of examined <i>S. aureus</i>	Staphylococcal protein A (SpA)		No. of examined <i>S. aureus</i>	Staphylococcal protein A (SpA)	
		No.	%		No.	%		No.	%		No.	%
Cows	56	42	75	29	22	75.86	85	64	75.29			
Buffaloes	12	9	75	9	5	55.56	21	14	66.67			
Total	68	51	75	38	27	71.05	106	78	73.58			

\*non toxigenic *S. aureus* using RPLA



**Photo (1):** Shows SDS profile analysis of amplified PCR products among the examined *S. aureus*. 3 isolates produced type a toxin (lanes 1, 3 and 7). 2 isolates produced type B toxin (lanes 4 and 8) by using polymerase chain reaction technique (PCR). Lane 2: standard *S. aureus* strain. Lane 5: *S. aureus* isolate produce c toxin as detected by RPLA. Lane 6: *S. aureus* negative for production of toxins as detected by RPLA.

#### 4. Discussion

*S. aureus* is involved in intramammary infections in bovine causing economic losses and milk-safety problems (Taverna *et al.*, 2007). Mastitis control is complex problem for which there are no simple solutions.

Bacteriological study of mastitic milk samples was carried out and results obtained revealed that staphylococcal species were isolated from 129 samples with the percentage of 23.29 % this percentage was calculated according to the total number of quarters (554) as cleared from Table (4). These results were nearly similar to those mentioned by Pankey *et al.* (1991) (25.4 %); Mahbub *et al.* (1996); Badia (2004) (27.21%) and Elgabry (2006) (21.2%). Among coagulase-positive *Staphylococcus* species: *S. aureus*, *S. hyicus* and *S. intermedius*. *S. aureus* is a major agent of bovine mastitis as mentioned by Schleifer (1986). The results obtained in Table (3) showed that 106 isolates were identified as *S. aureus* with an incidence of 19.13%, followed by 2.89% were identified as *S. intermedius* and 1.26% were identified as *S. hyicus*. These results goes in the direction which indicated that high incidence of staphylococcal mastitis was mainly due to *S. aureus*. The present results are in agreement

with Badia (2004); Ekman *et al.* (2004) and Elgabry (2006) who found that *S. aureus* isolates were of high incidence than the other types of *Staphylococcus*. High incidence of *S. aureus* may be attributed to that *S. aureus* has a wide spread during the different seasons of the year. Nickerson *et al.* (1995) recorded that *S. aureus* was known to be easily spread between animals so that one *S. aureus* case may lead to more cases. The invasion of *S. aureus* in the interstitial tissue of the mammary gland and the nature of capsular polysaccharide type 5 (CP5) probably help bacteria to withstand the host defense mechanism (Hensen *et al.* 2000).

A number of different phenotypic and genotypic techniques are available to classified *S. aureus* strains for epidemiological investigation (Wildemauwe *et al.*, 2010). One of the goals of this study was to explore the phenotypic characters including different virulence factors of *S. aureus* isolates. *S. aureus* is a major food borne pathogen due to its capability to produce a wide range of heat-stable enterotoxins (Peles *et al.*, 2007). Detection of staphylococcal enterotoxins is decisive for confirmation of an outbreak and determination of the enterotoxicogenicity of the strains. Since the recognition

of their antigenicity, large numbers of serological methods for the detection of enterotoxins in food and culture media have been proposed (Da Cunha *et al.*, 2007). Major virulence factors of *S. aureus* organism include enterotoxins (SEs) that cause both food poisoning and toxic shock syndrome. Recently, a novel SE tentatively designated SEL was identified in a bovine mastitis isolates, the toxin lacked emetic activity (Orwin *et al.*, 2003). A little as 0.1 µg of enterotoxins can be sufficient to produce food poisoning after incubation period which can be as short as 1 hour out of usually 4 - 6 hours (IASR, 2001).

Reverse passive latex agglutination test (RPLA) test was used in this study as a recent technique for detection of the presence of staphylococcal enterotoxins and this fact was in accordance with that mentioned by Schumacher *et al.* (1995) who confirmed the accuracy of commercial available RPLA for detection of enterotoxins. Results obtained in Table (5) showed that 68 out of 106 *S. aureus* isolates were found to be toxigenic with an incidence of 64.2% and distributed as follow: enterotoxin C were detected from 34 samples with an incidence of 32.1% followed by enterotoxin A from 19 samples with an incidence of 17.9% and enterotoxin B from 15 samples with an incidence of 14.2%. It is clear from previous results that the enterotoxin C is the predominant one, this observation were in agreement with that mentioned by Jorgensen *et al.* (2005) who found that SEC and sec were most common toxin detected in *S. aureus* isolates from bovine mastitis. Samah (2003) recorded that 16.6% isolates of 106 *S. aureus* isolates obtained from milk were enterotoxigenic type SEC producing isolates. In addition to that mentioned by Soriano *et al.* (2002) who found that obtained results showed the high incidence of the type C followed by type B and then type A.

*S. aureus* isolates were characterized as coagulase positive isolates produce endopigments when cultivated on nutrient agar. As shown in Table (6) toxigenic *S. aureus* isolates produced golden yellow, creamy and white colonies on agar, in percent of 69.11%, 27.94% and 2.94% respectively. Non toxigenic *S. aureus* isolates produced golden yellow and creamy colonies on agar in percent of 71.05% and 28.95% respectively. It is clear that golden yellow colony was the predominant pigment among *S. aureus* isolates. These results are in agreement with Elgabry (2006) who found that toxigenic *S. aureus* isolates produced golden yellow, creamy and white colonies on agar in percent of 64.1%, 29.5% and 6.4% respectively. 92.65% of toxigenic *S. aureus* isolates had hemolytic activity on sheep blood agar as shown in Table (7) and 92.1% of non toxigenic *S. aureus* isolates were hemolytic. Lam *et al.* (1995) and Aarestrup *et al.* (1999) showed that approximately 1/5 to 1/4 of the *S.*

*aureus* isolates of bovine mastitis do not present any detectable beta-hemolytic activity in primary cultures.

Out of 68 toxigenic *S. aureus* isolates 46 (67.65%) were DNase positive, while out of 38 non toxigenic *S. aureus* isolates 26 (68.42%) were DNase positive as shown in Table (8). Abd El-Salam (2003) recorded that all toxigenic strains of *Staphylococcus* were coagulase positive and DNase producers. Boerlin *et al.* (2003) illustrated that 71.8% of *S. aureus* isolates had DNase activity. It is clear from Table (9) that out of 68 isolates of toxigenic *S. aureus* isolates 66 (97.06%), were able to reduce tellurite to metallic tellurium producing a black coloration, and all non toxigenic *S. aureus* isolates (100%) were positive. *S. aureus* isolates was able to reduce tellurite to metallic tellurium with an incidence of 96.2% (Elgabry, 2006). Selective agars like modified Baird-Parker agar have been used successfully for the detection and identification of *S. aureus* and other coagulase-positive staphylococci (Roberson *et al.*, 1992). Three characteristic appearances were recorded among *S. aureus* isolates after having been grown on crystal violet agar medium, as shown in Table (10). Yellow colonies were detected in 23.53% of the isolates, and violet colonies were detected in 64.71% of the isolates, while white colonies were detected only in 11.76% from the toxigenic *S. aureus* isolates. It is clear that most of bovine isolates had violet colonies on the medium. The present results are in agreement with Wan *et al.* (1999).

Several rapid identification tests for *S. aureus* are commercially available and have been extensively in use. For instance, the slide staph plus kit from Bio-Merieux is an agglutination test used for the simultaneous demonstration of protein A, clumping factor and other surface antigens specific for *S. aureus* (Boerlin *et al.*, 2003). In the present study 51 out of 68 isolates of toxigenic *S. aureus* isolates (75%) showed SpA by agglutination test positive as shown in Table (11). Detection of toxigenic strains in *S. aureus* isolates using polymerase chain reaction technique (PCR) was illustrated in photo (1). The isolates proved to be toxigenic using RPLA were confirmed using PCR (detection of toxin C was not available) as recent technique. Results obtained showed that 100% agreement between the 2 tests RPLA & PCR. Zouharova and Rysanek (2008) found that the results of both methods were identical concerning SEB and SED. It was concluded that detection of SEs by PCR was a useful additional tool to support identification of Enterotoxigenic strains.

#### Corresponding Author:

Professor Dr. Jakeen Kamal Abdel Haleem El-Jakee

Professor of Microbiology and Head of the Microbiology Department, Faculty of Vet. Medicine Cairo University, Egypt.  
Phone: 0124395853  
Email: [jeljakee@yahoo.com](mailto:jeljakee@yahoo.com)

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6/1/2010

# Hydro-Thermal Safety Control of Karun-1 Dam under Unusual Reservoir Level Reduction

Mojtaba Labibzadeh<sup>1</sup>, Amin Khajehdezfuly<sup>1</sup>

<sup>1</sup>. Department of Civil Engineering, Faculty of Engineering, Shahid Chamran University, Ahvaz, Iran  
[Labibzadeh\\_m@scu.ac.ir](mailto:Labibzadeh_m@scu.ac.ir)

**Abstract:** Karun-1 dam safety was examined through carrying over a 3D finite elements analysis. The dam as well as its foundation and abutments have been modeled in a relatively exact manner. Furthermore, the vertical contraction joints were simulated in calculations. Hydrostatic, gravity and thermal effects have been taken into account as the load collections. 10m reduction of reservoir level from normal water level of the dam reservoir was applied in the modeling and the possibility of initiate and development of cracks in dam body was investigated by means of monitoring of principal stresses. The obtained results showed that mentioned possibility existed and the downstream face of the dam in vicinity of the abutments near to crest level probably experiences the tensile cracks. [Journal of American Science. 2010;6(11):179-184]. (ISSN: 1545-1003).

**Keywords:** Arch dam, Thermal, Contraction joints, Cracks, Dam safety

## 1. Introduction

Usually, after a service life of several decades, a considerable percentage of existing concrete dams, illustrate some kind of deterioration. Based on the studies performed examining the causes of this phenomenon, the ASR (Alkali-Silica Reaction) and unusual extreme loading such as earthquake excitations and reservoir level fluctuations are the main reasons of this dam stiffness and strength degradation (Swamy and Al-Asali, 1988; Ahmed et.al., 2003; Pedro, 1999). Sometimes mentioned degradation is concomitant with the occurrence of local cracks in dam body which can be a threat for the dam safety. Holding this issue in mind, the main objective of this paper is to investigate the possibility of damage occurrence in Karun-1 arch concrete dam due to unusual reduction in its reservoir level about to 10m from the normal water level. Karun-1 dam is a double curvature concrete dam located in northeastern of Khouzestan province of Iran in vicinity of Masjedsoleiman city which its construction were completed in 1976. The dam height is approximately 200m from the base level, its dam crest thickness is 6m and the thickness of the dam at base level is about 380m.

In this investigation, as well as the hydrostatic pressure of reservoir and dam weight, the thermal loads due to air temperature changes have been assessed in the modeling. Thermal loads has a major effects in arch concrete dam stability analysis (Sheibani and Ghaemian, 2006; Ardito, et. al, 2008; Léger and Leclerc 2007; Léger and Seydou 2009; Labibzadeh and Khajehdezfuly, 2010). Furthermore, for achieving the more and more accurate in analysis, the effect of vertical contraction joints in hydro-

thermal simulation of the dam was taken into account. Even though the latter issue was no major challenge for dam engineers in their analyses and designs, this factor can affect the dam safety analysis results significantly (Labibzadeh and Khajehdezfuly, 2010). In the past recent years, the amount of the rainfall has been decreased considerably in Iran specifically in Karun-1 dam water fall domain. Consequently, the reservoir volume of the dam has been reduced gradually. As the water level of the dam was decreasing, the safety control of Karun-1 dam became more highlighted due to the fact that the electrical energy generation of the dam has been increased recently thorough the development of the second phase of its power plant. It is the main reason of doing this research. The proposed study has been done by means of a relatively 3D exact simulation of the geometric, material behavior and boundary conditions of the dam. Principal stress tensors, displacement vectors were selected as stability indexes safety control and examined. It will be shown that under the reservoir level reduction up to 10m the possibility of initiate and development of cracks in downstream face of the dam exists.

## 2. Material and Methods

As it was mentioned in previous section, the hydrostatic, gravity and thermal loads have been simulated in the proposed model. The governing equation for heat transfer in three-dimensional region is (Reddy and Gartling, 2001):

$$\rho C \frac{\partial T}{\partial t} = \frac{\partial}{\partial x_i} \left( k_{ij} \frac{\partial T}{\partial x_j} \right) + Q \quad (1)$$

Where  $\rho$  = density  $\left(\frac{kg}{m^3}\right)$ ;  $C$  = specific heat  $\left(\frac{J}{kgK^0}\right)$ ;  $T$  = temperature of medium  $K^0$ ;  $t$  = time (s);  $k_{ij}$  = Cartesian components of conductivity tensor  $\left(\frac{W}{m^2 K^0}\right)$ ;  $Q$  = internal heat generation per unit volume  $\left(\frac{W}{m^3}\right)$  and ij summation on repeated subscripts (i,j=1,2,3).

The above mentioned equation with the following boundary conditions generates the mathematical model of heat transfer which in turn is the physical model:

$$T = T^* \text{ on } \Gamma_T \quad (2a)$$

$$- \left( k_{ij} \frac{\partial T}{\partial x_j} \right) n_i = -q_a + q_c + q_r \text{ on } \Gamma_q \quad (2b)$$

Where  $q_a$  = applied flux  $\left(\frac{W}{m^2}\right)$ ;  $q_c$  = convective flux  $\left(\frac{W}{m^2}\right)$ ;  $q_r$  = radiative flux  $\left(\frac{W}{m^2}\right)$  and  $n_i$  = Cartesian component of unit normal boundary vector.

In our problem the  $Q, \Gamma_T, \Gamma_q, T^*$  and  $q_a$  are illustrative of hydration, concrete-water interface, exposure surface, reservoir temperature and solar radiation flux respectively.

Implementing the thermal transfer equation (1) through the powerful numerical approximate method F.E.M results in:

$$M^e \dot{T} + K^e T = Q^e + q_a - CT + F_{hc} - RT + F_{hr} \quad (3)$$

Where  $\dot{T}$  denotes the rate of change of temperature with time and:

$$M^e = \oint_{\Omega_e} \rho C \psi \psi^T d\Omega \quad (4)$$

The equivalent thermal mass matrix of an element;

$$K^e = \oint_{\Omega_e} k \left( \frac{\partial \psi \partial \psi^T}{\partial x^2} + \frac{\partial \psi \partial \psi^T}{\partial y^2} + \frac{\partial \psi \partial \psi^T}{\partial z^2} \right) d\Omega \quad (5)$$

The equivalent thermal stiffness matrix of an element;

$$Q^e = \oint_{\Omega_e} \psi Q d\Omega \quad (6)$$

The equivalent internal heat generation vector of an element;

$$q_a = \int_{eq} \hat{\psi} q_a ds \quad (7)$$

The equivalent applied heat flux vector of an element;

$$C = \int_{eq} \hat{\psi} h_c ds \quad (8)$$

The equivalent convectional heat matrix of an element;

$$R = \int_{eq} \hat{\psi} h_r ds \quad (9)$$

The equivalent radiative heat matrix of an element;

$$F_{hc} = \int_{eq} \hat{\psi} h_c T_a ds \quad (10)$$

The equivalent convectional heat vector of an element;

$$F_{hr} = \int_{eq} \hat{\psi} h_r T_a ds \quad (11)$$

The equivalent radiative heat vector of an element;

In above equations:

$\psi$  is defined as the interpolation function in the element domain. The sign  $\wedge$  indicates the restriction of the interpolation function to an element face and the  $ds$  = element surface area.  $h_c$  is defined as the

convection coefficient  $\left(\frac{W}{m^2 K^0}\right)$  and implemented in

definition of the  $q_c$  (exchange of the heat by convection as a result of temperature differences between  $\Gamma_q$  and ambient temperature) through the Newton's cooling law:

$$q_c = h_c(T - T_a) \quad (12)$$

Where  $T$  = temperature of  $\Gamma_q$  boundaries and  $T_a$  = ambient temperature. These two temperatures are measured based on Kelvin degrees. In this paper,  $T_a$  is considered as the air temperature and  $T$  is the concrete temperature at the external surface of the dam.  $h_c$  was calculated in this study by the following relatively accurate formulation (Duffie and Beckman 1980):

$$h_c = 5.7 + 3.8V \quad (13)$$

$V$  = fluid speed (wind speed) (m/s).  $h_r$  represents the linearized radiation coefficient  $\left(\frac{W}{m^2}\right)$  and used in evaluating of exchange of the heat by the electromagnetic radiation (Stefan-Boltzman law) through the below manner:

$$q_r = eC_s(T^4 - T_a^4) \quad (14)$$

$e$  = emissivity of surface and  $C_s$  = Stefan-Boltzman constant  $[5.669 \times 10^{-8} \left(\frac{W}{m^2}\right)]$ . The relation (14) can be rewritten as follows to have a friendlier user form:

$$q_r = h_r(T - T_a) \quad (15)$$

Where  $h_r$  is defined as:

$$h_r = eC_s(T^2 + T_a^2)(T + T_a) \quad (16)$$

$q_a$  identifies the amount of absorbed solar energy by the body of system (in our study the dam) and described by the below equation:

$$q_a = aH \quad (17)$$

In which  $H$  = total amount of solar energy reaching the surface and  $a$  = solar absorptivity of the surface.

Rearranging and assembling the equation (3) over the entire domain yields the following tensorial form of the primarily ordinary differential equation (1):

$$MT\&+ \hat{K}T = \hat{F} \quad (18)$$

Where

$$\hat{K} = K + C + R \quad (19a)$$

$$\hat{F} = Q + q_a + F_{h_c} + F_{h_r} \quad (19b)$$

Equation (18) presents a system of nonlinear dependent differential equations because the components of tensor  $\hat{K}$  depends on the temperature values  $T$  and  $T_a$ . Since, in our study, the smallest period of considered time in calculation was set to a day (24 hours), so the variation of temperature with respect to time became very small value and therefore the first term of equation (18) was neglected. The sequence of solution has been summarized as in figure 1.

### 3. Results and Discussions

Following the method described in previous section, the Karun-1 arch concrete dam has been analyzed under the effect of 10m unusual reduction of its reservoir level from normal water level. The most dangerous situation which threatens the dam safety was obtained when the level of the lake came down to 480m from the free sea level. This means that the reservoir level reduces about to 10m from its normal water level. The counters of dam displacements at this level have been shown in figure 2.

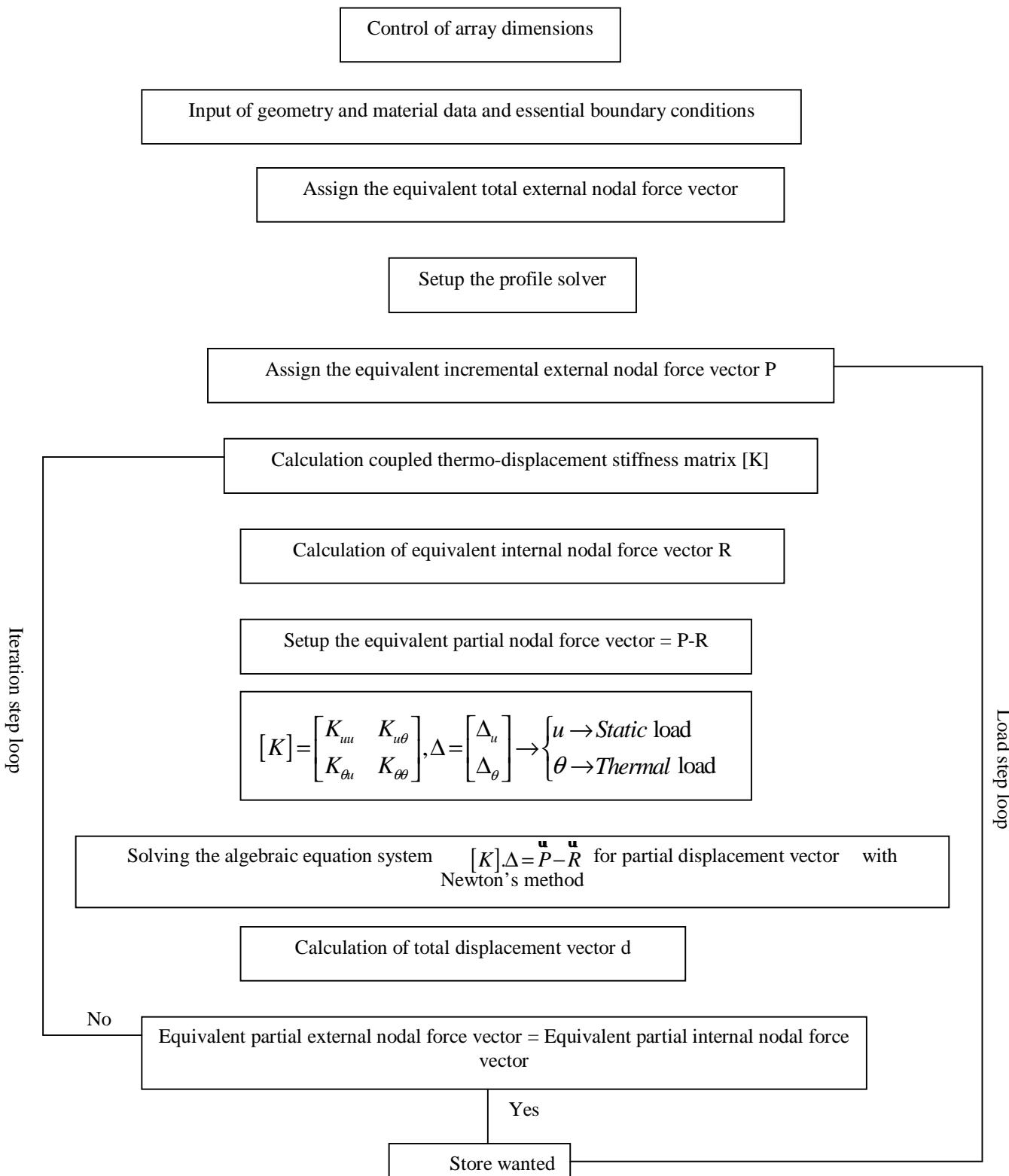


Figure1. The numerical solution of proposed problem

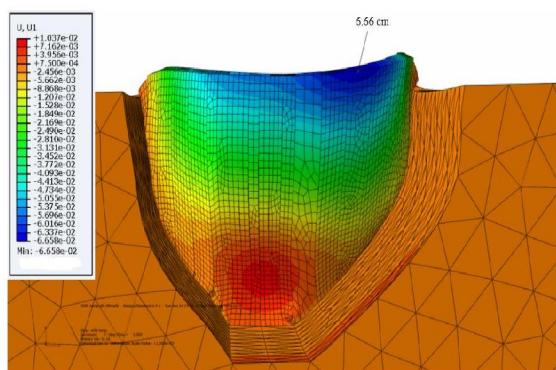


Figure 2. the Karun-1 dam deflections: the reservoir level= 480m

The face which is observed in figure 2 is the upstream face of the dam. As it can be seen from that picture, the largest displacement is about to 6.67 cm at crest level toward the upstream face. This value is not too large for the dam with the mentioned dimensions form the engineering point of view. In figure 3 the pattern of displacements at crest level of the dam was depicted.

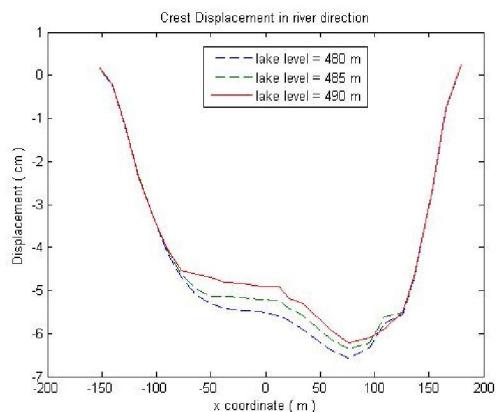


Figure 2. displacements in crown of dam

As it can be seen from figure 2 the displacements are not symmetric with respect to the middle of the crest. This is due to the fact that this arch dam has not the symmetric geometry and was designed with two different arch radius and centers at each level. Moreover, the biggest displacements obtained for the reservoir level equal to 480m. This confirms that the worse situation is occurred when the lake level of the dam decreased about 10m from the normal water level.

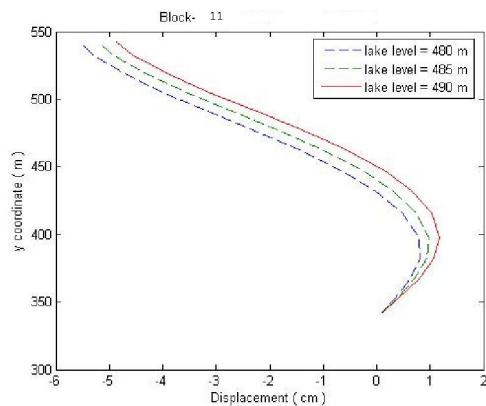


Figure 3. displacements along the height of the dam

The variation of deflections along the height of the block no.11 (the highest block of the dam) has been illustrated in figure 3. This picture verifies that under the reduction of reservoir water level, the lower parts of the dam deflect towards the downstream whereas the upper parts tend to move to upstream direction. This configuration of the dam cannot be captured in two dimensions and so in this study the three dimensional model has been used.

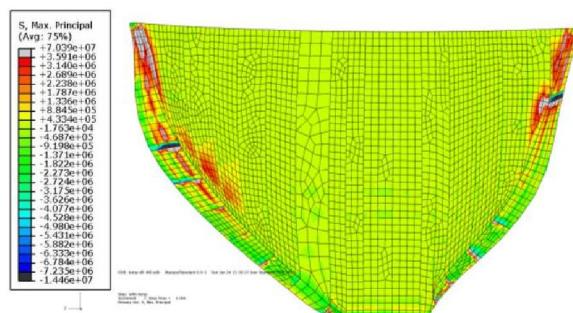


Figure 4. maximum principal stresses, downstream

For assessing the potential of cracking through the unusual reduction of water level, the attention should be focused on maximum and minimum stresses. The maximum stresses can inform us about the cracking due to tensile nature and minimum stresses can tell us about the cracking due to crushing of the concrete. The largest maximum stresses occurred on downstream face of the dam and has been shown in figure 4. From this graph it can be deduced that there are regions on downstream face near the abutments which tensile cracking can be occurred (the red color regions). It is worth to mention that the maximum allowable tensile strength of dam concrete can be considered as 3.5 MPa. So, author of this paper suggests that the operation of the dam should be organized in such a way that this amount of water

level reduction is avoided. The minimum principal stresses are depicted in the next figure. As it can be seen from it the crushing of concrete cannot be happen because the stress values are smaller than the compressive strength of mass concrete (40 MPa).

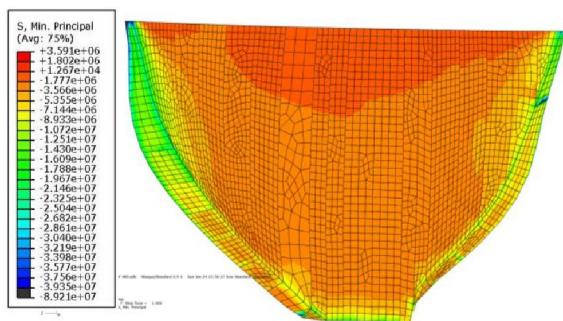


Figure 5. minimum principal stresses, downstream face.

#### 4. Conclusion

After reviewing the results of stress analysis of Karun-1 arch concrete dam it was cleared that the unusual reduction of the reservoir level dam can be the source of cracking on downstream face of the dam in tensile mode and such a reduction should be avoided by proper operation programming.

#### Acknowledgements:

Author is so grateful to Yaqub Arab and Ebrahim Barati Choobi, the head his assistant in Dam Stability Control Center of Water&Power Authority of Khuzestan Province of Iran.

#### Corresponding Author:

Dr. Mojtaba Labibzadeh  
Head of Civil Department,  
Faculty of Engineering, Shahid Chamran University,  
Ahvaz, Iran.



E-mail: [labibzadeh\\_m@scu.ac.ir](mailto:labibzadeh_m@scu.ac.ir)

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6/9/2010

# Prevalence of HBV Genotypes in Egypt among Hepatitis Patients

Iman A.El Aziz Khaled <sup>\*</sup>1, Ola M. Mahmoud<sup>1</sup>, Abeya F.Saleh<sup>1</sup>, and Emad A. Baioumi<sup>2</sup>

<sup>1</sup>Haematology &Blood Bank, <sup>2</sup>Tropical Medicine, Theodor Bilharz Research Institute (TBRI).Cairo, Egypt  
<sup>\*</sup>iman\_khaled@yahoo.com

**Abstract:** Phylogenetic analysis has led to the classification of hepatitis B virus into eight genotypes, designated A to H. The genotypes have differences in biological properties and show heterogeneity in their global distribution. These attributes of the genotypes may account not only for differences in the prevalence of hepatitis B virus mutants in various geographic regions, but also makes them responsible for differences in the clinical outcome and response to antiviral treatment in different population groups. Africa is one of the highly endemic regions of HBV with five genotypes (A-E) identified. Almost all patients in the Mediterranean area are infected with genotype D. However, there is little information of genotype distribution in Egypt. A total of 140 Egyptian patients with hepatitis B surface antigen (HBsAg) positive were enrolled in this study. Of the 140 patients, only 100 patients were HBV DNA positive and only these were included in the study. They were classified into 20 patients with acute hepatitis (AH), 75 patients with chronic active hepatitis (CAH) and 5 patients with hepatocellular carcinoma (HCC)]. HBV genotypes were determined using INNO-LiPA methodology which is based on the reversed hybridization principle. Results: This study showed that genotype D constituted 87% of the total infections (75% CAH, 7% AH & 5% HCC). The other 13% showed mixed infections of D/F. Conclusion: These findings show that the most prevalent genotype in Egypt is genotype D especially in CAH and HCC patients while the mixed type D/F is mostly encountered in AH. [Journal of American Science. 2010;6(11):185-190]. (ISSN: 1545-1003).

**Keywords:** Phylogenetic, Genotypes , Hepatocellular

## 1. Introduction

It is estimated that 350 million individuals are chronically infected with hepatitis B virus (HBV) and that more than 1 million die from cirrhosis and hepatocellular carcinoma (HCC) each year [1-3]. Approximately 5-10% of infected adults and 80-90% of children become chronic carriers of HBV [4]. HBV has been classified into eight genotypes (A-H) based on the sequence divergence of > 8% in the entire genome, which consists of about 3200 base pairs [5-7]. Different HBV genotypes have distinct geographical distributions. Genotype A is found mainly in Northwest Europe, the United States, India, and Sub-Saharan Africa. Genotypes B and C prevail in East Asia, while genotype D is common in the Mediterranean countries. Genotype E is only found in Africa and genotype F is found mainly in Central and South America. The distribution of HBV genotypes G and H still needs to be determined [8]. Africa is one of the highly endemic regions of HBV with five genotypes (A-E) identified: genotype A in Kenya [9], genotype D in Tunisia [10], genotype (A-D) in South Africa [11] and genotype E in Nigeria [12]. Apart from these reports, however, there is little information of genotype distribution in Egypt despite the importance of HBV infection in this region of Africa. According to Egyptian studies [13, 14], the prevalence of HBsAg in Egypt is of intermediate endemicity (2-8%). Nearly 2-3 million Egyptians are

chronic carriers of HBV. Structural and functional differences between genotypes can influence the severity, course and likelihood of complications and hepatitis Be antigen (HBeAg) seroconversion. In addition, HBV genotypes may be associated with differences in response to antiviral therapy. Some studies indicate that HBV genotypes respond differently to interferon in patients with chronic hepatitis B [15].

Several technologies have been developed for genotyping of HBV. Including direct sequencing [16], restriction fragment length polymorphism analysis [17], line probe assay [18], PCR using type specific primers [19], colorimetric point mutation assay [20], ligase chain reaction assay [21] and enzyme linked immunosorbent assay for genotype-specific epitopes [22]. Our aim in this study was to use the line probe assay (INNO-LiPA HBV Genotyping assay; Innogenetics N.V., Ghent, Belgium) to detect the most prevalent genotypes of HBV among Egyptian hepatitis patients

## 2. Patients and Methods

The study was approved by the ethical committee of Theodor Bilharz Research Institute (TBRI) (No 52) and informed consents were obtained from patients participating in this study. A total of 140 patients with hepatitis B surface antigen (HBsAg) positivity were enrolled in this

study. Of the 140 patients, only 100 patients were HBV DNA positive and those were included in our study and classified into: 20 patients with active hepatitis (AH) diagnosed by HBsAg and HBc-IgM, 75 patients with chronic active hepatitis (CAH) characterized by presence of HBsAg with increased alanine aminotransferase (ALT) level for more than 6 months, and 5 patients with hepatocellular carcinoma (HCC) diagnosed by ultrasonography. HBV was diagnosed depending on clinical data, liver function tests done by (Hitachi 902), HBV serum markers done by ELISA technique (Abbott AxSYM® HBsAg Assay) and HBV DNA by real time PCR (Two step RT-PCR using Applied Biosystem). Patients were excluded if they were co-infected with hepatitis C virus (HCV) or human immunodeficiency virus (HIV).

#### DNA Extraction

The QIAamp DNA extraction kit (QIAGEN GmbH) was used for DNA extraction from serum samples according to the manual. The extracted DNA was used for amplification in the LiPA procedures. LiPA analysis was performed within approximately 5 days following DNA extraction. If DNA extracts were not used immediately, they were stored at -20°C.

#### LiPA amplification and detection

HBV genotyping was performed for all PCR-positive samples by a reverse hybridization line probe assay (INNO-LiPA HBV Genotyping assay; Innogenetics NV, Ghent, Belgium [available for research use only, not for use in diagnostic procedures]) [23]. The extracted DNA was amplified by nested PCR according to the instructions of the manufacturer (Innogenetics) for amplification of the HBsAg region to provide a biotinylated product. The HBV genomic region amplified extends from nucleotides 415 to 824 for the outer primers and nucleotides 456 to 798 for the nested inner primers (the numbering is based on the sequence with GenBank accession number AY128092). These procedures in brief includes initial denaturation of the biotinylated PCR products [24], which were then incubated with a test strip for hybridization of the denatured amplicon to genotype-specific probes immobilized as parallel lines on each strip. Following hybridization, the strips were stringently washed and incubated with a streptavidin conjugate to allow color development from the biotinylated DNA bound to the strip.

#### Statistical analysis

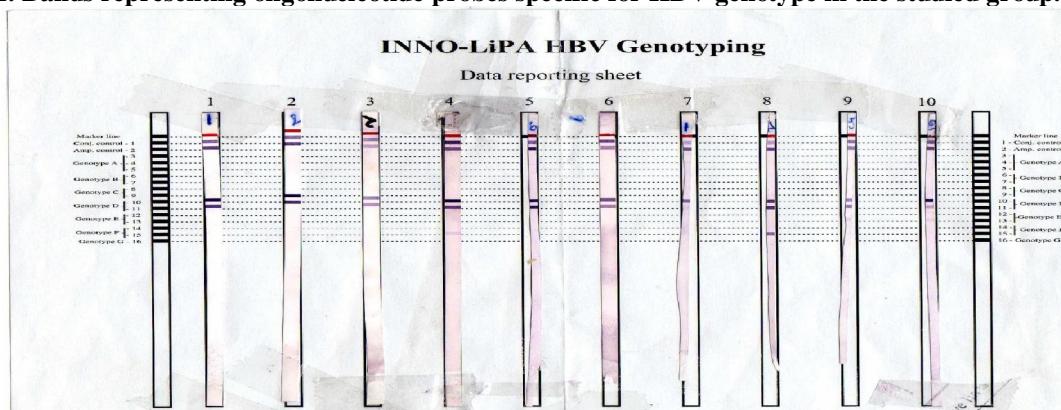
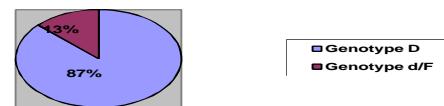
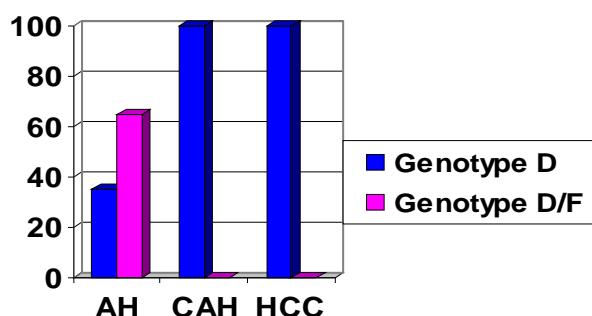
Analysis of data was carried out with the aid of SPSS package version 10.0 software (Chicago, Illions, USA) Parameters were compared using the Chi-square test. *P* values less than 0.05 were considered statistically significant.

### 3. Results

A total of 100 patients with a mean age of  $37.17 \pm 11.75$  years, including 25% females and 75% males, were enrolled in this HBV genotype study. Genotype detection by hybridization of the PCR products to the kit membrane strips was performed as described above. A representation of the membrane strip with all the immobilized control and genotype-specific oligonucleotide bands is shown in Figure 1. For all genotypes except genotype G, several reactive bands can indicate a specific genotype. Interpretation of the test strips was relatively straightforward; however, in certain cases faint bands appeared, and these made interpretation of the genotype unclear. The INNO-LiPA HBV genotyping strip contains 1 red marker line, 2 control lines, and 14 parallel probe lines. The conjugate control line is a control for the color development reaction and the amplification control line contains universal HBV probes to check for the presence of amplified HBV genomic material.

#### Distribution of HBV genotypes

This study showed that HBV infections in hepatitis patients are attributed predominantly to viral genotype D constituted 87% of the total infections. In addition, there was a relatively high prevalence of mixed infections (D/F) represented 13% among the studied group. No HBV genotypes A, B, C, E or G were found in our study (Figure 1,2). The Association between liver disease and the prevalence of HBV genotypes was as following: Genotype D was found significantly more often in patients with CAH and HCC than in patients with AH [75/75(100%), (5/5(100%) v (7/20 (35%)). Mixed infection (D/F) was only found in AH group [13/20 (65%)] (Figure 3).

**Figure 1.** Bands representing oligonucleotide probes specific for HBV genotype in the studied group.**Figure 2.** HBV genotype distribution in the studied group.**Figure 3.** The Association between liver disease and the prevalence of HBV genotypes.

#### 4. Discussion

Hepatitis B virus (HBV) infection is a global health problem with a continuously increasing burden in developing countries like Egypt. A greater demand for genotyping of patient strains of HBV is growing as specific clinical associations with each genotype becomes increasingly apparent

[25-27]. Presently, based on an intergroup divergence of 8% or more in the complete nucleotide sequence, HBV can be classified into eight genotypes A-H, and different HBV genotypes are dominant in various parts of the world [8]. Thus, it is imperative to collect more information on HBV genotypes from all over

the world to reach a decision concerning their clinical utility [28].

The most important finding in our results was the predominance of the genotype D as the predominant HBV genotype in the studied subjects (87%) followed by mixed genotype (D/F) that constituted 13%. These findings conform with other studies done in Egypt. Saudy et al (2003) studied the genotypes of HBV isolated from 100 serum samples of Egyptian carriers by sequencing and found that HBV genotype D was the most prevalent in Egypt [29], but did not detect mixed infection. Discrepancy may be correlated to the difference in sensitivity between the two methods used. In other words, sequence analysis provides information only on the majority strain, while LiPA appears to overcome this limitation by its sensitive detection of mixed genotypes [30]. On the other hand, Naito et al (2001) examined 2 serum samples positive for HBV DNA by primer specific to be of genotype D but they didn't find other genotypes as they only examined 2 serum samples [19]. A third study was done on 70 pediatric cancer patients suffering from hepatitis and were diagnosed as HBV infection. In this study, genotype D was reported as the predominant HBV genotype in the study subjects [31]. This study also concurs with previous studies, indicating that HBV genotype D prevails in the Mediterranean area, near and Middle East [32, 33, 34]. A similar study performed in Syria showed that 97% of the studied patients were of genotype D, and 72% were HBeAg negative [35]. Moreover, study in Turkey revealed that all 44 patients studied had genotype D [34]. Another study in Yemen demonstrated that genotype D was the dominant genotype in a settled population, while genotype A was found only in communities with continuing African links [36]. In addition, two studies in Iran revealed genotype D was the most prevalent HBV genotype [37, 38].

The clinical impact of HBV genotype D has been studied less extensively. However, initial studies have found that it may be associated with lower rates of sustained remission and HBsAg clearance and more severe liver disease compared with genotype A [39]. Emerging evidence suggests that patients with genotype D infection may develop fulminant hepatitis with high frequency [40]. A study from Syria and India indicated that genotype D is more often associated with HBeAg-negative chronic hepatitis B (CHB), more severe diseases and may predict the occurrence of HCC in young patients [35, 41, 42]. Several studies have reported lower response rates to interferon and pegylated interferon- therapy in patients with genotypes C and D than in those infected with

genotypes B and A [43,44]. Evidence suggests that the emergence of lamivudine resistance develop later and less frequent in patients with genotype D infection than in those with genotype A infection [45-47].

In this study, we reported a prevalence of mixed genotype infections D/F at an incidence of 65% in patients with AH. The existence of HBV genotype F in acute forms of liver disease suggests an association of genotype F with more severe and acute forms of liver disease. Mixed infection with two different HBV genotypes has been known since typing was done serologically [48, 49]. Mixed infection was accompanied by acute exacerbation of the chronic disease [31], and may be provoked by population migration [36, 50].

We therefore suggest that HBV genotyping become a routine exercise in clinical medicine and molecular epidemiology. As genotypes have different biological and epidemiological behavior, their detection and monitoring is more than just academic but also medically significant. Continued efforts for understanding HBV genotypes through international co-operation will reveal further virological differences of the genotypes and their clinical relevance. Furthermore, efforts to prevent mixed infections (super-infection or co-infections) in patients with chronic hepatitis B should not be overlooked, especially in areas endemic for HBV infection. Since a small number of subjects were employed in our investigation, we propose that large scale studies be conducted to substantiate our findings. Such studies could also provide more insight into the association between co-infection and disease exacerbations as well as shed light on the molecular, virological and host mechanisms underlying the pathogenesis of HBV-related disease

#### Corresponding author

Iman A.El Aziz Khaled

Haematology &Blood Bank, Theodor Bilharz Research Institute (TBRI).Cairo, Egypt  
[iman\\_khaled@yahoo.com](mailto:iman_khaled@yahoo.com)

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6/1/2010

# Cytogenetic Study of the Effect of *Schistosoma mansoni* Infection on Human Peripheral Blood Lymphocytes and the Role of -Carotene and Vitamin E in Modulating this Effect.

Mervat S.El-Ansary<sup>1</sup>, Iman A.Khaled<sup>2</sup>, Abeya F. Saleh<sup>2</sup>, Ola M.Mahmoud<sup>2</sup>, Emad A. Baioumi<sup>3</sup>, Heba A.Bakr<sup>4</sup>

<sup>1</sup>Immunology (Cairo University), <sup>2</sup>Haematology (TBRI), <sup>3</sup>Hepatology (TBRI), <sup>4</sup>Science (Ain Shams University), Cairo Egypt, [iman\\_khaled@yahoo.com](mailto:iman_khaled@yahoo.com)

**Abstract:** Aim: This study has been made to determine the potential genotoxicity of *Schistosoma mansoni* on lymphocytes of infected patients using different mutagenic end points. The protective role of antioxidants pro vitamin -carotene and vitamin E in minimizing these genotoxic effect was also studied. The study focused on the effect of schistosomiasis on the induction of sister chromatid exchange (SCEs) and other chromosomal aberrations. Patients and Methods: This work was conducted on 24 *Schistosoma mansoni* infected patients and 10 healthy adults as a control group. Lymphocytes from peripheral blood of patients and control group were used for culture and subsequent cytogenetic studies. Results: The results indicated that schistosomiasis was genotoxic in all examined tests. It induced a significant increase in the percentage of structural chromosomal aberrations and the frequency of SCEs. It also inhibited cell division and caused cell cycle delay. Lymphocyte cultures of *S. mansoni* patients treated with 10 µg/ml -carotene or 20 mg/ml vitamin E showed a significant decrease in the percentage of structural chromosomal aberrations and the frequency of SCEs. Conclusion: Schistosomiasis has a genotoxic effect on peripheral blood lymphocytes. The use of the antioxidants -carotene and vitamin E can be considered a promising approach not only toward inhibiting the genetic damage of schistosomiasis but also as prophylactic agents against infection with *S mansoni*. Furthermore, higher doses of antioxidant drugs, -carotene and vitamin E, should be tried as an adjuvants to conventional therapy in a trial to improve treatment of schistosomiasis. [Journal of American Science. 2010;6(11):191-202]. (ISSN: 1545-1003).

**Key words:** Schistosomiasis, -carotene, vitamin E, chromosomal aberration

## 1. Introduction

Schistosomiasis is a common parasitic disease, affecting millions of people, mostly in tropical and developing countries. One of the causative agents of the disease is a trematode worm, *Schistosoma mansoni* [1]. *Schistosoma mansoni* infects over 83 million people in Africa and the Middle East [2]. Egypt represents one of the most highly infected populations with schistosomes in the world [3] with an estimated prevalence of (33.7%-57.7%) in Upper Egypt [4].

Schistosomiasis has been suspected as a risk factor for various types of cancers e.g., bladder cancer, colorectal cancer and hepatic cancer. However, the mechanisms of the carcinogenesis are still unclear [5]. The fact that Schistosomiasis is found to have a mutagenic effect [6] and a co-mutagenic effect [7] may be one of those mechanisms.

The mechanisms of chromosomal aberrations involve the concepts of clastogens directly acting on DNA to produce strand breaks, and subsequently, the survival of these directly caused DNA strand breaks - or misrepairs of them -up to metaphase when they appear as chromosomal breaks or translocations [8]. Enzymes that continually repair DNA damage – frequently cannot counteract all of the oxidative attack, and the resulting damage may lead to genetic mutations that could contribute to carcinogenesis [9].

Many diseases are associated with oxidative stress; this is why the use of antioxidant rich food or antioxidant food supplements has become immensely popular. These antioxidants include enzymes like superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase, minerals such as selenium, manganese, copper, zinc and vitamins such as vitamins A, C and E beside compounds such as glutathione, uric

acid and flavonoids. These antioxidants protect, prevent or reduce the extent of oxidative destruction of cellular tissues. Elevated levels of lipid peroxidation products and the simultaneous decline of antioxidant defense mechanism has been suggested to be harmful through disruption of membrane lipid and damage of cellular organelles resulting in oxidative stress [10].

Carotenoids (provitamin A) and tocopherols (vitamin E) are lipid soluble antioxidants associated with decreased risk of several degenerative diseases [11].

Alpha tocopherol is the main form of vitamin E and this has been the most commonly studied dietary antioxidant supplement in clinical trials [12].

Vitamin E supplementation has been shown to take part in immunoregulation, antibody protection and resistance in planted tumor probably through increased tumourolytic effect of natural killer cells [13]. Also, it was reported that vitamin E protects against lipid peroxidation and prevents skin cancer [14, 15].

Dietary carotenoids play important roles in the promotion of human health as pro vitamin A, antioxidants and chemopreventive agents against certain types of cancers [16] and -carotene have gained prominence for their role against reactive oxygen species (ROS), protecting the organism against oxidative stress and consequently preventing damages and tissue lesions [17].

Sister chromatid exchange (SCEs) represents the interchange of DNA replication products at apparently homologous chromosomal loci. These exchanges involve DNA breakage and reunion. SCE technique affords the opportunity for cytological detection of DNA interchange. This technique is used as a sensitive means of monitoring DNA damage. It is useful for assessing the cytogenic impact of clastogenic agents on chromosomes. The increased resolution of SCE detection afforded by fluorescence or Giemsa technique has permitted localization of SCEs relative to chromosome-banding patterns. In human chromosomes, SCEs occur preferentially in Q-negative bands or at the junction of Q-positive and Q-negative regions [18].

Double stranded breaks (DSBs) are dangerous DNA lesions as they can lead to massive loss of genetic information and to chromosomal rearrangements [19]. In the past ten years, researchers in genome stability have observed that many kinds of cancer are associated with areas where human chromosomes break [20].

Chromosome fragmentation represents an efficient means of induced cell death and is a clinically relevant biomarker of mitotic cell death. Chromosome fragmentation serves as a method to eliminate genomically unstable cells. Paradoxically, this process could result in genome aberrations common in cancer [21].

Centric fission results when a metacentric or submetacentric chromosome splits at the centromere, giving rise to two stable telocentric products, isochromosomes, or ring chromosomes [22].

The aim of the present study was to investigate chromosomal aberrations and sister chromatid exchanges in peripheral blood lymphocytes of *Schistosoma* infected patients. The roles of -carotene and vitamin E as antimutagenic agents modulating the frequency of these chromosomal aberrations and sister chromatid exchange induced by schistosomiasis were also studied.

## 2. Materials and Methods

This study was conducted on 24 *schistosoma mansoni* infected patients (21 males and 3 females) with a mean age of 28.7 years who were submitted to Theodor Bilharz Research Institute, tropical medicine department. Lymphocytes from peripheral blood of patients were used for culture and subsequent cytogenetic studies. The results were compared to those of 10 healthy adults acting as a control group.

### Sample collection:

Ten ml of blood were collected, five of which were collected in a Na-heparin vacutainer for the performance of lymphocyte cultures and the other five ml were collected in gel containing vacutainer for IgG antischistosomal antibody titer detection and liver functions (ALT and AST).

### Stool analysis was also done.

For each culture, 25 metaphases were examined for chromosomal aberrations, 25 metaphases for sister chromatid exchanges, 100 metaphases for cell cycle kinetic and 1000 cells for mitotic index

### Cytogenetic parameters:

Chromosome preparation for human peripheral blood:

For each sample, six lymphocyte cultures were set up, two of them were treated with 10 µg/ml -carotene [13], another two were treated with 20 mg/ml vitamin E and the last two

cultures were used as controls. The two antioxidants were prepared as a suspension in 2% Cremophore-El under sterile conditions. Cultures were set up using whole heparinized venous blood.

One ml of blood was incubated in a culture medium consisting of 4 ml RPMI 1640 with L-glutamine + 1 ml fetal calf serum + 0.1 ml (penicillin + streptomycin) + 0.2 ml of phytohemagglutinin. The medium was also supplemented with 10 $\mu$ g/ml 5-bromodeoxyuridine (DrdU). Cultures were set up in 9 ml culture tubes for 72 hrs. The culture tubes were wrapped in aluminum foil, transferred to a thermostatically controlled incubator at 37°C, kept in a tilted position and shaken daily. All the materials used were sterile. Two hours prior to termination of the culture i.e. 70 hrs after initiation of culture, Colcemid (0.4  $\mu$ g/ml) was added and mixed thoroughly by shaking the tubes, then they were returned back to the incubator.

#### Harvesting and fixation of cells:

Each culture tube was centrifuged at 1000 rpm for 10 minutes. The supernatant was discarded and cell pellet disrupted by flicking the base of the tube. A hypotonic solution 0.075 M KCl (37°C) was added to give a light cloudy solution about (5-6 ml) and left to stand for (20-30) min. Half ml of fresh fixative (Methanol: glacial acetic acid (3:1) was added to each tube. The tube was centrifuged again at about 1000 rpm for 10 min. The supernatant was discarded and cell pellet was disrupted. The cells were then fixed in 4 ml methanol/glacial acetic acid (3:1) fixative for 10 minutes at room temperature. The fixative was changed twice and the cells were finally suspended in a small amount of fixative (0.5-1 ml). Two or three drops of the cell suspension were dropped onto a clean slide dipped in cold 70 % ethanol. The slides were flame dried.

#### Slide staining:

The principle of the fluorescent plus Giemsa (FPG) technique [23] with some modifications was followed for scoring SCEs. After complete drying, the slides were divided and marked into 2 parts; part stained in 50  $\mu$ g/ml of Hoechst 33258 dye for 15 minutes (protected from light). Slides were rinsed in distilled water, layered with 2 x SSC buffer (PH 7), cover slipped, immersed in clean wide Petri dishes full of 2 x SSC buffer (PH 7) and subjected to UV light e.g. predominantly 365 nm Hg line, 400 nm dichromic mirror for 90-120 minutes in a closed cabinet. Slides were rinsed in distilled water and immersed in 4 % Giemsa dye for 7 minutes. The second part was stained directly in 4% Giemsa stain for seven minutes as well as the 1st slides without

staining in Hoechst or exposed to U.V light for scoring of chromosomal aberrations.

#### Scoring of chromosomal aberrations:

For each patient or normal control, 25 metaphases were examined microscopically for chromosomal aberrations in -carotene, vitamin E treated tubes as well as in the untreated tubes. Only cells having well spread chromosomes with minimal overlapping were selected for scoring. Photographs were made of all types of abnormal metaphase Figures. Structural aberrations such as gaps, breaks, deletions, end to end association, centric fusion and centromeric attenuation were recorded. Also, metaphase spreads were examined for evident numerical aberrations including euploidy (endomitosis).

#### Scoring of sister chromatid exchanges:

For each patient or normal control the frequency of SCEs was recorded in 25 metaphase spreads in second division cells in -carotene, vitamin E treated tubes and untreated tubes. In some patients and because of the very low mitotic indices, we could not complete 25 metaphases and we calculated the mean of all found metaphases.

#### Mitotic activity:

The mitotic index was counted as the ratio of mitoses to interphase nuclei in 1000 cells.

#### Cell cycle kinetics:

Cell cycle analysis can be studied by calculating the replicative index (RI) [24], a derived index that reflects the relative contribution of each cell cycle to the sample population. In 100 consecutive metaphase cells, at each dose level, the number of first (M1), second (M2) and third or subsequent (M3) divisions were determined. The RI was calculated as follows: RI = 1M1 + 2M2 + 3M3 /100.

#### Stool examination and measurement of Schistosomal antibodies, ALT and AST:

Preparation and examination of patients stool specimens by saline stool smear was done according to (Haridy, 1979), and measurement of Schistosomal antibodies in human sera was done by ELISA technique using (*Schistosoma* IgG (EIA-3512) from DRG international inc., U.S.A.). ALT and AST were evaluated coloerimetrically in sera of patients and control by commercially available kits according to manufacturer's guidelines.

### Statistical analysis:

Statistical analysis was performed by using SPSS software for windows II version. Student (t) test was used to compare means of SCE, cell cycle kinetics while Chi square test was used for chromosomal changes and mitotic indices.

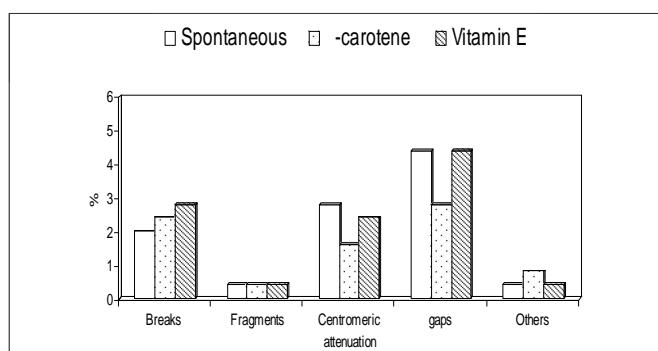
### 3. Results

The biochemical data (ALT level, AST level and antischistosomal antibody titer) of normal controls and patients are represented in Table 1.

**Table 1: Biochemical data (ALT level, AST level and antischistosomal antibody titer) of normal controls and patients.**

	Control group	Patient group
ALT	2.9+/- 0.57	6.1±1.26
AST	6.4+/- 1.19	13.85±1.99*
Serum level of specific IgG		2.77±0.34

\*P<0.05



**Figure (1): Effect of -carotene and vitamin E on the percentage of different types of structural chromosomal aberrations in peripheral blood lymphocytes of normal controls**



**Figure (2): illustrates a normal metaphase from peripheral blood lymphocytes.**

Chromosomal aberrations in peripheral blood lymphocytes:

Table 2 shows the effect of -carotene and vitamin E on the number and percentage of metaphases of different types of structural chromosomal aberrations, frequency of SCEs cell cycle kinetic (RI) and mitotic indices (MI) in peripheral blood lymphocytes of both normal controls and *Schistosoma mansoni*-infected patients.

The data of breaks and deletions were combined together as the two are related. Features of centric fusion, end to end association and ring chromosomes are combined together in one parameter and grouped under “other aberrations” category.

Only a chromosomal aberration of the structural type was found in patients’ cultures (Figs 3, 4,5,6)

The results point to the fact that infection with Schistosomiasis may induce structural chromosomal aberrations in peripheral blood lymphocytes. The number and percentage of different types of the induced chromosomal aberrations are recorded in Table 2.

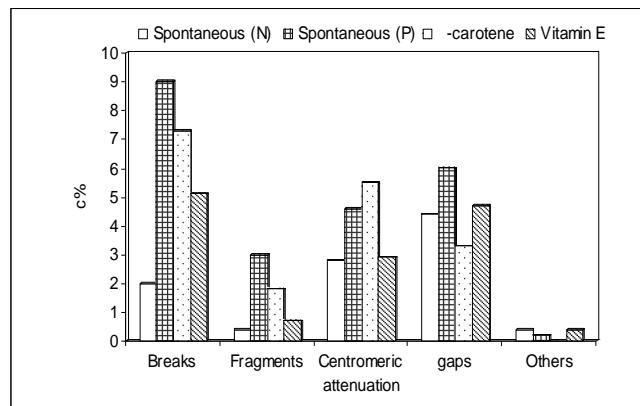
**Table 2: Effect of -carotene and vitamin E on the number and percentage of metaphases of different types of structural chromosomal aberrations, frequency of SCEs cell cycle kinetic (RI) and mitotic indices (MI) in peripheral blood lymphocytes of both normal controls and *Schistosoma mansoni*-infected patients.**

	Control			Patients		
	Non treated N=10 (250cells) N (%)	B carotene treated N=10 (250 cells) N (%)	Vitamin E treated N=10 (250cells) N (%)	Non treated N=20 (500cells) N (%)	B carotene treated N=11 (275cells) N (%)	Vitamin E treated N=11 (275cells) N (%)
structural chromosomal aberrations with gaps	24 (9.6)	19 (7.6)	28 (11.8)	99(19.8)**	47(17.1)*	34(12.4)
structural chromosomal aberrations without gaps	14 (5.6)	13(5.2)	17 (6.8)	73(14.6)**	40(14.5)**	23(8.4)
Breaks	5 (2)	6 (2.4)	7 (2.8)	45 (9) **	20 (7.3) *	14 (5.1)
Fragments	1 (0.4)	1 (0.4)	1 (0.4)	15 (3)	5 (1.8)	2 (0.7)
Centromeric attenuation	7 (2.8)	4 (1.6)	6 (2.4)	23 (4.6)	15 (5.5)	8 (2.9)
Gaps	11 (4.4)	7 (2.8)	11 (4.4)	30 (16)	9 (3.3)	13 (4.7)
others	1 (0.4)	2 (0.8)	1 (0.4)	1 (0.2)	0 (0)	1 (0.4)
Total aberrations with gaps	25 (10)	20 (8)	26 (10.4)	114(22.8)***	49(17.8) *	38(13.8)
Total aberrations without gaps	19 (5.6)	13 (5.2)	15 (6)	84 (16.8) ***	40(14.5)**	25(9.1)
SCE/cells	3.92±0.38	4.51±0.2	3.48±0.4	6.37±0.6**	5.91±0.6*	6.24±1.2*
RI	1.43±0.06	1.36±0.07	1.39±0.4	1.22±0.4**	1.31±0.06	1.36±0.11
MI	20.5±5.55	18.5±3.08	19.7±4.13	10.4±1.94***	11.45±2.31***	9.45±2.13***

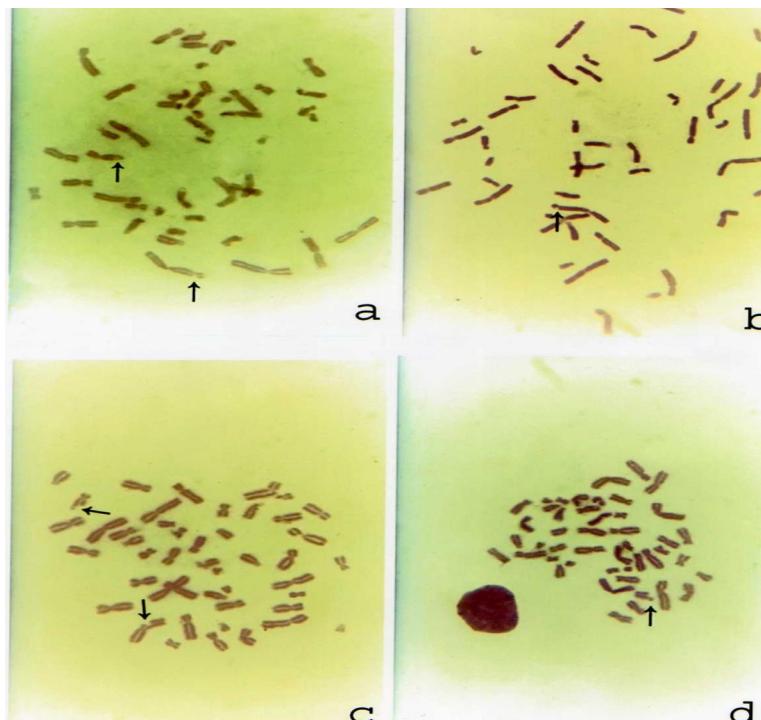
\* P< 0.005,    \*\* P< 0.01,    \*\*\* P < 0.001 in relation to spontaneous cultures of normal controls.

P< 0.005,    P< 0.01,    P < 0.001 in relation to spontaneous cultures of patients.

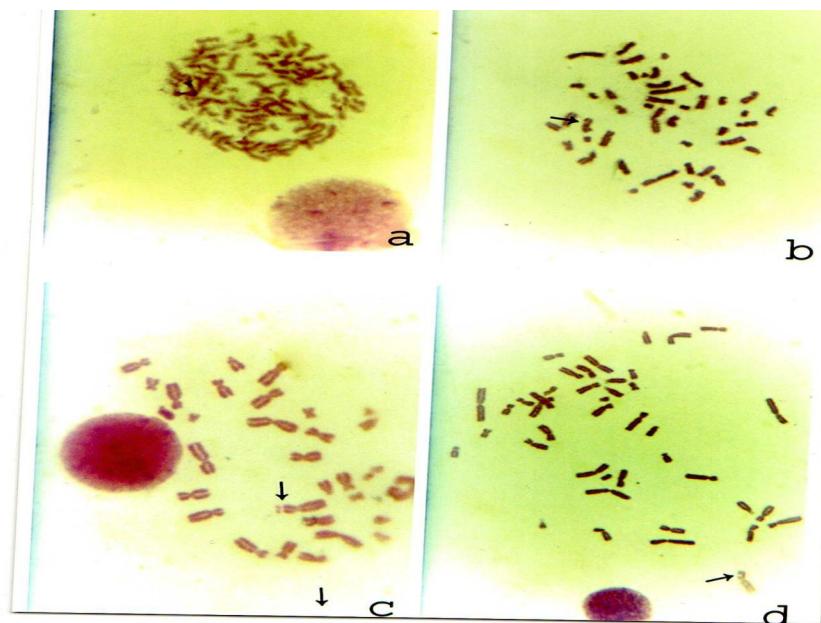
SCE: sister chromatid exchange. MI: mitotic index. RI: replicative index



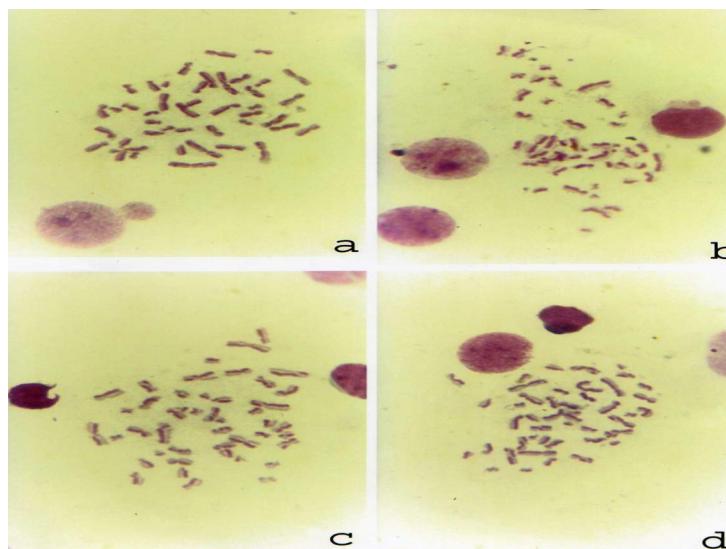
**Figure (3): Effect of -carotene and vitamin E on the percentage of different types of structural chromosomal aberrations in peripheral blood lymphocytes of *S. mansoni*-infected patients.**



**Figure(4):Metaphase plates from human peripheral blood lymphocytes showing a)Chromatid gaps b)& c)Chromatid break d) Fragments.**



**Figure(5): Metaphase plates from human peripheral blood lymphocytes showing a)Centromeric attenuation b) Chromatid gaps c) Chromosome gaps d)Chromatid gap**



**Figure(6) : Metaphases with sister chromatid exchange from human peripheral blood lymphocytes.**

In peripheral blood lymphocyte cultures of *S. mansoni* patients, spontaneous human lymphocyte cultures showed a significant increase in the number of metaphases with chromosomal aberrations even after excluding gaps ( $p<0.01$ ) and the total number of aberrations with and without gaps showed significant increase ( $p<0.001$ ). Breaks and deletions constituted the major part of these aberrations. The total number of breaks and deletions was significant at  $P<0.01$ . These findings run in

#### 4. Discussion

Schistosomiasis is considered the most important of the human helminthiases in terms of both morbidity and mortality. Advances in molecular genetics and immunology hold the promise to control the spread of schistosomiasis and to guide development of new tools to combat this tropical disease [25].

parallel with other studies [6, 26, 27, 28, 29, and 30].

Although some studies reported a significant increase in the number of gaps in *S. mansoni* infected mice [27, 29 and 6], the present study did not reveal any significant increase in the number of gaps. This may be due to the fact that there is not a clear idea about the nature of gaps. While some authors consider gaps to be chromosome fragile sites that are especially prone to forming non-staining gaps, constrictions or breaks in one or both of the chromatids on metaphase chromosomes either spontaneously or following partial inhibition of DNA synthesis [31], others considered the scoring of gaps to be highly subjective and therefore unsuitable indicator for mutagenic potential [32, 33, 34]. It was reported that chromatid gaps occur as two morphologically indistinguishable types; the clastogenic (DNA damage) and the tubagenic (non DNA damage) types. Thus the importance of recording gaps in assessing the mutagenic potential of a compound has been controversial [34].

Four to five sister chromatid exchange is considered within the normal distribution, 14-100 exchanges is not normal and presents a danger to the organism. SCE may be related to tumors [35].

Spontaneous lymphocyte cultures of patients showed a significant increase in the mean frequency of SCEs  $p<0.01$ . This finding was similar to what was reported previously in *S. mansoni* patients [26]. Data presented in this study demonstrate that a significant decrease in the mitotic index ( $p<0.001$ ) was observed in spontaneous lymphocytic cultures of *S. mansoni* patients. These results are in agreement with other findings [26, 7]. Also, spontaneous lymphocyte cultures of patients showed a significant decrease in the replicative index (RI) ( $p<0.01$ ) results which run in agreement with previous studies [26, 36].

How may schistosomiasis cause this genotoxic effect? In fact, the results of this study and the previous studies showed that schistosomiasis causes increase in the induction of chromosomal aberrations and the frequency of SCEs. Also, schistosomiasis causes a decrease in the mitotic index and a cell cycle delay in human somatic cells but the mechanism involved in this abnormalities is not totally clear. Suggested explanation is that disordered tryptophan metabolism occurring in schistosomal infection leads to an increase in the production of carcinogenic metabolites known to be genotoxic [36 and 37]. A second explanation is that schistosomiasis is accompanied by elevated enzymatic activities in serum, such as -glucuronidase ( g) [38], an enzyme known to enhance the metabolic activation of procarcinogens such as 2-aminoanthracin [36] and 3, 3 -dichlorobenzidine [39]. It was reported that the

active forms of these chemicals are able to induce SCEs in human blood lymphocytes [40]. A third explanation is the possible role of the immunization process in changing the type of sample cells [36]. The cause of the variability in the results of patients is unknown, however many factors such as disease duration, severity and patient immunity could be implicated in chromosomal aberrations and SCEs elevation of this type of infection [36]. Another explanation is the presence of Schistosoma toxins in the blood of infected patients and these toxins may cause a reduction in the cell growth and proliferation [41]. The last and most important explanation is that oxidation of DNA may lead to mutation (and hence to carcinogenesis); Free radicals can also damage DNA and result in mutations, altered capacity of cells to produce critical factors and derangement of the capacity to proliferate [42].

Oxidative stress may contribute to the development of fibrosis in the liver either through direct stimulation or by promoting the production of profibrotic cytokines. Furthermore, oxidative stress may also promote polarization of T-cell differentiation toward T helper 2 phenotype. Using experimental models of the disease, it has been shown that the granulomatous inflammatory response to *S. mansoni* eggs entrapped in the liver induces hepatic oxidative stress, with production of reactive oxygen species (ROS) and reduced anti-oxidant status of the organ. The ultimate result of ROS generation is killing of the parasite eggs; however, the process is potentially harmful for the host as production of ROS may initiate a fibrogenesis cascade in the liver or modulate tissue and cellular events responsible for progression of liver fibrosis. Thus, the pathophysiologic effect of ROS production associated with inflammatory response depend on a balance between opposing mechanisms that can either terminate the oxidative process or lead to increased generation of potentially harmful oxidants. The latter condition may promote the development of liver fibrosis, particularly in subjects with suboptimal antioxidant micronutrients status. The finding of an inverse relationship between serum retinol and intensity of *S. mansoni* infection and the finding of high levels of periportal fibrosis occurring with low antioxidant micronutrient concentrations suggest that micronutrients may have important roles in the differential morbidity patterns observed among communities who, otherwise, have comparable levels and intensities of *S. mansoni* infection [43]. The metamorphosis of normal

liver tissue to fibrotic tissue might give the chance for these mutagenic changes.

Evidence is accumulating in support of a role for ROS in the etiology of cancer. Inflammatory cells, such as neutrophils, macrophages, and eosinophils, are an important endogenous source of oxygen radicals. Stimulation of these cells by tumor promoters or by foreign bodies (parasites, bacteria, etc.) causes the release of ROS. In this context, it was found that H<sub>2</sub>O<sub>2</sub>/ myeloperoxidase system, which is the corner stone of the anti-microbial defense associated with inflammation, is activated in close contact with parasite eggs. The process although contributes to egg killing in vivo, yet, it causes accumulation of H<sub>2</sub>O<sub>2</sub>, superoxide anions and hydroxyl radicals in the host's tissues. It was reported that schistosome infection might have had suppressive effects on the host glutathione peroxidase (GPX) and glutathione S- transferase (GST) thereby minimizing the consumption of GSH either in eliminating H<sub>2</sub>O<sub>2</sub>, a reaction catalyzed by GPX, or in the conjugation of schistosomal toxins, a reaction catalyzed by GST. Therefore, the accumulation of free radicals and toxins is augmented with *S. mansoni* infection. Under such condition, the need for antioxidants increase and their presence may be crucial to eliminate the products of oxidative reactions and keep the ongoing immunological operations leading to destruction of eggs [44].

Another question is raised: How do these genotoxic changes lead to carcinogenesis? Zalata et al, (2005) suggested that the genotoxic agents produced endogenously through the course of schistosomiasis mansoni may play a role in colorectal cancer associated with schistosoma mansoni pathogenesis through the dysregulation of apoptosis by altering the expression pattern of Bcl-2 protein differently from non schistosoma associated colorectal carcinoma suggesting a different biological behavior [45].

The second part of our study included the antimutagenic effect of -carotene and vitamin E against the genotoxic hazards occurred by schistosomiasis. The treatment of cultures of peripheral blood lymphocytes of normal controls did not induce any remarkable changes from spontaneous (non-treated) cultures in normal controls in any parameter.

Also, -carotene did not decrease the aberrations and SCEs significantly when incubated with cultures of peripheral blood lymphocytes of Schistosoma-infected patients. The total numbers of metaphases with structural chromosomal aberrations with or without gaps or with SCEs were still higher in comparison to the spontaneous cultures of normal controls.

On the contrary, vitamin E decreased the aberrations caused by schistosomiasis when incubated with spontaneous blood cultures of *S. mansoni*-infected patients. The total number of metaphases with structural chromosomal aberrations with or without gaps decreased significantly ( $p<0.05$ ) compared with spontaneous cultures of infected patients. The frequency of SCEs was not influenced by the presence of vitamin E.

The improvement in cultures after vitamin E treatment compared to -carotene could be explained by the fact that while -carotene can enter the cell and protect against strand breaks but not against oxidized DNA bases [46], vitamin E is effective in preventing chromosomal damage [47], reduction of DNA fragmentation [48] and micronuclei formation in blood lymphocytes [49].

The replicative indices in the case of the two vitamins revealed an improvement in cell cycle kinetic but the increase was not significant compared with either spontaneous cultures in normal controls or patients.

Neither -carotene nor vitamin E improved the mitotic indices. The mitotic indices decreased significantly at  $p<0.001$  in all patient cultures whether incubated with either of the vitamins or not.

In our experiments, although an improvement in the cell cycle kinetics had occurred, the mitotic indices were still low. This may be due to the elimination of highly aberrated cells.

A possible interpretation of the role of -carotene or vitamin E against schistosome worms may be attributed to the antioxidative properties of these antioxidants, their metabolic modulator effect on some oxidases and the ability of these antioxidants to modulate the DNA repair mechanism by elimination of highly aberrated cells [48] or by gap junction-enhanced intracellular communication [50].

The use of -carotene or vitamin E may inactivate or reduce the schistosome glutathione peroxidase (GPX) enzyme activity, which is an important antioxidant enzyme protecting *S. mansoni* by reducing hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and peroxidized lipid [51, 52] thus damaging one key survival mechanism of the schistosomes.

As a conclusion to our study, -carotene and vitamin E have anti mutagenic effect. This anti mutagenic effect may be due to the antioxidant protection by scavenging DNA damaging free radicals or by acting as a modulator of the metabolism selectively inhibiting certain forms of

mixed function oxidases or lastly through modulation of DNA repair mechanism.

In conclusion, the present results indicates that schistosomiasis has a potential mutagenic effect, increases the level of chromosomal aberrations and SCEs, decreases the level of mitotic indices and causes cell cycle delay. The present results indicate that the investigated antioxidants pro-vitamin - carotene and vitamin E could improve the genetic damage induced by schistosomal pathogenesis.

We are thus justified to recommend giving antioxidant drugs like -carotene and vitamin E on regular basis to minimize the genotoxic hazards that may occur as a sequel of infection.

#### **Corresponding author**

Iman A.El Aziz Khaled  
Haematology &Blood Bank, Theodor Bilharz Research Institute (TBRI).Cairo, Egypt  
[iman\\_khaled@yahoo.com](mailto:iman_khaled@yahoo.com)

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6/1/2010

## BIOCHEMICAL PATTERN FOR HEPATITIS C AND ACUTE LYMPHOBLASTIC LEUKEMIA IN HUMAN SERA

Abulyazid<sup>1</sup>, I., Mohga S. Abdallah<sup>2</sup>, Hayate I. Sharada<sup>3</sup> and Sama H. Okasha<sup>4</sup>

<sup>1</sup>. Molecular Biology Department, Atomic Energy Authority  
<sup>2, 3, 4</sup>Chemistry Department, Faculty of Science, Helwan university

**Abstract:** Present experimental work aimed to show role of the molecular biology in diagnosis of hepatitis C liver disease (HCV) and acute lymphoblastic leukemia (ALL) which occur as a result of the disturbances of protein and enzymes fractions at the molecular level. The study carried out using vertical slab gel electrophoresis for detection of the protein pattern, catalase and peroxidase. Protein fractionation of the control samples produced 13 bands with Rf ranged between 0.17 and 0.96 and (amount, 3.14 - 7.24). Comparing hepatitis C with control one out of these 13 bands are completely disappeared at Rf 0.86 (amount 9.34). Ten bands appeared to be common bands in all HCV samples except one sample only nine common bands were produced while the band number ten was disappeared. The data showed that 5 characteristic bands were produced. One from these five bands determined at Rf 0.7 in all HCV sera samples except the first sample. Comparing leukemia samples with control only two were considered as common bands. These bands completely appeared in all sera samples. On the other side one band was completely disappeared in all leukemia samples. The rest bands distributed between different leukemia samples. 15 bands produced as characteristic bands. Electrophoresis pattern for catalase mentioned that six bands were produced in control samples. When hepatitis C compared with the control showed that two out these six bands were completely disappeared and other all HCV four bands considered as common bands. The amount of catalase enzyme completely decreased in all bands. In leukemia five common bands were produced with the appearance of one characteristic band, from the other side one band was disappeared. A documentation of peroxidase pattern data showed that tow common bands were appeared with Rf 0.1 and 0.33, the amount of these two bands were decreased when the amount of HCV compared with control in the same rows. In leukemia there is only one common band was produced with appearance of a three characteristic bands. [Journal of American Science. 2010;6(11):203-216]. (ISSN: 1545-1003).

**Keywords:** HCV, Acute lymphoblastic leukemia, Protein electrophoresis, catalase, peroxidase

### 1. Introduction

Hepatitis C virus (HCV) is a major human blood-borne pathogen that has infected almost 170 million people worldwide (Liu et al., 2010). Hepatitis C virus is a single positive stranded RNA virus, classified as family Flaviviridae, genus Hepacivirus. This virus can be differentiated by RNA sequence analysis in to at least 6 major genotypes and more than 100 subtypes Richter, (2002) and Ismail et al., (2004).

Hoofnagle, 1997 and Bièche et al., 2005 reviewed that the infection with HCV is often asymptomatic, but once established, chronic infection can cause inflammation of the liver (chronic hepatitis). This condition can progress to scarring (fibrosis), and advanced scarring (cirrhosis). In some cases, those with cirrhosis will go on to develop liver failure or other complications of cirrhosis, including liver cancer.

Egypt has the highest countrywide prevalence of hepatitis C virus in the world, with an estimated 8-

10 million among a population of 68 million having been exposed to the virus and 5–7 million active infections and the prevalence of antibodies to HCV is approximately 10-fold greater than in the United States and Europe (Ebeid and El-Bakry .., 2009). Frank et al., 2000 reported that HCV in Egypt associated with a high morbidity and mortality from chronic liver disease, cirrhosis, and hepatocellular carcinoma; the authors mentioned that Parenteral antischistosomal therapy had a major role in the spread of HCV throughout Egypt.

Leukemia originates from hematopoietic stem cells that lose their ability to differentiate normally for production of the mature blood cells (Battisti et al., 2008). The experimental studies reported that leukemia caused as a result of genetic changes include point mutations, gene deletions and rearrangements. This was able to cause disturbances in the gene expression without changing the DNA sequence (Melki and Clark, 2002). There were several types of leukemia. The acute lymphoblastic leukemia

(ALL) is a disease characterized by uncontrolled arrest in proliferation and maturation of lymphoid progenitor cells in bone marrow. This resulted in production of an excess of malignant cells (Battisti et al., 2008). The authors mentioned that the lymphoblasts replace the normal marrow elements, resulting in a marked decrease in the production of normal blood cells. Acute lymphoblastic leukemia (ALL) is the most common cancer found in the pediatric population and it accounts for more than 50% of the hematopoietic malignancies (Downing and Shannon, 2002 and Gaynon, 2005).

The present experimental work aimed to show role of the molecular biology in diagnosis of hepatitis C liver disease (HCV) and acute lymphoblastic leukemia (ALL) which occur as a result of the disturbances of protein and enzymes fractions at the molecular level. The study carried out using vertical slab gel electrophoresis for detection of protein, catalase and peroxidase patterns.

## 2. Material and Methods

This study was conducted in the nuclear research center, atomic energy authority, Inshas. Blood samples were taken as part of the experimental research. The study carried out on 29 cases divided into three groups. 10 patients diagnosed to have hepatitis C infection representing the HCV group, 9 diagnosed to have acute lymphoblastic leukemia representing the leukemia group and 10 were chosen from the same population and taken as control.

### Hepatitis C patients

Ten patients; (males) mean age  $30 \pm 6$  years, with hepatitis C infection. patients diagnosis depended completely on the historical review of each patient represented in elevation of serum transaminases, presence of anti-HCV antibodies. The patients with other autoimmune liver diseases were excluded from the study.

### Leukemia patients

Nine patients (males), mean age  $28 \pm 12$  years infected with acute lymphoblastic leukemia. The disease diagnosis depended completely on the historical review of each patient including clinical examination, complete blood picture, bone marrow aspiration . The patients with other diseases were excluded from the study.

### Control group

Ten healthy individuals (male), mean age  $27 \pm 7$  years. They were selected on the basis of general physical examination. They have no any disease of HCV, HBV and HIV. There was no previous history of hepatitis and/or blood related cancers in any

individual. They obey all the biochemical tests including liver functions (Alanine Transaminase (ALT), Aspartate Transaminase (AST) and Gamma Glutamyl Transferase (GGT), complete blood count and bone marrow examination, protein profile (Total protein and albumin) showing no evidence of any abnormal disturbances.

### Sample collection and assay:

Five milliliter blood samples were collected. Samples were collected on heparin (anti coagulant) in a sterile micro centrifuge tube and then centrifuged at  $400 \times g$  for five minutes. The relatively clear supernatant were divided into aliquots and frozen at  $-40^{\circ}\text{C}$  till the assay time.

Total protein was determined according to Bradford, 1976, then Protein electrophoresis methods and procedures were taken from the book Gel electrophoresis of proteins ( Hames, 1990). Resolving Gel (8%) solution were prepared for Native Poly acrylamide gel electrophoresis (PAGE) by mixing 14.4 ml distilled water, 8.1 ml of Acrylamide/Bis (30% T, 2.67% C) stock solution, and 7.5 ml Tris (1.5M, pH8.8). The total volume of the solution was 30 ml. To this solution 150 $\mu\text{l}$  of 10% APS, freshly prepared, and 30  $\mu\text{l}$  of TEMED were added prior to pouring into the gel plate assembly. Then proper comb was inserted into the assembly to form wells in which samples were loaded. Electric current of 50 mA was applied on Serum protein samples under cooling conditions at  $4^{\circ}\text{C}$  for about 4 hours. At the end of the run, electrical current was stopped and the gels were stained overnight then photographed after destaining.

Native protein gel was stained for catalase pattern depending on Gregory and Fridovich, (1974); Siciliano and Shaw, (1976) and Baker and Manwell, (1977). Peroxidase pattern was determined as Native protein gel then it was stained for using certain stain prepared according to Siciliano and Shaw, (1976) ; Misra and Fridovich, (1977) ; Shimoni, M. (1994) and Rescigno et al., (1997).

### Data analysis

Gel plate was photographed, scanned and then analyzed using a gel pro Analyzer (Version 3.1 Media Cybernetics USA) for the analysis of tested samples. This program is a comprehensive computer software application designed to determine the relative fragmentation, the molecular weights and the amounts of protein as well as scanned graphical presentation of the fractionated bands of each lane.

The similarity index (S.I.) compares patterns within, as well as, between Control, hepatitis C and leukemia samples using the formula:  $S.I. = (2 Nab/Na + Nb) / (Nei and Li, 1979)$ . Where, Na and Nb are the

number of bands in individuals a and b and Nab is the number of shared bands between a and b. The similarity values were converted into genetic distance (D) using the formula:  $D = 1 - S$ .

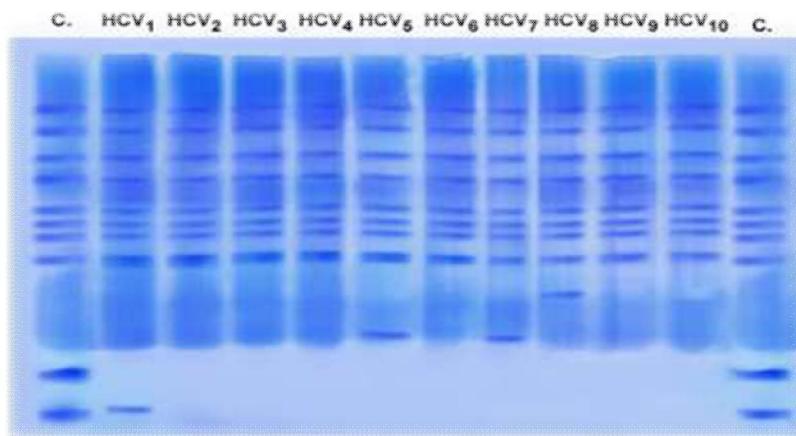
### 3. Results

I-Protein electrophoresis of control and hepatitis C liver disease:

The protein pattern in sera of the control and HCV patients was revealed in table (1) and graphically illustrated in Fig. (1). protein fractionation of the control samples produced 13 bands with Rf ranged between 0.17 and 0.96 and (amount, 3.14 - 7.24). Comparing hepatitis C with control one out of these 13 bands are completely disappeared at row r18 with Rf 0.86 (amount 6.39) while bands in row r19 were disappeared in all HCV sera samples except first one; appeared with low amount (4.63) when compared with control at Rf 0.95. A documentation of protein in HCV sera showed that ten bands appeared to be common bands

at rows r1, r3, r5, r6, r7, r8, r10, r11, r12, r13 and r17 in all HCV samples except HCV7 9 common bands were produced while the band number ten was disappeared at r8. The data showed that 5 characteristic bands were produced., HCV4 at r4 with Rf 0.26 (amount 3.39), HCV9 at r15 with Rf 0.68 (amount 4.24), HCV 10 at r2 and r14 at Rf 0.19 and 0.64 with amount 7.7 and 3.01 respectively, the five one appeared in r16 with Rf 0.7 and amount range (6.1 - 12.7) in all HCV sera samples except the first sample

The similarity indices between the control samples & hepatitis C samples (Table: 2) showed high value, S.I range (0.81- 0.96). from the other side, by comparing the infected sera samples with each other, the similarity index again recorded high values (range: 0.85-1).



**Fig. (1):** The protein pattern detected in sera of control and different HCV patients.

**Table (1):** protein pattern in human sera of Control and HCV samples

Lanes	Control Lane 1		HCV <sub>1</sub> Lane 2		HCV <sub>2</sub> Lane3		HCV <sub>3</sub> Lane4		HCV <sub>4</sub> Lane 5	
Rows	(Rf.)	Amount %	(Rf.)	Amount %	(Rf.)	Amount%	(Rf.)	Amount %	(Rf.)	Amount %
R <sub>1</sub>	0.17	9.2% (6.32)	0.17	13.1% (7.59)	0.17	14.03% (9.21)	0.17	14.8% (9.71)	0.18	14.5% (10.4)
R <sub>2</sub>										
R <sub>3</sub>	0.24	10.4% (7.12)	0.24	7.9% (4.59)	0.24	10.45% (6.86)	0.24	9.7% (6.34)	0.24	8.5% (6.1)
R <sub>4</sub>									0.26	4.7% (3.39)

R <sub>5</sub>	0.3	6.7% (4.59)	0.3	8.2% (4.75)	0.3	6.40% (4.2)	0.3	6.8% (4.46)	0.3	6.2% (4.46))
R <sub>6</sub>	0.36	7.6% (5.19)	0.36	6.6% (3.84)	0.36	6.43% (4.22)	0.36	7.4% (4.87)	0.36	7.5% (5.33)
R <sub>7</sub>	0.41	9.1 (6.2)	0.41	6.7% (3.89)	0.41	6.20% (4.07)	0.41	5.4% (3.51)	0.41	5.3% (3.81)
R <sub>8</sub>	0.45	6.2% (4.27)	0.45	8.3% (4.84)	0.45	6.26% (4.11)	0.45	7.9% (5.15)	0.45	6.9% (4.95)
R <sub>9</sub>										
R <sub>10</sub>	0.48	4.8% (3.3)	0.48	5.4% (3.16)	0.48	5.94% (3.9)	0.48	5.9% (3.9)	0.48	5.3% (3.76)
R <sub>11</sub>	0.51	4.6% (3.14)	0.51	4.5% (2.61)	0.51	5.03% (3.3)	0.51	5.4% (3.5)	0.51	4.3% (3.09)
R <sub>12</sub>	0.54	5.6 (3.86)	0.54	6.3% (3.65)	0.54	5.54% (3.64)	0.54	6.3% (4.09)	0.54	5.3% (3.8)
R <sub>13</sub>	0.6	5.2% (3.52)	0.6	12.6% (7.32)	0.6	9.40% (6.17)	0.6	9.4% (6.13)	0.6	9.5% (6.81)
R <sub>14</sub>										
R <sub>15</sub>										
R <sub>16</sub>					0.7	13.37% (8.78)	0.7	10.2% (6.66)	0.7	11.6% (8.31)
R <sub>17</sub>	0.79	10.6 (7.24)	0.79	12.4% (7.22)	0.79	10.95% (7.19)	0.79	10.8% (7.09)	0.79	10.2% (7.28)
R <sub>18</sub>	0.86	9.4% (6.39)								
R <sub>19</sub>	0.95	10.5% (7.2)	0.95	8.0% (4.63)						

Note: number between practices expressed the real mass of protein

**Table (1):** continued

HCV <sub>5</sub> Lane 6		HCV <sub>6</sub> Lane 7		HCV <sub>7</sub> Lane 8		HCV <sub>8</sub> Lane 9		HCV <sub>9</sub> Lane 10		HCV <sub>10</sub> Lane 11		Control Lane 12	
(Rf.)	Amount %	(Rf.)	Amount %	(Rf.)	Amount %	(Rf.)	Amount %	(Rf.)	Amount %	(Rf.)	Amount %	(Rf.)	Amount %
0.17	15.2% (10.4)	0.17	15.7% (10.7)	0.17	14.4% (9.71)	0.17	16.9% (11)	0.17	14.2% (10.98)	0.17	13.9% (9.01)	0.17	9.2% (6.32)
								0.19	9.9% (7.7)				
0.24	10.0% (6.67)	0.24	8.8% (5.96)	0.24	9.7% (6.56)	0.24	9.0% (5.86)	0.24	8.3% (6.42)	0.24	9.4% (6.09)	0.24	10.4% (7.12)
0.3	6.9% (4.57)	0.3	7.2% (4.93)	0.3	6.5% (4.42)	0.3	6.6% (4.31)	0.3	6.3% (4.92)	0.3	6.9% (4.49)	0.3	6.7% (4.59)
0.36	8.2% (5.43)	0.36	6.6% (5.5)	0.36	8.3% (5.58)	0.36	7.3% (4.74)	0.36	5.3% (4.1)	0.35	7.3% (4.76)	0.36	7.6% (5.19)
0.41	5.3% (3.54)	0.41	6.8% (4.6)	0.41	6.1% (4.14)	0.41	8.4% (5.44)	0.41	5.5% (4.03)	0.41	6.2% (4)	0.41	9.1% (6.2)

0.45	6.5% (4.32)	0.45	7.0% (4.74)			0.45	7.0% (4.53)	0.45	6.0% (4.65)	0.45	7.2% (4.69)	0.45	6.2% (4.27)
0.48	5.8% (3.4)	0.48	4.7% (3.18)	0.48	4.9% (3.3)	0.48	5.6% (3.66)	0.48	3.9% (3.04)	0.48	5.8% (3.74)	0.48	4.8% (3.3)
0.51	5.9% (3.9)	0.51	4.7% (3.22)	0.51	5.2% (3.54)	0.51	4.6% (3.01)	0.51	4.5% (3.5)	0.51	4.8% (3.11)	0.51	4.6% (3.14)
0.54	4.8% (3.2)	0.54	5.6% (3.82)	0.54	6.4% (4.35)	0.54	5.1% (3.29)	0.54	4.8% (3.75)	0.54	6.4% (4.12)	0.54	5.6% (3.86)
0.6	8.1% (5.36)	0.6	9.8% (6.66)	0.6	8.9% (6)	0.6	9.1% (5.9)	0.6	8.1% (6.3)	0.6	9.3% (6.04)	0.6	5.2% (3.52)
								0.64	3.9% (3.01)				
						0.68	6.5% (4.24)						
0.7	12.5% (8.32)	0.7	12.0% (8.2)	0.7	18.8% (12.7)	0.7	9.3% (6.1)	0.7	8.9% (6.88)	0.7	10.4% (6.76)		
0.79	10.9% (7.23)	0.79	11.1% (7.55)	0.79	10.8% (7.29)	0.79	13.8% (8.98)	0.79	10.3% (7.97)	0.79	12.3% (8)	0.79	10.6% (7.24)
												0.86	9.4% (6.39)
												0.95	10.5% (7.2)

Rf: rate of flow

Note: number between practices expressed the real mass of protein

**Table (2):** Protein pattern similarity index (SI) and genetic distance (Gd) in sera of control and different HCV samples.

S.I												
Lane	C.	HCV <sub>1</sub>	HCV <sub>2</sub>	HCV <sub>3</sub>	HCV <sub>4</sub>	HCV <sub>5</sub>	HCV <sub>6</sub>	HCV <sub>7</sub>	HCV <sub>8</sub>	HCV <sub>9</sub>	HCV <sub>10</sub>	
G.d	C.	0.96	0.88	0.88	0.85	0.88	0.88	0.83	0.85	0.81	0.88	
	...	...	...	...	...	...	...	...	...	...	...	
	HCV <sub>1</sub>	0.04	...	0.92	0.92	0.88	0.92	0.92	0.87	0.88	0.85	0.92
	HCV <sub>2</sub>	0.12	0.08	...	1	0.96	1	1	0.96	0.96	0.96	0.92
	HCV <sub>3</sub>	0.12	0.08	0	...	0.96	1	1	0.96	0.96	0.96	0.92
	HCV <sub>4</sub>	0.15	0.12	0.04	0.04	...	0.96	0.96	0.92	0.92	0.89	0.96
	HCV <sub>5</sub>	0.12	0.08	0	0	0.04	...	1	0.96	0.96	0.92	1
	HCV <sub>6</sub>	0.12	0.08	0	0	0.04	0	...	0.96	0.96	0.92	1
	HCV <sub>7</sub>	0.17	0.13	0.04	0.04	0.08	0.04	0.04	...	0.92	0.92	0.96
	HCV <sub>8</sub>	0.15	0.12	0.04	0.04	0.08	0.04	0.04	0.08	...	0.89	0.96
	HCV <sub>9</sub>	0.19	0.15	0.08	0.08	0.11	0.08	0.08	0.08	0.11	...	0.92
	HCV <sub>10</sub>	0.12	0.08	0	0	0.04	0	0	0.04	0.04	0.08	...

## II. Protein electrophoresis of control and leukemia

Protein pattern in sera of the control and leukemia patients was revealed in table (3) and graphically illustrated in Fig. (2). Protein fractionation of the control samples produced 12 bands with Rf ranged between 0.08 and 0.73 and (amount, 3.46 - 15.2). Comparing leukemia samples

with control only two of these 12 bands were considered as common bands these bands completely appeared in all sera samples. On the other side one band was completely disappeared in all leukemia samples at row r3 with Rf 0.14 (amount, 8.59). The rest nine bands distributed between different leukemia samples. 15 bands produced as characteristic bands ; two characteristic bands were

produced at row r4 with Rf 0.18 (amount, 8.2 and 7.27) for samples number five and seven respectively, at row r7 only one was detected in the four sample of leukemia with Rf 0.25 (amount, 7.42), the same occurred at row r11 one band was determined with Rf 0.45 (amount, 10.1) for sample number 6, another two were appeared at row r12 with Rf 0.5 (amount, 8.74 and 8.3 ) in samples number five and seven respectively, while in the row r16 five characteristic bands were documented at Rf 0.7 (amount range: 5.2 and 7.82) for sera samples for patients number one, two, five, seven and nine. The rest four bands were fractionated at row r17 with Rf

0.78 and the bands mostly had the same amount (amount range: 5.71 And 6.27) in the first four samples.

The similarity indices between the control & acute lymphoblastic leukemia samples showed low value, S.I range (0.47-0.82) the data indicating to the severe effect of leukemia on the protein fractions (Table:4). From the other side, by comparing the infected sera samples of leukemic patients with each other, the similarity index again recorded (range: 0.33-0.95).

**Fig. (2):** Graphic illustration of protein pattern of Control and different leukemia patients.

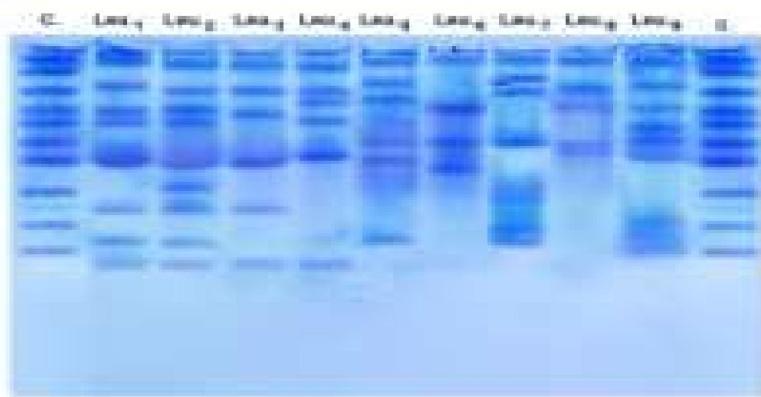


Table (3): Protein pattern of Control and acute lymphoblastic leukemia sera samples

		(6.16)									
R <sub>16</sub>			0.7	9.4% (6.81)	0.7	7.5% (5.49)					
R <sub>17</sub>	0.73	6.3% (5.98)									
R <sub>18</sub>			0.78	7.9% (5.71)	0.78	8.0% (5.89)	0.78	10.8% (5.85)	0.78	12.0% (6.27)	

**R<sub>f</sub>:** rate of flow

Table (3): continued

Leukemia <sub>5</sub> Lane 6		Leukemia <sub>6</sub> Lane 7		Leukemia <sub>7</sub> Lane 8		Leukemia <sub>8</sub> Lane 9		Leukemia <sub>9</sub> Lane 10		Control Lane 11	
Rf.	amount%	Rf.	Amount%	Rf.	Amount%	Rf.	Amount%	Rf.	Amount%	Rf.	Amount%
0.08	5.4% (3.7)	0.08	7.10% (3.05)	0.08	4.30% (3.24)	0.08	7.96% (3.58)	0.08	4.2% (3.04)	0.08	4.2% (3.96)
0.12	9.7% (6.59)	0.12	18.22% (7.83)	0.12	10.50% (7.92)	0.12	15.99% (7.19)	0.12	10.5% (7.7)	0.2	7.9% (7.54)
										0.14	9.0% (8.59)
0.18	12.1% (8.2)			0.18	10.96% (7.27)						
0.23	11.6% (7.9)			0.23	13.92% (10.5)	0.23	19.35% (7.7)	0.23	10.0% (7.34)	0.23	8.5% (8.14)
	0.28	24.43% (10.5)			0.28	19.75% (8.88)	0.28	10.3% (7.53)	0.28	7.6% (7.27)	
0.33	11.6% (7.86)						0.33	12.3% (8.99)	0.33	15.9% (15.2)	
0.37	10.7% (7.25)	0.37	26.76% (11.5)	0.37	13.79% (10.4)	0.37	19.64% (8.83)	0.37	15.2% (11.1)	0.37	12.7% (12.1)
0.43	14.5% (9.87)				0.43	17.30% (7.78)	0.43	13.1% (9.56)	0.43	11.0% (10.5)	
	0.45	23.50% (10.1)									
0.5	12.9% (8.74)			0.5	11.00% (8.3)						
				0.54	8.33% (6.28)				0.54	6.8% (6.48)	
				0.59	9.32% (7.03)				0.59	3.6% (3.46)	
				0.65	10.34% (7.08)			0.65	10.5% (7.66)	0.65	6.5% (6.16)
0.7	11.5% (7.82)			0.7	7.54% (5.69)			0.7	6.9% (5.02)		
							0.73	7.1% (5.21)	0.73	6.3% (5.98)	

**R<sub>f</sub>:** rate of flow

Note: number between practices expressed the real mass of protein

**Table (4):** The protein pattern similarity index (SI) and genetic distance (Gd) in sera of control and Acute lymphoblastic leukemia sera samples.

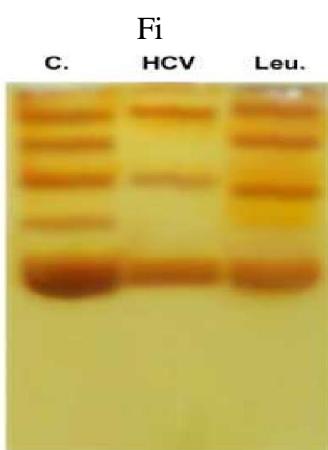
		S.I										
		lanes	control	leuk <sub>1</sub>	leuk <sub>2</sub>	leuk <sub>3</sub>	leuk <sub>4</sub>	leuk <sub>5</sub>	leuk <sub>6</sub>	leuk <sub>7</sub>	leuk <sub>8</sub>	leuk <sub>9</sub>
G.d	control	....	0.67	0.73	0.63	0.53	0.57	0.47	0.64	0.67	0.82	
	leuk <sub>1</sub>	0.33	....	0.95	0.88	0.75	0.67	0.43	0.53	0.67	0.74	
	leuk <sub>2</sub>	0.27	0.05	....	0.82	0.71	0.63	0.40	0.60	0.63	0.70	
	leuk <sub>3</sub>	0.37	0.13	0.18	....	0.71	0.50	0.50	0.47	0.77	0.59	
	leuk <sub>4</sub>	0.47	0.25	0.29	0.29	....	0.63	0.33	0.35	0.62	0.59	
	leuk <sub>5</sub>	0.43	0.33	0.37	0.50	0.38	....	0.43	0.74	0.67	0.74	
	leuk <sub>6</sub>	0.53	0.57	0.60	0.50	0.67	0.57	....	0.40	0.73	0.53	
	leuk <sub>7</sub>	0.36	0.47	0.40	0.53	0.65	0.26	0.60	....	0.50	0.60	
	leuk <sub>8</sub>	0.33	0.33	0.38	0.23	0.38	0.33	0.27	0.50	....	0.75	
	leuk <sub>9</sub>	0.18	0.26	0.30	0.41	0.41	0.26	0.47	0.40	0.25	....	

#### Catalase electrophoresis:

Catalase pattern of control serum, hepatitis C and leukemia were shown in Table (5) and Figures (3) Electrophoresis pattern for catalase mentioned that six bands were produced in control samples with Rf range (0.05, 0.58), (amount range: 6.88-25.2). When hepatitis C compared with the control showed that two out these six bands were completely disappeared in r3 and r6 and other all HCV four bands considered as common bands. The amount of catalase enzyme completely decreased in all bands with the respect of control ones.

In leukemia five common bands were produced at rows r1, r2, r3, r6 and r7 with the appearance of one characteristic band at r5 with Rf 0.33 (amount, 10.7), from the other side one band was disappeared completely at r4 when compared with control. Similar to hepatitis C all bands of acute lymphoblastic leukemia was decreased in its amount.

The similarity indices between the control samples & hepatitis C samples showed high value (SI=0.8) and control samples with leukemia showed the highest value (SI=0.83) indicating that low obvious effect of hepatitis C virus & leukemia in the catalase activity of human body.



**Fig (3):** Catalase bands detected in serum of control, hepatitis C & Leukemia respectively

**Table (5):** Catalase pattern of serum of control, hepatitis C and leukemia

Lanes:	Control, Lane 1		HCV, Lane 2		Leukemia, Lane 3	
Rows	(Rf.)	amount %	(Rf.)	amount%	(Rf.)	amount%
R <sub>1</sub>	0.05	9.7% (6.88)	0.05	8.4% (3.35)	0.05	6.2% (3.53)
R <sub>2</sub>	0.097	16.3% (11.6)	0.097	21.1% (8.42)	0.097	16.4% (9.35)
R <sub>3</sub>	0.19	11.6% (8.24)			0.19	15.1% (8.61)
R <sub>4</sub>	0.3	15.6% (11.1)	0.3	23.3% (9.3)		
R <sub>5</sub>					0.33	18.5% (10.6)
R <sub>6</sub>	0.43	11.5% (8.19)			0.43	12% (6.89)
R <sub>7</sub>	0.58	35.3% (25.2)	0.58	47.2% (18.8)	0.58	31.8% (18.2)

Rf: rate of flow; Note: number between practices expressed the real mass of protein

**Table (6):** Catalase similarity index (S.I) and genetic distance (G.d) between control, hepatitis C and leukemia samples.

		S.I		
		Control	HCV	Leukemia
G.d	Control		0.8	0.83
	HCV	0.2		0.6
	Leukemia	0.17	0.4	

### Peroxidase pattern

The peroxidase pattern of the serum isolated from control, hepatitis C and leukemia were shown in Table (7) and in Fig. (4). A documentation of peroxidase pattern data showed that the peroxidase fractionation in control appeared as three bands with Rf 0.1, 0.25 & 0.33 with amount (10.5, 12.8 & 11.1). When hepatitis C compared with the control, it was observed that two common bands were appeared at Rows (r<sub>2</sub>, & r<sub>5</sub>), with Rf 0.1 and 0.33 (amount, 4.37 and 6.2) respectively the amount of these two bands were decreased when the amount compared with control in the same rows.

When leukemia samples compared with the control, data showed there is only one common band was produced at r<sub>7</sub> with Rf 0.33 (amount 13.9), with appearance of a three characteristic bands in the rows r<sub>1</sub> at Rf 0.07 (amount, 8.06), r<sub>3</sub> at Rf 0.13 (amount 9.98) and r<sub>4</sub> at Rf 0.22 (amount, 12.4). On the other hand, the amount of the leukemia common band was higher than that of control.

The similarity index between the control, hepatitis C and leukemia recorded in the table (8). By comparing hepatitis C with control, the similarity index recorded values (SI=0.57). Also, comparing between the control and leukemia the similarity index recorded

values (SI=0.3).indicating the effect of hepatitis C virus & leukemia on the peroxidase fractions and failure in overcomes effect of this diseases.

**Fig(4):** Peroxidase bands detected in serum of control, hepatitis C & Leukemia respectively

**Table (7):** Peroxidase pattern of serum of control, hepatitis C and leukemia

Lanes:	Control		HCV		Leukemia	
	(Rf.)	(amount)	(Rf.)	(amount)	(Rf.)	(amount)
R <sub>1</sub>			0.07	11.4% (3.43)	0.070	18.2% (8.06)
R <sub>2</sub>	0.1	30.53% (10.5)	0.1	15.7% (4.73)		
R <sub>3</sub>					0.13	22.5% (9.98)
R <sub>4</sub>					0.22	27.96% (12.4)
R <sub>5</sub>	0.25	37.2% (12.8)	0.25	20.6% (6.2)		
R <sub>6</sub>			0.29	51.3% (15.8)		
R <sub>7</sub>	0.33	32.27% (11.1)			0.33	31.3% (13.9)

**Rf:** rate of flow.

**Note:** number between parentheses expressed the real mass of protein.

**Table (8):** Peroxidase similarity index (S.I) and genetic distance (G.d) between control, hepatitis C and leukemia samples.

G.d	Lanes	Control	HCV	S.I
	Control		0.57	0.3
	HCV	0.43		0.25
	Leu	0.7	0.75	

### 3. Result

The proteome is the total complement of proteins expressed within a cell, a tissue or an organism. Proteins rather than genes or mRNAs represent the key players in the cell. Proteomics is the study of proteins, including their expression level, post-translational modification and interaction with other proteins, on a large scale. Expression levels of proteins determine the cellular phenotype and its plasticity in response to external signals. Since not all proteins are expressed at all times, but are dependent on physiological and environmental factors, proteomics can provide an excellent global view of disease processes at the protein level (Hütter et al., 2009 and Oie 2009).

#### I. Protein electrophoresis of control, HCV and leukemia patients

Data in the present study indicated that the specific protein bands of Hepatitis C and leukemia samples differed (through disappearance in some protein bands or appearance of new ones) after comparing with the control (there was a great difference in the effect of the leukemic disease among patients' sera samples themselves. While in HCV the data indicating that the viral infection had low mutagenic effect on the protein pattern and mostly no-significant differences appeared of the different

samples among themselves. The disappearance in certain protein bands of Hepatitis C & leukemia sera may be attributed to the effects of oxidative stress (translational modification and interaction with other proteins) which inhibit the synthesis and expression process of these deleted proteins (qualitative effect). In addition, even the bands remained it usually differs in the amount of protein and this may be explained as translational modification could not inhibit the synthesis of this protein type, but it may be affected only on the quantitative level. Protein is an essential nutrient made up of building-block chemicals called amino acids. Protein provides energy and is needed for the body to make new cells, to maintain and rebuild muscles, to carry other nutrients, to act as messengers in the body, and to support the immune system. Low levels may be seen in severe malnutrition and with conditions that cause malabsorption. Changes in total protein levels may be seen with chronic inflammation or infections such as viral hepatitis. They may be caused by bone marrow disorders such as different types of leukemia and multiple myeloma (Dhinaa, and Palanisamy, 2010).

Choi and James, 2006 reported that oxidative stress has emerged as a key player in the development and the progression of many pathological conditions,

including HCV-induced pathogenesis of liver. Oltra et al., 2001 and Al-Gayyar et al., 2007 mentioned that there was a relationship between leukemia and oxidative stress. Leukemic cells produce higher amounts oxidative stress (ROS) than non-leukemic cells.

Hawkins et al., 2009 and Rahmanto et al., 2010 described protein to be a major quantitative target for oxidative stress as a result of their abundance in cells (proteins compose ca. 70% of the dry mass of cells), plasma, and most tissues and their rapid rates of reaction with many oxidants.

Omar et al., 1995 founded that serum protein electrophoresis showed a significant decrease in albumin and increase in alpha-1, beta and gamma globulin levels in HCV infected group as compared to seronegative and anti-HCV positive groups, a finding that may reflect an increased burden on the liver. The significant increase in gamma globulins in the anti-HCV positive as well as the combined anti-HCV and HBsAg positive rous may be due to the increase in one or more of the immunoglobulins, necessitating immunoglobulin typing, an observation being currently investigated.

According to Battisti et al, 2008 Protein oxidation, determined by protein carbonyl content in serum of ALL patients observed that there was a significant difference between the patients and the controls. The protein carbonyl content was increased in the just diagnosed patients, when compared to the controls. These in the serum of patients with chronic leukemia and acute lymphoblastic leukemia and of bone marrow transplant recipients.

In acute lymphoblastic leukemia (ALL) show elevated levels of oxidatively modified DNA lesions. Supportive of the finding those oxidative events are largely responsible for spontaneous mutagenesis, and strongly implicating such damage in the etiology of cancer. Oxidative mechanisms have been shown to have a potential role in a cell becomes malignant by DNA mutation, activation of proto-oncogenes and inactivation or loss of tumor suppressor genes (Evans et al., 2004 and Kong et al., 2009).

## **II. Isozymes electrophoretic pattern of control, HCV and leukemia.**

### **II.I Catalases group**

Catalase is an enzyme present in the cells of plants, animals and aerobic (oxygen requiring) bacteria. Catalase is located in a cell organelle called the peroxisome. The enzyme very efficiently promotes the conversion of hydrogen peroxide to water and molecular oxygen. Catalase has one of the highest turnover rates for all enzymes: one molecule of catalase can convert 6 million molecules of hydrogen peroxide to water and oxygen each minute. The

significantly decreased capacity of a variety of tumours for detoxifying hydrogen peroxide is linked to a decreased level of catalase (Valko, et al., 2006).

Data in present study indicating that two bands were completely disappeared while, the amount of the enzyme completely decreased in all bands. In leukemia five common bands were produced with the appearance of one characteristic band, on the other hand one band was completely disappeared. In leukemia sera catalase amount appeared to be low when compared with control. Inhibition the synthesis and expression process of these deleted bands may refer to qualitative change. In addition, differs in the amount of the enzymes may be explained as translational modification could not inhibit the synthesis of this catalase type, but it may be affected only on the quantitative level.

There was a possible link between decreased catalase activity and increased levels of cellular alterations. The oxidative damage supported the idea that there was a persistence of oxidative stress in acute lymphoblastic leukemia (Battisti et al., 2008). Patients with chronic HCV infection are under the influence of oxidative stress associated with lower levels of antioxidant enzymes. Ebeid and El-Bakry., 2009 mentioned that the level of catalase was decreased in group of children with chronic hepatitis C, in comparison to the healthy children.

CAT activity in total blood of ALL patients just diagnosed was reduced when compared to controls. Battisti, et al 2008 suggested that oxidative damage accumulates in biological molecules during aging and that oxidative stress is relevant to the aging process. However, the antioxidant capacity of tissues decreases during aging. This phenomenon indicates a disturbance of the protective role of these enzymes against free radicals in ALL. These findings are in accordance with earlier studies of Oltra et al., 2001 confirmed decreased CAT activities in the lymphocytes of lymphocytic leukemia patients. The results are also in agreement with the reports of Sentürker et al., 1997, who demonstrated reduced CAT activities in the lymphocytes of ALL patients, and Madej et al. 1988 who found a decreased activity of these enzymes during the development of the leukemic process in mice.

### **ii.iii. Peroxidase group**

Glutathione peroxidase (GSH-Px) enzymes are the most important hydrogen peroxide ( $H_2O_2$ )-removing enzymes in mammalian cells ( Nagwa, et al., 2010). Valko et al., 2006 reviewed that GSH-Px acts in conjunction with the tripeptide glutathione (GSH), which is present in cells in high (micromolar) concentrations. The substrate for the catalytic reaction

of GSH-Px is H<sub>2</sub>O<sub>2</sub>, or organic peroxide ROOH. GSH-Px decomposes peroxides to water (or alcohol).

Data in present study indicating that oxidative stress seemed to have effect on peroxidase enzyme activity. This effect appeared on HCV and leukemia samples when compared with control. The data show disappearance in some bands in HCV samples and even the bands remained it usually differs in the amount and this may be explained as oxidative stress may be inhibit or decreases preoxidase enzyme activity. On the other hand appearance of some bands of peroxidase enzymes in leukemia samples when compared with control. The data may explain as the role of peroxidase enzymes in the protection of cell from oxidative damage.

In hepatitis C virus GSHPx depleted when compared to the control. The viral activity is enhanced by redox imbalance and peroxidation with virus expression in chronically infected cells. The increase in peroxidation appears to contribute to disease progression with reduction of antioxidant activity that favors further viral replication and potentiates carcinogenesis (Stehbens., 2004). The author founded that Antioxidants suppress viral infections. Therefore, large doses of primary antioxidants should be the initial therapy to restore and thereafter to maintain serum and tissue concentrations at high normal values. Optimal plasma and tissue levels of all antioxidants require review, because when under severe stress, are too low to prevent pathological cellular changes.

Levent, et al., 2006 reviewed that the reduction in the amount of superoxide dismutases (SOD), and GSH-Px reflects both a decrease in the synthesize capacity of liver, and the antioxidant defense power of the patients. It can be argued that increased lipid peroxidation is caused by the inflammation related to viral infection and decreased the antioxidant levels may be an early marker of the oxidative stress. Lipid peroxides formed can be chemotactic for the neutrophils causing increased inflammation, which further drives oxidant-mediated injury in the liver.

GSH-Px activities were significantly increased in ALL when compared to the controls. Devi et al., 2000 reported that antioxidant enzyme activities showed significant increased red cell GSH-PX activity in leukemia patients. These scavenging enzymes play an important role in the protection of cell from oxidative damage. The authors mentioned that GSH-Px is known to detoxify lipid peroxides and thereby inhibit lipid peroxidation, thus although the generation of superoxide anion was increased the observed normal malonaldehyde (MDA) levels in patients could be due to the increased protective response of antioxidant enzymes. Alternatively, increased GSH-PX activity could also maintain normal MDA levels through

utilization of lipid peroxide as substrates and produce alcohols as by products.

## Conclusions

Proteins and enzymes were involved in cell proliferation, invasion, angiogenesis, metastasis, inflammation, synthesis, energy and metabolism. We recommended using common and characteristic bands as an investigation diagnostic biomarker as these bands reflects the physiological and pathological state where many proteins and enzymes in the resistant cell lines were found to have increased or decreased abundances, reflecting changes in both gene expression/regulation and protein degradation for identifying more selective targets for therapeutic intervention. Also more studies in different etiologies and a larger number of subjects should be considered.

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6/1/2010

## Serum resistin levels and haemostatic changes in experimentally induced diabetic and high fat fed rats

Mohammad I. Hoseen, Mai M .Hassan, Dalia I. Abd-Alaleem and Eman M. Faragallah.

Department of physiology, Faculty of medicine, Zagazig University.

**Abstract:** Adipose tissue is considered as an active endocrine gland that affects many aspects of body homeostasis. Adipose tissue derived molecules “adipokines” regulate energy homeostasis, dietary behavior, as well as insulin sensitivity and immunity; it refers to leptin, adiponectin, resistin, apelin, visfatin and omentin.

Resistin is a cysteine-rich adipokine that is released by adipocytes and macrophages and has been involved in the development of insulin resistance in rodents. Moreover a strong link between diabetes, hypercoagulability and thrombogenesis, had been recognized for decades.

Aim: In a trial to identify any possible relationship between resistin levels and some haemostatic changes in streptozotocin-induced diabetic and high fat diet-fed rats (HFD); the present work had been carried out.

Design: A total number of 40 adult male albino rats were divided into 2 main groups: Group I (n= 24): To study the effect of streptozotocin-induced type 1 diabetes and was further divided into 3 equal subgroups (n= 8 in each) and survived for 30 days: Ia: (control group), Ib: (experimental diabetic non-treated group (by a single i.p. injection of streptozotocin (65mg/Kg B.W), Ic (experimental diabetic group treated with insulin).

Group II (n= 16) : To study the effect of high fat diet and was further divided into 2 equal subgroups (n= 8 in each) and survived for 7 weeks: IIa: (control group), IIb (high fat diet fed (58% fat).

In all groups, serum levels of glucose, insulin, resistin, total cholesterol(TC), triglycerides (TG), HDL, LDL, BT, WBCT, PT, aPTT, plasma fibrinogen level, plasma D-dimmers level and platelet count were measured.

Results: The results of this study showed a significant decrease in serum resistin levels ( $p<0.001$ ) in streptozotocin-induced diabetic group in comparison with its control group and insulin-treated group. Moreover, no significant correlation could be detected between resistin levels and any of measured parameters in these groups except the significant positive correlation with body weight at the end of experimental period.

In addition, our study revealed a significant increase in serum resistin levels ( $p<0.001$ ) in HFD-fed group in comparison with its controls, which was correlated positively and significantly with body weight, serum glucose levels, insulin levels and HOMA-IR index ( $p<0.001$ ), atherogenic lipid profile and markers of hyper-coagulability (except for platelet count)

Conclusion: No role for resistin in metabolic and haemostatic changes in type 1 diabetic rats was detected. Although, hyperresistinemia may represent a link between metabolic signals, atherosclerosis, and hypercoagulability in type 2 diabetic rats. However, further studies are needed to clarify this relationship in human cardiovascular diseases. [Journal of American Science. 2010;6(11):217-227]. (ISSN: 1545-1003).

**Keywords:** Resistin, Streptozotocin, high fat, diabetes, haemostasis

### 1. Introduction

Obesity is associated with an array of health problems in adult and pediatric population. Understanding the pathogenesis of obesity and its metabolic sequelae has advanced rapidly over the past decades (American Diabetes Association, 2007).

Adipose tissue represents an active endocrine organ that, in addition to regulating fat mass and nutrient homeostasis, releases a large number of bioactive mediators (adipokines) that signal to organs of metabolic importance including brain, liver, skeletal muscle, and immune system, thereby modulating haemostasis, blood pressure, lipid and glucose metabolism, inflammation, and atherosclerosis (Rabe et al., 2008).

In (2001), Steppan et al. discovered a novel adipocyte-derived hormone called resistin, which was expressed exclusively in white adipose tissue as a member of a family of cysteine-rich proteins called resistin-like molecules.

Intra-peritoneally administered resistin augments blood glucose and plasma insulin levels and limits the hypoglycemic response to insulin infusion, furthermore, resistin suppresses insulin-stimulated glucose uptake in cultured adipocytes, and this effect is prevented by exposure to anti-resistin antibodies (steppan et al., 2001).

Hence, these data suggest that resistin could contribute to the insulin resistance observed in

obesity by decreasing insulin sensitivity (Rajala et al., 2003, Muse et al., 2007).

In fact, obese subjects show a reduced insulin-stimulated skeletal muscle glucose uptake as well as an impaired insulin-evoked vasodilatation (Baron, 1994) and these observations have suggested that the pathophysiological mechanisms linking obesity to the development of cardiovascular diseases could go beyond the classical metabolic derangements, so, much effort has been made to understand the interaction between insulin resistance and vascular function, with particular emphasis on adipocyte-derived hormones and their effects on vascular homeostasis (verma et al., 2003).

Resistin has been shown to selectively impair the effect of insulin on endothelial nitric oxide synthase (eNOS) enzymatic activity and through this mechanism resistin can reduce insulin-evoked vasorelaxation (Gentile et al., 2008). Moreover, in high fat-fed rats, resistin levels correlate negatively with vascular nitric oxide (NO) levels even after correction of insulin measurements, which suggests a direct inhibitory role of resistin on NO secretion (Li et al., 2007).

Also, plasma resistin levels were reported to be associated with many inflammatory markers including C-reactive protein, tumor necrosis factor-alpha (TNF- $\alpha$ ) and interleukin-6 (IL-6) (Silswal et al., 2005, Stofkova, 2010). Considering the crosstalk between inflammatory pathways and the insulin signaling cascade, resistin may represent a link between metabolic signals, inflammation and atherosclerosis (Lehrke et al., 2004, Daniel et al., 2010).

In general, diabetes is associated with an excessive risk of cardiovascular events (Williams et al., 2002), in that the coagulation system is switched towards a pro-thrombotic state involving, increased blood coagulation, decreased endothelial thrombo-resistance and pro-inflammatory state (Palomo et al., 2006), thereby increasing the risk of micro-vascular disease as well as macro-vascular diseases (Lender and Sysko, 2007).

So, this study was designed in a trial to clarify any possible relationship between resistin levels and some haemostatic changes in streptozotocin-induced diabetic rats and high fat diet-fed rats.

## **2. Material and Methods**

This study was conducted on 40 healthy, adult, male albino rats weighing 200–260 gm (animals were obtained from faculty of medicine animal house and the animal experiments were approved by the local ethics committee). The rats had free access to water and chow and are kept at room temperature. All rats received standard chow (25.8 % protein, 62.8 %

carbohydrate and 11.4 % fat (Ahren and Scheurink, 1998) except the rats in high fat-fed group, which received high-fat chow (16.4% protein, 25.6% carbohydrate, and 58.0% fat (a total 23.4 kJ/g) in the form of cotton seed oil added to the laboratory chow diet (Cha et al., 2000), (Diets were obtained from faculty of agriculture, Zagazig university).

The animals were divided into 2 main groups:

Group I: To study the effect of streptozotocin-induced type 1 diabetes on the measured parameters. This group was further divided into 3 equal subgroups and survived for 30 days:

Group Ia: "Control group (n=8)". Each rat was intra-peritoneally (i.p.) injected with 0.2 mmol/L Na citrate (0.1 mL).

Group Ib: "Experimental diabetic non-treated group (n=8)". Diabetes was induced by single intra-peritoneal injection of freshly prepared solution of streptozotocin (sigma) 65 mg/kg of body weight dissolved in 0.2 mmol/L sodium citrate, at PH 4.5 (Lutz and Pardridge, 1993) and maintained for 30 days (Toba et al., 2009).

Three days later, diabetes induction was confirmed through measurement of blood glucose level in each animal (from blood sampled from the tail vein) with the One Touch Ultra Glucometer (Yves and Theo, 2007) and rats with blood glucose levels more than 250 mg/dl were selected for experiments (Coskun et al., 2004). The rats were provided with 10% glucose solution after 6 hours of streptozotocin administration for the next 48 hours.

Group Ic: "Experimental diabetic group treated with insulin (n=8)". These animals were treated with regular (R) and NPH (N) insulin (2UR at diagnosis of diabetes and then 1R/3N at 6 P.M and 1R/1N at 9 A.M daily subcutaneously for 30 days after induction of diabetes (Sivitz et al., 1998).

Group II: To study the effect of high fat diet (HFD) on the measured parameters. This group was further divided into 2 equal subgroups:

Group IIa: "control group (n=8)", which was fed a standard chow for 7 weeks.

Group IIb: "High fat diet fed group (n=8)". These rats were fed a high-fat chow for 7 weeks.

For all groups, body weight was recorded per week, and at the end of the study period.

Haemostatic measurements:

-Determination of bleeding time (BT) according to Martin, (1981).

-Determination of whole blood clotting time (WBCT) according to Quick, (1966).

-Determination of prothrombin time (PT) according to Ansell, (1992).

-Determination of activated partial thromboplastin time (aPTT) according to Ansell, (1992).

-Estimation of plasma fibrinogen levels according to Cooper and Douglas, (1991).

-Estimation of plasma D-dimmers levels according to Declerck et al. (1987).

#### Biochemical and Hormonal measurements:

- Estimation of serum glucose levels according to Trinder, (1969).

- Estimation of serum insulin levels according to Reaven, (1991).

-Estimation of serum resistin levels: by enzyme-linked immunoassay kits from Linco Research Inc., (USA) according to Steppan and Lazar, (2004).

-Estimation of serum total cholesterol (TC) levels according to and Allain et al. (1974).

--Estimation of serum triglycerides (TG) levels according to Naito, (1989).

-Estimation of serum high density lipoproteins (HDL) levels according to Warnick et al. (1983)

-Estimation of serum low density lipoproteins (LDL) levels according to Friedwald et al. (1972).

-Determination of platelet count according to Brecher et al. (1953).

#### Calculations:

The homeostasis model of assessment of insulin resistance (HOMA-IR) = fasting blood glucose (mmol/l) x fasting insulin (uIU/ml)/22.5 was calculated as an index of insulin resistance (Matthews et al., 1985) in group II.

#### Statistical analysis:

Data were presented as mean  $\pm$  SD. Statistical significance was determined by unpaired Student's "t" test, P values less than 0.05 were considered to be significant. The correlations between parameters were analyzed using Pearson,s correlation.

In statistical analysis, SPSS version 10.0 programs for Windows (SPSS Inc. Chicago, IL, USA) was used.

### 3. Results

**Table 1:** Body weight and the metabolic parameters of the studied groups:

	Group Ia	Group Ib	Group Ic	Group IIa	Group IIb
Initial BW (gr)	222.5 $\pm$ 16.1	219.4 $\pm$ 18.9	224.6 $\pm$ 21	213.7 $\pm$ 14.8	225.5 $\pm$ 19.3
Final BW (gm) r=0.73*	252.3 $\pm$ 16.5 r=0.73*	192 $\pm$ 14.8*** r=0.86**	251.3 $\pm$ 17.5 r=0.82*	242.5 $\pm$ 15.1 r=0.92**	339 $\pm$ 19.4*** r=0.97***
Glucose (mg/dl) r=0.31	75.6 $\pm$ 6.6 r=0.31	411.5 $\pm$ 97.6*** r=0.19	79.4 $\pm$ 5.5 r=0.12	86.1 $\pm$ 8.7 r=0.65	262.5 $\pm$ 37.4*** r=0.98***
Insulin (uIU/ml) r=0.55	19.7 $\pm$ 2.5 r=0.55	1.24 $\pm$ 0.36*** r=0.5	66 $\pm$ 9.2*** r=0.64	18.4 $\pm$ 3.1 r=0.03	46.1 $\pm$ 4.8*** r=0.97***
HOMA-IR				4.13 $\pm$ 0.47 r=0.03	30.28 $\pm$ 7.26*** r=0.99***
Resistin (ng/ml)	7.2 $\pm$ 0.77	3.99 $\pm$ 0.2***	7.4 $\pm$ 0.6	7.6 $\pm$ 0.46	14 $\pm$ 0.78***
TC (mg/dl) r=0.32	115.25 $\pm$ 6.5 r=0.32	223.6 $\pm$ 24.2*** r=0.45	110.5 $\pm$ 8.5 r=0.39	113.75 $\pm$ 7.8 r=0.04	208.6 $\pm$ 26*** r=0.73*
TG (mg/dl) r=0.35	49.75 $\pm$ 5.3 r=0.35	82.6 $\pm$ 6.5*** r=0.37	51.5 $\pm$ 7.5 r=0.33	54.25 $\pm$ 7.5 r=0.25	188.25 $\pm$ 12.5*** r=0.9**
HDL (mg/dl) r= -0.52	64.4 $\pm$ 10.2 r= -0.52	35.5 $\pm$ 4.2*** r= -0.25	54.9 $\pm$ 10.2 r= -0.22	40.9 $\pm$ 4.2 r= -0.05	36.1 $\pm$ 4.4* r= -0.91**
LDL (mg/dl) r=0.53	40.9 $\pm$ 13.3 r=0.53	171.6 $\pm$ 24.3*** r=0.47	47.4 $\pm$ 13.7 r=0.25	62.03 $\pm$ 7.4 r=0.01	134.9 $\pm$ 27.7*** r=0.75*

r=correlation coefficient versus resistin levels.

\*=significant (P<0.05).

\*\*=significant (P<0.01).

\*\*\*=significant (P<0.001).

**Table 2:** The haemostatic parameters of the studied groups:

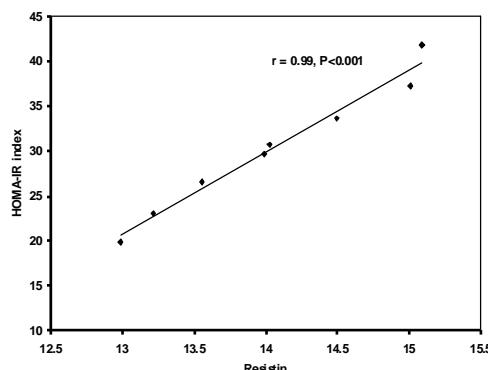
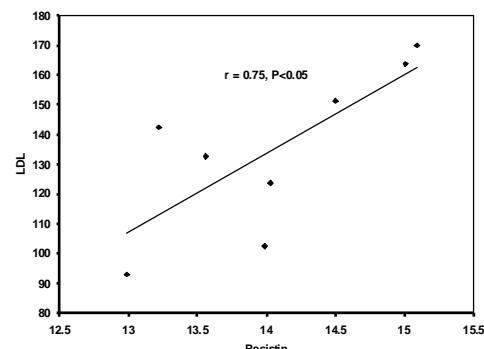
	Group Ia	Group Ib	Group Ic	Group IIa	Group IIb
BT (sec)	214.4±26.5 r= -0.33	150±19.4*** r= -0.52	202.5±35.6 r= -0.38	212.5±25.2 r= -0.31	170.6±14.5*** r= -0.97***
WBCT (sec)	236.1±21.6 r= -0.002	165.1±27*** r= -0.16	220.75±32.4 r= -0.69	228.3±28.7 r= -0.28	166±14.2*** r= -0.88**
PT (sec)	13.3±0.85 r= -0.06	10.25±1.7*** r= -0.17	13.55±0.65 r= -0.25	13.2±1 r= -0.29	10.6±1.28*** r= -0.8*
aPTT (sec)	24.1±2.7 r= -0.2	13.6±4.5*** r= -0.37	23±2.4 r= -0.22	25.6±3.8 r= -0.02	14.7±4.5*** r= -0.93**
Fibrinogen (mg/dl)	272.2±44.6 r=0.52	512.4±89.6*** r=0.19	287.2±46.2 r= -0.59	286.6±56.1 r=0.17	438.9±66.2*** r=0.84**
D-Dimmers (mg/dl)	171.7±34.6 r=0.49	255.1±20.6*** r=0.6	170.8±15.9 r=0.28	147.75±27.6 r= 0.02	216.87±27*** r=0.73*
Platelet cou (1000/mm <sup>3</sup> )	211.9±17.8 r=0.55	302±21.7*** r=0.05	226.4±19.1 r=0.02	220.4±20.4 r=0.08	216.1±13.8 r= 0.43

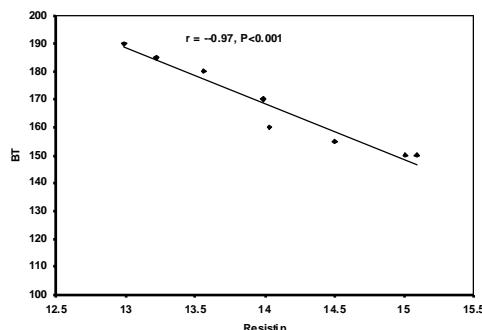
r=correlation coefficient versus resistin levels.

\*=significant (P&lt;0.05).

\*\*=significant (P&lt;0.01).

\*\*\* =significant (P&lt;0.001).

**Figure (1):** Correlation between serum resistin levels and HOMA IR index in group IIb.**Figure (2):** Correlation between serum resistin levels and serum levels of LDL in group IIb.



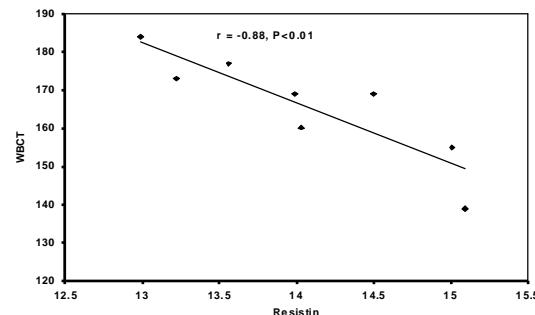
**Figure (3):** Correlation between serum resistin levels and bleeding time in group IIb.

The metabolic, hormonal and the haemostatic parameters of the groups are summarized in table 1&2, respectively. There was a significant positive correlation between the final body weight and serum resistin levels in all groups ( $p<0.05$ ,  $p<0.01$ ,  $p<0.05$ ,  $p<0.01$ ,  $p<0.001$ , respectively).

As regards group I, the results of this study showed a significant decrease in serum insulin and resistin ( $p<0.001$ ) levels in group Ib in comparison with that of control group (Ia) in association with increased blood glucose levels ( $p<0.001$ ). In addition, there was a significant increase ( $p<0.001$ ) in the serum levels of TC, TG and LDL while the serum levels of HDL were significantly decreased ( $p<0.001$ ) in the same group. Concerning the haemostatic parameters in group Ib, there was a significant decrease ( $p<0.001$ ) in BT, WBCT, PT, aPTT while plasma fibrinogen levels, D-dimmers and platelets count were significantly increased ( $p<0.001$ ). Moreover, all these metabolic and haemostatic disturbances were normalized in insulin treated group.

Finally, no significant correlation could be detected between serum resistin levels and metabolic, hormonal or haemostatic measured parameters in this group.

As regards group II, group IIb showed a significant increase ( $p<0.001$ ) in body weight, serum glucose levels, insulin levels, HOMA-IR, TC levels, TG levels, and LDL levels, in addition to the significant decrease ( $p<0.001$ ) in HDL levels in comparison with that of controls (IIa). Moreover, our study revealed a significant increase ( $p<0.001$ ) in serum resistin levels in this group in comparison with that of controls, which was correlated positively and significantly ( $p<0.001$ ) with body weight, serum glucose levels, insulin levels and HOMA-IR. Also, a significant positive correlation was found between serum resistin levels and serum levels of TC ( $p<0.05$ ), TG ( $p<0.01$ ) and LDL ( $p<0.01$ ) while a



**Figure (4):** Correlation between serum resistin levels and WBCT time in group IIb.

significant negative correlation ( $p<0.05$ ) between its levels and serum levels of HDL was reported.

Moreover, BT, WBCT, PT and aPTT were found to be significantly decreased ( $p<0.001$ ), while, plasma fibrinogen and D-dimmers levels were found to be significantly increased ( $p<0.001$ ). However, no significant difference in the platelets count was found. Furthermore, serum resistin levels were found to be correlated negatively and significantly with BT ( $p<0.001$ ), WBCT ( $p<0.01$ ), PT ( $p<0.05$ ), aPTT ( $p<0.01$ ) and positively with plasma fibrinogen ( $p<0.01$ ) and D-dimmers ( $p<0.05$ ) levels.

#### 4. Discussions

The results of this study showed a significant decrease in serum insulin and resistin levels in streptozotocin-induced diabetic group in comparison with that of control group and insulin-treated group, in association with increased blood glucose levels. This decrease in serum resistin levels could be attributed to the weight loss that occurs in type1 diabetes, as resistin levels were positively and significantly correlated with the body weight in this study, which is in agreement with that of Stroubini et al. (2009).

In addition, our results indicated that there was a significant increase in the serum levels of total cholesterol, triglycerides and LDL while the serum levels of HDL were significantly decreased in streptozotocin-induced type 1 diabetes which is defined as an atherogenic lipid profile (Vergè, 2009).

Concerning the haemostatic parameters in group Ib, there was a significant decrease in BT, WBCT, PT, aPTT while D-dimmers, platelets count and plasma fibrinogen levels were significantly increased, denoting a hyper-coagulable state (Khatun et al., 1999). Moreover, all these metabolic (Sivitz et al., 1998) and haemostatic (Sobel, 2003, Nishikawa et al., 2008) disturbances were normalized in insulin treated group.

In addition, no significant correlation could be detected between serum resistin levels and either metabolic or haemostatic measured parameters in this group.

So, it can be concluded that resistin has no role in type 1 diabetes as regards haemostatic changes, however, these changes could be attributed to the presence of high levels of LDL particles, as these particles have atherogenic properties (Skryme-Jones et al., 2000). Also, lowered HDL may play a role as HDL has antioxidative, anti-inflammatory, anti-thrombotic and vasorelaxant properties, all of which are potentially anti-atherogenic (Link et al., 2007). Therefore, it can be concluded that this dyslipidemia is associated with an increased cardiovascular risk in type 1 diabetes (Vergè, 2009).

As regards group HFD group, it was found to show marked increase in body weight, insulin resistance and dyslipidemia in rats. These results are in accordance with those of Willett, (2002) and Schaal et al. (2009).

Moreover, our study revealed a significant increase in serum resistin levels in this group in comparison with that of controls, which correlate positively and significantly with body weight, serum glucose levels, insulin levels and HOMA-IR index, while no significant correlation could be found between its levels and those measured parameters in controls except a significant positive correlation with body weight.

These results are supported by that of Azuma et al. (2003) and Silha et al. (2003) who reported that the mean circulating resistin levels in obese subjects is increased about four folds compared with lean subjects and by Stroubini et al. (2009), who reported that resistin levels were elevated in many experimental models of obesity and decreased after weight loss.

Moreover, de Luis et al. (2009) demonstrated that resistin concentrations were related to the total fat mass in patients with metabolic syndrome.

Concerning resistin-insulin relationship, our results are supported by those who concluded that, administration of antiresistin antibody improved insulin action and glucose metabolism in mice with diet-induced obesity (Steppan et al., 2001). While, infusion of recombinant resistin to rats rapidly induces hepatic IR and increases hepatic glucose production (Rajala et al., 2003). Also, ablation of the resistin gene in mice decreases fasting glucose through reducing gluconeogenesis, while resistin administration in these resistin-deficient mice increases hepatic glucose production (Banerjee et al., 2004).

Resistin primarily exerts its glucoregulatory effect by stimulating hepatic glucose output (Rangwala et al., 2004). As elevation of circulating resistin in rodents, either acutely (Muse et al., 2007) or chronically (as following diet-induced obesity) (Muse et al., 2004), leads to marked decreases in hepatic insulin sensitivity.

Moreover, the findings of this study are in line with that of Kushiyama et al. (2005), who found that transgenic mice with hepatic resistin over-expression exhibit significant hyperglycemia, hyperlipidemia, fatty liver, and pancreatic islet enlargement, when fed an HFD. These effects may be due to resistin-induced impairment of glucose homeostasis and insulin action, thus modulating one or more steps in the insulin signaling pathway and possibly playing a role in the pathogenesis of insulin resistance (Muse et al., 2004).

The majority of in vivo studies showed that resistin has a negative effect on insulin signaling in the liver (Qi et al., 2006).

In contrast with our results, no significant correlation was found between resistin levels and glucose levels in high fat-fed rats (Li et al., 2007), and also in patients with type 2 diabetes mellitus (T2DM) (Mojiminiyi and Abdella, 2007). Also, some studies have observed significant low resistin mRNA levels in adipose tissue in different obese mouse models, such as db/db, or high-fat-diet-induced obesity, and in rat models characterized by IR (Way et al., 2001).

The mechanism whereby resistin decreases insulin sensitivity involves several impacts. First, resistin reduces adenosine 5'-monophosphate-activated protein kinase activity in skeletal muscle, adipose tissue, and liver. In addition, insulin receptor substrate-1 (IRS-1) and IRS-2 protein levels and phosphorylation states, as well as protein kinase B activity, were decreased in hyperresistinemic animal tissues. These alterations decrease tissue insulin sensitivity that results in glucose intolerance, hyperinsulinemia, elevated free fatty acid (FFA) levels, and hypertriglyceridemia (Rajala et al., 2003).

Secondly, the resistin-induced reduction in IRS-1 and IRS-2 elevates mRNA levels of gluconeogenetic enzymes, such as glucose-6-phosphatase (G-6-Pase) and phosphoenolpyruvate carboxykinase, thus suggesting a direct resistin induction of insulin resistance in the liver (Moon et al., 2003).

Thirdly, it was found that resistin decreased glycogen synthase (GS) activity both in the presence or absence of insulin, this suggests that resistin directly down-regulates GS activity (Ferrer et al., 2003).

Insulin signaling in pancreatic islets plays an important role in the maintenance of -cell functions and glucose-induced insulin secretion in islets of pancreas (Otani et al., 2004). Therefore, the inhibition of insulin signaling could underlie the impairment of glucose-induced insulin secretion by resistin (Nakata et al., 2007).

Moreover, it was observed that there is a positive correlation between resistin levels and C-reactive protein (CRP) (Kunnari et al., 2006). Accordingly, the correlation between fasting glucose and resistin levels might be explained by this inflammatory state (Bo et al., 2005).

As regards the lipid profile in the high fat diet-fed group, our results revealed a significant increase in serum levels of total cholesterol, triglycerides and LDL, while serum levels of HDL were significantly decreased (atherogenic lipid profile). Also, a significant positive correlation was found between serum resistin levels and serum levels of total cholesterol, triglycerides and LDL while a significant negative correlation between its levels and serum levels of HDL was reported.

These results are supported by those of Mojiminiyi and Abdella (2007), who concluded that resistin was correlated positively and significantly with atherogenic lipid profile in type 2 diabetic patients, and in patients with metabolic syndrome (de Luis et al., 2009).

On the contrary to our results, Qi et al. (2008), found no significant correlation between resistin levels and lipid profile parameters except a negative correlation with HDL levels only in patients with metabolic syndrome.

Moreover, it was reported that resistin directly increases the endothelial expression of adhesion molecules, vascular wall adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1) that play central roles in the early stage of the atherogenic processes (Burnett et al., 2005), it also promotes lipid accumulation in human macrophages by up-regulating CD36 cell surface expression, which is one of the scavenger receptors in macrophages involved in the uptake of modified LDL (Xu et al., 2006). Based on these data, resistin is supposed to induce atherosclerosis by mediating endothelial hyperactivity in response to the systematic inflammatory condition in human (Tsukahara et al., 2009).

At the cellular level, resistin has also been shown to exert potent pro-inflammatory properties by up-regulating pro-inflammatory cytokines, probably via the nuclear factor- $\kappa$ B (NF- $\kappa$ B) pathway, and resistin can induce inflammation in animal models (Stofkova, 2010). Besides this inflammatory induction, resistin also promotes proliferation and

activation of human smooth muscle cells and endothelial cells, (Calabro et al., 2004) and induces angiogenic responses in endothelial cells, in part via phosphorylation and activation of different phosphate-kinase pathways (Mu et al., 2006).

Accordingly, it was reported that resistin may influence angiogenesis, not only in adipose tissue but also at other sites, so that systemic concentrations of resistin may contribute to the development of vascular disease (Robertson et al., 2009).

Hence, considering the expression of resistin by mononuclear cells and that obesity and T2DM are states of low-grade inflammation with activated inflammatory cascades, resistin may indeed present a molecular link between metabolic signals, inflammation and atherosclerosis (Kadoglou et al., 2007).

As insulin resistance is associated not only with hyperinsulinemia and hyperglycemia but also with other disorders such as abnormal lipid profile (Ding et al., 2005). These findings indicate a link between lipid profile and insulin sensitivity, since systemic excess of FFAs impairs the ability of insulin to stimulate glucose metabolism, contributing to whole-body insulin resistance (Schaalan et al., 2009).

In addition, several studies reported that resistin is implicated in the control of lipolysis (Rae et al., 2007). Also, in the humanized resistin mice, resistin was found to increase hormone sensitive lipase (HSL) activity by inducing white adipose tissue (WAT) inflammation and enhance the phosphorylation of HSL at its activating protein kinase-A (PKA) site (Qatanani et al., 2009).

Taken together, it could be postulated that the significant relationship between serum resistin levels and atherogenic lipid profile may be due to a direct effect in addition to induction of insulin resistance.

In relation to the haemostatic changes in the same group (group IIb), BT, WBCT, PT and aPTT were found to be significantly decreased, while, plasma fibrinogen and D-dimers levels were found to be significantly increased (indicating hypercoagulable state). However, no significant difference in the platelets count was found. Furthermore, serum resistin levels were found to be correlated negatively and significantly with BT, WBCT, PT and aPTT and positively with plasma fibrinogen and D-dimmers levels.

Our findings are in line with those of other investigators who concluded that, resistin is an emerging cardiovascular risk factor implicated in T2DM (Kershaw and Flier, 2004). Furthermore, the patients with myocardial infarction showed higher plasma resistin levels especially those with coronary

heart disease (CHD) when compared with the controls (Burnett et al., 2006).

Hyperfibrinogenaemia is associated with an increased prevalence and incidence of primary and recurrent CHD and thrombosis (Mc Dermott et al., 2003) and correlates with measures of obesity in several studies (Woodward et al., 1997).

Also, Menzaghi et al. (2006) reported a significant positive correlation between resistin levels and fibrinogen levels in insulin resistant patients.

Furthermore, it was found that, resistin has been shown to selectively impair the effect of insulin on endothelial nitric oxide synthetase (eNOS) enzymatic activity and indicate a mechanism through which resistin can reduce insulin-evoked vasorelaxation (Gentile et al., 2008).

Moreover, resistin induces serine protease (Akt)-dependent endothelial NO dysfunction through the inhibition of IRS-1 signaling pathway and IRS-1 itself is present in a lower amount in cells challenged with insulin and pretreated with resistin suggesting that resistin interferes with the insulin-stimulated IRS-1-dependent signaling pathway, acting both on the IRS-1 protein and on its ability to activate phosphatidyl inositol tri-phosphate kinase (PI3K) (Palanivel et al., 2006).

Moreover, in high fat diet-fed rats, resistin levels correlate negatively with vascular NO levels even after correction of insulin levels, which suggests a direct inhibitory role of resistin on NO secretion (Li et al., 2007). And since endothelial NO has a crucial role not only in modulating vascular tone but also in anti-atherogenic protection (Myazaki et al., 2003) by inhibiting inflammation, oxidation, vascular smooth muscle cell proliferation and migration, it can be speculated that endothelial NO dysfunction induced by resistin could also participate to the enhanced atherosclerotic process that occurs in obese subjects (Gentile et al., 2008).

In vitro studies of Takahashi et al. (2006), who described that resistin activates endothelial cell directly by promoting endothelin-1 (ET-1) release and expression of ET Jung et al. (2006). In addition, Li et al. (2007) reported a significant positive correlation between resistin levels and ET levels in high fat-fed rats.

Furthermore, Li et al. (2007) concluded that chronic administration of resistin in rats produced a significant increase in plasminogen activator inhibitor-1 (PAI-1) and Von Wellebrand factor (VWF). Moreover, they also reported that, diet-induced hyperresistinemia in rats correlated positively with levels of PAI-1 and VWF even after correction of insulin levels. In addition, Qi et al. (2008) reported that, resistin was correlated positively with PAI-1 levels in women with

metabolic syndrome. In fact, the increased levels of PAI-1 found in obesity may predispose to micro- and macro-vascular, arterial and venous thrombosis (Lundgren et al., 1996).

## 5. Conclusion:

No role for resistin in metabolic and haemostatic changes in type 1 diabetic rats was detected. Although, hyperresistinemia may represent a novel link between metabolic signals, atherosclerosis, and hypercoagulability in type 2 diabetic rats. However, further studies are needed to clarify this relationship in human cardiovascular diseases.

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6/5/2010

# Analytic Investigation and Numeric Prediction for Biodynamic Response of the Seated Human Body

Mostafa A. M. Abdeen<sup>a</sup>, W. Abbas<sup>b</sup>

<sup>a</sup> Dept. of Eng. Mathematics and Physics, Faculty of Eng. Cairo University, Egypt

<sup>b</sup>Eng. Physics and Mathematics Dept., Faculty of Eng. (Mataria), Helwan University, Egypt

[msotafa\\_a\\_m\\_abdeen@hotmail.com](mailto:msotafa_a_m_abdeen@hotmail.com); [wael\\_abass@hotmail.com](mailto:wael_abass@hotmail.com)

**Abstract:** The biodynamic response behaviors of seated human body subject to whole-body vibration have been widely investigated. The biodynamic response characteristics of seated human subjects have been extensively reported in terms of apparent mass and driving-point mechanical impedance while seat-to-head vibration transmissibility has been widely used to characterize response behavior of the seated subjects exposed to vibration. These functions (apparent mass, driving-point mechanical impedance) describe "to-the-body" force-motion relationship at the human-seat interface, while the transmissibility function describes "through-the-body" vibration transmission properties. The current study proposed a 4-DOF analytic biomechanical model of the human body in a sitting posture without backrest in vertical vibration direction to investigate the biodynamic responses of different masses and stiffness. Following the analytical approach, numerical technique developed in the present paper to facilitate and rapid the analysis. The numerical analysis used here applies one of the artificial intelligence technique to simulate and predict the response behaviors of seated human body for different masses and stiffness without the need to go through the analytic solution every time. The Artificial Neural Network (ANN) technique is introduced in the current study to predict the response behaviors for different masses and stiffness rather than those used in the analytic solution. The results of the numerical study showed that the ANN method with less effort was very efficiently capable of simulating and predicting the response behaviors of seated human body subject to whole-body vibration. [Journal of American Science. 2010;6(11):228-239]. (ISSN: 1545-1003).

**Keywords:** Biodynamic response, Analytic seated human body model, Numerical simulation model, Artificial Neural Network.

## 1. Introduction

The biodynamic responses of seated human occupant exposed to vibration have been widely characterized to define frequency-weightings for assessment of exposure, to identify human sensitivity and perception of vibration, and to develop seated body models [1]. The biodynamic response of the human body exposed to vibration have been invariably characterized through measurement of force motion relationship at the point of entry of vibration "To-the-body response function", expressed as the driving-point mechanical impedance (DPMI) or the apparent mass (APMS) and transmission of vibration to different body segments "Through-the-body response function", generally termed as seat-to-head transmissibility (STHT) for the seated occupant. Considering that the human body is a complex biological system, the "To-the-body" response function is conveniently characterized through non-invasive measurements at the driving point alone.

The vast majority of the reported studies on biodynamic response to whole-body vibration have considered vibration along the vertical axis alone. In many of the early studied, such as those conducted by

Coermann [2], Vogt [3], and Suggs [4], the numbers of subjects was usually relatively small, and only sinusoidal excitation was used, not generally representative of the type of excitation and levels of vibration usually encountered in practice. In many of these studies, the feet of the subjects were either not supported or supported but not vibrated, a condition not common in most driving situations. Fairley and Griffin [5], reported the vertical apparent mass of 60 seated subjects including men, women and children, which revealed a large scatter of data presumably owing to large variations in the subject masses. Boileau et al. [6] investigated the relationships between driving point mechanical impedance and seat-to-head transmissibility functions based upon 11 reported one dimensional lumped parameter models.

The majority of the models showed differences in frequencies corresponding to peak magnitudes of the two functions, which were expressed as resonant frequencies. Toward [7], summarized that a support of the back caused higher resonance frequency and slightly lower peak magnitude of the APMS response for subjects sitting on a horizontal plane. Wang et al. [8], study the

vertical apparent mass and seat-to-head transmissibility response characteristics of seated subjects are derived through measurements of total biodynamic force at the seat pan, and motions of the seat pan and head along the applied input acceleration direction, using 12 male subjects. The data were acquired under three different back support conditions and two different hands positions representative of drivers and passengers-like postures. Steina et al.[9], analyzed apparent mass measurements in the y- direction with a group of 13 male test subjects exposed to three excitation intensities.

In early studies, various biodynamic models have been developed to depict human motion from single-DOF to multi-DOF models. These models can be divided as distributed (finite element) models, lumped parameter models and multi-body models. The distributed model treats the spine as a layered structure of rigid elements, representing the vertebral bodies, and deformable elements representing the intervertebral discs by the finite element method. Multi-body human models are made of several rigid bodies interconnected by pin (two-dimensional) or ball and socket (three-dimensional) joints, and can be further separated into kinetic and kinematic models. It is clear that the lumped-parameter model is probably one of the most popular analytical methods in the study of biodynamic responses of seated human subjects, though it is limited to one-directional analysis. However, vertical vibration exposure of the driver is our main concern. Therefore, this paper carries out a thorough survey of literature on the lumped-parameter models for seated human subjects exposed to vertical vibration.

The lumped parameter models consider the human body as several rigid bodies and spring-dampers. This type of model is simple to analyze and easy to validate with experiments. However, the disadvantage is the limitation to one-directional analysis. Coermann [2], measured the driving-point impedance of the human body and suggested 1-DOF model. Suggs et al. [4], developed a 2-DOF human body. It was modeled as a damped spring-mass system to build a standardized vehicle seat testing procedure. A 3-DOF analytical model for a tractor seat suspension system is presented by Tewari et al. [10]. It was observed that the model could be employed as a tool in selection of optimal suspension parameters for any other type of vehicles. Boileau et al. [11] used an optimization procedure to establish a 4-DOF human model based on test data.

It is quite clear from the literature mentioned previously the amount of effort (experimentally or analytically) required to accurately investigate and understand the biodynamic response behaviors of

seated human body subject to whole-body vibration of different types and magnitudes. This fact urged the need for utilizing new technology and techniques to facilitate this comprehensive effort and at the same time preserving high accuracy.

Artificial intelligence has proven its capability in simulating and predicting the behavior of the different physical phenomena in most of the engineering fields. Artificial Neural Network (ANN) is one of the artificial intelligence techniques that have been incorporated in various scientific disciplines. Ramanitharan and Li [12] utilized ANN with back-propagation algorithm for modeling ocean curves that were presented by wave height and period. Abdeen [13] developed neural network model for predicting flow depths and average flow velocities along the channel reach when the geometrical properties of the channel cross sections were measured or vice versa. Allam [14] used the artificial intelligence technique to predict the effect of tunnel construction on nearby buildings which is the main factor in choosing the tunnel route. Allam, in her thesis, predicted the maximum and minimum differential settlement necessary precautionary measures. Azmathullah et al. [15] presented a study for estimating the scour characteristics downstream of a ski-jump bucket using Neural Networks (NN). Abdeen [16] presented a study for the development of ANN models to simulate flow behavior in open channel infested by submerged aquatic weeds. Mohamed [17] proposed an artificial neural network for the selection of optimal lateral load-resisting system for multi-story steel frames. Mohamed, in her master thesis, proposed the neural network to reduce the computing time consumed in the design iterations. Abdeen [18] utilized ANN technique for the development of various models to simulate the impacts of different submerged weeds' densities, different flow discharges, and different distributaries operation scheduling on the water surface profile in an experimental main open channel that supplies water to different distributaries.

## 2. Problem Description

To investigate the biodynamic response behaviors of seated human body subject to whole-body vibration (sinusoidal wave with amplitude  $5 \text{ m/s}^2$ ), analytical and numerical techniques will be presented in this study. The analytical model and its results will be described in detail in the following sections. The numerical models presented in this study utilized Artificial Neural Network technique (ANN) using the data and the results of the analytical model to understand the biodynamic response behaviors and then can predict the behaviors for

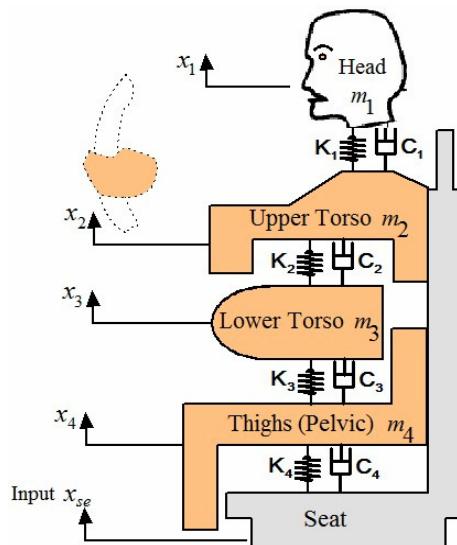
different data of the human body without the need to go through the analytical solution.

### 3. Analytical Model

#### 3.1 Biomechanical modeling

The human body in a sitting posture can be modeled as a mechanical system that is composed of several rigid bodies interconnected by springs and dampers. (Boileau and Rakheja [11]. This model as shown in Fig. 1 consists of four mass segments interconnected by four sets of springs and dampers. The four masses represent the following four body segments: the head and neck ( $m_1$ ), the chest and upper torso ( $m_2$ ), the lower torso ( $m_3$ ), and the thighs and pelvis in contact with the seat ( $m_4$ ). The mass due to lower legs and the feet is not included in this representation, assuming they have negligible contributions to the biodynamic response of the seated body. The stiffness and damping properties of thighs and pelvis are ( $k_4$ ) and ( $c_4$ ), the lower torso are ( $k_3$ ) and ( $c_3$ ), upper torsos are ( $k_2$ ) and ( $c_2$ ), and head are ( $k_1$ ) and ( $c_1$ ).

The equation of motion of the human body can be obtained as follows:



**Figure 1:** Biomechanical Boileau and Rakheja 4-DOF model.

$$\left\{ \begin{array}{l} m_1 \ddot{x}_1 = -c_1(\dot{x}_1 - \dot{x}_2) - k_1(x_1 - x_2), \\ m_2 \ddot{x}_2 = c_1(\dot{x}_1 - \dot{x}_2) + k_1(x_1 - x_2) \\ \quad - c_2(\dot{x}_2 - \dot{x}_3) - k_2(x_2 - x_3), \\ m_3 \ddot{x}_3 = c_2(\dot{x}_2 - \dot{x}_3) + k_2(x_2 - x_3) \\ \quad - c_3(\dot{x}_3 - \dot{x}_4) - k_3(x_3 - x_4), \\ m_4 \ddot{x}_4 = +c_3(\dot{x}_3 - \dot{x}_4) + k_3(x_3 - x_4) \\ \quad - c_4(\dot{x}_4 - \dot{x}_{se}) - k_4(x_4 - x_{se}). \end{array} \right. \quad (1)$$

The system equations of motion, equation (1), for the model can be expressed in matrix form as follows:

$$[\mathbf{M}]\{\ddot{\mathbf{x}}\} + [\mathbf{C}]\{\dot{\mathbf{x}}\} + [\mathbf{k}]\{\mathbf{x}\} = \{\mathbf{f}\} \quad (2)$$

Where  $[\mathbf{M}]$ ,  $[\mathbf{C}]$ , and  $[\mathbf{k}]$  are  $n \times n$  mass, damping, and stiffness matrices, respectively;  $\{\mathbf{f}\}$  is the force vector due to external excitation.

$$[\mathbf{M}] = \begin{bmatrix} m_1 & 0 & 0 & 0 \\ 0 & m_2 & 0 & 0 \\ 0 & 0 & m_3 & 0 \\ 0 & 0 & 0 & m_4 \end{bmatrix},$$

$$[\mathbf{C}] = \begin{bmatrix} c_1 & -c_1 & 0 & 0 \\ -c_1 & c_1 + c_2 & -c_2 & 0 \\ 0 & -c_2 & c_2 + c_3 & -c_3 \\ 0 & 0 & -c_3 & c_3 + c_4 \end{bmatrix},$$

$$[\mathbf{k}] = \begin{bmatrix} k_1 & -k_1 & 0 & 0 \\ -k_1 & k_1 + k_2 & -k_2 & 0 \\ 0 & -k_2 & k_2 + k_3 & -k_3 \\ 0 & 0 & -k_3 & k_3 + k_4 \end{bmatrix}.$$

And,

$$\{\mathbf{f}\} = \begin{Bmatrix} 0 \\ 0 \\ 0 \\ c_4 \dot{x}_{se} + k_4 x_{se} \end{Bmatrix}.$$

By taking the Fourier transformation of equation (2), the following matrix form of equation can be obtained:

$$\{X(j\omega)\} = [ [\mathbf{K}] - \omega^2 [\mathbf{M}] + j\omega [\mathbf{C}] ]^{-1} \{F(j\omega)\} \quad (3)$$

Where,  $\{X(j\omega)\}$  and  $\{F(j\omega)\}$  are the complex Fourier transformation vectors of

$\{x\}$  and  $\{f\}$ , respectively.  $\omega$  is the excitation frequency. Vector  $\{X(j\omega)\}$  contains complex displacement responses of n mass segments as a function of  $\omega$  ( $\{X_1(j\omega), X_2(j\omega), X_3(j\omega), \dots, X_n(j\omega)\}$ ).

$\{F(j\omega)\}$  consists of complex excitation forces on the mass segments as a function of  $\omega$  as well.

### 3.2 Biodynamic response of human body

The biodynamic response of a seated human body exposed to whole-body vibration can be broadly categorized into two types. The first category "To-the-body" force motion interrelation as a function of frequency at the human-seat interface, expressed as the driving-point mechanical impedance (DPMI) or the apparent mass (APMS). The second category "Through-the-body" response function, generally termed as seat-to-head transmissibility (STHT) for the seated occupant.

The DPMI relates the driving force and resulting velocity response at the driving point (the seat-buttocks interface), and is given by [1]:

$$Z(j\omega) = \frac{F(j\omega)}{V(j\omega)} = \frac{F(j\omega)}{\dot{X}(j\omega)} \quad (4)$$

Where,  $Z(j\omega)$  is the complex DPMI,  $F(j\omega)$  and  $V(j\omega)$  or  $\dot{X}(j\omega)$  are the driving force and response velocity at the driving point, respectively.  $\omega$  is the angular frequency in rad/s, and  $j = \sqrt{-1}$  is the complex phasor.

Accordingly, DPMI for the model can be represented as:

$$\text{DPMI}(j\omega) = \left| \left( c_4 + \frac{k_4}{j\omega} \right) \frac{X_4(j\omega)}{X_0(\omega)} - \left( c_4 + \frac{k_4}{j\omega} \right) \right| \quad (5)$$

In a similar manner, the apparent mass response relates the driving force to the resulting acceleration response, and is given by [19]:

$$\text{APMS}(j\omega) = \frac{F(j\omega)}{a(j\omega)} \quad (6)$$

Where,  $a(j\omega)$  is the acceleration response at the driving point

The magnitude of APMS offers a simple physical interpretation as it is equal to the static mass of the human body supported by the seat at very low frequencies, when the human body resembles that of a rigid mass. The above two functions are frequently used interchangeably, due to their direct relationship that given by:

$$\text{APMS}(j\omega) = \frac{\text{DPMI}(j\omega)}{j\omega} \quad (7)$$

APMS for the model can be represented as:

$$\begin{aligned} \text{APMS}(j\omega) &= \left| \frac{\text{DPMI}(j\omega)}{j\omega} \right| = \\ &\left| \left( c_4 + \frac{k_4}{-\omega^2} \right) \frac{X_4(j\omega)}{X_0(\omega)} - \left( c_4 + \frac{k_4}{-\omega^2} \right) \right| \end{aligned} \quad (8)$$

The biodynamic response characteristics of seated occupants exposed to whole body vibration can also be expressed in terms of seat-to-head transmissibility (STHT), which is termed as "through-the-body" response function. Unlike the force-motion relationship at the driving-point, the STHT function describes the transmission of vibration through the seated body. The STHT response function is expressed as:

$$H(j\omega) = \frac{a_H(j\omega)}{a(j\omega)} \quad (9)$$

Where,  $H(j\omega)$  is the complex STHT,  $a_H(j\omega)$  is the response acceleration measured at the head of seated occupant, and  $a(j\omega)$  is the acceleration response at the driving point. According to equation (9) seat-to-head transmissibility for the model is:

$$\text{STHT}(j\omega) = \frac{X_1(j\omega)}{X_0(\omega)} \quad (10)$$

The above three functions have been widely used to characterize the biodynamic responses of the seated human subjects exposed to whole body vibration.

### 4. Analytic Results and Discussions

On the basis of anthropometric Boileau data [19], the proportion of total body weight estimated for different body segments is 7.5% for the head and neck, 40.2% for the chest and upper torso, 12.2% for the lower torso, and 18.2% for the thighs and upper legs. For a seated driver with mean body mass, maintaining an erect back not supported posture, 78% of the weight was found to be supported by the seat. The biomechanical parameters of the human model (Stiffness, Damping) are listed in Table 1.

**Table 1:** The biomechanical parameters of the Boileau and Rakheja model.

Stiffness Coefficient (N/m)	Damping (N.s/m)	coefficient
$k_1 = 310000$	$c_1 = 400$	
$k_2 = 183000$	$c_2 = 4750$	
$k_3 = 162800$	$c_3 = 4585$	
$k_4 = 90000$	$c_4 = 2064$	

#### 4.1 Response behaviors of the human body

In the following subsections the effect of body's mass, stiffness coefficient, and damping coefficient on the response behaviors of the human body (STHT, DPMI, and APMS) will be investigated using the analytical solution presented in the current study.

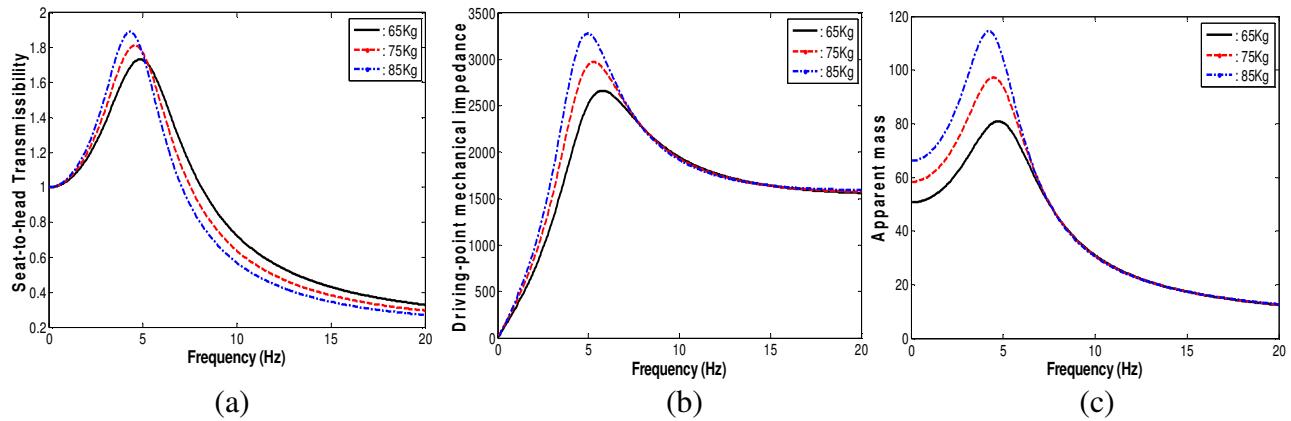
##### 4.1.1 Effect of human body's mass

Three different total body masses (65, 75, and 85 kg) are used to investigate the effect of mass on the response behaviors of human body (STHT, DPMI and APMS) as shown in Fig. 2 (a, b, and c) respectively. From these figures, one can see that by

increasing the human body mass, the biodynamic response characteristics of seated human body (STHT, DPMI, and APMS) are increased.

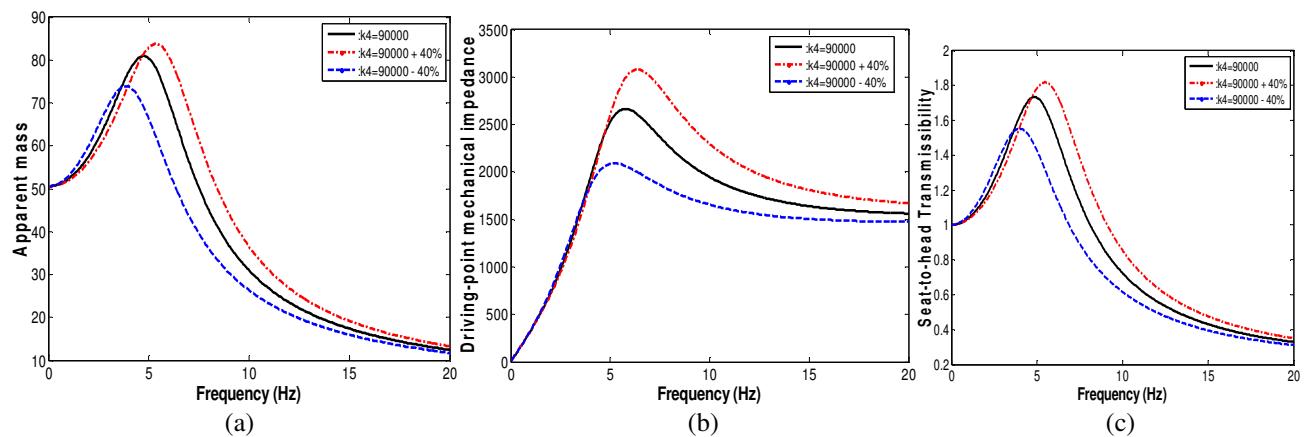
##### 4.1.2 Effect of stiffness coefficient

Three different values of pelvic stiffness  $k_4$  (Boileau value (B.V.), B.V. +40%, and B.V. -40%) are used to investigate the effect of pelvic stiffness on the response behaviors of human body (STHT, DPMI and APMS) as shown in Fig. 3 (a, b, and c) respectively. From these figures, it is clear that by increasing the pelvic stiffness, the biodynamic response characteristics of seated human body (STHT, DPMI, and APMS) are increased.



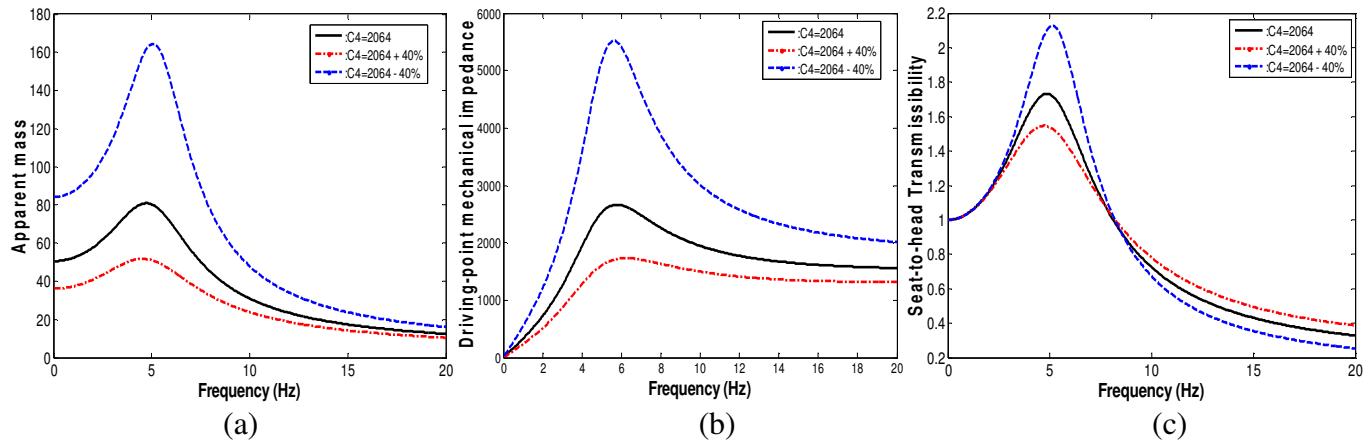
**Figure 2:** Effect of human body's mass on the biodynamic response behaviors (Analytic Results)

((a))STHT, (b) DPMI and (c) APMS)



**Figure 3:** Effect of stiffness coefficient on the biodynamic response behaviors (Analytic Results)

((a)) STHT, (b) DPMI and (c) APMS)



**Figure 4:** Effect of damping coefficient on the biodynamic response behaviors (Analytic Results)

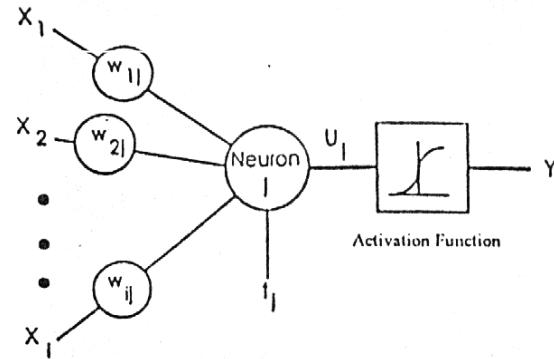
((a) STHT, (b) DPMI and (c) APMS)

#### 4.1.3 Effect of damping coefficient

Three different values of pelvic damping coefficient  $C_4$  (Boileau value (B.V.), B.V. +40%, and B.V. -40%) are used to investigate the effect of pelvic damping coefficient on the response behaviors of human body (STHT, DPMI and APMS) as shown in Fig. 4 (a, b, and c) respectively. From these figures, it is clear that by increasing pelvic damping coefficient, the biodynamic response characteristics of seated human body (STHT, DPMI, and APMS) are decreased.

#### 5. Numerical Model Structure

Neural networks are models of biological neural structures. Abdeen [13] described in a very detailed fashion the structure of any neural network. Briefly, the starting point for most networks is a model neuron as shown in Fig. 5. This neuron is connected to multiple inputs and produces a single output. Each input is modified by a weighting value ( $w$ ). The neuron will combine these weighted inputs with reference to a threshold value and an activation function, will determine its output. This behavior follows closely the real neurons work of the human's brain. In the network structure, the input layer is considered a distributor of the signals from the external world while hidden layers are considered to be feature detectors of such signals. On the other hand, the output layer is considered as a collector of the features detected and the producer of the response.



**Figure 5:** Typical picture of a model neuron that exists in every neural network

#### 5.1 Neural Network Operation

It is quite important for the reader to understand how the neural network operates to simulate different physical problems. The output of each neuron is a function of its inputs ( $X_i$ ). In more details, the output ( $Y_j$ ) of the  $j^{\text{th}}$  neuron in any layer is described by two sets of equations as follows:

$$U_j = \sum(X_i w_{ij}) \quad (11)$$

And

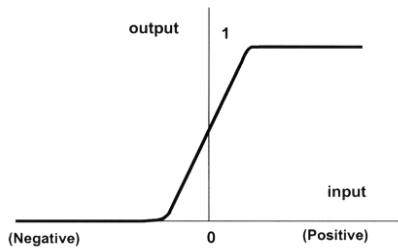
$$Y_j = F_{th}(U_j + t_j) \quad (12)$$

For every neuron,  $j$ , in a layer, each of the  $i$  inputs,  $X_i$ , to that layer is multiplied by a previously established weight,  $w_{ij}$ . These are all summed together, resulting in the internal value of this operation,  $U_j$ . This value is then biased by a

previously established threshold value,  $t_j$ , and sent through an activation function,  $F_{th}$ . This activation function can take several forms such as Step, Linear, Sigmoid, Hyperbolic, and Gaussian functions. The Hyperbolic function, used in this study, is shaped exactly as the Sigmoid one with the same mathematical representation, as in equation (13), but it ranges from  $-1$  to  $+1$  rather than from 0 to 1 as in the Sigmoid one (Fig. 6)

$$f(x) = \frac{1}{1 + e^{-x}} \quad (13)$$

The resulting output,  $Y_j$ , is an input to the next layer or it is a response of the neural network if it is the last layer. In applying the Neural Network technique, in this study, Neuralyst Software, Shin [20], was used.



**Figure 6:** The Sigmoid Activation Function

## 5.2 Neural Network Training

The next step in neural network procedure is the training operation. The main purpose of this operation is to tune up the network to what it should produce as a response. From the difference between the desired response and the actual response, the error is determined and a portion of it is back propagated through the network. At each neuron in the network, the error is used to adjust the weights and the threshold value of this neuron. Consequently, the error in the network will be less for the same inputs at the next iteration. This corrective procedure is applied continuously and repetitively for each set of inputs and corresponding set of outputs. This procedure will decrease the individual or total error in the responses to reach a desired tolerance.

Once the network reduces the total error to the satisfactory limit, the training process may stop. The error propagation in the network starts at the output layer with the following equations:

$$w_{ij} = w_{ij}^+ + LR(e_j X_i) \quad (14)$$

And,

$$e_j = Y_j (1 - Y_j) (d_j - Y_j) \quad (15)$$

Where,  $w_{ij}$  is the corrected weight,  $w_{ij}^+$  is the previous weight value, LR is the learning rate,  $e_j$  is the error term,  $X_i$  is the  $i^{th}$  input value,  $Y_j$  is the output, and  $d_j$  is the desired output.

## 6. Numerical Simulation Cases

To fully investigate numerically the biodynamic response behaviors of seated human body subject to whole body vibration, several simulation cases are considered in this study. These simulation cases can be divided into two groups to simulate the response behaviors due to changing of human body's mass and stiffness respectively. From the analytic investigation, it is clear that the effect of damping coefficient is opposite to the effect of stiffness coefficient on the response behaviors of the human body. So in the numerical analysis, the effect of stiffness coefficient will be studied only in addition with the effect of human body's mass.

### 6.1 Neural Network Design

To develop a neural network model to simulate the effect of mass and stiffness on the biodynamic response behaviors of seated human body, first input and output variables have to be determined. Input variables are chosen according to the nature of the problem and the type of data that would be collected. To clearly specify the key input variables for each neural network simulation group and their associated outputs, Tables 2 and 3 are designed to summarize all neural network key input and output variables for the first and second simulation groups respectively.

It can be noticed from Tables 2 and 3 that every simulation group consists of three simulation cases (three neural network models) to study the effect of mass and stiffness on the seat-to-head transmissibility (STHT), driving point mechanical impedance (DPMI) and apparent mass (APMS).

**Table 2:** Key input and output variables for the first neural network simulation group (effect of human body's mass)

Simulation Case	Input Variables					Output
STHT						STHT
DPMI	$m_1$	$m_2$	$m_3$	$m_4$	Frequency	DPMI
APMS						APMS

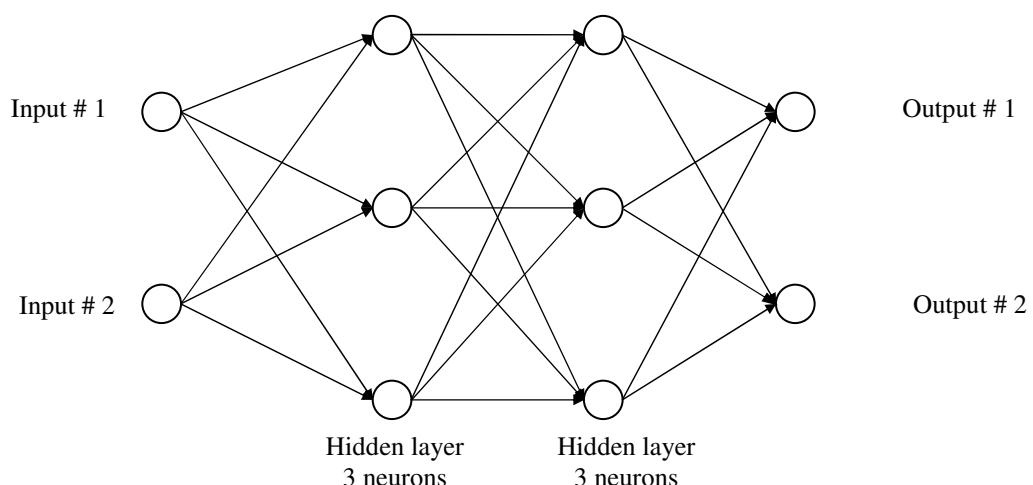
**Table 3:** Key input and output variables for the second neural network simulation group (effect of stiffness coefficient)

Simulation Case	Input Variables		Output
STHT	k <sub>4</sub>	Frequency	STHT
DPMI			DPMI
APMS			APMS

Several neural network architectures are designed and tested for all simulation cases investigated in this study to finally determine the best network models to simulate, very accurately, the effect of mass and stiffness based on minimizing the

Root Mean Square Error (RMS-Error). Fig. 7 shows a schematic diagram for a generic neural network. The training procedure for the developed ANN models, in the current study, uses the data from the results of the analytical model to let the ANN understands the behaviors. After fitting finally the ANN models, these models are used to predict the biodynamic response behaviors for different masses and stiffness rather than those used in the analytic solution.

Table 4 shows the final neural network models for the two simulation groups and their associate number of neurons. The input and output layers represent the key input and output variables described previously for each simulation group.



**Figure 7:** General schematic diagram of a simple generic neural network

**Table 4:** The developed neural network models for all the simulation cases

Simulation Group		No. of Layers	No. of Neurons in each Layer					
			Input Layer	First Hidden	Second Hidden	Third Hidden	Output Layer	
First Group (mass)	STHT	5	5	6	4	2	1	
	DPMI		5	6	4	-	1	
	APMS							
Second Group (Stiffness)	STHT	4	2	5	3	-	1	
	DPMI							
	APMS							

**Table 5:** Parameters used in the developed neural network models

Simulation Group		Training Epochs	MPRE	RMS-Error
First Group (mass)	STHT	45931	1.213	0.0015
	DPMI	7560	2.609	0.0022
	APMS	7174	3.743	0.0023
Second Group (Stiffness)	STHT	14012	3.449	0.0014
	DPMI	100185	3.938	0.002
	APMS	101463	1.644	0.0012

The parameters of the various network models developed in the current study for the different simulation models are presented in table 5. These parameters can be described with their tasks as follows:

Learning Rate (LR): determines the magnitude of the correction term applied to adjust each neuron's weights during training process = 1 in the current study.

Momentum (M): determines the "life time" of a correction term as the training process takes place = 0.9 in the current study.

Training Tolerance (TRT): defines the percentage error allowed in comparing the neural network output to the target value to be scored as "Right" during the training process = 0.001 in the current study.

Testing Tolerance (TST): it is similar to Training Tolerance, but it is applied to the neural network outputs and the target values only for the test data = 0.003 in the current study.

Input Noise (IN): provides a slight random variation to each input value for every training epoch = 0 in the current study.

Function Gain (FG): allows a change in the scaling or width of the selected function = 1 in the current study.

Scaling Margin (SM): adds additional headroom, as a percentage of range, to the rescaling computations used by Neuralyst Software, Shin (1994), in preparing data for the neural network or interpreting data from the neural network = 0.1 in the current study.

Training Epochs: number of trials to achieve the present accuracy.

Percentage Relative Error (PRR): percentage relative error between the numerical results and actual measured value and is computed according to equation (16) as follows:

$$\text{PRE} = \frac{(\text{Absolute Value (ANN\_PR}) - \text{AMV})}{\text{AMV}} * 100 \quad (16)$$

Where:

ANN\_PR: Predicted results using the developed ANN model

AMV : Actual Measured Value

MPRE: Maximum percentage relative error during the model results for the training step.

## 7. Numeric Results and Discussions

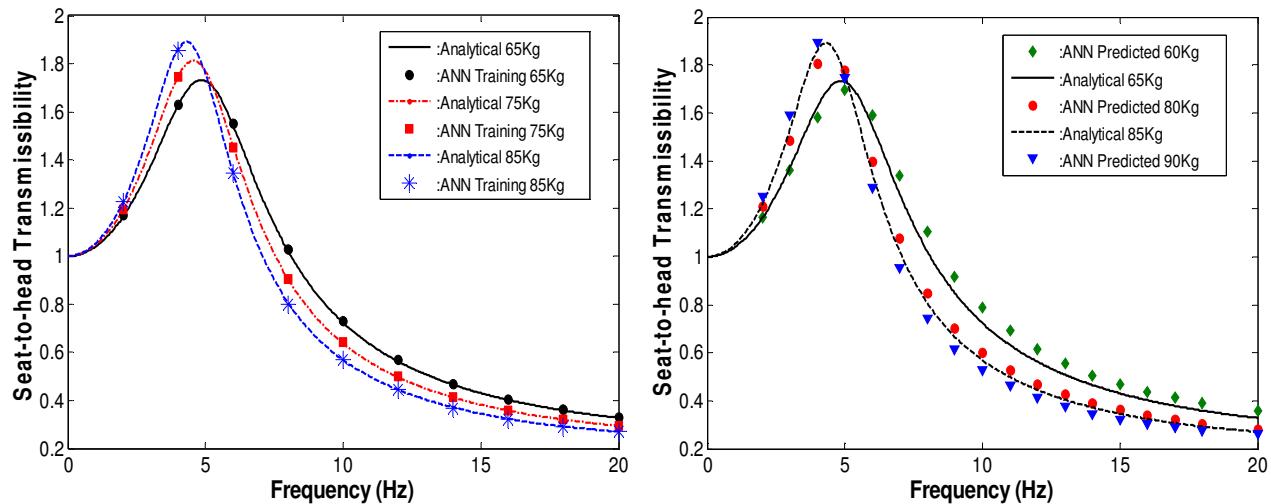
Numerical results using ANN technique will be presented in this section for the two groups (six models) to show the simulation and prediction powers of ANN technique for the effect of human body's mass and stiffness coefficient on the biodynamic response behaviors (STHT, DPMI and APMS) subject to whole-body vibration.

### 7.1 Effect of human body's mass

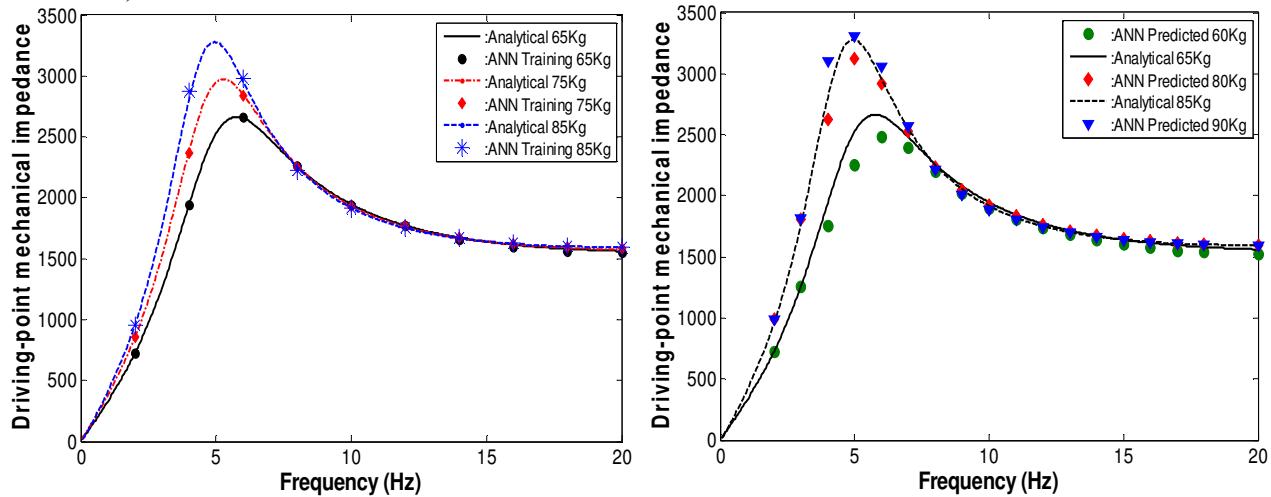
Three ANN models are developed to simulate and predict the effect of human body's mass on the biodynamic response behaviors (STHT, DPMI and APMS). Figures 8, 9, and 10 show the ANN results and analytical ones for different human body's masses. From ANN training figures (Left), it is very clear that ANN understands and simulates very well the biodynamic response behaviors. After that the developed ANN models used very successfully and efficiently to predict the response behaviors for different masses rather than those used in the analytic solution as shown in the predicted figures of ANN results (Right).

### 7.2 Effect of stiffness coefficient

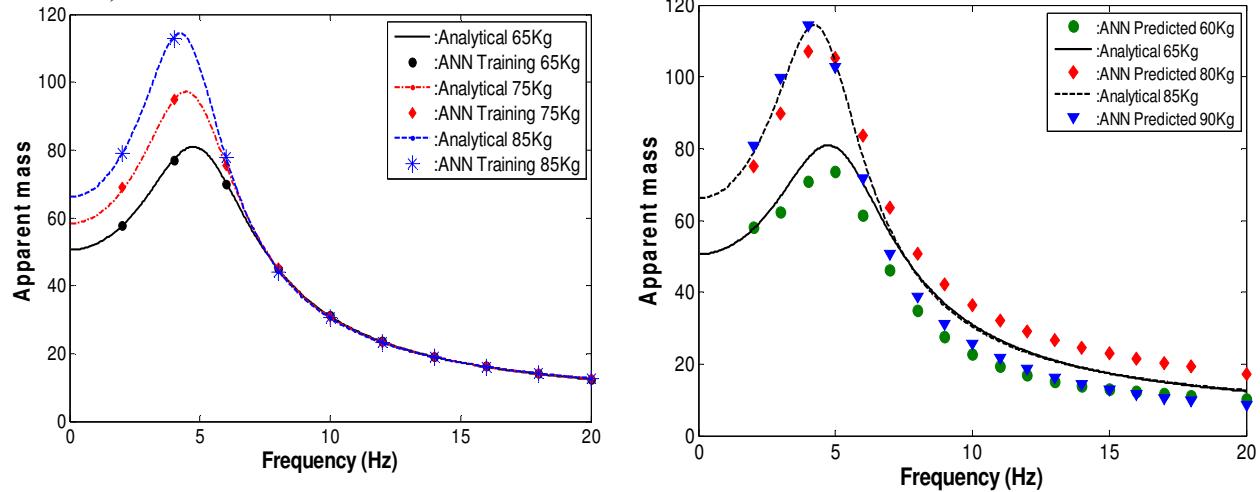
Another three ANN models are developed in this sub-section to simulate and predict the effect of stiffness coefficient ( $k_4$ ) on the biodynamic response behaviors (STHT, DPMI and APMS). Figures 11, 12, and 13 show the ANN results and analytical ones for different values of  $k_4$ . From ANN training figures (Left), it is very clear that ANN understands and simulates very well the biodynamic response behaviors. After that the developed ANN models used very successfully and efficiently to predict the response behaviors for different values of  $k_4$  rather than those used in the analytic solution as shown in the predicted figures of ANN results(Right).



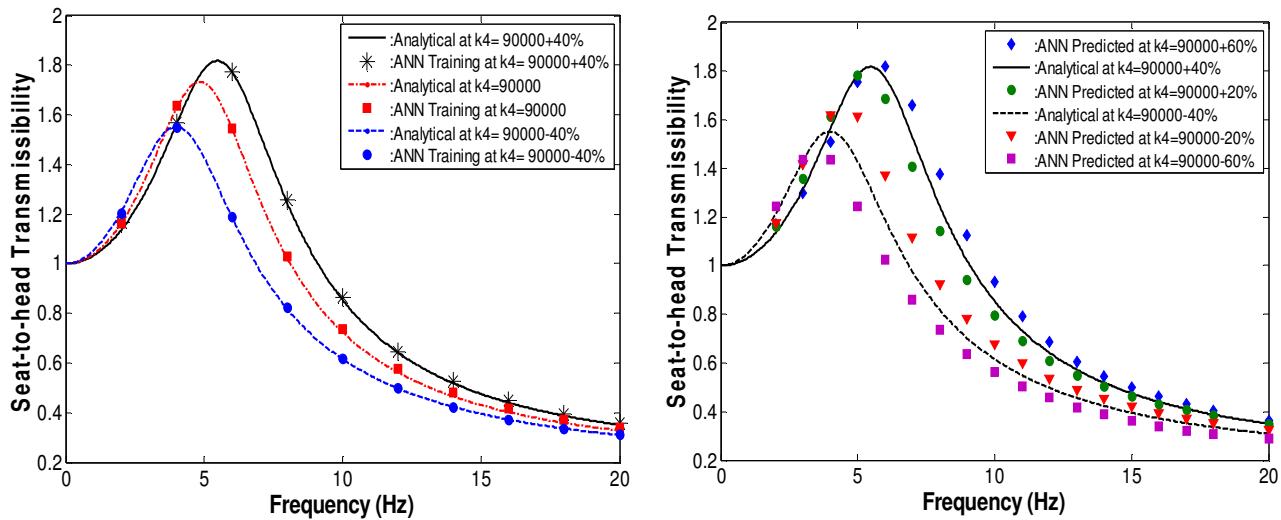
**Figure 8:** ANN results for the effect of human body's mass on STHT (Left : ANN Training, Right : ANN Prediction)



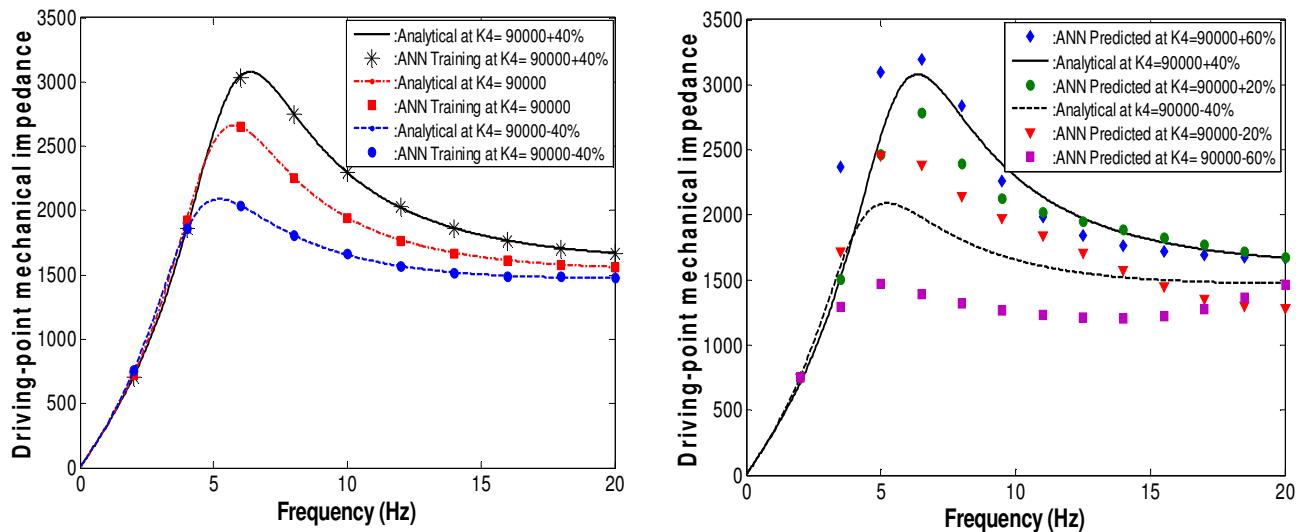
**Figure 9:** ANN results for the effect of human body's mass on DPMI(Left : ANN Training, Right : ANN Prediction)



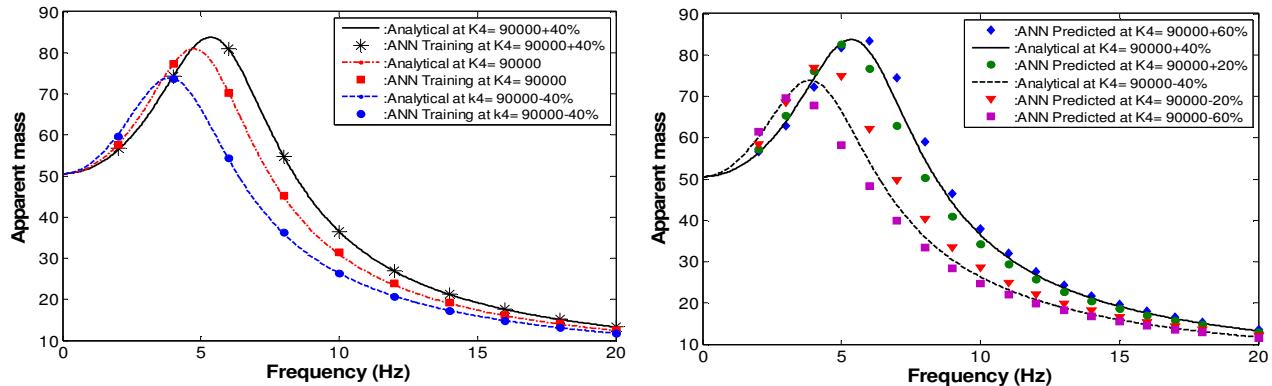
**Figure 10:** ANN results for the effect of human body's mass on APMS(Left : ANN Training, Right : ANN Prediction)



**Figure 11:** ANN results for the effect stiffness coefficient on STHT(Left : ANN Training, Right : ANN Prediction)



**Figure 12:** ANN results for the effect stiffness coefficient on DPMI(Left : ANN Training, Right : ANN Prediction)



**Figure 13:** ANN results for the effect stiffness coefficient on APMS(Left : ANN Training, Right : ANN Prediction)

## 8. Conclusions

Based on the analytical investigation conducted in the course of the current research, it could be concluded that the change in human body's mass, pelvic stiffness, and pelvic damping coefficient give a remarkable change in biodynamic response behaviors of seated human body (direct proportional for human body's mass and pelvic stiffness coefficient and inverse proportional for pelvic damping coefficient.)

Based on the results of implementing the ANN technique in this study, the following can be concluded:

1. The developed ANN models presented in this study are very successful in simulating the effect of human body's mass and stiffness on the biodynamic response behaviors under whole-body vibration.
2. The presented ANN models are very efficiently capable of predicting the response behaviors at different masses and stiffness rather than those used in the analytic solution.

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5/6/2010

# Design Synthesis of New Peptide Derivatives and Evaluated DNA Binding Activity, Anticancer and Antimicrobial Activity.

A. A. EL-HENAWY

Chemistry Department, Faculty of Science, Al-Azhar University, Nasr City, Cairo-Egypt.

**Abstract:** Recently, sulfonamides have been reported to show significant antitumor activity in vitro and/or in vivo. There are a variety of mechanisms for the anticancer activity. The present work reports the synthesis some novel peptide sulfadiazine derivatives using novel method, this may play a role in their anticancer activity. All the newly synthesized compounds were evaluated for DNA binding activity and antimicrobial activity, some synthesized compounds showed high DNA binding activity and antimicrobial activity. Some selected compounds were evaluated for anticancer activity against breast cancer cell line (MCF7) in vitro. All selected compounds showed interesting cytotoxic activities compared to a reference drug. [Journal of American Science. 2010;6(11):240-249]. (ISSN: 1545-1003).

**Keywords:** Peptide; Anticancer; Antimicrobial; DNA Binding

## 1. Introduction

Sulfonamides posses many types of biological activities and representatives of this class of pharmacological agents are widely used in clinic as antibacterial [1], antithyroid [2], diuretic [3,4], hypoglycaemic [5] and anti-carbonic anhydrase [3,6]. From other studies, Sulfadiazine derivatives have been reported to show considerable antitumor activity [7,8]. Also, aryl/heteroaryl sulfonamides may act as antitumor agents through several mechanisms, such as disruption of microtubule assembly, angiogenesis inhibition, perturbation in the G1 phase, functional suppression of the transcriptional activator NF-Y, and most important suggested mechanism by inhibition of carbonic anhydrase isozymes(CA) [9-13]. After wildly evaluation, Sulfonamides were found act as carbonic anhydrase (CA) inhibitors [14].

On other hand, the structural changes of DNA Based on the interaction of small molecular weight ligands with DNA (deoxyribonucleic acid) have attracted attention in the medicinal design of anticancer and anti-AIDS drugs [15-22]. Moreover, peptide derivatives pose anti tumour effect [23-25].

In the light of these facts, the present study aim to syntheses of peptides series of sulfadiazinylacetyl derivatives, and test the influence of these compounds toward the DNA binding affinity, anticancer activity and antimicrobial activity.

## 2. Results and discussion:

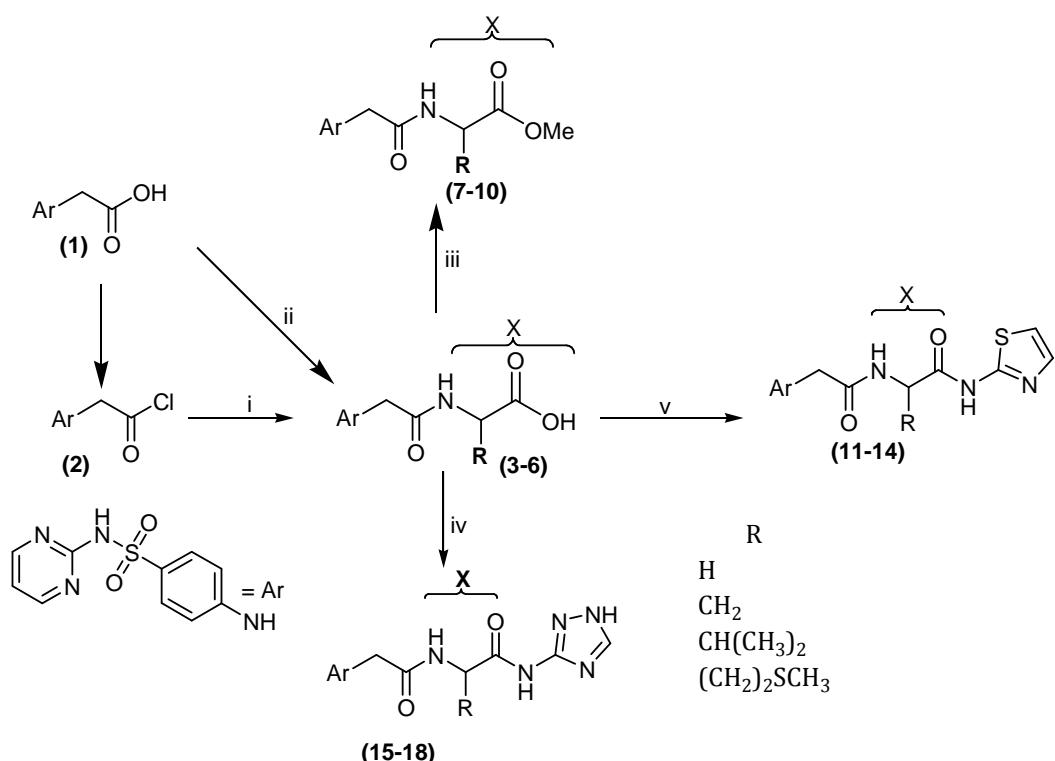
### 2.1. Chemistry:

In this paper, aimed to prepare bio peptide molecules contain sulfonamide moiety using novel method. This method is convenient, high yield, high purity and completing chemical reactions in short

time comparable with other methods which take several hours to days.

The synthetic route designed for the pseudo-peptide derivatives (**3-22**), was summarized in (Scheme 1,2). The reaction of acid chloride (**2**) with amino acids as nucleophiles, might seem hard to conduct. The reason might be due to characteristics of amino acids. Since, amino acids are amphoteric compounds, in solution a dipolar ion ( $\text{^NH}_3\text{CH}(\text{R})-\text{COO}^-$ ) is formed by a proton transformation from the carboxyl group to the nitrogen atom of amino group [26]. The amphoteric natures of amino acids decrease the electron density on nitrogen atom. Thus, the zwitterions amino acids possess lower nucleophilicity than amines and are difficult to react with acid chloride. In order to facilitate the reaction, adding an organic base such as triethylamine(TEA) to improve the reaction rate. The formations of sulfadiazinylacetyl dipeptides (**3-6, 19**) were achieved by the reaction of (**2**) with suitable type of amino acids (Scheme 1,2) in tetrahydrofuran (THF/ TEA) media, or by heating Sulfadiazinyl acetic (**1**) with amino acids at 250 °C (Scheme 1,2).

The IR spectra of dipeptide derivatives (**3-6,19**) displayed a broad bands for (NH) and (OH) absorption between  $\nu=3157-3382\text{cm}^{-1}$ . The (CO) group of carboxyl group occurred between  $\nu=1672-1698\text{ cm}^{-1}$ . Characteristic  $^1\text{H}\text{NMR}$  of dipeptide derivatives (**3-6,19**) were displayed peaks between  $\delta=11.82-12.65\text{ ppm}$  for the proton of the OH group, which disappeared immediately when treated with  $\text{D}_2\text{O}$ . Also, when compounds (**3-6,19**) were reacted with thionyl chloride (molar ratio) in methanol give the corresponding sulfadiazinylacetyl dipeptide methyl esters (**7-10** and **20**; Scheme 1,2).



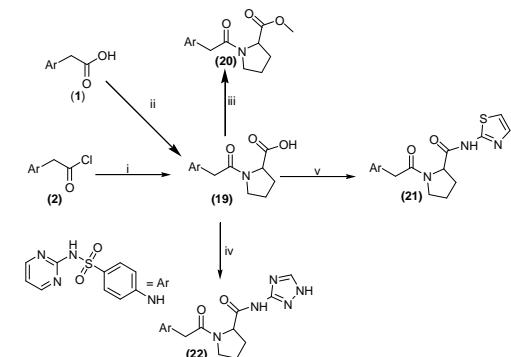
(SCHEME 1)

The IR spectra of dipeptide methyl esters derivatives (**7-10** and **20**) disappeared a bands for (OH) of carboxyl group. <sup>1</sup>H NMR characteristic of dipeptide methyl esters derivatives (**7-10** and **20**) were showed a peaks between  $\delta$ =3.59-3.76 ppm for a proton (CH<sub>3</sub>) of methoxy group. Sulfadiazinylacetyl dipeptidyl-N<sup>2</sup>-thiazol derivatives (**11-14** and **21**; Scheme 1,2) and Sulfadiazinylacetyl dipeptidyl-N<sup>3</sup>-4H-1,2,4-triazol derivatives (**15-18** and **22**; Scheme 1,2) were prepared by the reaction of compounds (**3-7** and **18**) with 2-aminothiazole and/or 3-amino-1,2,4-triazol in presence of dicyclohexylcarbodiimide (DCC/ THF) media. <sup>1</sup>H NMR spectra showed that, The imine protons (**NH**) of dipeptidyl-N<sup>2</sup>-thiazol derivatives (**11-14** and **21**), and dipeptidyl-N<sup>3</sup>-4H-1,2,4-triazol derivatives (**15-18** and **22**) were affected by the deshielding effect of CO group, and the chemical shift was  $\delta$ = 9.91-13.33 ppm, which disappeared immediately when treated with D<sub>2</sub>O. The results of chemical analyses of the synthesized compounds were summarized in Tables (1-3).

*Table (1): Showed physical data and elemental analysis of the synthesized compounds (3-22).*

*Table (2): Showed of <sup>1</sup>H-NMR in (CDCl<sub>3</sub>) results of synthesized compounds (3-22).*

*Table (3): Showed of IR and mass spectroscopy results of synthesized compounds (3-22).*



## 2.2. Biological activity:

### 2.2.1. DNA Binding Assay:

The mechanism of several antitumor compounds and antitumor antibiotics depend on their interaction with DNA. In this work, the antitumor activities of the newly synthesized compounds were determined using DNA binding assay and methyl green DNA displacement assay[27,28]. In this method, a fixed amount of the ligand is spotted on the RP-18 TLC plates, followed by addition of known amount of DNA on the same spot. The plate was then developed and the position of unbound DNA was determined by

spraying the plates with anisaldehyde reagent. The free DNA was detected as a blue spot ( $R_f$ , MeOH–H<sub>2</sub>O, 8:2) on RP-18 TLC. It was demonstrated that, when DNA was mixed with compounds known to interact with it, e.g. ethidium bromide, the complex was retained at the origin. Compounds with high binding affinity to DNA remained on the base line or migrated for a very short distance, while compounds with poor binding affinity did not cause DNA to be retained at the origin [27].

#### **2.2.2. Methyl Green-DNA Displacement Assay:**

Methyl green reversibly binds polymerized DNA forming a stable complex at neutral pH [28]. The maximum absorption for the DNA-methyl green complex is 642-645 nm. This colorimetric assay was used to measure the displacement of methyl green with DNA by compounds, which having the ability to bind with DNA. The degree of displacement was determined spectrophotometrically by measuring the change in the initial absorbance of the DNA-methyl green solution in the presence of reference compound.

Table (4): Showed DNA binding activity of compounds (3-22) using methyl green DNA displacements assay as IC<sub>50</sub> (concentration required for 50% decrease in the initial absorbance of the DNA/methyl green solution).

Results from (Table 4) indicate that, Compounds (3, 6, 7, 8 and 10) showed the highest affinity for DNA, which was demonstrated by retaining the complex at the origin or by migrating for a very short distances, and by measuring IC<sub>50</sub>. Compounds (5, 11, 18, 21 and 22) showed moderate affinity, while compound (12,14 and 15) showed weak affinity.

From these results, the following points can be concluded. The combination of some amino acids with acetylSulfadiazinyl (3-6 and 19), showed high to moderate activity, except that combined with  $\beta$ -alanine and proline residue gave inactive against DNA. Also, the esterification of C-terminal amino acids of the synthesized compounds (7-10, and 20) gave high activity compounds, except that combined with valine(9) and proline (20) residue gave inactive compounds. Moreover, when elongation of the peptide chain by introduced withdrawing moieties such as thiazoles (11-14 and 21) and\ or triazoles (15-18 and 22) showed moderate to week activity against DNA, except that contained  $\beta$ -alaninethiazole (11), valinetriazoles (16) and Methionintriazoles (17) residue gave inactive compounds.

#### **2.2.2. In vitro anticancer screening :**

In the present work, four newly synthesized compounds (3,10,12 and 17) were selected to evaluate their in vitro growth inhibitory activities

against breast cancer cell line (MCF7). Doxorubicin was used as the reference drug in this study. The response parameter calculated was IC<sub>50</sub> value (concentration required for 50% inhibition of cell viability).

The four selected compounds, were carefully selected to be representatives for all the twenty newly Synthesized compounds, which covering all structural variations in these work. These analogs, being of acetylsulfadiazinea attached to (i) Free amino acids "Sulfadiazinyl-acetyl glycine" (3) which represented Dipeptide series. (ii) Amino acid methyl esters "Sulfadiazinylacetyl-L-Methionine methyl ester" (10), which represented Dipeptide methyl ester series.(iii) Amino acid-N2-thiazols "Sulfadiazinyl-acetyl - $\beta$ -alaninyl-N2-thiazol" (12) and Amino acid-N3-4H-1,2,4-triazols "Sulfadiazinylacetyl -L-valinyl-N3-4H-1,2,4-triazol" (17), which represented Dipeptide series attached with withdrawing moieties.

*Table (5): showed the cytotoxic activity of the synthesized compounds in vitro as IC<sub>50</sub> (concentration required for 50% inhibition of cell viability) compared to the reference drug Doxorubicin.*

From Table (5), it was found that, all the tested compounds showed significant antitumor activities compared to reference compound. Also, variation in biological activity between the synthesized compounds was not very high. Moreover, dipeptide the most potent compound. furthurmore, dipeptide methyl ester more potent than those dipeptides has containing withdrawing moieties.

#### **2.2.3.Anti-microbial activity:**

All the synthesized compounds were screened for antimicrobial activities by paper disc diffusion technique (29-33). The tested micro-organism strains were: S. aureus (ATCC-9144), S. epidermidis (ATCC-155), E. coli (ATCC-25922), K. pneumoniae (ATCC-11298), A. niger (ATCC-9029), A. fumigatus (ATCC-46645).

Table (6): Showed the anti-microbial activity of the synthesized compounds and reference drug.

The result from (Table 6) showed that, most synthesized compounds were active against all tested micro-organisms with the range of MICs values for S. aureus(11.1-28.4  $\mu$ g /ml), S. epidermidis (15.1-28.2  $\mu$ /ml), K. pneumoniae (14.6-33.8  $\mu$ g /ml), E. coli (12.6-25.3  $\mu$ g/ml), A. niger (11.6-32.2  $\mu$ g /ml) and A. fumigates(13.4-32.3  $\mu$ g/ml). The compounds (3, 6, 7, 8, 9, 13, 15, 16, 20) were exhibit in vitro anti-microbial activity against all micro-organisms at MICs of (11.1 -33.8  $\mu$ g/ml). Compounds (10, 15 and 16) were exhibit in vitro anti-microbial activity

against most micro-organisms at MICs of (18.7-32.2 µg/ml).

From the previous results, it was concluded that, sulfadiazinylacetyl combined with amino acid residues have antimicrobial activity against all tested microorganisms (**3-6and19**). Moreover, esterification of C-terminal of amino acids, and/or when elongation of peptide chain by introducing withdrawing moieties gave biologically active compounds against all tested micro-organisms (**7-10 and20**), excepted that containing methionine methyl ester moiety (**10**), valinetriazol(**17**) and methionine triazol(**18**) moiety.

**In conclusion**, the present work was aimed to design sulphonamide peptide derivatives using novel method; it was clearly observed that the compounds containing free peptide moieties and peptide methyl ester moieties exhibit significant anticancer activity, DNA binding activity and antimicrobial activity. When these compounds attached with withdrawing group lead to decrease anticancer activity, DNA binding activity and antimicrobial activity. SO, the further modification of these compounds may be promising candidates for clinically useful drug agents.

### 3.EXPERMINTAL:

Melting points were taken on a Griffin melting point apparatus and are uncorrected. Thin layer chromatography (Rf) for analytical purposes was carried out on silica gel and developed with benzene-ethyl acetate (6:1) using iodine-KI (20%) solution as spraying agent. Benzidine, ninhydrin, and hydroxamate tests used for detection reactions. The IR spectra of the compounds were recorded on a Perkin-Elmer spectrophotometer model 1430 as potassium bromide pellets and frequencies are reported in cm-1. The <sup>1</sup>H NMR spectra were observed on a Varian Genini-300 MHz spectrometer and chemical shifts ( $\delta$ ) are in ppm. The mass spectra were recorded on a mass spectrometer HP model MS-QPLO00EX (Shimadzu) at 70 eV. Elemental analyses (C,H,N) were carried out at the Microanalytical Centre of Cairo University, Giza, Egypt.

#### 3.1. Synthesise:

##### 3.1.1.Preparation of Sulfadiazinylacetyl dipeptides (**3-6 and19**):

*General Procedures:*

##### *Mothed A:*

A mixture of amino acids (1.5 equiv) was dissolved in water (25 ml), THF (15 ml) mixture and triethylamine (2 ml) was added, followed by portionwise addition of sulfadiazinylacetyl chloride (**2**; 1 equiv) during 30 min. The temperature of the

reaction mixture during the addition was kept at 10°C. Stirring continued for 2 hrs. at 20°C. (THF) was removed by concentration of the reaction mixture under reduced pressure; water (30 ml) was added and acidified with 2 M HCl to pH=5. The crude products were filtered and recrystallized from Ethanol. All the products (**3-6 and 19**) were chromatographically homogeneous by iodine and benzidine development.

##### *Mothed B:*

A mixture of amino acids (0.01 mol) and sulfanadiazine (**1**, 0.01 mol) was fused at 250 °C in an oil bath for 15 min. Fused mass was dissolved in ethanol and poured onto cold water, the solid obtained was recrystallized from ethanol.

##### 3.1.2.Preparation Sulfadiazinylacetyl dipeptide methyl esters (**7-10 and 20**):-

*General Procedures:*

A suspension of coupling reaction products (**3-7**; 1 equiv) in absolute methanol (150 ml) was cooled to -10°C and pure thionylchlorid (1 equiv) was added dropwise during one hour. The reaction mixture was stirred for an additional 34 hrs. at room temperature, and then kept overnight when the solvent was removed by vacuum distillation. The residual solid material was recrystallized from Ethanol. All the products (**7-10 and 20**) were chromatographically homogeneous by iodine and benzidine development.

##### 3.1.3.Preparation of Sulfadiazinylacetyl dipeptidyl-N<sup>2</sup>-thiazol(**11-14and 21**):

*General Procedures:*

To a solution of 2-aminothiazol (1.5 equiv) was dissolved in THF, the solution was stirred for 30 min. at 20°C, and cooled to 0 °C, dipeptide (**3-6and 19**;1equiv) in THF (50 ml) and DCC (1 equiv) were added to the above mixture. The reaction mixture was stirred for 6 h. at 0°C and for another 12 hrs. at room temperature. The crude material is diluted with EtOAc and washed with sat. aq. Na<sub>2</sub>CO<sub>3</sub> ( $\times 2$ ) and brine ( $\times 1$ ), dried over sodium sulfate, evaporated, and purified by Ethanol to give desired products. The products (**11-14and 21**) were to be chromatographically homogeneous by iodine and benzidine development.

##### 3.1.4. Preparation of Sulfadiazinylacetyl dipeptidyl-N<sub>3</sub>-4H-1,2,4-triazol (**15-18 and 22**):

*General Procedures*

To a solution of 1H-1,2,4-triazol-3-amine (1.5 equiv) was dissolved in THF, the solution was stirred for 30 min. at 20°C, and cooled to 0°C, dipeptide (**3-6 and 19**;1equiv) in THF (50 ml) and DCC (1 equiv) were added to the above mixture. The reaction mixture was stirred for 6 hrs. at 0°C and for another 12 hrs. at room temperature. The crude material is diluted with EtOAc and washed with aqueous

Na<sub>2</sub>CO<sub>3</sub> ( $\times 2$ ) and brine ( $\times 1$ ), dried over sodium sulfate, evaporated, and purified by Ethanol to give desired products. The products (**15-18 and 22**) were to be chromatographically homogeneous by iodine and benzidine development.

### 3.2. Biological screening:

#### 3.2.1. Evaluation of the degree of DNA binding:

##### 3.2.1.1. DNA binding assay on TLC plates:

Analyses of the DNA binding affinity of the tested compounds were predeveloped first using methanol-water (8:2). The tested compounds were then applied (5 mg/ml in methanol) at the origin, followed by the spotting of DNA (1 mg/ml in methanol-water mixture (8:2) at the same positions at the origin. Ethidium bromide was used as a positive control. After complete spotting, the plates were developed with the same solvent system, and the positions of DNA were visualized by spraying the plates with anisaldehyde, which produces a blue colour with DNA. The intensity of the colour was proportional to the quantity of DNA added to the plate.

##### 3.2.1.2. Colorimetric assay for the degree of DNA binding:

DNA\methyl green complex (20 mg) was suspended in 100 ml of 0.05M tris-HCl buffer (pH 7.5) containing 7.5 mM MgSO<sub>4</sub> and stirred at 37 °C with a magnetic stirrer for 24 hrs. The calculated amounts of samples were placed in Eppendorf tubes, and (200  $\mu$ l) of the DNA-methyl green solution was added to each tube. The samples were incubated in dark at room temperature, and after 24 hrs. the final absorbance of each sample was determined at (642-645) nm. The results were recorded in form of the IC<sub>50</sub> for each compound, which is the sample concentration required to produce 50% decrease in the initial absorbance of the DNA-methyl green complex. Ethidium bromide was used as a positive control. The molar concentration required for 50% decrease in the initial absorbance of the DNA-methyl green complex was calculated and the results are given in Table 4.

### 3.3.2. Anticancer screening:

Antitumor screening was performed at the National Cancer Institute, Cancer Biology Department, Cairo, Egypt. Cytotoxic activity was measured *in vitro* for the selected synthesized compounds (3, 10, 15 and 18) using Skehan et al method(34). Cells were plated in 96-multiwellmicrotiter plate (104 cells/well) for 24 hrs. before treatment with the compounds to allow attachment of cell to the wall of the plate. Test compounds were dissolved in DMSO and diluted with saline to the appropriate volume.

Different concentrations of the compound under test (0, 1, 2.5, 5, and 10 mg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compound(s) for 48 hrs. at 37 °C and in atmosphere of 5% CO<sub>2</sub>. After 48 hrs., cells were fixed, washed and stained with SRB (Sulfo-Rhodamine-B) stain. Excess stain was washed with 1% acetic acid and attached stain was recovered with Tris EDTA buffer. Colour intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve for breast tumour cell line after the specified time. The molar concentration required for 50% inhibition of cell viability (IC<sub>50</sub>) was calculated and the results are given in Table 5.

### 3.3.3. Anti-microbial screening

Biological activities were carried out at the biogenetic engineering center, molecular biology unit, Al-Azhar University, Nasr city, Egypt. The anti-bacterial activity of the synthesized compounds was tested against two Gram- positive bacteria S. aureus (ATCC-9144) and S. epidermidis (ATCC-155), two Gram-negative bacteria E. coli (ATCC-25922), and K. pneumoniae (ATCC-11298) and two fungi namely A. niger (ATCC-9029)and A. fumigatus (ATCC-46645) using nutrient agar medium.

#### 3.3.3.1. Paper disc diffusion technique:

The sterilized (autoclaved at 120 °C for 30 min) medium (40-50° C) was incubated (1ml/100ml. of medium) with the suspension (105 cfu ml-1) of the micro-organism (matched to McFarland barium sulphate standard) and poured into a petridish to give a depth of 3-4 mm. The paper impregnated with the test compounds ( $\mu$ g/ml-1 in methanol) was placed on the solidified medium. The plates were pre-incubated for 1 h at room temperature and incubated at 37 °C for 24 and 48 hrs. for anti-bacterial and anti-fungal activities, respectively. Ciprofloxacin (100  $\mu$ g/disc) were used as standard for antibacterial and anti-fungal activity respectively. The observed zone of inhibition is presented in Table 6.

#### 4.3.3.3Minimum inhibitory concentration (MIC):

MIC of the compound was determined by agar streak dilution method (35). A stock solution of the synthesized compound (100  $\mu$ g/ml-1) in methanol was prepared and graded quantities of the test compounds were incorporated in specified quantity of molten sterile agar (nutrient agar for anti-bacterial activity and sabouraud dextrose agar medium for anti-fungal activity). A specified quantity of the medium (40-50 °C) containing the compound was poured into a Petri dish to give a depth of 3-4 mm and allowed to solidify. Suspension of the micro-organism was prepared to contain approximately (105cfu ml-1) and applied to plates with serially

diluted compounds in dimethyl formamide to be tested and incubated at 37°C for 24 and 48 hrs. for bacteria and fungi, respectively. The MIC was considered to be the lowest concentration of the test

substance exhibiting no visible growth of bacteria or fungi on the plate. The observed MIC is presented in (Table6).

**Table (1):** Physical data for the compounds (3-22):

Cpd. No.	X	Yield %	M.P. °C	Color	$[\alpha]_D^{20}$	$R_f$	Molecular Formula (M.Wt.)	Elemental Analysis		
								Calculated/Found		
		C	H	N						
<b>3</b>	Gly	80	195-97	brown	—	0.55	$C_{14}H_{15}N_5O_5S$ 365	46.02	4.10	19.17
<b>4</b>	$\beta$ -Ala	83	160-62	brown	—	0.60	$C_{15}H_{17}N_5O_5S$ 379	46.09	4.14	19.22
<b>5</b>	L-Val	83	182-84	brown	+116	0.68	$C_{17}H_{21}N_5O_5S$ 407	47.49	4.48	18.46
<b>6</b>	L-Met	75	191-93	brown	+160	0.64	$C_{17}H_{21}N_5O_5S_2$ 439	47.55	4.52	18.55
<b>7</b>	Gly- OMe	50	200-02	yellow	—	0.69	$C_{15}H_{17}N_5O_5S$ 379	50.12	5.15	17.19
<b>8</b>	$\beta$ -Ala- OMe	57	176-78	yellow	—	0.80	$C_{16}H_{19}N_5O_5S$ 393	50.11	5.20	17.29
<b>9</b>	L-Val- OMe	45	210-13	yellow	+215	0.87	$C_{18}H_{23}N_5O_5S$ 421	46.46	4.78	15.94
<b>10</b>	L-Met- OMe	48	222-24	yellow	+179	0.86	$C_{18}H_{23}N_5O_5S_2$ 453	46.50	4.82	15.93
<b>11</b>	Gly- thiazol	80	247-49	brown	—	0.89	$C_{17}H_{17}N_7O_4S_2$ 447	46.85	4.48	18.46
<b>12</b>	$\beta$ -Ala- thiazol	75	179-81	brown	—	0.65	$C_{18}H_{19}N_7O_4S_2$ 461	47.67	5.11	18.48
<b>13</b>	L-Val- thiazol	88	240-42	brown	+160	0.94	$C_{20}H_{23}N_7O_4S_2$ 489	51.30	4.84	17.81
<b>14</b>	L-Met- thiazol	77	167-78	brown	+184	0.79	$C_{20}H_{23}N_7O_4S_3$ 521	47.68	4.12	17.80
<b>15</b>	Gly- triazol	83	274-76	gray	—	0.84	$C_{16}H_{17}N_9O_4S$ 431	46.05	4.44	16.62
<b>16</b>	$\beta$ -Ala- triazol	75	197-99	brown	—	0.90	$C_{17}H_{19}N_9O_4S$ 445	48.89	4.26	16.22
<b>17</b>	L-Val- triazol	67	140-42	brown	+102	0.81	$C_{19}H_{23}N_9O_4S$ 473	46.84	4.15	16.44
<b>18</b>	L-Met- triazol	82	154-56	brown	+193	0.87	$C_{19}H_{23}N_9O_4S_2$ 505	49.07	4.70	16.65
<b>19</b>	L-Pro	78	189-91	brown	+130	0.67	$C_{17}H_{19}N_5O_5S$ 405	49.12	4.74	20.04
<b>20</b>	L-Pro- OMe	30	208-10	yellow	+170	0.88	$C_{18}H_{21}N_5O_5S$ 419	45.18	4.41	20.03
<b>21</b>	L-Pro- thiazol	89	236-38	brown	+213	0.95	$C_{20}H_{21}N_7O_4S_2$ 487	49.28	4.31	24.95
<b>22</b>	L-Pro- triazol	92	248-50	brown	+180	0.92	$C_{19}H_{21}N_9O_4S$ 471	49.27	4.34	24.93
								50.37	4.69	17.28
								50.36	4.72	17.27
								51.55	5.01	16.70
								51.54	5.05	16.73
								49.28	4.31	20.12
								49.27	4.34	20.11
								48.40	4.45	26.75
								48.35	4.49	26.77

\* Crystallization solvent: Ethanol (**3-22**).

Where Gly= Gycine,  $\beta$ -Ala =  $\beta$ -Alanine, Val= Valine, Met= Methionine, Pro= Proline.

**Table2:** Results of the chemical analyses ( $^1\text{H}$ NMR spectra).

Cpd.	$^1\text{H}$ NMR spectra [DMSO-d6(ppm) $\delta$ ] of compounds
3	12.65(s, 1H, OH-COOH, exchangeable $D_2O$ ), 8.90(1H, NH- $\text{NHSO}_2$ , exchangeable $D_2O$ ) 8.63 (s, 1H, NH- $\text{NHCH}_2\text{COOH-Gly}$ , exchangeable $D_2O$ ) 8.49 (d, 2H, $\text{CH}$ -primidine), 7.71-7.07 (m,5H,[4H, Ar-H+1H,CH-primidine]), 5.64 (1H, NH- $\text{NHCH}_2\text{CO}$ exchangeable $D_2O$ ), 4.24 (d,2H, $\text{CH}_2\text{Gly}_1$ ), 4.08 (d,2H, $\text{NHCH}_2\text{CO}$ ).
4	11.82 (s, 1H, OH-carboxylic, exchangeable $D_2O$ ), 8.89(s, 1H, NH- $\text{NHSO}_2$ , exchangeable $D_2O$ ), 8.49 (d, 2H, $\text{CH}$ -primidine), 7.73-7.10 (m,6H,[4H, Ar-H + 1H, $\text{CH}$ -primidine + 1H, CON $\text{HCH}_2\text{CH}_2$ exchangeable $D_2O$ ]), 5.93(s, 1H, NH- $\text{NHCH}_2\text{CO}$ , exchangeable $D_2O$ ), 4.09 (d,2H, $\text{CH}_2\text{-NHCH}_2\text{CO}$ ), 3.77-3.65(m,2H, $\text{CH}_2\text{-NHCH}_2\text{CH}_2\text{COOH}$ ), 2.56(t, 2H, $\text{CH}_2\text{-CH}_2\text{CH}_2\text{COOH}$ ).
5	11.92 (s, 1H, OH- carboxylic, exchangeable $D_2O$ ), 8.86(s, 1H, NH- $\text{NHSO}_2$ , exchangeable $D_2O$ ), 8.47 (d, 2H, $\text{CH}$ -primidine), 8.27(d, 1H, NH- $\text{NHCH}$ -Val, exchangeable $D_2O$ ), 7.69-6.98(m,5H,[4H-Ar-H + 1H, $\text{CH}$ -primidine]), 5.92 (s, 1H, NH- $\text{NHCH}_2\text{CO}$ exchangeable $D_2O$ ), 4.16 (d, 2H, $\text{NHCH}_2\text{CO}$ ), 2.03-1.99 (m,2H,[1H,CH- $\text{CH}(\text{CH}_3)_2$ COOH& 1H,CH- $\text{CHCH}(\text{CH}_3)_2$ ]), 0.98 (s, 6H,2 $\text{CH}_3$ -( $\text{CH}_3)_2$ ).
6	12.50 (s, 1H, OH-carboxylic, exchangeable $D_2O$ ), 8.90 (s, 1H, NH- $\text{NHSO}_2$ exchangeable $D_2O$ ), 8.53(d,2H, $\text{CH}$ -primidine), 7.72-7.00(m,6H, [4H, Ar-H + 1H, $\text{CH}$ -primidine + 1H, NH- $\text{NHCH}$ -Met, exchangeable $D_2O$ ]), 5.09 (s, 1H, NH- $\text{NHCH}_2\text{CO}$ , exchangeable $D_2O$ ), 4.59(t, 1H, CH- $\text{NHCH}_2\text{COOH}$ ), 4.13(d,2H, $\text{CH}_2\text{-NHCH}_2\text{CO}$ ), 2.67 (t, 2H, $\text{CH}_2\text{-CH}_2\text{SCH}_3$ ), 2.35(s,3H, $\text{CH}_3$ ), 2.24 (dt, 2H, $\text{CH}_2\text{-CH}_2\text{CH}_2\text{SCH}_3$ ).
7	8.90 (s, 1H, NH- $\text{NHSO}_2$ exchangeable $D_2O$ ), 8.75(s, 1H, NH- $\text{NHCH}_2\text{COOCH}_3$ , exchangeable $D_2O$ ), 8.49 (d,2H, $\text{CH}$ -primidine), 7.72-7.07(m,5H, [4H, Ar-H + 1H,CH-primidine]), 5.64 (s, 1H, NH- $\text{NHCH}_2\text{CO}$ , exchangeable $D_2O$ ), 4.31 (s, 2H, $\text{CH}_2\text{-CH}_2\text{COOCH}_3$ ), 4.05 (d, 2H, $\text{CH}_2\text{-NHCH}_2\text{CO}$ ), 3.75 (s, 3H, $\text{CH}_3\text{-COOCH}_3$ ).
8	8.39 (d, 2H, $\text{CH}$ -primidine), 8.00 (s, 1H, NH- $\text{NHSO}_2$ ), 7.72-6.56(m,6H,[4H, Ar-H + 1H,CH-primidine + 1H, NH- $\text{NHCH}_2\text{CH}_2$ exchangeable $D_2O$ ]), 4.00 (s, 1H, NH- $\text{NHCH}_2\text{CO}$ , exchangeable $D_2O$ ), 3.95(2H- $\text{CH}_2\text{CONH}$ ),3.67(s,3H, $\text{CH}_3\text{-COOCH}_3$ ), 3.60-3.58(m, 2H, $\text{CH}_2\text{-CH}_2\text{CH}_2\text{COOCH}_3$ ),2.52(t,2H, $\text{CH}_2\text{-CH}_2\text{CH}_2\text{COOCH}_3$ ).
9	8.39 (d,2H, $\text{CH}$ -primidine), 8.00 (s, 1H, NH- $\text{NHSO}_2$ ), 7.72-6.56(m,6H,[4H, Ar-H + 1H,CH-primidine + 1H,NH- $\text{NHCHCOOCH}_3$ , exchangeable $D_2O$ ]), 5.00 (s, 1H, NH- $\text{NHCH}_2\text{CO}$ , exchangeable $D_2O$ ), 4.42(d,1H,CH- $\text{NHCH}_2\text{COOCH}_3$ ), 3.95(s, 2H- $\text{CH}_2\text{-NHCH}_2\text{CO}$ ), 3.67(s,3H, $\text{CH}_3\text{-COOCH}_3$ ), 3.14-3.04(m, 1H,CH- $\text{CH}(\text{CH}_3)_2$ ), 1.02 (d,6H,( $\text{CH}_3)_2$ ).
10	8.90 (s, 1H, NH- $\text{NHSO}_2$ ,exchangeable $D_2O$ ), 8.49-8.48 (d, 2H,CH-primidine), 7.72-7.07(m,6H, [4H, Ar-H + 1H, $\text{CH}$ -primidine + 1H, NH- $\text{NHCHCOOCH}_3$ - Met. exchangeable $D_2O$ ]), 5.66(s, 1H, NH- $\text{NHCH}_2\text{CO}$ exchangeable $D_2O$ ), 4.79(t,1H, CH- $\text{NHCH}_2\text{COOCH}_3$ ), 4.05(d, 2H, $\text{CH}_2\text{-NHCH}_2\text{CO}$ ) 3.76(s,3H, $\text{CH}_3\text{-COOCH}_3$ ),2.67 (t,2H, $\text{CH}_2\text{-CH}_2\text{SCH}_3$ ), 2.34 (s, 3H, $\text{CH}_3\text{-SCH}_3$ ), 2.24-2.18 (m,2H, $\text{CH}_2\text{-CH}_2\text{CH}_2\text{SCH}_3$ ).
11	13.26(s, 1H, CONH-thiazole,exchangeable $D_2O$ ), 8.90(s, 1H, NH- $\text{NHSO}_2$ ), 8.64 (d,2H,CH-primidine),8.50(s, 1H, NH-CON $\text{HCH}_2\text{-Gly}$ exchangeable $D_2O$ ), 7.52-6.95(m,7H, [4H, Ar-H + 1H,CH-primidine +2H, CH-thiazole]), 5.05 (s, 1H, NH- $\text{NHCH}_2\text{CO}$ , exchangeable $D_2O$ ), 4.39 (s,2H, $\text{CH}_2\text{CONH-Gly}$ ), 4.00 (s, 2H, $\text{NHCH}_2\text{CO}$ ).
12	12.82(s, 1H, NH-CONHthiazole,exchangeable $D_2O$ ), 8.90(s, 1H, NH- $\text{NHSO}_2$ ), 8.55 (d, 2H,CH-primidine), 7.71-7.04(m,8H, [4H, Ar-H + 1H,CH-primidine +2H, CH-thiazole+ 1H, NH- $\text{NHCH}_2\text{CH}_2$ exchangeable $D_2O$ ]), 5.46 (s, 1H, NH- $\text{NHCH}_2\text{CO}$ , exchangeable $D_2O$ ), 4.08 (s,2H, $\text{CH}_2\text{-NHCH}_2\text{CO}$ ), 3.79 (dt, 2H, $\text{CH}_2\text{-NHCH}_2\text{CH}_2\text{CO}$ ), 2.79(t,2H, $\text{CH}_2\text{-NHCH}_2\text{CH}_2\text{CO}$ ).
13	13.09(s, 1H, NH-CONH-thiazole, exchangeable $D_2O$ ), 8.90(s, 1H, NH- $\text{NHSO}_2$ ), 8.50-8.49(d,2H,CH-primidine), 7.67-7.09(m,8H, [4H, Ar-H + 1H,CH-primidine +2H, CH-thiazole + 1H, NH-CONHCH, exchangeable $D_2O$ ]),5.03 (s, 1H, NH- $\text{NHCH}_2\text{CO}$ , exchangeable $D_2O$ ), 4.83(s,1H, CH- $\text{CHCONH}$ ), 4.31 (d,2H, $\text{CH}_2\text{-NHCH}_2\text{CO}$ ),2.55-2.47(m, 1H,CH- $\text{CH}(\text{CH}_3)_2$ ), 1.08 (d,6H,2 $\text{CH}_3$ -( $\text{CH}_3)_2$ ).
14	13.32(s, 1H, NH- CONH-thiazole,exchangeable $D_2O$ ), 8.90(s, 1H, NH- $\text{NHSO}_2$ ), 8.54-8.52(d,2H, $\text{CH}$ -primidine), 7.66-9.65 (m,8H, [4H, Ar-H + 1H,CH-primidine +2H, CH-thiazole+ 1H, NH-CONHCH,exchangeable $D_2O$ ]), 4.89 (s, 1H, NH- $\text{NHCH}_2\text{CO}$ , exchangeable $D_2O$ ), 4.64(t,1H, CH- $\text{CONHCH}_2\text{CH}_2\text{CH}_2$ ), 4.26(d,2H, $\text{CH}_2\text{-NHCH}_2\text{CO}$ ), 2.77(t,2H, $\text{CH}_2\text{-CH}_2\text{SCH}_3$ ), 2.34(s, 3H, $\text{CH}_3\text{-SCH}_3$ ), 2.23(dt, 2H, $\text{CH}_2\text{-CH}_2\text{CH}_2\text{SCH}_3$ ).
15	9.92 (s, 1H, NH-CONH-triazole), 8.90(s, 1H, NH- $\text{NHSO}_2$ ), 8.70(s,1H, NH-NH-triazole), 8.57(s, 1H, NH- CONHCH, exchangeable $D_2O$ ), 8.50(d, 2H,CH-primidine), 7.69-7.05(m,6H,[4H,Ar-H + 1H,CH-primidine +1H, CH-triazole]), 5.62 (s, 1H, NH- $\text{NHCH}_2\text{CO}$ , exchangeable $D_2O$ ), 4.31 (d,2H, $\text{CH}_2\text{-CONHCH}_2$ ), 4.12 (s,2H, $\text{CH}_2\text{-NHCH}_2\text{CO}$ ).
16	9.91(s, 1H, NH-CONH- triazole,exchangeable $D_2O$ ), 8.90 (s, 1H, NH- $\text{NHSO}_2$ ), 8.68 (s, 1H, NH- triazole), 8.48 (d,2H,CH-primidine), 7.68-7.03(m,7H,[4H, Ar-H + 1H,CH-primidine+ 1H, CH-triazole+ 1H, NH- $\text{NHCH}_2\text{CH}_2\text{CONH}$ exchangeable $D_2O$ ]), 5.60 (s, 1H, NH- $\text{NHCH}_2\text{CO}$ , exchangeable $D_2O$ ), 4.09 (d,2H, $\text{CH}_2\text{-NHCH}_2\text{CO}$ ), 3.75 (dt,2H, $\text{CH}_2\text{-CH}_2\text{CH}_2\text{CONH}$ ), 2.77 (t,2H, $\text{CH}_2\text{-CH}_2\text{CONH}$ ).
17	10.79(s, 1H, NH-CONH- triazole,exchangeable $D_2O$ ), 9.03 (s, 1H, NH- $\text{NHSO}_2$ ), 8.89 (s, 1H, NH-triazole), 8.58 (d,2H,CH- primidine), 7.03-7.73(m,7H, [4H, Ar-H + 1H,CH-primidine +1H, CH-triazole+ 1H, NH- $\text{NHCHCONH}$ exchangeable $D_2O$ ]),4.91 (s, 1H, NH- $\text{NHCH}_2\text{CO}$ , exchangeable $D_2O$ ), 4.35(s, 1H,CH,CH- $\text{CHCONH-Val}$ ),4.17 (d, 2H, $\text{CH}_2\text{-NHCH}_2\text{CO}$ ), 1.91-1.83 (m,1H, CH- $\text{CH}(\text{CH}_3)_2$ ), 0.86 (d,6H,2 $\text{CH}_3$ -( $\text{CH}_3)_2$ ).
18	11.03(s, 1H, NH-CONH- triazole,exchangeable $D_2O$ ), 9.95 (s,1H, NH- triazole), 8.89 (s, 1H, NH- $\text{NHSO}_2$ ), 8.56 (d, 2H,CH- primidine), 7.66-7.00 (m,7H, [4H, Ar-H + 1H,CH-primidine +1H, CH-triazole+ 1H, NH- $\text{NHCHCONH}$ exchangeable $D_2O$ ]), 5.19 (s, 1H, NH- $\text{NHCH}_2\text{CO}$ , exchangeable $D_2O$ ), 4.49 (m, 3H, [ 2H, $\text{CH}_2\text{-CH}_2\text{CONH}$ + (1H, CH- $\text{CHCONH}$ ]), 2.67 (t,2H, $\text{CH}_2\text{-CH}_2\text{SCH}_3$ ), 2.36 (s,3H, $\text{CH}_3$ ), 2.19-2.12 (m,2H, $\text{CH}_2\text{-CH}_2\text{CH}_2\text{SCH}_3$ ).
19	11.86(s, 1H, OH-carboxylic, exchangeable $D_2O$ ), 8.89 (s, 1H, NH- $\text{NHSO}_2$ ), 8.53(d, 2H, $\text{CH}$ -primidine),7.69-7.03(m,5H,[4H, Ar-H + 1H, $\text{CH}$ -primidine]), 5.16 (t, 1H,CH- $\text{CH}_2\text{-Pro}$ ), 5.09 (s, 1H, NH- $\text{NHCH}_2\text{CO}$ , exchangeable $D_2O$ ), 4.50 (s, 2H, $\text{CH}_2\text{-NHCH}_2\text{CO}$ ), 3.81-3.61(m, 2H, $\text{CH}_2\text{-CH}(\text{CH}_2)_2\text{CH}_2\text{NCO-Pro}$ ), 2.37-2.35 (m, 2H, $\text{CH}_2\text{-CH}_2\text{CH}_2\text{NCO-Pro}$ ), 2.11-2.01(m, 2H, $\text{CH}_2\text{-CH}_2\text{CH}_2\text{CH}_2\text{NCO-Pro}$ ).
20	8.89 (s, 1H, NH- $\text{NHSO}_2$ ), 8.55-8.54(d,2H,CH-primidine),7.72-7.06(m,5H,[4H, Ar-H + 1H,CH-primidine]), 5.21 (s, 1H, NH- $\text{NHCH}_2\text{CO}$ , exchangeable $D_2O$ ),4.60(t,1H,CH- $\text{CH}_2\text{-Pro}$ ),4.01(d,2H, $\text{CH}_2\text{-CH}_2\text{CO}$ ),3.79-3.62(m,2H, $\text{CH}_2\text{-CH}(\text{CH}_2)_2\text{CH}_2\text{NCO-Pro}$ ), 3.59(s,3H, $\text{CH}_3\text{-COOCH}_3$ ), 2.44-2.42 (m, 2H, $\text{CH}_2\text{-CH}_2\text{CH}_2\text{NCO-Pro}$ ), 2.23-1.95(m, 2H, $\text{CH}_2\text{-CH}_2\text{CH}_2\text{CH}_2\text{NCO-Pro}$ ).
21	13.33(s, 1H, NH-CONH-Thiazole,exchangeable $D_2O$ ), 8.90 (s, 1H, NH- $\text{NHSO}_2$ ), 8.50-8.49(d,2H,CH-primidine),7.67-7.13(m,7H, [4H, Ar-H + 1H,CH-primidine +2H, CH-thiazole]), 5.21 (s, 1H, NH- $\text{NHCH}_2$ , exchangeable $D_2O$ ), 5.04(t,1H,CH- $\text{CH}_2\text{-Pro}$ ), 4.21(s, 2H, $\text{CH}_2\text{-CO}$ ), 3.92-3.57(m, 2H, $\text{CH}_2\text{-CH}(\text{CH}_2)_2\text{CH}_2\text{NCO-Pro}$ ), 2.37-2.11 (m,2H, $\text{CH}_2\text{-CH}_2\text{CH}_2\text{CH}_2\text{NCO-Pro}$ ), 2.10-2.00(m,2H, $\text{CH}_2\text{-CH}_2\text{CH}_2\text{CH}_2\text{NCO-Pro}$ ).
22	10.91(s, 1H, NH-CONH-triazole,exchangeable $D_2O$ ), 10.11 (s, 1H, NH-triazole), 8.90 (s, 1H, NH- $\text{NHSO}_2$ ), 8.53 (d, 2H,CH- primidine),7.72-7.04(m,6H, [4H, Ar-H + 1H,CH-primidine +1H, CH-triazole]), 5.01 (s, 1H, NH- $\text{NHCH}_2$ , exchangeable $D_2O$ ), 4.40-4.34(t,1H,CH- $\text{CH}_2\text{-Pro}$ ),4.05 (s, 2H, $\text{CH}_2\text{-NHCH}_2\text{CO}$ ), 4.01-3.45(m, 2H, $\text{CH}_2\text{-CH}(\text{CH}_2)_2\text{CH}_2\text{NCO-Pro}$ ), 2.39-2.211 (m, 2H, $\text{CH}_2\text{-CH}_2\text{CH}_2\text{CH}_2\text{NCO-Pro}$ ), 2.10-2.02(m, 2H, $\text{CH}_2\text{-CH}_2\text{-CH}_2\text{CH}_2\text{CH}_2\text{NCO-Pro}$ ).

**Table 3:** Results of the chemical analyses (IR and Mass spectra):

Compd.	IR ( $\nu_{\text{max}}$ cm $^{-1}$ ) Selected bands	MS [M $^{+}$ ]
<b>3</b>	(b)3350cm $^{-1}$ (OH); 3235 (NH); 1672 (CO);1350 (SO $_2$ ).	(365,1.68)
<b>4</b>	(b)3364 (OH); 3157 (NH);3094 (CH-aromatic), 1690 (CO), 1355,1152 (SO $_2$ NH).	(380,M+1,16.9)
<b>5</b>	(b)3370 (OH);3245 (NH);2888 (CH-ali), 1690 (CO), 1352,1152 (SO $_2$ ).	(407, 11.1)
<b>6</b>		(439,1.04)
<b>7</b>	3150 (NH), 1672 (CO), 1352,1152 (SO $_2$ ).	(393, 5.07)
<b>8</b>	3154 (NH), 1695 (CO), 1342,1155 (SO $_2$ ).	(423,M+2,7.97)
<b>9</b>	3151 (NH); 1692 (CO).	(423, M+2,7.97)
<b>10</b>	3145 (NH); 1695 (CO).	(453, 11.25)
<b>11</b>	3265 (NH); 1720 (CO).	(448,M+1,24.3)
<b>12</b>	3260 (NH); 1722 (CO).	(462, M+1,1.02)
<b>13</b>	3198 (NH); 1723 (CO).	(489, 1.11)
<b>14</b>	3335 (NH);1718(CO).	(521,2.45)
<b>15</b>	3352 (NH); 1708 (CO).	(431,0.01)
<b>16</b>	3345(NH); 1702 (CO).	(445,0.89)
<b>17</b>	3358(NH); 1708(CO).	
<b>18</b>	3265 (NH),1656 (CO).	(505, 2.08)
<b>19</b>	3382 (OH); 3147 (NH) 1698 (CO).	(407,M+2,3.14)
<b>20</b>	3145 (NH) 1690(CO).	(420, M+1,1.86)
<b>21</b>	3358 (NH) 1690 (CO).	(489,M+2, 26.3)
<b>22</b>	3350 (NH) 1693 (CO).	

**Table 4.** DNA binding activity of compounds (**3-20**) using methyl green DNA displacements assay.

NO.	IC <sub>50</sub> ( $\mu\text{g}/\text{ml.}$ )*	IC <sub>50</sub> ( $\mu\text{M.}$ )**	NO.	IC <sub>50</sub> ( $\mu\text{g}/\text{ml.}$ )*	IC <sub>50</sub> ( $\mu\text{M.}$ )**
<b>EtBr</b>	81 $\pm$ 0.02	205.58	<b>3</b>	15 $\pm$ 0.005	41.09
<b>4</b>	ND	ND	<b>5</b>	49 $\pm$ 0.004	120.39
<b>6</b>	19 $\pm$ 0.01	43.28	<b>7</b>	11 $\pm$ 0.03	29.02
<b>8</b>	16 $\pm$ 0.02	40.71	<b>9</b>	ND	ND
<b>10</b>	20 $\pm$ 0.01	44.15	<b>11</b>	21 $\pm$ 0.01	46.98
<b>12</b>	69 $\pm$ 0.01	149.67	<b>13</b>	ND	ND
<b>14</b>	83 $\pm$ 0.01	169.73	<b>15</b>	78 $\pm$ 0.01	180.97
<b>16</b>	ND	ND	<b>17</b>	ND	ND
<b>18</b>	21 $\pm$ 0.01	41.58	<b>19</b>	ND	ND
<b>20</b>	ND	ND	<b>21</b>	22 $\pm$ 0.002	45.17
<b>22</b>	33 $\pm$ 0.001	70.00			

ND) Not determined (Compounds having IC<sub>50</sub> value > 100  $\mu\text{g}/\text{ml.}$ ).\*) IC<sub>50</sub> Values: Represented IC<sub>50</sub> obtained from three independent determinations (mean  $\pm$  SD) required for 50% decrease in the initial absorbance of DNA-methyl green solution.\*\*) IC<sub>50</sub> values: Concentration required for 50% decrease in the initial absorbance of DNA-methyl green solution.

**Table 5:** Growth inhibitory action (IC<sub>50</sub>) of the tested compounds against (MCF7) human cell lines.

NO.	IC <sub>50</sub> ( $\mu$ g/ml.)*	IC <sub>50</sub> ( $\mu$ M.)**
<b>3</b>	0.97	2.66
<b>10</b>	1.48	3.26
<b>12</b>	2.37	5.14
<b>17</b>	3.65	7.71
<b>DOX</b>	0.9	1.54

\*) IC<sub>50</sub> Values: represent IC<sub>50</sub> obtained from three independent determinations.

\*\*) IC<sub>50</sub> values: concentration causing 50% inhibition of cell viability.

**Table 6:** Anti-microbial activity of the synthesized compounds.

Comp No.	In vitro activity-zone of inhibition in mm (MIC in $\mu$ g/ml)										
	S.Aureus (ATCC-9144)		S.epidermidis (ATCC-155)		K.Pneum-oniae (ATCC-11298)		E.coli (ATCC-25922)		A.niger (ATCC-9029)		A.fumigatus(ATCC-46645)
	A	M.I.C	A	M.I.C	A	M.I.C	A	M.I.C	A	M.I.C	
<b>3</b>	16	28.4	18	15.1	13	33.8	13	15.6	17	26.2	35
<b>6</b>	14	27.9	19	15.2	19	18.9	22	25.3	15	18.8	13
<b>7</b>	18	22.6	19	30.1	19	11.6	25	21.8	18	19.4	14
<b>8</b>	22	11.1	27	20.1	20	14.6	17	14.8	21	18.8	27
<b>9</b>	26	17.3	16	17.2	26	19.1	18	22.6	19	20.2	24
<b>10</b>	15	23.8	7	—	7	—	9	—	11	32.2	14
<b>13</b>	26	22.4	12	27.4	16	22.9	16	17.3	19	20.2	19
<b>15</b>	18	18.9	13	18.8	15	32.9	14	21.8	19	30.2	22
<b>16</b>	22	21.6	17	15.8	16	11.2	22	12.6	13	11.6	15
<b>17</b>	17	23.8	19	22.8	20	17.2	21	15.6	22	31.1	7
<b>18</b>	25	18.9	16	18.8	17	19.1	7	—	25	21.8	22
<b>20</b>	19	14.9	19	28.1	20	15.2	21	12.6	17	11.9	15
<b>Ciprofloxacin</b>	29	0.2	31	0.39	30	0.1	29	0.3	—	—	—

Inhibition zone in millimeters.

Inhibition zone diameter: (7-13 mm) Weak active;  
(14-20 mm) Moderate active;  
(21-42 mm) High active.

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6/1/2010

# Amelioration Productivity of Sandy Soil by using Amino Acids, Sulphur and Micronutrients for Sesame Production

Salwa, A.I. Eisa; Mohsen. M. Abass and S.S. Behary

Soils, Water and Environment Research Institute, ARC, Egypt

**Abstract:** A field experiment was carried out at Ismailia Agricultural Research Station, Ismailia Governorate, Egypt for two successive summer seasons 2008 and 2009 using sesame (Giza 32) to study the effect of elemental sulphur as soil application, amino acids and micronutrients (Fe, Zn, Mn) as foliar spray and their interactions by concentration ( $A_0$ ) zero and ( $A_1$ ) 2 g/l, for amino acids, micronutrients Fe, Zn and Mn were added as mixture at rates of zero ( $T_0$ ) & 450, 225, 225  $\mu\text{g g}^{-1}$  ( $T_1$ ) and 900, 450, 450  $\mu\text{g g}^{-1}$  ( $T_2$ ). While elemental sulphur was added at the rates of zero ( $S_0$ ) & 0.5 Mg S/fed ( $S_1$ ) and 1.0 Mg S/fed ( $S_2$ ), on sesame yield, its components and seed quality. Data indicated that, plant height (cm) significantly affected by all applied treatments. The tallest plant height (213.3 cm) achieved upon treating by  $A_1 + 1.0 \text{ Mg S/fed} + 900, 450, 450 \mu\text{g g}^{-1}$  (Fe, Zn, Mn) by rate of increases 48.0% over control. Data also show that there were significantly increases in the whole plant weight with increasing application of sulphur as soil application and micronutrients as foliar spray. The highest plant weight were achieved upon treating by  $A_1 + 1.0 \text{ Mg S/fed} + 900, 450, 450 \mu\text{g g}^{-1}$  (Fe, Zn, Mn) by rate of increases 50.04% over control. A significantly increase in the seed sesame yields, the highest seed yields were achieved upon treating by  $A_0 + 1.0 \text{ Mg S/fed} + 900, 450, 450 \mu\text{g g}^{-1}$  (Fe, Zn, Mn) by rate of increases 89.80% over control. For seed quality data show that an increases in P and K%, Fe, Zn and Mn  $\mu\text{g g}^{-1}$ , oil and protein%, oil and protein yield upon treating by  $A_1 + 1.0 \text{ Mg S/fed} + 900, 450, 450 \mu\text{g g}^{-1}$  (Fe, Zn, Mn). Generally, a combined application of amino acid with micronutrient Fe, Zn, Mn in the presence of elemental sulphur significantly increased the sesame yield; improved nutrition and increased seed quality; except the seeds yield whither the highest amount for seeds yield occurred in absence of amino acids; it was probably related to the physiological actions of amino acids and micronutrients. [Journal of American Science. 2010;6(11):250-257]. (ISSN: 1545-1003).

**Key words:** Amino acids- sulphur-micronutrients-sesame-sandy soil.

## 1. Introduction

Micronutrients are elements which are essential for plant growth, but are required in quite smaller amounts than those of the primary nutrients, nitrogen, phosphorus and potassium. They play an indispensable role in cell division and development of meristematic tissues, stimulate photosynthesis, respiration, energy and nucleotide transfer reactions and fasten the plant maturity (Marschner, 1998). Although micronutrients are needed in relatively very small quantities for adequate plant growth and production, their deficiencies induce a great disturbance in the different physiological and metabolic processes inside the plant.

Amino acids can directly or indirectly influence the physiological activities of the plant. Functionally, amino acids especially L- amino acids rather than D- amino acids are involved in the enzymes responsible for the structural photosynthesis process. Also, amino acids have act as chelating effect on micronutrients, when applied together with micronutrients, the absorption and transportation of micronutrients inside the plant is easier (Ibrahim, 2007). The requirement of amino acids in essential quantities is well known as a mean to

increase yield and overall quality of crops. The application of amino acids for foliar spray is based on their requirement by plants in general and critical stages of growth in particular. Plants absorb amino acids through stomata and are proportionate to environment temperature that controls the opening mechanism of the plant stomata. Also amino acids are fundamentals ingredients in the process of protein synthesis. About 20 important amino acids are involved in the process of each function (Ewais *et al.*, 2005). Khalil *et al.*, (2008) found that foliar spray of both amino acids and micronutrients together on onion plants could improve the onion yield and its components.

In oil seeds, sulphur plays a significant role in the quality and development of the seeds. Therefore, crops of oil seeds require a higher quantity of sulphur for proper growth, development for high yields (Mandal *et al.*, 1993). Ceccotti (1996) recorded that sulphur plays an important role in the primary and secondary plant metabolism as a component of proteins, glucosinolates and other compounds that related to several parameter

determining the nutritive quality of crops. Moreover, sulphur is an integral part of acyl-coenzyme A that helps synthesis of more fatty acid (Lal *et al.*, 1995).

Sesame (*Sesamum indicum*, L) is one of the most important crops grown for oil production in Egypt. The crop is grown for its seeds, which contain 50-60% oil, 8% protein, 5.8% water, 3.2% crude fiber, 18% carbohydrate, 5.7% ash and it is very rich in minerals such as Ca, P and vitamin E Dasharath *et al.* (2007). Also, sesame oil has a very high level of unsaturated acids, which is assumed to have reducing effect on plasma cholesterol, as well as on coronary heart disease (Agboola, 1979). Sesame seeds have a positive amino-acid structure- high level of methionine and low level of lysine; this makes it an excellent protein complement to other plant proteins. Obiajunwa *et al.* (2005) reported that Nigerian sesame seeds are rich in essential minerals and trace elements that promote well being in humans. Minerals are unique nutrients because of their important role in metabolism. They are essential part of many important enzymes and they also play roles as catalysts and antioxidants. They added that at a daily consumption rate of 100 g, the values of all the elements in sesame seed fall within the US recommended Dietary Reference Intakes (DRIS) (National Academy of Sciences, 1998). Neelam Sharma *et al.*, (2007) found that sesame genotype contained high value of protein content in the range of 18.60 to 27.57 %. The moisture content was in the range of 3.14 to 4.40 % and ash content ranged from 4.20 to 6.20 %. Sesame seeds are good source of methionine (2.32 to 3.77 g / 16 g N) and tryptophan (1.03 to 1.95 g / 16 g N). Oxalate content varied from 475.32 to 987.19 mg / 100g, while the range of oil content was 32.00 to 50.36. In spite of its importance, little attention has been paid for its nutrient requirements, especially in newly reclaimed sandy soils. So the main target of the current investigation is to study the efficiency of amino acid, micronutrients Fe, Mn and Zn applied with different rates in the presence of sulphur on the yield, seed quality for sesames plant in sandy soils.

## 2. Materials and Methods

A field experiment was carried out at Ismailia Agricultural Research Station, for two successive summer seasons 2008 and 2009 using sesame (Giza 32) to study the effect of amino acids, micronutrients (Fe, Zn, Mn) and elemental sulphur and their interaction on sesame yield, its components and seed quality. Some physical and chemical characteristics of the studied soil are presented in Table (1) which, were determined according to Klute (1986) and Page *et.al.*, (1982) .The experimental design was a randomized complete block with four replicates. The plot area was 10.5 m<sup>2</sup> (3 m width and 3.5 m length). The plots were ploughed twice in two ways and received superphosphate (15% P<sub>2</sub>O<sub>5</sub>) at

rate 30 kg P<sub>2</sub>O<sub>5</sub>/fed and elemental sulphur at the rates (S<sub>0</sub>) zero, (S<sub>1</sub>) 0.5 Mg S/fed and (S<sub>2</sub>) 1.0 Mg S/fed. Nitrogen and potassium fertilizers were added to all plots in two equal doses during the growing period in the form of ammonium nitrate (33.5% N) and, potassium sulphate (48% K<sub>2</sub>O) at rates of 100 kg N/fed and 50 kg K<sub>2</sub>O/fed, respectively.

The treatments of amino acids were (A<sub>0</sub>) zero and (A<sub>1</sub>) 2 g/l, while micronutrients Fe, Zn and Mn were added as mixture at rates of zero (T<sub>0</sub>) & 450, 225, 225 µg g<sup>-1</sup> (T<sub>1</sub>) and 900, 450, 450 µg g<sup>-1</sup> (T<sub>2</sub>). They were added as foliar application at two times after 30 and 60 days from planting.

At harvest, plant samples were separated and seeds dried at 70 °C ground in a Willy mill and digested with H<sub>2</sub>SO<sub>4</sub> and H<sub>2</sub>O<sub>2</sub> according to Parkinson and Allen (1975). The digested samples were analyzed for N, P and K; Fe, Zn, Mn by using Inductively Coupled Plasma Spectrometer (ICP) plasma 400 according to Cottenie *et al.*, (1982). Oil of sesame seeds was determined according to A.O.A.C (1975). The obtained data (average of two seasons) were statistically analyzed according to S.A.S (2001).

**Table (1): Some physical and chemical properties of the tested soil.**

Characteristics	Value
§ Particle size distribution (%):	
Coarse sand	78.0
Fine sand	14.7
Silt	4.8
Clay	2.5
Texture class	Sandy
§ Chemical analysis:	
pH (1: 2.5, soil suspension)	7.7
Total carbonates (%)	0.41
Organic matter (%)	0.21
EC <sub>e</sub> dS m <sup>-1</sup> , soil paste	2.69
Soluble cations (meq/l)	
Ca <sup>++</sup>	6.96
Mg <sup>++</sup>	4.76
Na <sup>+</sup>	14.60
K <sup>+</sup>	0.62
Soluble anions (meq/l)	
CO <sub>3</sub> <sup>=</sup>	-
HCO <sub>3</sub> <sup>-</sup>	2.74
Cl <sup>-</sup>	15.40
SO <sub>4</sub> <sup>=</sup>	8.80
Available N (µg g <sup>-1</sup> )	10.80
Available P (µg g <sup>-1</sup> )	4.60
Available K (µg g <sup>-1</sup> )	69.0
Available Fe (µg g <sup>-1</sup> )	2.50
Available Zn (µg g <sup>-1</sup> )	1.20
Available Mn (µg g <sup>-1</sup> )	1.58

### 3. Results and Discussion

Plant height (cm) for sesame yield:

Data in Table (2) show the effect of amino acids, sulphur and micronutrients on plant height for sesame yield. Data indicated that there were increases in the plant height (cm) by sulphur application, foliar application of Fe, Mn, Zn and/or amino acids. Generally, these increases were more obvious with foliar application of amino acids. The tallest plant height (213.3 cm) were achieved upon treating by amino acids 2g/l + 1.0 Mg S/fed + 900, 450, 450  $\mu\text{g g}^{-1}$  Fe, Zn, Mn, by rate of increases 48.0% over control. The statistical analysis for the data indicated that plant height significantly affected by all applied treatments. Such results are in agreement with those recorded by Ibrahim *et al.* (2007) in faba bean found that foliar application of both amino acids and micronutrients significantly increased plant height, number of branches, leaf area as well as number of pods per plants and consequently the faba bean seed yield. This explained that foliar application of amino acids affects positively the plant growth and crop yield through :- 1) their role in quick nutrient absorption and systemic transportation through the aerial parts of plants. 2) rapidly metabolized with subsequent formation of biologically useful substances i.e. chlorophyll and plant growth regulators. 3) Nutritional and reconstituent function with formation of proteins and carbohydrates. 4) Catalyst and biostimulant action on the activities of main enzyme systems. 5) Hormone like action of equilibrium and synergistic action with endogenous plant growth regulators. 6) Better transport and use of micronutrients. 7) Regulation of water equilibrium.

**Table (2): Plant height (cm) for sesame yield as affected by amino acids, sulphur and micronutrients**

Treatment	A <sub>0</sub>	A <sub>1</sub>	Means
	Plant height (cm)	Plant height (cm)	
T <sub>0</sub> S <sub>0</sub>	144.1	156.1	150.1
T <sub>0</sub> S <sub>1</sub>	163.5	165.8	164.7
T <sub>0</sub> S <sub>2</sub>	172.6	180.1	176.4
T <sub>1</sub> S <sub>0</sub>	151.3	163.6	157.5
T <sub>1</sub> S <sub>1</sub>	178.0	191.7	184.9
T <sub>1</sub> S <sub>2</sub>	188.3	193.8	191.1
T <sub>2</sub> S <sub>0</sub>	164.3	167.4	165.9
T <sub>2</sub> S <sub>1</sub>	198.0	198.8	198.4
T <sub>2</sub> S <sub>2</sub>	210.8	213.3	212.1
Means	174.5	181.2	

T<sub>0</sub> = zero Fe, Zn, Mn      T<sub>1</sub> = 450, 225, 225  $\mu\text{g g}^{-1}$

T<sub>2</sub> = 900, 450, 450  $\mu\text{g g}^{-1}$       S<sub>0</sub> = zero sulphur

S<sub>1</sub> = 0.5 Mg S/fed      S<sub>2</sub> = 1.0 Mg S/fed

A<sub>0</sub> = without amino acids      A<sub>1</sub> = 2 g/l amino acid

L.S.D <sub>0.05</sub> for	Plant height
A (amino acids)	5.58**
T (micronutrients)	2.31**
A × T	3.27**
S (sulphure)	2.31**
A × S	3.27**
T × S	2.58**
A × T × S	3.65**
C.V	0.91

\*\*highly significant \* Significant

Sesame yields:

Data in Table (3) show the effect of amino acids, sulphur and micronutrients on seed weight (Kg/ha) and whole plant weight (Mg/ha) for sesame yield. Data indicated that there were significantly increases in the seed weight with increasing application of sulphur and micronutrients as foliar spray. Increasing the addition rate of S from 0.5 to 1.0 Mg S/fed and Fe, Zn, Mn from 450, 225, 225  $\mu\text{g g}^{-1}$  to 900, 450, 450  $\mu\text{g g}^{-1}$  led to significantly increase the seed sesame yields. These increases were more obvious when amino acids sprayed in combination by rate of 2 g/l. The highest seed yields were achieved upon treating by A<sub>0</sub> + 1.0 Mg S/fed + 900, 450, 450  $\mu\text{g g}^{-1}$  (Fe, Zn, Mn) by rate of increases 89.80% over control Fig (1). The statistical analysis for the data indicated that seed weight significantly affected by all applied treatments. Data also show that there were significantly increases in the whole plant weight with increasing application of sulphur and micronutrients as foliar spray. The highest plant weight were achieved upon treating by A<sub>1</sub> + 1.0 Mg S/fed + 900, 450, 450  $\mu\text{g g}^{-1}$  (Fe, Zn, Mn) by rate of increases 50.04% over control Fig (1). The statistical analysis for the data indicated that plant weight significantly affected by S and micronutrients (Fe, Zn and Mn). The response of sesame yield to S application may be due to its effect on soil pH and increasing the availability of most of the nutrient elements, especially in sandy soil and presence of micronutrient and /or amino acids.

**Table (3): Sesame yield as affected by amino acids, sulphur and micronutrients**

Treatment	Seeds weight (Kg/ha)		Means	Whole plant weight (Mg/ha)		Means
	A <sub>0</sub>	A <sub>1</sub>		A <sub>0</sub>	A <sub>1</sub>	
T <sub>0</sub> S <sub>0</sub>	608	594	601	11.08	11.87	11.48
T <sub>0</sub> S <sub>1</sub>	665	698	682	13.15	13.32	13.24
T <sub>0</sub> S <sub>2</sub>	748	821	785	13.98	14.65	14.32
T <sub>1</sub> S <sub>0</sub>	620	698	659	11.99	13.20	12.60
T <sub>1</sub> S <sub>1</sub>	833	956	895	14.77	15.49	15.13
T <sub>1</sub> S <sub>2</sub>	872	1006	939	15.11	16.00	15.56
T <sub>2</sub> S <sub>0</sub>	778	800	789	12.79	13.54	13.17
T <sub>2</sub> S <sub>1</sub>	1052	1037	1045	16.48	17.12	16.80
T <sub>2</sub> S <sub>2</sub>	1154	1076	1115	17.62	17.81	17.72
Means	814	854		14.11	14.78	

L.S.D <sub>0.05</sub> for	Seeds weight	Whole plant weight
A (amino acid)	11.15**	N.S
T (micronutrients)	4.62**	3.16**
A × T	6.54**	
S (sulphure)	4.62**	3.16**
A × S	6.54**	
T × S	5.16**	
A × T × S	7.30**	
C.V	9.3	15.18

N.S. Not significant \*\*highly significant \* significant

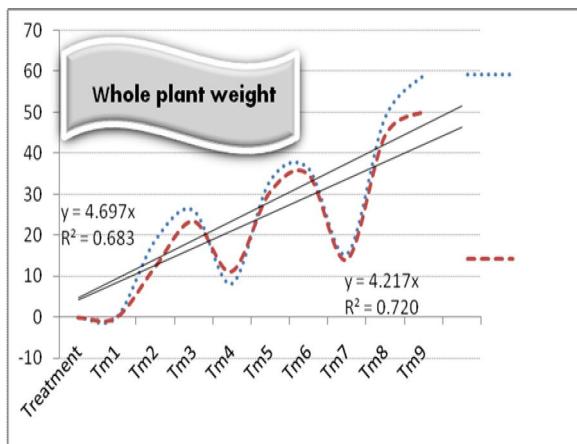
In this connection, Yadav *et al.* (1996) reported that S application significantly increased seed and straw yields of sesame. Fathi *et al.* (1999) observed that the application of elemental sulphur resulted in a significant increase in sesame yield. A positive response of sesame yield to S fertilization was associated with the rate of 1.0 ton S/fed. These results were confirmed with Salwa Eisa *et al.* (2009). On the other hand, Bekhetta (2004), on wheat who found that foliar application for both amino acids and micronutrients led to obvious increases of grain and straw. This is because amino acids help to increases chlorophyll concentration in plant leading to higher degree of photosynthesis. This makes crops mush green and leads to more accumulation of the dry matter and subsequently increases the crop yield.

#### Seed quality:

#### Macro and micronutrients contents:

The effect of sulphur application, amino acids and Fe, Zn, Mn on the contents of macro and micronutrients in the yield of sesame seeds was recorded in Table (4) and (5). Data showed that Increasing the addition rate of S from 0.5 to 1.0 Mg S/fed and Fe, Zn, Mn from 450, 225, 225  $\mu\text{g g}^{-1}$  to 900, 450, 450  $\mu\text{g g}^{-1}$  led to significantly increase in the P and K percentage and

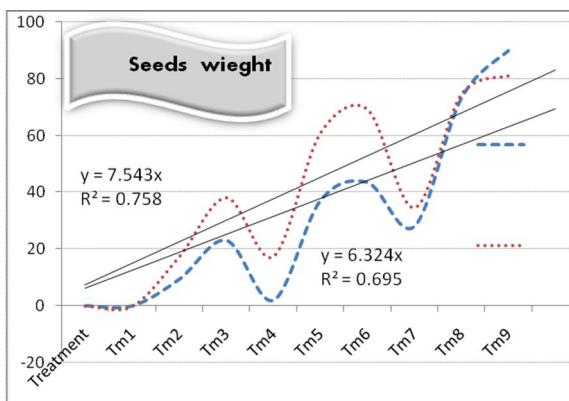
(Fe, Zn, Mn)  $\mu\text{g g}^{-1}$  in seed sesame yields. These increases were more obvious when amino acids sprayed in combination by rate of 2 g/l. The highest seed yields were achieved upon treating by A<sub>1</sub> + 1.0 Mg S/fed + 900, 450, 450  $\mu\text{g g}^{-1}$  (Fe, Zn, and Mn). The corresponding values were 0.56 and 2.45% for P and K. The corresponding values were 333, 78 and 33  $\mu\text{g g}^{-1}$  for Fe, Zn, and Mn. This is because the presence of acid form amendments as mineral sulphur, which improves the physical and chemical properties of soil, lowers soil PH, which well-known effects upon increasing the availability of elements in soil. Also, amino acids have act as chelating effect on micronutrients, when applied together with micronutrients, the absorption and transportation of micronutrients inside the plant is easier (Ibrahim, 2007). These results were confirmed with obtained by Khalil, *et al.* (2008) and Salwa Eisa *et al.* (2009). The statistical analysis for the data indicated that P% significantly affected by S and T& K% significantly affected by T treatments & Fe  $\mu\text{g g}^{-1}$  significantly affected by all treatments & Zn  $\mu\text{g g}^{-1}$  significantly affected by all treatments except A where Mn  $\mu\text{g g}^{-1}$  significantly affected by A, T and S treatments.



L.S.D <sub>0.05</sub> for	P %	K %
A (amino acids)	N.S	
T (micronutrients)	0.02**	0.09*
A × T		
S (sulphure)	0.02**	N.S
A × S		
T × S		
A × T × S		
C.V	3.69	4.20

N.S. Not significant    \*\*highly significant

\* significant



$Tm_1 = T_0 S_0$     $Tm_2 = T_0 S_1$     $Tm_3 = T_0 S_2$     $Tm_4 = T_1 S_0$   
 $Tm_6 = T_1 S_2$     $Tm_7 = T_2 S_0$     $Tm_8 = T_2 S_1$     $Tm_9 = T_2 S_2$

**Fig (1): Rate of increases for whole plant weight and seeds sesame weight.**

**Table (4): Macronutrients content on Sesame seeds as affected by amino acids, sulphur and micronutrients**

Treatment	P %		Means	K%		Means
	A <sub>0</sub>	A <sub>1</sub>		A <sub>0</sub>	A <sub>1</sub>	
T <sub>0</sub> S <sub>0</sub>	0.50	0.50	0.50	2.30	2.31	2.30
T <sub>0</sub> S <sub>1</sub>	0.51	0.52	0.51	2.34	2.34	2.34
T <sub>0</sub> S <sub>2</sub>	0.52	0.52	0.52	2.36	2.37	2.36
T <sub>1</sub> S <sub>0</sub>	0.52	0.51	0.51	2.29	2.32	2.30
T <sub>1</sub> S <sub>1</sub>	0.53	0.54	0.53	2.36	2.36	2.36
T <sub>1</sub> S <sub>2</sub>	0.53	0.54	0.53	2.40	2.38	2.39
T <sub>2</sub> S <sub>0</sub>	0.52	0.54	0.53	2.41	2.43	2.42
T <sub>2</sub> S <sub>1</sub>	0.54	0.55	0.53	2.42	2.44	2.43
T <sub>2</sub> S <sub>2</sub>	0.55	0.56	0.54	2.44	2.45	2.44
Means	0.52	0.53		2.36	2.37	

#### Oil and protein content:

The effect of sulphur application, amino acids and Fe, Zn, Mn on the oil and protein % and oil, protein yield contents of in the yield of sesame seeds was recorded in Table (6). Data showed that application of amino acids and micronutrients as foliar application in the absence of S led to a slight increase in seed oil % and oil yield content. In other words, applications of sulphur increase seed oil content, oil yield content either in the presence or absence of amino acid and micronutrients. Also, data show that, slight increases of oil % for all treatment over control, and there is no significant difference in oil % between the treatments. This mainly, because it is genetically controlled. The highest seed oil % content was obtained upon treating by 1.0 Mg S/fed + 900, 450, 450  $\mu\text{g g}^{-1}$  (Fe, Zn, and Mn) in the presence or absence of amino acids, where for oil yield the highest seed oil yield content was obtained upon treating by 1.0 Mg S/fed + 900, 450, 450  $\mu\text{g g}^{-1}$  (Fe, Zn, and Mn). This is because the addition of elemental sulphur plays a significant role in the quality and development of the oil seeds. With this respect, Fathi (1999) recorded that S application alone up to 1.0 ton S/fed, significantly increased seed oil content of sesame. Similar observation was obtained by Salwa Eisa *et.al.*(2009). Data also showed that Increasing the addition rate of S to 1.0 Mg S/fed and Fe, Zn, Mn to 900, 450, 450  $\mu\text{g g}^{-1}$  in the presence of amino acids led to increase in the protein % and protein yield contents. This is because plants make their proteins by synthesizing them from amino acids, which are produced by complex biochemical processes starting with the elements of nitrogen, carbon, oxygen and hydrogen. This process consumes biological and biochemical energy. Foliar application of pre-formed amino acids gives the plant its requirements and thereby saving biological energy (Ibrahim, 2007).

**Table (5): Micronutrients content of Sesame seeds**

Treatment	Fe ( $\mu\text{gg}^{-1}$ )		Means	Zn ( $\mu\text{gg}^{-1}$ )		Means	Mn ( $\mu\text{gg}^{-1}$ )		Means
	A <sub>0</sub>	A <sub>1</sub>		A <sub>0</sub>	A <sub>1</sub>		A <sub>0</sub>	A <sub>1</sub>	
T <sub>0</sub> S <sub>0</sub>	186	206	196	43	44	43.5	13	14	13.5
T <sub>0</sub> S <sub>1</sub>	208	210	209	48	49	48.5	14	15	14.5
T <sub>0</sub> S <sub>2</sub>	246	251	249	48	50	49.0	18	19	18.5
T <sub>1</sub> S <sub>0</sub>	254	258	256	54	54	54.0	21	21	21.0
T <sub>1</sub> S <sub>1</sub>	260	266	263	59	59	59.0	24	25	24.5
T <sub>1</sub> S <sub>2</sub>	278	285	282	59	62	60.5	26	27	26.5
T <sub>2</sub> S <sub>0</sub>	289	298	294	64	45	54.5	28	28	28.0
T <sub>2</sub> S <sub>1</sub>	300	305	303	69	70	69.5	29	32	30.5
T <sub>2</sub> S <sub>2</sub>	304	333	319	76	78	77.0	31	33	32.0
Means	258	268		58	57		23	24	

L.S.D <sub>0.05</sub> for	Fe	Zn	Mn
A (amino acids)	14.02**	N.S	6.65*
T (micronutrients)	5.81**	4.33**	2.75**
A × T	8.22*	6.13**	
S (sulphure)	5.81**	4.33**	2.75**
A × S	8.22*	6.13**	
T × S	6.49**	4.84**	N.S
A × T × S	9.18**	6.84**	
C.V	1.54	5.28	8.29

N.S. Not significant \* significant \*\*highly significant

**Table (6): Oil & protein% and oil& protein yield in sesame seeds**

Treatment	Oil %		Means	Protein %		Means
	A <sub>0</sub>	A <sub>1</sub>		A <sub>0</sub>	A <sub>1</sub>	
T <sub>0</sub> S <sub>0</sub>	52	52	52.0	16.37	16.87	16.65
T <sub>0</sub> S <sub>1</sub>	54	53	53.5	16.50	16.93	16.68
T <sub>0</sub> S <sub>2</sub>	54	53	53.5	16.50	16.93	16.81
T <sub>1</sub> S <sub>0</sub>	52	52	52.0	16.43	16.87	16.80
T <sub>1</sub> S <sub>1</sub>	53	54	53.5	16.50	17.12	16.87
T <sub>1</sub> S <sub>2</sub>	55	55	55.0	16.56	17.18	16.93
T <sub>2</sub> S <sub>0</sub>	53	54	53.5	16.93	17.25	17.15
T <sub>2</sub> S <sub>1</sub>	54	54	54.0	17.00	17.31	17.16
T <sub>2</sub> S <sub>2</sub>	56	56	56.0	17.12	17.37	17.24
Means	53.6	53.6		16.65	17.09	

Treatment	Oil yield (Kg/ha)		Means	Protein yield (Kg/ha)		Means
	A <sub>0</sub>	A <sub>1</sub>		A <sub>0</sub>	A <sub>1</sub>	
T <sub>0</sub> S <sub>0</sub>	316	309	312.5	99	100	99.5
T <sub>0</sub> S <sub>1</sub>	359	370	364.5	110	118	114.0
T <sub>0</sub> S <sub>2</sub>	404	435	419.5	123	139	131.0
T <sub>1</sub> S <sub>0</sub>	310	363	336.5	98	118	108.0
T <sub>1</sub> S <sub>1</sub>	442	516	479.0	137	164	150.5
T <sub>1</sub> S <sub>2</sub>	480	553	516.5	144	173	158.5
T <sub>2</sub> S <sub>0</sub>	412	432	422.0	132	138	135.0
T <sub>2</sub> S <sub>1</sub>	568	560	564.0	179	180	179.5
T <sub>2</sub> S <sub>2</sub>	646	603	624.5	198	187	192.5
Means	437.4	460.1		135.5	146.3	

L.S.D <sub>0.05</sub> for		Oil %	Protein %	Oil yield	Protein yield
A (amino acids)			1.04**	10.7**	10.6**
T (micronutrients)	1.82**		0.43**	4.45**	4.40**
A × T				6.29**	6.23**
S (sulphure)	1.82**	N.S		4.45**	4.40**
A × S				6.29*	N.S
T × S	N.S			4.97**	4.92**
A × T × S				7.02**	6.96**
C.V	2.37	1.79		0.69	2.18

N.S. Not significant    \*\*highly significant    \* significant

The statistical analysis for the data indicated that oil % significantly affected by micronutrients and elemental sulphur treatments & oil yield significantly affected by all treatments & protein % significantly affected by amino acids and micronutrients treatments and protein yield significantly affected by all treatments except amino acids + sulphure (A×S).

#### 4. Conclusion

From above mentioned, results and under the conditions of this experiment, a combined application of the amino acids and micronutrients in the presence of elemental sulphur increased significantly sesame yield and improved seed quality; except the seeds yield whither the highest amount for seeds yield occurred in absence of amino acids; was probably related to the physiological actions of amino acids and micronutrients. In addition to improving sesame nutrition and quality, reduction in the dose of amino acids and micronutrients applied by foliar spray is of economic importance and may also lead to ecological benefits.

#### Corresponding author

Salwa, A.I. Eisa  
Soils, Water and Environment Research Institute, ARC,  
Egypt

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5/28/2010

# Comparative Study of Software Engineering Processes in Egyptian Cmmi Companies

Alaa El-Din Hamouda and Mohammad Abdрабو Elwahsh  
 Computers & Systems Engineering Dept., Al-Azhar University Cairo, Egypt.  
[Alaa\\_ham@giga.net](mailto:Alaa_ham@giga.net), [eng.md.elwahsh@gmail.com](mailto:eng.md.elwahsh@gmail.com)

**Abstract:** The Egyptian government has paid special attention to the software industry as Egypt to provide it with a competitive advantage that makes this emerging industry promising. Thus, the State has supported the Egyptian companies to make use of the Capability Maturity Model Integration (CMMI). Since 2009, more than thirty companies obtained the CMMI at different levels. However, these companies suffer from lack of a mechanism to exchange experience and information among themselves although they could be similar in the culture of their engineers and perhaps in the nature and size of their software projects. So, we provide in this research a survey to gauge the quality of methods, tools and processes used in these Egyptian companies winning the CMMI. Then we analyzed the results to reach the recommendations aimed at enriching the software industry in Egypt. [Journal of American Science. 2010;6(11):258-263]. (ISSN: 1545-1003).

**Keywords:** CMMI in Egypt, software engineering processes, survey.

## 1. Introduction

In 1993, the Software Engineering Institute (SEI) released the Capability Maturity Model Integration (CMMI) with five staged maturity levels as a means to both appraise maturity level and guide process improvement efforts for software organizations. This model has since been widely accepted around the world, especially in Egypt where the CMMI has helped many software companies [1, 2, 3, 4, and 5].

CMMI combines software engineering, systems engineering, integrated products and procurement to design and improve all types of processes. CMMI has become an international standard for devising software development processes and is credited with helping Egypt rise rapidly to become among the world's software exporters. CMMI provides guidance to improve organizations processes and ability to manage the development, acquisition, and maintenance of products or services. The process areas are grouped into four categories: Process Management, Project Management, Engineering, and Support [6, 7, 8, 9, and 10].

Around the world, there are fast growing CMMI companies. Many countries use the CMMI model extensively e.g. India, China, Japan, Australia, Russia, USA, S.Korea, France, Germany, Brazil, Argentine, Canada and Taiwan. For example in Taiwan seven companies hold Level 2 accreditation, two have Level 3 accreditation and one(IBM Taiwan) has already achieved Level 5 accreditation. In 2009 more than 500 companies in the U.S. were certified to CMMI standard [11, 12, and 14].

Today, use of CMMI in Software industry in Egypt has been increasing to improve software

processes. By June 2009, thirty-one software companies achieved CMMI accreditation levels, from Level 2 to Level 5. One of the problems that face CMMI companies in Egypt is lack of conferences that enable specialists to meet to share their experience about software engineering processes. Also, there is lack of researches that reflect the experiences and provides comparative studies. So, we made the CMMI Survey in 2009 to help organizations identify the best practices and enhance the maturity of their processes. By investigating most of the organizations that have been appraised, processes automation, success factors, keep performance indicator (KPI), benefits of CMMI implementation are identified [13].

## 2. Survey Design

We designed a Survey for CMMI companies in Egypt. The target of the survey is to make a comparative study of the process implementation, best practices, tools, and techniques used in these companies. The target of these questions is to give informative details about the CMMI companies to get clear and transparent information about the software industry in Egypt [13]. These survey requirements were divided into twelve sections, covering most areas of operations in the maturity model with the aim of measuring the capacity of the second and third division levels. The results were as follows: The first section was to get general information about the company characteristics in terms of size and structure, other sections of the survey focus on processes implementation and tools used for different process areas of levels two and three. Level two includes

Project Management, Requirement Management, Measurements and Analysis, Quality Assurance and Configuration Management. Level three includes Technical Solutions, Product Integration, Risks Management, Testing, Decision Analysis and Resolutions , Process Improvement, and CMMI satisfaction[3,9].

The survey was launched in 2009. Through Software Engineering Competence Center (SECC) in the Egyptian Ministry of Communications, the thirty-one Egyptian CMMI companies were requested to fill the survey to answer 65 questions addressing different sections of programming activities in Egypt. Then four experts from software engineering processes and CMMI section were contacted to define the key points which Egyptian companies need to relay their experience. Based on these needs we divided the survey as follows:

- 1) General Information
- 2) Individual Evaluation System
- 3) Engineering Processes Group
- 4) Project Management
- 5) Requirement Management
- 6) Measurements and Analysis
- 7) Quality Assurance
- 8) Technical Solution and Product Integration
- 9) Testing
- 10) Risks
- 11) Process Improvement
- 12) Satisfaction with CMMI based processes

### **3. Survey Implementation**

This survey was sent to 31 companies in Egypt that obtained the Capability Maturity Model Integration (CMMI) at different levels: eighteen companies were granted the second level (58.2%), ten received third level (32.3%), one got level four (3.2%), and two obtained the fifth level (5.6%).

From the results of the survey, we find that 14 companies participated as follows: 8 companies got the second level (57.1%) got the third level (28, 6%), and two got the fifth level (14.3%). most companies responded to all questions included in the survey. However, some vague points were not answered by few companies.

### **4. Results and Evaluations**

#### **A. General Information**

As a result of evaluation, the following rates were obtained from responding companies:

- ✓ A percent of 58.3 % have projectized structures, while 41.7 % are matrix organizations. This is striking if we bear in mind that most companies and projects are individual and small-scale projects. The importance of the adoption of the Matrix Organization is demonstrated when the

companies are large, including a multiplicity of departments and skills. But in a small institution, it is usually advisable to adopt a system of Projectized Organization, where the responsibilities and tasks are more specific and there is speed in decision-making, flexibility in management, and easy follow-up on the other hand. Please note that in medium-sized companies (where the number of employees is less than fifty) 50% of the employees use the matrix model. So we recommend that these medium-sized companies adopt a projectized system.

- ✓ 36% of the companies got the Capability Maturity Model Integration (CMMI) before 2006, 36.4% before 2007, and 27.6% by the year 2008.
- ✓ All companies on the second level have valid plans to get the third level in a year or two, which indicates:
  - a. Growing awareness of the importance of quality systems and the positive repercussions on companies.
  - b. Companies are satisfied with returns resulting from the application of quality systems (Return on Investment).
- ✓ All the companies that got the third level in re-evaluation (reappraisal) after expiry of the first evaluation aim at higher level of the CMMI Levels 4 and 5 but this is impaired by the financial constraints, as the Egyptian Government supports only the companies that plan to get the second and third levels.
- ✓ The ISO 9001 certification was a good start for Egyptian companies, as 43% of the participants, received the ISO certification before they got the CMMI. This can be explained as the required specifications in ISO 9001 focus on the administrative side, at the same time the CMMI standards focus on the specifications of the technical operations, especially in the third level and above. This is why we recommend the companies that got the ISO certification or wishing to start a quality journey to follow the experience gained and the methodology proposed by [Chanwoo, 2006].

#### **B. Individual Evaluation System**

Individual Evaluation is a key success factor for organization. Having good measures and processes for performance evaluation affects the employee's satisfaction and turn over rate. In 55% of the companies, where the individual survey system was applied the direct manager alone filled the survey. This reflects two facts:

- a. Companies need performance indicators which truly reflect the real level of performance of engineers.
- b. Companies need to design questionnaires on specific scientific basis which reflect the level of performance of software engineers from different points of view. For example, designing a system of individual performance appraisal method based on the Three-Hundred and Sixty Degrees method can be a good choice. Hence it is recommended that researchers in the field of software engineering would address this need and give it priority in their research.

#### C. Engineering Process Group (EPG)

At the beginning of the processes improvement initiative, there are usually some important questions, e.g. How many people are needed for the EPG? Should they be dedicated process engineers or normal software engineers who spend some time in processes? What are the criteria for EPG member selection? The paragraphs below help the decision maker through highlighting the actual performance.

- ✓ 83% of the companies that dealt with the survey are of small and medium SME.
- ✓ 53.8% of the companies prefer to have the engineering team dedicated to this work size (Static EPG), while in 46.2% of the companies, EPG members are originally working in software projects (such as a systems analyst, developer, tester) and those who deal with process improvement tasks would, take over these functions to complement their own tasks (Virtual EPG).

The average overall efforts to improve operations in companies have a monthly rate of 1.25 employees. This will be useful later to show the amount of spending on improving processes, calculate the resulting returns and consequently access the return on expenditure (Return on Investment, ROI).

#### D. Project Management (PM)

Project managers face challenges of selecting the suitable quick and detailed estimation techniques and the adopted software life cycle. They are also required to select the project management tools and decide about the meetings frequency. Challenge facing the project manager to select the appropriate method to estimate the size of the product backend forums and then estimate the cost and time, has emerged from the questionnaire which also revealed that:

- ✓ 42.9% of the companies use the Microsoft Professional project management (Microsoft Project Professional).

- ✓ 36% use the Microsoft Advanced Project Management (Microsoft Enterprise Project).
- ✓ 21.1% are using a spreadsheet (Excel sheet). These rates are consistent with the nature of projects which are based on dealing with the user.
- ✓ 53.8% Use Case Points (Use Case Point) as a tool to estimate the size of projects.
- ✓ 38.2% use point of the task (Function Point), and 8% use COSMIC tool.
- ∅ 78.6% have a preliminary technical assessment (Initial Estimation Technique), and 21.4% do not.

#### E. Requirement Management

A percent of 46.2% of the companies use spreadsheet software for management of requirements. However, complex software systems steadily increase the list of requirements which makes it difficult to manage and follow-up. It is also difficult to link design and test programs and schemes corresponding to each requirement (Traceability Matrix). Accordingly, we recommend, in such case, use of special programs to manage the requirements of software systems to enhance the efficiency of the management process requirements.

#### F. Measurements and Analysis (M&A)

The number of key performance indicators (KPI) in projects and institutions in general was great compared to company sizes. So as, each KPI has a cost for managing it (e.g. collecting KPI values, verifying their validity, and analyzing them), we recommend training specialists to calculate the cost of these indicators (cost/benefit analysis) in order to be able to take the right decision for selection of numbers and quality.

In 50% of the companies, key performance indicators for the (KPIs) are less than 4 and more than 7, and 33% less than 4, in 17% it is more than 4, this is compared to the number of key performance indicators where in 64.2% of companies the number of KPI is between 4 and 7, and in 30.8% the number of is less than 4, and in 23% is more than 7. We find that 85.7% of the companies take advantage of the actual analysis of performance indicators to improve operations.

#### G. Quality Assurance (QA)

How many process QA engineers are needed? Is it useful to get QA approval before a project is closure?

When should QA issues be escalated? The results below express the real situation in Egyptian CMMI Companies. The senior management support to a quality assurance is an essential element in the commitment of staff operations that are in line with quality systems. It has been found that 57.1% of the companies need to get the approval of the project manager of quality assurance team (QA approval) at the end of any project, and in 71.4% of the companies, the number of quality assurance engineers was more than three. These are good promising an indicator of the manager's concern to stress the value of the quality of operations and give it direct support.

#### H. Technical Solution and Product Integration

For developers, it is important to determine how unit testing is performed and to define dedicated positions for architecture, analysis, and design are used. It is equally important to define who is responsible for support documentation. The results of that survey are given below.

- ✓ 78.6% of the companies perform automatic unit testing, and 57.1% perform the testing manually. This reduces efficiency as much as it lowers the productivity of the developer/tester. These companies are recommended to adopt unit testing and general testing. We also recommend training developers on tools unit testing automatically, and training testers to use automated testing tools.
- ✓ 27.3% of the companies use dot-net (Dot Net) as a tool for program development. 27.3% use the Java language (Java), 9.1% use the Oracle (Oracle) language, while 36% use other languages such as Delphi (Delphi).
- ✓ There is no specialization in technical writing where developers and testers take the responsibility of preparing documents associated with the product code. This is, in most cases, not accomplished professionally which reduces companies' efficiency. So, we recommend that company's employee technical writer who would be responsible for documentation especially since the cost incurred may be less than the cost of employing a developer or tester.

#### I. Testing

- ✓ It is noted that the checking bugs (Bugs), and problem issues (Issues) are checked manually in 57.1% of the companies. Perhaps this makes it difficult to follow-up and may affect the quality of products and processes. Hence, we propose that companies automate this process, either through

their own software or through ready-made software.

- ✓ Manuscript rapid testing (rapid testing script) is very important to examine applications in a limited time. The importance of this testing is highlighted in the maintenance phase of software systems. However, we find that 71.4% of the companies do not have this facility. Therefore, we recommend its provision for testers and training them to use it.
- ✓ The rate of developers to testers in the companies was at an average of one laboratory for each 3.7 developers. This reflects the distribution of effort in software projects, where this ratio is severely limited if compared to the ratio of 1: 2 approximately [17] in COCOM I and COCOM II. This indicates a weak interest in testing the products adequately. So, it is recommended to invest more effort in testing products which would improve the quality and increase competitiveness of Egyptian software.
- ✓ 78% of the companies perform unit tests manually, and 57.1% do screening tests manually. This reduces efficiency as much as it reduces the productivity of the developer/tester. Thus, we recommend companies to adopt mechanical unit testing. We also recommend training developers to perform the Tools unit test automatically, and training examiners to use automated testing tools.

#### J. Risks

54.5% of the companies use spreadsheets to manage risks, 18.2% use radar (Radar), and 27.3% use other programs such as (Microsoft Project Server) for the management of risks.

#### K. Overall Satisfaction with CMMI

- ✓ Generally, the companies applying the CMMI are satisfied which is a good sign. However, there are complaints from the complexity of operations there is an urgent need to review the operations approved by each company in the system to alleviate any burden carried by these processes (Process overhead). To accomplish this, we recommend that companies work on some ideas inspired by the Agile Models and trying to integrate them in their quality system which is compatible with the CMMI, especially as most companies are small and thus need flexible and simple processes.
- ✓ 21% of the projects in the companies do not follow the processes set forth by the CMMI quality system. When these companies were asked, the answer was that pressure from

- customers to get the product forces them not to follow the internal CMMI quality systems. This in fact represents a threat to the quality of processes and then the quality of products in these projects. So, it is proposed that companies apply another Simple Process to speed the completion of work such as the Agile Model, and thus Subject all company projects whether complex processes or simple operations, to the internal quality system according to the standard set by the companies to follow the appropriate processes.
- ✓ The percentage of delay in software projects delivery date was 58% and the average percentage of projects costing more than the approved budget is estimated by 45.5%. This is consistent with global figures estimated by 75% and 50% respectively. It is observed that most international projects that suffer from delays and cost increase are large-scale projects, while the projects in the Egyptian companies are not huge. According to [18], the most important reasons at the global level are:
    - a. poor planning and management
    - b. changing objectives of projects during their implementation
    - c. Non-participation of senior management in the follow-up projects and consequently not giving enough support.

We find that the second and third reasons may not apply directly to the Egyptian companies which are relatively small in size. Also, the number of projects is limited, which reduces the likelihood of changing objectives and lack of support from senior management. Thus, the primary cause is the most influential and therefore it is recommended to raise the skills of project managers through training to use the specific and effective methods of project management software.

## 5. Conclusion

Through this paper we are providing the results of a questionnaire to investigate the processes and Tools used in CMMI software companies in Egypt. By analyzing the results and conclusion obtained, we recommend that:

1. Small and medium companies adopt a structural Projectized Organization process, where the responsibilities and tasks are more specific and there is speed in decision-making and flexibility in management and follow-up.
2. Training the responsible personal to estimate the cost of key performance indicators (analysis of the relationship between expenses and profit) in order to be able to reach a wise decision for selection of

performance indicators with respect to quality and quantity.

3. Carrying performance unit tests and general tests automatically. Also, training developers to perform the Tools unit tests automatically, and testers to automated testing tools.
4. Automation of the follow-up bugs (Bugs), and issues (Issues) either through their own software or through ready-made software's.
5. Training testers to prepare and implement rapid testing (rapid testing script) to support the maintenance of software products.
6. With the steady complexity of the code systems and growth of the list of requirements which is difficult to manage and follow-up, it becomes difficult to link it to design, test programs and plans corresponding to each requirement (Traceability Matrix). Preparation and use of special programs to manage the requirements of software systems, to enhance the efficiency of the management process requirements.
7. Exerting more effort in the work of a good testing of products in order to increase the quality and competitiveness of Egyptian software.
8. Companies employ a technical writer, especially since the cost incurred is less than that of employee a programmer or tester.
9. Enhancing skills of project managers through training on the unique and effective methods of project management software.
10. Companies prepare new classifications for simple operations (Simple Process) in order to be able to speed the completion of work by following the Agile model and thus subject all projects to their special quality system (both for complex or simple operations) according to the standard they set.
11. Companies holding ISO 9001 or wishing to start a trip to ISO quality should follow the experience gained and the methodology proposed in [19]. The ISO 9001 certification was a good start for many Egyptian companies. Forty-three percent of the participating companies received a certificate of the ISO before they got the Capability Maturity Model. This can be explained by the required specifications in both, where the ISO 9001 focuses on the administrative side, at the same time the Capability Maturity Model identifies standards of technical operations, notably in the third level and above.
12. There is an urgent need to review the operations approved by each company in its system, and for this process said companies try to make use of Agile models and integrate them in their quality system which is compatible with the CMMI to decrease the Process overhead,

especially as most companies are small and need to be flexible, and simple.

13. Researchers in the field of software engineering should study to find indications of a genuine performance which truly reflects the performance of engineers to design by questionnaires on the basis of special scientific programmed software which describe the performance of engineers from different points of view, for example, designing a system to assess individual performance depending on the method of Three Hundred and Sixty Degrees.

### **Future Work**

After publication of this paper we shall supply the participating companies with the results through the Egyptian Ministry of Communications represented by SECC. Through participation in Arab conferences we hope that government's private institutions Adopt these recommendations and spread them in their countries.

### **Acknowledgments**

We thank the Software Engineering Competence Center (SECC) especially Dr. Gamal Aly and Abeer Khedr for their support, and Dr. Mohammad Zaki for his valuable comments; we also thank all individuals who took the time to assist us in this survey. Finally, we thank Horizon Software Company as a sponsor of this work.

### **Corresponding author**

Alaa El-Din Hamouda  
 Mohammad Abdurbo Elwahsh  
 Computers & Systems Engineering Dept., Al-Azhar University Cairo, Egypt.  
[Alaa\\_ham@giga.net](mailto:Alaa_ham@giga.net), [eng.md.elwahsh@gmail.com](mailto:eng.md.elwahsh@gmail.com)

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5/29/2010

# Margin Assessment and Fracture Resistance of Adhesively Luted Ceramic Crowns

Jylan F. ElGuindy<sup>1</sup>, Dina H. Mostafa<sup>\*2</sup> and Mona A. El Agroudi<sup>1</sup>

<sup>1</sup>Fixed Prosthodontics Department and <sup>2</sup>Biomaterials Department, Faculty of Oral and Dental Medicine, Cairo University, Cairo, Egypt.  
 \*dinamostafa@hotmail.com

**Abstract:** Objectives: The aim of this study was to investigate the effect of different adhesive systems on the vertical marginal gap distance and the fracture resistance of lithium disilicate based crowns.

Methods: Forty premolars were prepared to receive forty e-max crowns. The crowns were divided into 4 groups (N=10 each) according to the adhesive luting systems. Group (U): using RelyX Unicem resin cement (self-adhesive system). Group (V): Variolink II (total-etch system). Group (GU) and group (GV): application of G-bond (self-etch) on dentin preceded previously used adhesive systems. A stereomicroscope was used to record the vertical marginal gap distance before and after cementation. The crowns were subjected to cyclic loading and fracture resistance test. Data were statistically analyzed using One-way Analysis of Variance (ANOVA) SPSS 15.0. A scanning electron microscope was used to qualitatively examine the dentin/resin interface. Results: Groups (GU) ( $67.6 \pm 5.8 \mu\text{m}$ ) and (GV) ( $68 \pm 6.4 \mu\text{m}$ ) recorded the significantly lowest vertical marginal gap, followed by group (V) ( $82 \pm 6.8 \mu\text{m}$ ). Group (U) showed the highest marginal inaccuracy ( $114 \pm 6.4 \mu\text{m}$ ). Group (GU) recorded the significantly highest fracture resistance ( $2840.5 \pm 3.8 \text{ N}$ ), followed by group (GV) ( $2411.3 \pm 3.3 \text{ N}$ ) and group (V) ( $2365.8 \pm 3.6 \text{ N}$ ). Group (U) showed the lowest results ( $2270.9 \pm 3.4 \text{ N}$ ). Conclusions: Ceramic restorations luted with total-etch system offer better vertical marginal gap distance and fracture resistance than restorations luted with self-adhesive system. Treatment of the dentin surface prior to the application of the bonding system is efficient. [Journal of American Science. 2010;6(11):264-273]. (ISSN: 1545-1003).

**Keywords:** Adhesives, marginal gap, fracture resistance, all-ceramics

## 1. Introduction

Success of any indirect dental restorations depends on many factors among them the cementation techniques and procedures. Dental cement must act as a barrier against microleakage, holding the tooth and restoration together mechanically and/or chemically. The behavior of the cement and bonding systems is complex and partly depends on the properties and quality of the component parts of each system.<sup>1</sup> An ideal dental adhesive should be able to wet, infiltrate dentin and provide a durable bond between the unhomogeneity of enamel and dentin and the restoration.<sup>2</sup> The permeability of dentin to adhesive agents depends on the resin infiltration of both dentinal tubules and intertubular dentin, however, resin infiltration into intertubular dentin can occur only if the mineral phase of dentin is removed by acid conditioners.<sup>3</sup> Knobloch et al 2007, reported a résumé of the modification done through the bonding agent generations.<sup>4</sup> Total-etch technique including dry and wet techniques, rely on etching the dentin and removal of the smear layer. This technique involves a separate etch and rinse step followed by priming and application of the bonding resin, it is said to be a time

consuming technique.<sup>5</sup> Self-etching technique rely on etching the dentin using non rinse acidic monomers that simultaneously condition and prime, in one step, incorporating the smear layer within the hybrid layer so that it becomes one single layer.<sup>6</sup> RelyX Unicem (3M ESPE, Seefeld, Germany) the self-adhesive, universal resin cement without surface pretreatment has been introduced. It is based on a novel initiation technology using new monomer and filler. The organic matrix consists of newly developed multifunctional phosphoric acid methacrylate, which, can react with the basic fillers in the luting cement and the hydroxyapatite of the hard tooth tissue. This cement quickly neutralizes during the curing process, to switch from a hydrophilic to a hydrophobic state. This unique switch allows the material to adapt to the tooth structure while in the hydrophilic state, yet provide for ongoing dimensional stability with the restoration after converting to the hydrophobic matrix.<sup>7</sup>

Today operators have the choice between water based and resin based cements, which can be used with or without adhesives.

They should however choose the most efficient system i.e. pretreatment of the dentin surface is advisable.<sup>8</sup> A strong bond has been reported to improve marginal adaptation and increases fracture resistance of the tooth and the restoration.<sup>9</sup>

An increased demand for aesthetic restorations makes ceramic the material of choice as anterior and posterior restorations. Consequently, all-ceramic crown may be considered an alternative restoration for highly esthetic areas.<sup>10</sup> Several types of all-ceramic materials have been developed for posterior crowns, including castable ceramics, leucite-reinforced ceramics, lithium disilicate, aluminum oxide ceramics, and zirconium oxide ceramics.<sup>11,12</sup> Several factors influence the compressive strength testing of a clinical ceramic crown, such as preparation design, ceramic material, crown thickness, method of luting, cyclic loading, and thermal cycling.<sup>13</sup> The majority of failures of all ceramic crowns are initiated at the inner surface of the crown where it is subjected to maximum tensile stress and this is intensified by the presence of flaws and cracks.<sup>14</sup> Resin luting agents providing durable resin bonds significantly strengthen ceramic materials by "healing" minor surface defects.<sup>15</sup> Adhesive resin cements must have the ability to bond to both tooth structure and restoration, otherwise, poor bond quality at either the ceramic-cement or dentin-cement interface can significantly reduce the fracture resistance.<sup>13</sup>

The purpose of this study was to investigate the effect of the adhesive bonding system (total-etch versus self-etch) on the vertical marginal gap distance and the fracture resistance of lithium disilicate based crowns and to evaluate the effect of dentin pre-treatment preceding each cement on the results.

## 2. Materials and Methods

### 1- Sample preparation:

Forty recently extracted caries free maxillary first premolars of similar size were collected and stored in water in a refrigerator to avoid dehydration until used. The teeth were embedded in acyclic resin blocks (Melioident, Bayer Dental, Newbury, UK) up to 2mm below the cemento-enamel junction. To secure the tooth in the resin block, a hole in the root was drilled where an orthodontic wire was placed and protruded for mechanical interlocking within the resin. Using an industrial lathe machine, the teeth were prepared for all ceramic preparation with standardized dimensions of 6 degrees angle of convergence.<sup>16</sup> The preparation had 5mm occluso-cervical height, 3mm occlusal diameter, 6mm cervical diameter, 1.2 mm shoulder finish line and 130 degrees occlusal angle.

### 2- Crowns construction:

A total number of forty crowns were constructed. For the purpose of standardization, two counter dies (stainless steel) were constructed, one counter die provided 0.8mm space for core construction and the other provided 1.2mm space for veneer application. Forty impressions of the prepared teeth were made with polyvinyl siloxane impression material (Imprint II, 3M, ESPE, Minnesota, USA) using custom made trays and poured into stone dies (Degussa, AG, Frankfurt, Germany). The stone dies were trimmed, and die spacer was applied (Vita zahnfabrik, Bad Sackingen, Germany) followed by separating medium application (Bego, Bremer, Bremen, Germany). Direct wax pattern (Schuler-dental, GmbH & Co, Kohn, Germany) was made on the stone dies. Waxed crown samples were constructed, and then the first counter die was applied to provide 0.8mm space for core construction. Verification of the thickness was performed using a caliper. Spruing, investing using E-max Press Vest Speed investment (Ivoclar, Vivadent, Schaan, Liechtenstein) and wax elimination following manufacturer's instructions was performed. E-max Press ingots medium opacity (Ivoclar, Vivadent, Schaan, Liechtenstein) were heat pressed in the EP 600 furnace (EP600 Combi, Ivoclar, Vivadent, AG FL-9494, Schaan, Liechtenstein) following manufacturer's instructions forming the full thickness core. The cores were replaced onto the dies for full thickness veneering application, using the second counter die which provides 1.2mm space for veneer application (0.4mm thickness). The E-max Ceram veneering (Ivoclar, Vivadent, Schaan, Liechtenstein) was mixed, then applied conventionally and fired according to the manufacturer's direction. Adjustment to the final 1.2mm thickness was verified prior to over glaze at 750°C. Finally, the samples were ultrasonically cleaned in an ultrasonic bath (Vitasonic, Vita Zahnfabrik, Bad Sackingen, Germany) for five minutes and then examined carefully for any crack or defect. The fitting surface of the E-max crowns was etched for 2 minutes with hydrofluoric acid (Ivoclar, Vivadent, Schaan, Liechtenstein) then silane treated (Monobond-S, Vivadent, Schaan, Liechtenstein) according to the manufacturer recommendation.

### 3- Samples assignment:

The forty crowns were assigned to 4 groups (N = 10 each) according to the adhesive luting systems employed. Table (1) shows the

different systems used, their main components and application protocols.

**Group (U): Self-etch system:**

The self-adhesive approach was employed using RelyX Unicem resin cement (U) according to the manufacturer's instruction to lute the ceramic crowns.

**Group (V): Total-etch system:**

The dentin was etched with 35% phosphoric acid for 15 seconds, rinsed, gently air dried, and followed by Excite DSC bonding agent application (Ivoclar, Vivadent, Schaan-Liechtenstein). Variolink II resin cement (Ivoclar, Vivadent, Schaan, Liechtenstein) was mixed according to the manufacturer's instructions and applied to the internal walls of the crowns for cementation.

**Group (GU):**

An application of G-bond (G) on untreated dentin preceded the use of RelyX Unicem resin cement (U) for luting the crowns.

**Group (GV):**

It consisted of the application of G-Bond (G), the self-etch adhesive, on untreated dentin according to the manufacturer's instructions, and then Variolink II resin cement was used for cementation of the crowns.

**4- Ultrasonic cementation of the crowns:**

An ultrasonic seating tip (Sonic Flex, KaVo, America Corporation, Lake Zurich, IL, USA) was used to seat the crowns on the dentin surface. The ultrasonic unit was set at a power 2 and was turned on each time for five seconds to minimize the up heating of the tip. The total ultrasonic seating time was 30 seconds,<sup>17</sup> where pressure was applied on several points of the surface. Then light curing was done multidirectional according to the manufacturer's instructions. The cemented crown samples were stored in distilled water for 24 hours at room temperature, and then subjected to 500 thermal cycles between 5°C and 55°C with dwell time of 30 seconds.

**Vertical marginal gap distance assessment before and after cementation:**

A stereomicroscope (SZ-PT-Olympus, Tokyo, Japan) was used to measure the vertical marginal gap in microns at the tooth/ceramic interface for the ceramic crowns being non-luted which recorded a mean marginal gap of 58 µm. The cemented crown samples were examined for vertical marginal gap distance after cementation, to monitor

the interfacial vertical marginal gap distance in microns at the tooth/ceramic interface multiple measurements were made at eight different regions of the crown circumference. This is done to compare between the 4 groups regarding the effect of different adhesive systems on the marginal gap.

**Fracture resistance test after cyclic loading:**

All samples were individually and vertically mounted in the lower fixed compartment of a computer operated materials testing machine (Model LRX-plus; Lloyd Instruments Ltd., Fareham, Hampshire, UK) with a load cell of 5 kN and data were recorded. The samples underwent pre-loading in a cyclic manner (10000 cycles).<sup>18</sup> The load was cycled between a specified minimum 30 N to prevent lateral dislocation of load applicator and a maximum 300 N, this reflects normal occlusal and chewing forces.<sup>19</sup> This testing protocol was based on previous reports of physiological load levels during chewing (clinical realistic). The rate of cyclic loading was a compromise between physiological chewing rates (masticatory cycle 0.8-1.0 , approximately 1 Hz).<sup>20</sup> A layer of rubber was placed between the loading tip and the occlusal surface of the crown samples to achieve homogenous stress distribution and minimization of the transmission of local force peaks.

After load cycling, the crowns were then statically compressively loaded until fracture at a cross head speed of 1mm/min with the same steel rod, which had been used in cyclic pre-loading procedure placed centrally at the occlusal surface of the crowns. The load-deflection curves were recorded with computer software (Nexgen; Lloyd Instruments Ltd). The compressive load required to cause fracture was recorded for each specimen in Newtons.

**Statistical analysis:**

Data were presented as mean and standard deviation (SD) values. Paired t test was used to compare vertical marginal gap before and after cementation. One-way Analysis of Variance (ANOVA) was used to compare between the 4 groups regarding the effect of different adhesive systems on the mean fracture resistance and marginal gap distance of the four groups. Tukey's post-hoc test was used for pair-wise comparison between the means when ANOVA test was significant. The significance level was set at P < 0.05. Statistical analysis was performed with SPSS 15.0 (Statistical Package for Scientific

Studies for Windows. SPSS, Inc., Chicago, IL, USA.)

Visual examination for the mode of fracture:  
All specimens were visually examined to determine the mode of fracture.

#### Scanning electron microscopic examination at dentin/resin interface:

For morphologic evaluation of the dentin/resin interfaces by SEM (Jeol, XL, Phillips, Holland), eight mandibular premolars were collected. The teeth were prepared by sectioning the crown perpendicular to the long axis of the tooth using a low speed diamond disc under water coolant to remove occlusal enamel and expose a flat dentinal surface. The teeth were then embedded in self-cured acrylic resin, using a cylindrical Teflon mold, such that the long axis of the tooth was perpendicular to the surface of the mold. The dentinal surfaces were abraded with 360/grit silicone carbide paper under running water to create a flat, uniform, smooth dentinal surface. The teeth were selected as representative samples for each group with its prementioned protocol of adhesive application to the dentin surface but without the crowns. A split Teflon mold was used (with a central hole of 3mm diameter and 2mm depth) for resin cement application. Representative samples (two teeth) for each of the 4 groups were sectioned longitudinally through the dentin-resin interface perpendicular to the bonded surface of each tooth, using a low speed rotary cutting machine under copious water coolant. After the surfaces were polished with soflext polishing discs, they were immersed in 6-mol/liter hydrochloric acid (HCl) for 30 seconds to demineralize any minerals within the hybrid layer that was not protected by resin infiltration. This was followed by rinsing the specimens with water for one minute. The specimens were then immersed in 1% sodium hypochlorite (NaOCl) for 10 minutes to dissolve all exposed collagen beneath the hybrid layer, and then thorough rinsing with water was performed for 5 minutes.<sup>21</sup> The specimens were dehydrated in ascending concentration of alcohol, subjected to critical point drying and then all specimens were gold sputtered. The hybrid layer and the resin tags at dentin/resin interfaces of these specimens were observed with SEM at magnification 1000 X.

### 3. Results

The means, standard deviation values and results of paired t-test presented in Table 2, revealed that there was a statistically significant increase in mean gap distance after cementation in all groups.

Vertical marginal gap distance after cementation for the four groups

The means, standard deviation values and results of ANOVA and Tukey's tests are presented in Table (3). Group (U) showed the statistically significant highest mean gap distance. This was followed by group (V). There was no statistically significant difference between group (GU) and group (GV), which showed the statistically significantly lowest means gap distance.

#### Fracture resistance

The means, standard deviation values and results of ANOVA and Tukey's tests are presented in Table (4). Group (GU) showed the statistically significantly highest mean fracture resistance. This was followed by group (GV) then group (V). Group (U) showed the statistically significantly lowest mean fracture resistance value.

#### Visual mode of failure

Visual examination of all groups showed a longitudinal fracture through the crown continuous with the tooth structure. Figure 1 is a representative sample showing the longitudinal fracture.

#### Scanning Electrons Micrograph

Scanning electron micrograph for self-etch adhesive approach group (U) presented in Figure 2a revealed indistinct resin tag formation. Typical well-formed resin tags were not prominent. G-bond application to dentin prior self-etch adhesive system in group (GU), presented in Figure 2b, resulted in increased resin tag formation which are connected with resin infiltrated dentin surface. Long, thick coagulated pattern was evident. Total-etch approach of group (V), Figure 2c, revealed the presence of hybrid layer with numerous long, tubular resin tags forming a bundler appearance. They are connected with resin infiltrated dentin surface in a rough pattern, resinous lateral branches connecting adjacent resin tags. A gap free attachment at the interface was evident. G-bond application to dentin prior to Variolink (GV), presented in Figure 2d, resulted in increased resin tag formation in a bundler appearance. They are connected with resin infiltrated dentin surface. Resinous branches with long, thick coagulated pattern were evident.

**Table 1: The components and application protocols of the dentin adhesive system**

<b>Adhesive system (Manufacturer)</b>	<b>Main component</b>	<b>Application protocol</b>
RelyX Unicem resin luting cement (3M ESPE, Seefeld, Germany)	Methacrylated phosphoric ester, dimethacrylates, inorganic fillers, fumed silica, stabilizers and initiators.	(Self-adhesive approach) the cement was supplied in a capsule, which was activated and mixed for 10 seconds using Rotomix (3 M ESPE )
Variolink II resin luting cement(Ivoclar, Vivadent, Schaan, Liechtenstein)	Urethan Dimethacrylate (UDMA) and Bis phenol Glycidial methacrylate (BisGMA), inorganic fillers barium glass filler and silicon dioxide filler, Ytterbium triflouride, catalysts stabilizers and pigments.	2 paste system (total-etch approach) Base: Urethane dimethacrylate, Catalyst: Dimethacrylates.
Exite (Ivoclar, Vivadent, AG FL-9494 Shaan/Liechtenstein )	2-Hydroxyethylmethacrylate (HEMA), dimethacrylates, phosphoric acid acrylate, silicon dioxide, initiators and stabilizers in an ethanol solution	Apply to moist dentin for 10 seconds. Dry gently for 1-3 seconds. Light cure for 20 seconds.
G-Bond (GC corporation, Tokyo, Japan)	Methacryloyloxyethyl Trimellitate (4-MET), phosphoric ester monomer, UDMA, acetone and camphorquinone	Apply to entire dried surface. Leave undisturbed for 10 seconds. Dry thoroughly under maximum air pressure for 5 seconds. Light cure for 10 seconds.

**Table 2: The means and standard deviation values of paired t-test of the vertical marginal gap distance in micrometers before and after cementation**

	U	V	GU	GV
<b>Before cementation</b>	$58 \pm 6.7$			
<b>After cementation</b>	$114 \pm 6.4$	$82 \pm 6.8$	$67.6 \pm 5.8$	$68 \pm 6.4$
<b>P-value</b>	<0.001*	<0.001*	<0.001*	<0.001*

\*: Significant at P &lt; 0.05

**Table 3: Mean and standard deviation of the vertical marginal gap distance in micrometers of the different groups after cementation**

Group U (RelyX Unicem)		Group V (Variolink with Excite)		Group GU (RelyX Unicem with G-bond)		Group GV (Variolink with G-bond)		<i>P</i> -value
Mean	SD	Mean	SD	Mean	SD	Mean	SD	
114 <sup>a</sup>	$\pm 6.4$	82 <sup>b</sup>	$\pm 6.8$	67.6 <sup>c</sup>	$\pm 5.8$	68 <sup>c</sup>	$\pm 6.4$	<0.001*

\*: Significant at P &lt; 0.05, Means with different letters are statistically significantly different according to Tukey's test

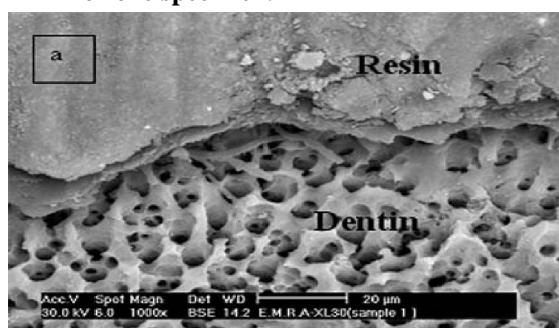
**Table 4: Mean and standard deviation of the fracture resistance in Newton of the different groups**

Group U (RelyX Unicem)		Group V (Variolink with Excite)		Group GU (RelyX Unicem with G-bond)		Group GV (Variolink with G-bond)		<i>P</i> -value
Mean	SD	Mean	SD	Mean	SD	Mean	SD	
2270.9 <sup>d</sup>	±3.4	2365.8 <sup>c</sup>	±3.6	2840.5 <sup>a</sup>	±3.8	2411.3 <sup>b</sup>	±3.3	<0.001*

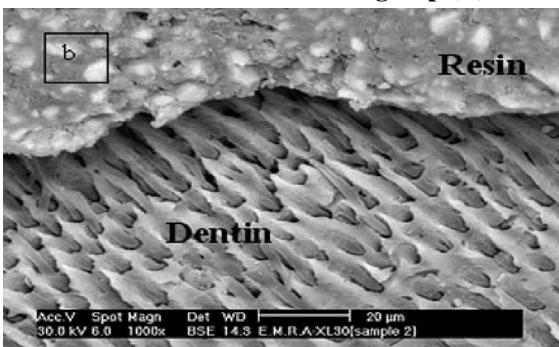
\*: Significant at  $P < 0.05$ , Means with different letters are statistically significantly different according to Tukey's test



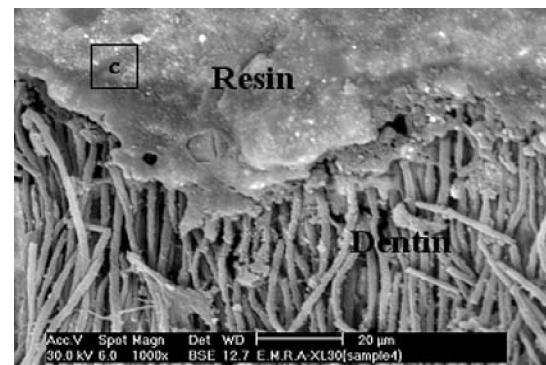
**Figure 1:** A representative longitudinal fracture for one specimen.



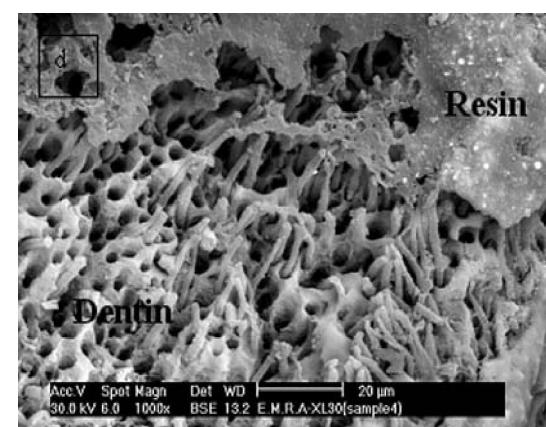
**Figure 2a:** SEM micrograph X 1000 of dentin/resin interface of group (U).



**Figure 2b:** SEM micrograph X 1000 of dentin/resin interface of group (GU)



**Figure 2c:** SEM micrograph X 1000 of dentin/resin interface of group (V).



**Figure 2d:** SEM micrograph X 1000 of dentin/resin interface of group (GV)

#### 4. Discussion:

Natural teeth have been used in this study, which are difficult in standardization as they show a large variation depending on age, and anatomy. Several studies used steel or resin dies for the fracture testing of crowns as they include standardized preparation and identical physical quality of materials used. However, prepared teeth made of steel or resins do not reproduce the real force distribution that occurs on crowns cemented on natural teeth. Dentin has a lower elastic modulus than steel; therefore, the inner crown surface shows a greater shear stress every time the tooth is subjected to deformation.<sup>10</sup> In natural teeth no uniform force distribution is expected between ceramic and tooth structure due to their different moduli of elasticity.<sup>22</sup> However if they are well bonded together they will act as one unit i.e. the stronger part strengthening the weaker part. This bonding is realized by the type and the efficiency of the bonding system. In this study, the bonding was done with several systems to investigate efficiency. To begin with, all crowns were silanated before cementation to improve the adhesion between the ceramic crowns and the luting systems.<sup>23</sup> Ultrasonic cementation technique was selected in this study to benefit from the acoustic energy applied to increase the flow of the luting cement with subsequent decrease in its viscosity (thixotropy). This acoustic energy results in decreasing the number and size of the voids in the luting cement, hence, increasing the adaptation of the material. Moreover, any medium or object in the path of an ultrasonic beam is subjected to a radiation force, which tends to push the luting cement in the direction of the propagating waves. This may cause redistribution and aggregation of the filler particles of the cement leading to optimum particle size distribution.<sup>17</sup> All the bonding systems used in this study were dual cured cements, their polymerization reaction is chemically and photo-initiated. This ensures higher conversion rate of curing, leading to better mechanical properties, i.e. the force will be distributed over a large area, as the whole assembly: the crown, the adhesive and the tooth structure would act as one unit.<sup>24</sup> This was confirmed by the visual mode of fracture where the failure was within the long axis of the crown and the tooth structure, which may demonstrate effective adhesive systems on the fracture resistance (Figure 1). From the limitation of this research, is that lateral forces were not duplicated. Axial loading was only applied in the testing method which interprets the presence of longitudinal failure mode. Actually, the forces existing in the clinical situation are multidirectional so by utilizing other testing method using chewing simulators, other fracture modes may be available.

The fracture resistance of the dentin-ceramic bonded system is affected by many factors among them are the composition of the restorative material, as well as the composition, the consistency and the flow properties of the bonding system. The used ceramic system is a new core glass ceramic material "E-max-press" containing lithium disilicate crystal as a strengthening agent. The ceramic crowns restoring the teeth showed a high fracture resistance values for all groups in varying degree. All groups had the same restorative material and were done with a similar controlled design. The only different parameter was the bonding system. The bonded interface can be the weakest area of tooth-colored restorations if it is exposed to the oral cavity i.e. increased marginal gap distance. Improving the bonding durability of luting resins to dentin and to the restoration leads to the reinforcement of brittle restorations and the longevity of bonded restorations in fixed prosthodontics.<sup>25</sup>

Comparing the results of the gap distance before cementation, all groups were insignificantly different from each other with an acceptable gap distance of approximately  $58 \pm 6.7 \mu\text{m}$ .<sup>26</sup>

After cementation, the magnitude of gap differed with the different groups i.e. the introduction of the bonding system and the cement. RelyX Unicem showed the highest gap distance ( $114 \pm 6.4 \mu\text{m}$ ) with the lowest fracture resistance ( $2270.9 \pm 3.4 \text{ N}$ ). This may be due to several factors: 1- the high viscosity of the RelyX Unicem noticed during application, due to the presence 9% amount of fillers, which lead to high film thickness. For effective chemical bonding, the distance between the adhesive and substrate must be less than few Angstroms. Increase in adhesive thickness is susceptible to large amount of voids preventing intimate contact between the tooth structure and the bonding system (Figure 2a), which may help in initiating crack. The bulk properties of the tooth substrates (dentin) and restorative substrate (ceramic) are much stronger than the bond strength of the cement/restorations. Therefore, cracks that form generally remain in the bonded interface zone. As cracks grow, they contribute to stress concentrations or stress redistributions within the substrates. The final failure may often extend for short distances through portions of tooth structure or restorative material explaining the accelerating failure rate.<sup>27,28</sup> In addition, the presence of voids at the interface can lead to bending of the restoration under force application, which will accelerate

failure of the restoration. The more the resin thickness the more contraction stresses of the bonding system, the less the maturing bond to walls. As long as the polymerizing material can flow before reaching the gel point, contraction stress can be dissipated. This suggests that a thin layer of resin bonded to the ceramic surface may, in some situations, act to reinforce the ceramic material, i.e. there will be no gap between the restoration/cement/tooth structure, the force induced is a compressive force. 2- RelyX Unicem is a self-etch adhesive maintaining the smear layer on the dentin preventing the adhesion between dentin and the adhesive (Figure 2a).<sup>29</sup> Since the self-etch approach uses acidic adhesive co-monomers, which dissolve the inorganic phase of dentin, and simultaneously primes and infiltrates the dentin matrix without removing the smear layer, it leads to fewer exposed collagen fibrils.<sup>30</sup> Adhesive stability is related to the effective coupling of the co-monomers with the infiltrated substrate.<sup>31</sup> 3- The pH of the acid used in any adhesive system is related to its success in bonding with dentin. RelyX Unicem contains phosphoric acid ester with higher pH than phosphoric acid acrylate used for the total-etch technique of the Variolink II and Excite system, as reported by the manufacturer, and therefore it has a lower bonding capacity.

Variolink II with Excite adhesive bonding system gave better vertical marginal gap distance and better fracture resistance than RelyX Unicem. Variolink II is a total-etch contributing to complete removal of the smear layer with dentin. Moreover, etch-and-rinse adhesive system is applied directly on the demineralized dentin collagen. The maintenance of the structural integrity of these structures during and after etching should greatly improve the final stability of the hybrid layer, as the collagen in the dentin matrix is preserved (Figure 2c).<sup>32</sup>

Treatment of dentin with G-bond improved the gap distance between the restoration, the tooth structure, and the fracture resistance. G bond was applied for groups GU and GV before the application of the RelyX Unicem and Variolink II. The values of fracture resistance were higher and significantly different for the pretreated groups than for the untreated groups (Table 4). This may be due to the presence of 4MET (methacryloyloxyethyl Trimellitate) formulated with fillers in the G-bond system .These components produce nano particles responsible for the formation of extremely thin layer of nano interaction zone containing insoluble calcium compound with dentin characterized by the exposure of little amount of collagen fibrils.<sup>33</sup> According to the adhesion-decalcification concept, the less soluble the calcium salt of an acidic molecule, the more intense

and stable the molecular adhesion to a hydroxyapatite-based substrate.<sup>34</sup> In addition, the absence of HEMA (Hydroxy Ethyl Methacrylic acid) in group (U) rendered the bonding system less sensitive to water uptake. HEMA creates a hydrogel within the hybrid layer and adhesive resin in some cases. The hydrogel may provide a channel for water permeation that has the potential to affect the durability of bonds. Thus, HEMA free adhesives have been proposed as RelyX Unicem and G-Bond. The omission of HEMA from the adhesive blends has been considered advantageous in removing water, separating it from the other components upon solvent evaporation.<sup>35</sup> Figures 2b and 2d for the treated dentin with G-bond, showed increased resin tags formation in a bundler appearance, which are connected with resin infiltrated dentin surface. Resinous branches with long, thick coagulated pattern were evident after the application of the G-bond system. Although the present investigation may be rather close to the clinical situation, the prospective clinical trial remains the final instrument to definitely answer the raised question regarding the appropriate adhesive system to enhance the fracture resistance and marginal fidelity of E-max crowns.

**CLINICAL IMPLICATIONS:** Within the limitation of this study total-etch adhesives are more preferred than self-etch adhesives for luting all -ceramic crowns. However, treatment of dentin surface prior to the application of any bonding agent is of great importance to enhance the fracture resistance and marginal fidelity of E-max ceramic crowns.

## 5. Conclusion

From the present study, the following can be concluded:

- 1- Bonded ceramic restorations with total-etch system offer better vertical marginal gap distance and fracture resistance than bonded restorations with self-adhesive system.
- 2- Treatment of the dentin surface, with all in one self-etch adhesive, prior to the application of the bonding system is very efficient.
- 3- The bonding system affects the vertical marginal gap distance and the fracture resistance of all- ceramic restorations.

**Corresponding author**

Dina H. Mostafa

Biomaterials Department, Faculty of Oral and Dental Medicine, Cairo University, Cairo, Egypt

[dinamostafa@hotmail.com](mailto:dinamostafa@hotmail.com)**5. References**

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6/1/2010

# Do Desensitizers Affect the Retention of Questionable Preparations?

Jylan F. ElGuindy<sup>1</sup>, Dina H. Mostafa<sup>\*2</sup> and Rana M Sherif<sup>1</sup>

<sup>1</sup>Fixed Prosthodontics Department and <sup>2</sup>Biomaterials Department, Faculty of Oral and Dental Medicine, Cairo University, Cairo, Egypt.  
\*dinamostafa@hotmail.com

**Abstract:** Objectives: This study aimed to investigate the effect of different desensitizers on the retention of short and over-converged preparations. Methods: Eighty molars were prepared with 3mm occluso-cervical height and 24 degrees convergence angle. Nickel-chromium copings were cast with a loop at the occlusal surface for tensile loading after cementation. The copings were assigned to two groups (N=40 each) according to the cement used. Group 1: resin cement (Duolink). Group 2: glass-ionomer (Ketac-Cem). Each group was assigned to four subgroups (N=10 each) according to desensitizers used prior each cement. Subgroup I: control (untreated), subgroup II: Gluma Comfort + Desensitizer, subgroup III: Oxalate (Bisblock) and subgroup IV: Fluoride varnish (Flor-Opal). The retention was determined by uniaxial tensile mode of force. Two-way Analysis of Variance (ANOVA) SPSS 16.0 was used to assess cements, desensitizers and their interactions on copings retention. Results: Resin group: Oxalate ( $212.10 \pm 7.41$ N) showed the significant highest mean of retention, followed by Gluma ( $201.52 \pm 6.93$ N), then control ( $177.52 \pm 6.14$ N). Fluoride ( $153.80 \pm 6.03$ N) recorded the lowest mean. Glass-ionomer group: control ( $135.54 \pm 4.58$ N) and Oxalate ( $132.62 \pm 4.84$ N) recorded the significant highest mean, followed by Gluma ( $126.84 \pm 4.75$ N). Fluoride ( $101.96 \pm 6.34$ N) recorded the lowest mean values. Conclusions: With questionable preparations, fluoride desensitizer drastically affected the retention of both cements. Oxalate and Gluma enhanced the retention with resin cement. Oxalate desensitizer can be efficiently used with glass-ionomer. [Journal of American Science. 2010;6(11):274-283]. (ISSN: 1545-1003).

**Keywords:** retention; Nickel-chromium coping; occlusal surface; Fluoride varnish

## 1. Introduction

Several factors play role in the maintenance of fixed prosthesis in service, such as the retentive and resistive capacity of the prepared tooth, the characteristic properties of the luting cements and sealing of dentin prior to cementation of cast restorations to decrease post-cementation sensitivity.<sup>1,2</sup>

The retentive capacity of the prepared tooth is influenced by many features including; the angle of occlusal convergence and the occluso-gingival (OG) height of the preparation. According to Tylman, the angle of convergence for ideal fixed partial denture preparation should be between 2-5 degrees.<sup>3</sup> Clinically, this ideal taper is seldom achieved. In a previous study, the mean angle of convergence of crown preparations made by final-year dental students was reported to be of 21 degrees.<sup>4</sup> In another study, the mean angle of convergence of crown preparations made by general dental practitioners and by specialists was reported to be 20 degrees.<sup>5</sup> However, in some situations such as mal-aligned teeth, near parallelism of the preparation walls is difficult to achieve without over cutting of the mal-aligned tooth to align its proximal walls with those of other abutments. As for teeth with short clinical

crowns, short preparation will result in relative reduction of crown retention. Clinically, a short crown may require lengthening before preparation. This lengthening procedure involves periodontal surgery and adds to the total cost and complexity of the treatment.<sup>6</sup>

Dental luting cements form the link between fixed restorations and the supporting tooth structure. Mechanical interlocking and chemical bonding are desirable factors in the fixation mechanisms of luting cements, and are critical for achieving suitable retention for metallic cast crowns.<sup>1</sup> Luting cements play a pivotal role in sealing the margins and overcoming preparation design errors.<sup>7</sup> The introduction of new strong forms of dental cements represents one of the most important changes in materials that relates to retention of fixed prosthesis.<sup>8</sup> When abutment tooth preparations have questionable retentive potential the use of an appropriate resin cementing medium in conjunction with a reliable bonding procedure can help to overcome retention problems.<sup>3</sup>

Dentin reduction and exposure of prepared tooth surface can lead to increased dentin permeability and subsequent pulpal irritation. Richardson et al, reported that approximately 1 to 2

million dentinal tubules are exposed during an average tooth preparation for a posterior crown.<sup>9</sup> Bränström's hydrodynamic theory speculated that any dentin stimulus can be transmitted back to nerve receptors resulting in fluid movement in the dentinal tubules with stimulation of the odontoblasts, which elicited a response by nerve fibers and resulted in pain.<sup>10</sup> During crown cementation, the luting agent is forced into the patent tubules before it sets and displaces an equal amount of dentinal fluid, leading to excessive hydrostatic pressure and irritation of pulpal tissues.<sup>11</sup> The smear layer evident after tooth preparation was also demonstrated to be in effective against luting agent irritation.<sup>12</sup>

Therefore the use of various dentin desensitizing agents after crown preparation or before cementation has been shown to be an effective clinical treatment in reducing sensitivity.<sup>13</sup> Desensitizers obturate exposed dentinal tubules with a resinous material and block tubular fluid flow thus reducing pain sensation.<sup>14</sup> Although the application of desensitizing has gained popularity, but unfortunately their effect on crown retention is still somewhat unclear and contradictory.

The purpose of this study was to investigate the effect of different desensitizers on the retention of short and over-converged preparations of crowns cemented with two adhesive luting agents.

## 2. Materials and Methods

### Teeth preparation:

Eighty caries free mandibular molars of similar sizes were collected and stored in water in a refrigerator to avoid dehydration until used. The teeth were embedded in acrylic resin blocks up to 2 mm below the cemento-enamel junction. To secure the tooth in the resin blocks, a hole in the root was drilled where an orthodontic wire was placed and protruded for mechanical interlocking within the resin. Using an industrial lathe machine the teeth were prepared for full veneer preparation with standardized dimensions of 24 degrees angle of convergence (wall angle of 12 degrees) as the convergence angle equals the sum of the taper of two opposing preparation walls.<sup>5</sup> The preparation had 3 mm occluso-cervical height, 3 mm occlusal diameter, 6 mm cervical diameter, 1 mm shoulder finish line and flat occlusal plane.

### Cast coping construction:

Eighty impressions for the prepared teeth were made with polyether impression material (Impergum, 3M ESPE, Germany) using custom made trays, Figure 1, and poured into stone die (Moldaroc, Bayer dental Lever Kusen, W Germany). The stone dies were trimmed, and die spacer was applied (Isocera BEGO). Direct wax pattern (Kerr, Mfg Co.

Rommelus, Mich) was made in the form of coping with a loop attached to the occlusal surface to allow tensile load testing after cementation. The wax patterns were cast in nickel chromium (Ni-Cr) alloys (Wiron 99, BEGO Bremer, Germany); according to the manufacturer's instructions, then were sprued and invested with Begoral phosphate bonded investment specific for the Ni-Cr alloy (BEGO Bermer, Germany). Wax burn out was carried out and casting was completed using an induction casting machine (Fornax 35 casting machine, BEGO Bremer, Germany). To simulate the construction conditions for ceramo-metallic restorations, after casting and divesting, the copings were subjected to the ceramic firing cycles recommended for the Vita VMK95 ceramics in the porcelain furnace. The fitting surface of the copings was sandblasted with 50 µm aluminum oxide particles for 15 seconds under a pressure of 60 PSI at a standard distance of 1cm away from the nozzle of the sandblasting machine (BEGO, Bremer, Germany). Before cementation, it was verified that the castings were not retentive as the copings were separated from the tooth preparations without any resistance when the samples were held upside down.

### Coping cementation:

The 80 copings were assigned to 2 groups (N=40 each) according to the adhesive luting cement presented in Table 1. Group 1 (R): Resin cement (Duolink).

Group 2 (G): Glass-ionomer cement (Ketac-Cem). Each group was then divided into 4 subgroups (N=10 each) according to desensitizers used prior each cement, Table 1.

Subgroup I: Untreated control (CON).  
 Subgroup II: Gluma comfort + Desensitizer (GLU).  
 Subgroup III: Oxalate (OXA). Subgroup IV: Flor-Opal (FLU).

### RCON

The dentin was etched with Uni-Etch etchant followed by One-step Plus bonding agent application. Duolink resin cement was mixed according to the manufacturers instructions and applied to the internal walls of the copings for cementation.

### R GLU

The dentin was etched with Gluma Etch followed by Gluma Comfort Bond + Desensitizer application following the manufacturers instructions, then Duolink resin cement was used for copings cementation.

### R OXA

The dentin was etched with Uni-Etch etchant, followed by Bisblock application following the manufacturers instructions. The adhesive One-step Plus was then applied followed by Duolink resin cement.

#### R FLU

Flor-Opal varnish was applied to the prepared dentin surface following the manufacturers instructions, then Duolink resin cement was used for copings, cementation.

#### G CON

Glass-ionomer cement (Ketec-Cem) was mixed according to the manufactures instructions and applied to the internal walls of the copings for cementation.

#### G (GLU,OXA and FLU)

Desensitizers were applied to the prepared tooth surface in a similar way as with the resin groups, and then Ketec-Cem glass- ionomer cement was used for copings cementation.

Each coping was first placed onto its corresponding tooth with finger pressure, and then a standardized static load of 5 kgN was applied with a specially fabricated loading device, Figure 2. The excess cement was removed while loading was maintained for 15 minutes to ensure complete setting of the cement. The eighty cemented samples were stored in water at 37°C in an incubator (Torre Picenardi, Italy) for 48 hours. To simulate the oral conditions thermocycling of the cemented samples was performed for 500 cycles between 5°C to 55°C + 2°C with a dwell time of 30 seconds in each water bath.<sup>15</sup>

#### **Coping Retention Test Procedure:**

The assembled teeth and copings were mounted on a computer controlled materials testing machine (Model LRX-Plus; Lloyd instruments Ltd., Fareham, UK) with a load cell of 5KN (Kilo Newton), and data were recorded using computer software (Nxygen-MT; Lloyd Instruments). Samples were secured to the lower fixed compartment of the testing machine by tightening screws. Coping retention was determined by uniaxial tensile mode of force using a specially fabricated attachment with a rigid metallic hook attached to the upper movable compartment of testing machine traveling at cross-head speed of 5mm/min. The hook was designed to grip the loop of the copings, Figure 3. The tensile load required to dislodge the cemented copings was recorded in Newton (N). The mean values and standard deviations for each group of both tested alloys were calculated and statistically analyzed.

#### **Statistical Analysis**

Tensile strength data were presented as mean and standard deviation (SD) values. Data were explored for normality using Kolmogorov – Smirnov test, and no significant departures from normality were observed (all P-values > 0.05). Homogeneity of variances among the groups was tested using Levene test, and the variances were found to be homogenous (P-value > 0.05).

Regression model with Two-way Analysis of Variance (ANOVA) was used in testing significance for the effect of cement, desensitizers and their interaction on mean retention. Tukey's post-hoc test was used for pair-wise comparison between the means when ANOVA test was significant. The lack of Fit test revealed P-value = 0.524 which means that the model adequately fits to describe the relationship between dependent and independent variables. Residual plots (Observed Predicted standardized residuals) for the dependent variable were produced. The points representing the residuals lie close to a line indicating a normal probability plot of the residuals. The significance level was set at P = 0.05. Statistical analysis was performed with SPSS 16.0® (Statistical Package for Scientific Studies) for Windows.

#### **3. Results**

The results showed that the regression model is fit to describe the relationship between the studied variables. Cement, desensitizers and their interaction had a statistically significant effect on mean retention, Table 2.

The means, standard deviation (SD) values and results of ANOVA test presented in Table 3, revealed that there was a statistically significant difference between the different interactions. Tukey's test showed that group resin (R) subgroup oxalate (OXA) showed the statistically significant highest mean of retention, followed by (R) subgroup Gluma (GLU), (R) subgroup control (CON) then (R) subgroup fluoride (FLU) that showed the lowest mean retention values. There was no statistically significant difference between group glass-ionomer (G) subgroup control (CON) and (G) subgroup oxalate (OXA), which showed high retention values, followed by (G) subgroup Gluma (GLU). Group glass-ionomer (G) subgroup fluoride (FLU) showed the statistically significant lowest mean retention. Resin cement showed statistically significant higher mean of retention than glass-ionomer cement, Table 4.

**Table (1): Different cements and desensitizers used in the study.**

Materials (Manufacturer)	Ingredients	Application protocol
Bisco, Inc, IL 60193 Schaumburg, U.S.A	<b>Uni-Etch</b> Phosphoric acid (32% Benzalkonium chloride (BAC) (0.1-1%)	Apply for 15 seconds washing with water, then gentle air drying for 2-3 seconds
Biso, Inc, IL 60193 Schaumburg, U.S.A	<b>One step plus:</b> Bisphenyle dimethacrylate (15-40%) Hydroxyethyl methacrylate (15-40%). Acetone (40-70%) Dental glass (1-10%).	Apply to the prepared tooth surface; use gentle air stream to evaporate the solvent. Light cure for 10 seconds.
Duolink resin luting cement (Bisco, Inc, IL 60193 Schaumburg) U.S.A	<b>Base:</b> - Bisphenol A.diglycidyl methacrylate (5-30%). - Triethylene glycol dimethacrylate (5-20%). - Glass filler (50-80%) - Urethane dimethacrylate (5-15%). <b>Catalyst:</b> - Bis-GMA (<31%). - Triethylene glycol Dimethacrylate (<21%) - Glass filler (<65%)	A dual-syringe delivery system is used for dispensing of equal amounts of base and catalyst. Light cure for 40 seconds
Ketac-Cem glass – ionomer cement (ESPE – Seefeld W. Germany).	<b>Powder:</b> - Glass powder - poly carboxylic - pigments <b>Liquid:</b> - Water - tartaric acid - conservation agents.	Mix a full one scope of powder to two drops of liquid for 30 seconds.
Gluma Comfort Bond + Desensitizer (Heraeus Kulzer, Inc 10504 Armonk, NY USA)	<b>Etchant:</b> 20%, phosphoric acid, blue dye: <b>Matrix:</b> UDMA HEMA 4-META Maleic acid polycarboxylic acid ester gluteraldehyde <b>Filler:</b> None	Etch the tooth surface for 20 seconds then wash with water. Use gentle air stream to remove excess moisture for 1-2 seconds. Leave dentin surface moist and shiny apply Gluma Comfort + Desensitizer with a brush in copious amount. Leave undisturbed for 15 seconds then use gentle air blast to evaporate the solvents. Light cure for 20 seconds.
Bisblock desensitizer (Bisco, Inc. IL 60193 Schaumburg, U.S.A).	Oxalic acid (1-4%)	Apply on etched tooth surface and leave for 30 seconds to allow calcium oxalate crystals formation Wash with water and leave the surface slightly moist for wet bonding.
Flor-Opal varnish desensitizer (Ultradent Products, Inc, 505 West 10200 South, South Jordan Utah 84095, U.S.A).	50 mg sodium fluoride in alcohol and natural resin suspension.	Supplied in the form of two syringes inter connected together for mixing their contents. Air drying the tooth surface their express a small bead of varnish from Fx Flex tip. Brush the varnish on the tooth surface then spray with water to harden.

**Table (2): Results of regression analysis.**

Source of variation	Sum of Squares	df	Mean Square	P-value
Corrected Model	104184.428	7	14883.490	<0.001*
Cement	76867.601	1	76867.601	<0.001*
Desensitizers	22465.034	3	7488.345	<0.001*
Cement x Material	4851.120	3	1617.265	<0.001*

\*: Significant at P < 0.05, R Squared = 0.976 (Adjusted R Squared = 0.974)

**Table (3): The means, standard deviation (SD) values in Newton, results of comparison between the different interactions**

	Mean	SD	P-value
Resin Control	177.52 <sup>c</sup>	6.14	<0.001*
Resin Gluma	201.52 <sup>b</sup>	6.93	
Resin Oxalate	212.10 <sup>a</sup>	7.41	
Resin Fluoride	153.80 <sup>d</sup>	6.03	
Glass-ionomer Control	135.54 <sup>e</sup>	4.58	
Glass-ionomer Gluma	126.84 <sup>f</sup>	4.75	
Glass-ionomer Oxalate	132.62 <sup>e</sup>	4.84	
Glass-ionomer Fluoride	101.96 <sup>g</sup>	6.34	

\*: Significant at P < 0.05, Means with different letters are statistically significantly different according to Tukey's test result

**Table (4): The means, standard deviation (SD) values in Newton and results of comparison between the retention of the two cements**

Resin		Glass-ionomer		P-value
Mean	SD	Mean	SD	
186.24	23.70	124.24	14.30	<0.001*

\*: Significant at P < 0.05



**Figure 1:** Polyether impression for the prepared tooth surface in perforated Copper tray



**Figure 2:** Metal coping sample within the cementing device



**Figure 3:** representative coping during tensile testing.

#### 4. Discussion:

In the oral environment, failure of retention of crowns and fixed partial dentures occurs under a combination of masticatory forces repeated over time. These are mainly direct compressive forces and some resultant shear lateral forces. In addition, there is a small component of tensile force. Most laboratories testing for crown retention, however, uses direct tensile force.<sup>6,8</sup> Consequently, one may state that the findings of this study, which used direct tensile loading, can be considered to relate directly to the clinical situation.

In the present study, prepared teeth with 24 degrees occlusal convergence angle and 3mm occluso-cervical height, were selected to approximate certain clinical situations. In this manner, the contribution of the cement to retention of the crown was better assessed.

Our study shows that with questionable retentive preparations oxalate and Gluma enhanced the retention of copings luted with resin cement. Only oxalate desensitizer can be efficiently used with glass-ionomer.

In this study the high values of retention recorded for group (R) subgroup (OXA) were attributed to the unique application technique and mechanism of action of BisBlock which is designed to prevent intra-tubular fluid movement causing dentinal sensitivity. BisBlock was applied on the dentin after surface decalcification by acid etching and rinsing, where the top 5-10 $\mu$ m of the matrix became depleted from calcium. Thus the oxalic acid was privileged to diffuse deeper into the dentinal tubules till reaching the calcium ions of dentin (mineralized

matrix) and dentinal fluid forming insoluble calcium oxalate crystals.<sup>16</sup> This caused blockage of dentinal fluid movement thus eliminating sensitivity and reducing outward fluid movement during the subsequent bonding procedure. This "subsurface tubular occlusion" left the dentin surface unobstructed for the infiltration of the adhesive into the demineralized collagen matrix (the top 5-10 $\mu$ m). The adhesive penetrated inbetween the calcium oxalate crystals and entrapped them during its polymerization. Thus, preventing dislodgment of these crystals and prepared the surface for bonding. This unique mechanism of action might have provided tubular occlusion, plus formation of resin tags inside the dentinal tubules and subsequently enhanced the retention.

These results were consistent with Pashley et al 2001, and Jalalian et al 2009, who found that different formulations of potassium oxalate produced significant reduction in hydraulic conductance resulting in less permeable and acid resistant dentin surface.<sup>16,17</sup>

In this study, oxalate was incorporated into a compatible system (Uni Etch etchant and one step plus adhesive) to obtain the best performance. Uni Etch is a 32% phosphoric acid ( $H_3PO_4$ ) supplied as semi-gel etchant. It has the advantage of leaving no silica debris on the etched surface that impede the flow of primer and/or resin over the surface or into the dentinal tubules.<sup>18</sup> The percentage of phosphoric acid also represented a major contributing factor for the success of Bisblock because if the content has exceeded 32% this might have reduced the oxalate affectivity by additional etching, leading to hydrolytic degradation of the resin dentin bond and oxalate crystals.<sup>19</sup>

One step plus is a total etch two step bonding agent (Etch and Rinse) containing Bisphenol dimethacrylate (BPDM) with some hydrophilicity that enhances the wettability of the adhesive. Hydroxyethyl methacrylate (HEMA) being a wetting agent and promoter for the infiltration of the adhesive into the tooth structure, allowed intimate contact between the adhesive and the collagen fibers.<sup>20</sup> It also contains acetone as organic solvent that displaced water from the dentin surface and from the moist collagen network, thus promoting the infiltration of the polymerizable monomers into the open dentinal tubules and the nanospaces in the collagen network.<sup>21,22</sup> The organic solvent being volatile was easily eliminated by air jet so that only the polymerizable monomer remained.<sup>23</sup> However, this study is inconsistent with the findings of Abou El Dahab, 2007, who declared that oxalate treatment decreased

the bond strength of resin cement. The author attributed the results to the incompatibility between the oxalate desensitizers and adhesives used in the study, which had a low pH and high fluoride content.<sup>24</sup> The Low pH values might have increased the solubility of calcium oxalate crystals in the dentinal tubules and transformed them into calcium and oxalate ions. This was according to le Chatelier's principle that once calcium oxalate crystals are exposed to high  $H_3O^+$ , more calcium oxalate dissolves into calcium and oxalate ions to compensate for the depletion of oxalate ions and maintain the equilibrium constant.<sup>25</sup> The free fluoride ions from the adhesives might have also interacted with calcium and phosphate ions on dentin surfaces to form spherical globules of calcium fluoride ( $CaF_2$ ). The presence of these spherical globules at the bonded interface and in the adhesive layer acted as stress raisers that would create debonding at lower stresses than would occur in their absence and will hinder the adhesive infiltration and hybridization of demineralized dentin.<sup>26</sup> In the present study the adhesive used (One Step Plus) was with almost pH 4.6 and low fluoride content (70 ppm). The results of our study is also contradictory to the findings of Nadu, who reported that oxalate has a low occlusive effect due to its solubility in oral fluids.<sup>27</sup> However One Step Plus adhesive used penetrated in between the oxalate crystals and entrapped them during polymerization and thus prevented their dislodgment.

The high values of retention recorded of group (R) subgroup (GLU) could be attributed to the unique mechanism of action of Gluma Comfort Bond + Desensitizer as explained by Dijkman et al and Schupbach et al. They postulated that the Gluteraldehyde compound of Gluma was responsible for the intrinsic blockage of dentinal tubules. It reacted with serum albumin present in the dentinal fluid by coagulation, causing setting up of multiple septa [walls] that blocked the flow of fluids in the tubules which is referred to as interdental sealing and thus counteracting the hydrodynamic mechanism of dentin hypersensitivity.<sup>28,29,30</sup>

Another possible explanation of these results can be related to gluteraldehyde as a naturally occurring cross-linker that bonds covalently to collagen fibers and straightens the collapsed ones. This efficiently stabilized the dentin collagen, helped in its rewetting, reduced its marginal contraction gap and subsequently improved the adhesive strength.<sup>31</sup>

The Combination of a resin adhesive with a desensitizing agent seems to be contradictory at first sight, since effective adhesives are expected to seal the etched dentin surface by inter-peritubular hybridization and by resin tag formation in the opened dentinal tubules. However Jacobsen and Finger when examined the resin tags formed with Gluma bonding system postulated that the occluding protein precipitates were apparently not tight, but permeable for monomers.<sup>32</sup> It might be speculated that a similar combined sealing effect of protein precipitation and resin penetration might occur to the adhesive Gluma Comfort Bond + Desensitizer on the etched dentin surface producing a regular dentin surface sealing. The high retention values can be also attributed to 4-methacryloyloxyethyl-trimellitate anhydride (4-META) which is a wetting agent contained in adhesive Gluma Comfort Bond + Desensitizer and has the ability to chemically adhere to metal and calcium ions producing good bond with tooth structure.<sup>33</sup> The combined water and ethanol solvents might have served to the deep penetrating action of the Gluma. The ethanol being a water chaser and solvents for monomers ensures a good infiltration of the adhesive, better sealing and good bond strength. Also it doesn't evaporate too quickly, which might affect the bond strength, while the water act as a re-wetting agent that prevents the collapse of collagen fibers.<sup>34</sup>

However, contradictory findings were demonstrated by, Assis et al, 2006 who declared that Gluma desensitizer caused reduction of bond strength of resin cement.<sup>35</sup> But in their studies they used Gluma desensitizer which is a non polymerizable desensitizer that is not capable of forming a bond with the resin cement. While, in our study we used Gluma Comfort bond + Desensitizer which is an adhesive bonding agent with desensitizing effect that reacted with resin and even enhanced retention.

Surprisingly, the low retention values of fluoride group, maybe attributed to the superficial mechanism of action of fluoride varnish where it reacted with calcium of hydroxyapatite and formed CaF<sub>2</sub> crystals, which are loosely bound spherical crystals. The presence of these CaF<sub>2</sub> crystals at the bonded interface and within the adhesive layer, might have acted as stress raisers that would create debonding at lower stresses than would occur in their absence.<sup>24,26</sup>

The low retention values can be also related to the fact that fluoride varnish doesn't polymerize with the cement to increase bond strength. Yim et al, explained that a non polymerizable desensitizer would fill in the surface irregularities of dentin and

prevent mechanical interlocking of the cement, thus decreasing the bond strength.<sup>36</sup>

Sodium fluoride varnish being a non polymerizable desensitizer it might have negatively affected the glass-ionomer bond strength. This postulation is in agreement with Yim et al, who explained that a non polymerizable desensitizer might have filled in and smoothed the surface irregularities, thus precluding any ability of glass-ionomer to lock into surface irregularities and form chelation bonding.<sup>36</sup> The interaction of sodium fluoride with dentin surface and formation of CaF<sub>2</sub>, might have also deprived glass-ionomer from calcium ions needed for chelation.<sup>37</sup>

The significant higher tensile loads obtained for Ni-Cr crowns cemented with resin cements ( $186.24 \pm 23.70$  N) compared to that with glass-ionomer luting agent ( $124.24 \pm 14.30$  N) were attributed to the ability of dentin desensitizers to polymerize with resin cement and subsequently enhancing the crowns retention. This finding is in agreement with Yim et al, who declared that, crown retention was not enhanced when using glass-ionomer cement and polymerizable dentin desensitizer, and was lowered when a non polymerizable one was used. While the use of resin cement with desensitizing agent capable of polymerizing to the cement provides the greatest retentive strength.<sup>36</sup>

## 5. Conclusion

Within the limitations of this study, it was concluded that teeth with questionable retentive preparations, fluoride desensitizer drastically affected the retention of copings luted with both resin and glass-ionomer cements. The use of oxalate and Gluma enhanced the retention of copings luted with resin cement. Oxalate desensitizer can be efficiently used with glass-ionomer cement.

## Corresponding author

Dina H. Mostafa  
Biomaterials Department, Faculty of Oral and Dental Medicine, Cairo University, Cairo, Egypt  
[dinamostafa@hotmail.com](mailto:dinamostafa@hotmail.com)

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6/2/2010

## Accuracy of working casts and dies produced by fast-setting polyvinyl siloxane impressions

Mona El-Agroudi MD DDS<sup>1</sup> and Eman Essam, MD DDS<sup>2</sup>

<sup>1</sup> Assistant professor of Fixed Prosthodontics, Faculty of Oral and Dental Medicine, Cairo University. , Egypt.

<sup>2</sup> Lecturer of Fixed Prosthodontics, Faculty of Oral and Dental Medicine, Al Azhar University. , Egypt.

**Abstract:** Purpose: This study aimed to evaluate the effect of spacer on the accuracy of working casts and dies produced from fast-setting polyvinyl siloxane impressions.

Materials and Methods: Twenty Impressions of the mandibular arch of a modified Dentoform master model incorporating a stainless steel circular crown preparation were made, using a fast-set Polyvinyl siloxane (Affinis perfect impressions Coltene/Whaladent AG) using 2-step impression technique with and without a spacer. Gypsum working casts and dies were produced from the poured impressions. Measurements of the master model and working casts were carried out including anteroposterior (AP) and cross-arch (CA) dimensions. The stainless steel circular crown preparation incorporated within the master model was also measured in buccolingual (BL), mesiodistal (MD), and occlusogingival (OG) dimensions and compared to measurements from recovered gypsum dies. Linear measurements were made using a measuring stereomicroscope. Results: Double impression technique without spacer showed statistically significant higher mean percent relative change than double impression technique with spacer. With each technique, the means percent relative change in die measurements showed statistically significant higher mean values than cast measurements. There was no statistically significant difference between means percent relative change in the BL and MD dimensions which showed the statistically significant highest mean values. The means percent relative change in the OG dimension showed the statistically significant lowest mean value. Conclusion: Accuracy of fast-setting polyvinyl siloxane impression material was favorably affected with the use of spacer, as the space resulted from contraction of the putty material was not enough to produce accurate detail reproduction by the light material. The working dies; from the fast- setting polyvinyl siloxane impression material without spacer demonstrated an increase in (mesio-distal and bucco-lingual) dimensions, while for cast dimensions, there was no difference between the two techniques. [Journal of American Science. 2010;6(11):284-292]. (ISSN: 1545-1003).

**Keywords:** dies; fast-setting; polyvinyl siloxane

### 1. Introduction

Understanding accuracy of impression materials is required. Impressions register and reproduce the prepared tooth form and the surrounding oral tissues<sup>1</sup>. Elastomers were developed as a replacement of natural rubbers during world war II then they were modified chemically and physically for dental use. At first Polysulfide rubbers existed exclusively followed by condensation silicones, Polyether, and then Polyvinyl siloxane. It is relative to clinical<sup>2,3</sup> and laboratory factors<sup>4,5</sup>. Elastomers are subjected to dimensional changes. Polymerization involving cross linking of the polymer chain can result in reduction of the spatial volume<sup>5</sup>. The reaction continues for some time after the final set clinically<sup>5</sup>. Effect of temperature is another variable<sup>6</sup>.

Impression techniques have been categorized as monophase and dual phase. Techniques using monophase materials are made in a single step using a medium viscosity material. Techniques that use dual phase materials such as

putty and light body wash method may be accomplished in 1-step or in 2-step (1-step and 2-step putty/light body techniques). In the one step technique the putty and wash material are mixed in the same time. The light body material is syringed around the prepared teeth and the tray containing the putty is seated and stabilized with minimal pressure until the impression materials are set and polymerized. In the 2-step putty/light body technique a stock tray is painted with adhesive and the putty material produces a tray similar to that of acrylic resin. One precaution is to select a tray closely fitting the arch form thus reducing the amount of impression material and facilitate seating of the loaded tray intraorally. Another method is to make a preliminary putty impression intraorally and selectively relieve the putty and details are recorded by the light body only.

There is a potential difficulty with this technique as it is practically impossible to control the bulk and even amount of wash material. Moreover, further modifications to this technique include the use

of polyethylene spacer. The addition type silicones have been reported to be the most accurate and dimensionally stable<sup>7, 8</sup>. Some authors claim that the impression materials has improved to such an extent that accuracy can be controlled by the technique rather than the material itself.<sup>9, 10</sup> Others report that the technique does not affect accuracy.<sup>11, 12</sup> Several techniques have been proposed. The Putty/wash 1 step technique, Putty/ wash 2 step and the Putty/wash with polyethylene spacer<sup>[13-15]</sup> or a resin spacer<sup>16</sup>. Nissan et al reported that the polyvinyl siloxane 2 step Putty/wash impression technique is the most accurate, as a uniform cross sectional bulk of 2mm is provided.<sup>13</sup> Nissan et al<sup>13</sup> and Lee et al<sup>17</sup> used different quantitative analysis, moreover in the 1/step and the2/step the light body should cover the entire preparation but this cannot be accomplished clinically<sup>11</sup>. The 2/step technique has been reported to be more accurate than the 1/step technique<sup>13</sup>. With the 2/step technique the impression with the light body is made after the putty has polymerized and contracted therefore any further contraction in the light body results in minimal dimensional change<sup>13</sup>. The 1/step putty/light body technique has been criticized because of the uncontrolled bulk of the light body material<sup>14</sup>, by diminishing the volume of the polymerizing material at each stage the final contraction will be reduced and the accuracy of the impression can be improved .The impression techniques and the different protocols used to asses the accuracy of Impression materials explain the contradictory results reported in the literature.

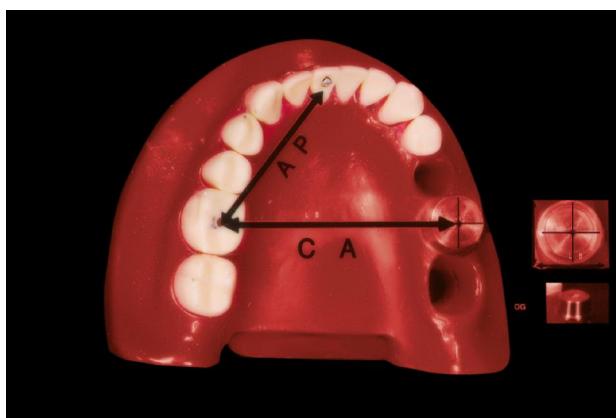
When clinical circumstances necessitate the use of fast-setting elastomeric impression materials; patients, practitioners, and dental office staff can benefit from the shorter setting times,<sup>18</sup> as time saving usually relates to reduced costs and increased patient comfort. Also, impression making is generally an uncomfortable procedure, and for some patients with a strong gag reflex, it presents a severe problem. Reducing the time the impression material remains intraorally is an improvement from the patient's perspective. The purpose of this study was to assess the effect of vacuum-formed resin sheet spacer on the accuracy of fast-setting polyvinyl siloxane impression material. Impressions were taken with and without spacer. Different methods of accuracy assessment were also used and compared. Thus, the research hypotheses were that no differences existed in accuracy among the two techniques which are with and without spacer, and secondly, no differences were existed between the two assessments.

## 2. Material and Methods

This study evaluated the accuracy of a polyvinyl siloxane rubber base impression material (Affinis perfect impressions Coltene/Whaledent AG), indirectly through recovered gypsum casts from impressions made on a master model. The tested variables included the use of double impression technique with a spacer and double impression technique without a spacer. (Table 1). The master model utilized was similar to that used in previous studies,<sup>[18-20]</sup> consisting of a Dentoform mandibular arch (Model 1362; Columbia Dentoform, Long Island City, NY) with some modifications. It contained a removable stainless steel complete crown preparation in the position of the mandibular right first molar (Figure 1). The complete crown preparation was machined with 12-degree angle of convergence with a gingival shoulder finish line. In addition two stainless steel inserts were placed, one on the central fossa of the occlusal surface of the left first molar, and one on the lingual surface of the mandibular left central incisor to provide reference points for measuring cross-arch(CA) and anteroposterior(AP) dimensions. Thus, reference mark 1 is placed on the lingual surface of the mandibular left central incisor; reference mark 2 on the central fossa of the left first molar; and reference mark 3 on the center of diameter of the stainless steel prepared crown representing the mandibular right first molar.

**Table [1]:** Variables tested

Tested variables	
Group 1	Double impression technique with spacer [n=10]
Group 2	Double impression technique without spacer[n=10]



**Figure 1:** Dentoform mandibular arch with removable stainless steel complete crown preparation



**Figure 2:** Spacer pressed onto the model

A total of twenty impressions were taken, and divided into two equal groups according to the selected variables, each consisting of 10 impressions.

Group 1: Ten casts were made from individual impressions of the master arch form, using the double step impression technique. A large, mandibular, disposable plastic impression tray (President Disposable Impression Tray; Coltene Whaledent, Cuyahoga Falls, Ohio) was used for all impressions. This was a rigid, perforated tray with the ability to resist distortion expected during seating and removal of the tray. A soft clear ethyl vinyl acetate spacer of thickness 1mm (Pro-Form; Dental Resources Inc, Delano, Minn) was pressed onto the master model using vacuum formed machine (Figure 2). For retention of the impression material the tray adhesive provided by the manufacturer was painted in the fitting surface of the tray. To standardize the seating position and centering of the tray during impression making on the master model, positioning guides were constructed with light polymerized acrylic resin material (Triad Tru Tray, VLC; Dentsply Intl, York, Pa). [Table 2] The type I high viscosity polyvinyl siloxane impression material [Affinis perfect impressions] was used according to manufacture instructions to make the impressions. The trays were loaded with the material then seated over the master model , centered according to the positioning guides, and left to polymerize [for 2.20 minutes at 37 0c in 100% humidity]. After the putty impression material is completely polymerized, the impression and the spacer were removed from the cast, and then the

spacer was removed from the putty impression. The light body polyvinyl siloxane material [Affinis fast light body] was then injected onto the complete crown preparation and occlusal reference points, and Syringed into the impressions evenly and the tray was reseated over the master model again and left for polymerization. Once removed, the impression was visually inspected to determine the reproduction of the details, rinsed for 10 seconds under running water to simulate the removal of saliva and other contaminants and then air dried. To form the working casts, type IV stone (Prima-Rock; Whip mix, Louisville, Ky) in the form of 70 gm packages was used. The stone was mixed using 14 ml of distilled water, first by hand for 15 seconds, and then vacuum-mixed for an additional 30 seconds (Combi-Mix; Whip mix). The stone was vibrated into the impression, filled to the level of the tray borders, and the excess material utilized to provide mechanical retention. The cast was allowed to set at room temperature in air for 60 minutes. To enable ease of separation of the stone working die, a stone separator (Super-Sep; Kerr Lab, Orange, Calif) was painted over the site. The cast was boxed and then a base was added using a type III dental stone (Microstone; Whip Mix). The base was allowed to bench set for one hour. The recorded stone cast with base was separated from the impression and left to set for 24 hours in ambient air for measurement.

Group 2: Ten casts were produced from individual impressions of the master model using the double step impression technique without a spacer, following the previously mentioned steps.

#### Measurements of casts' accuracy:

The master model and each recovered cast were measured in an ordered sequence, namely dimension A, the distance between reference marks 1 and 2; dimension B, the distance between reference marks 1 and 3; and dimension C, the distance between reference marks 2 and 3. The A, B, and C dimension measurements were made using a binocular measuring microscope (Nikon Measurescope 20; Nikon, Tokyo, Japan) capable of measuring to 0.001 mm. These measurements were repeated three times to determine the mean for each dimension. All measurements were made by the same investigator. The measurements were carried out at ambient room temperature and humidity ( $22.1^{\circ} 0 \pm 0.2^{\circ} \text{C}$  and 60% -10 %).

#### Measurements of dies accuracy:

The stainless steel and working dies were positioned on a custom fabricated stainless steel device, to assess the BL, MD, and OG dimensions. The cross-arch (CA) dimension was assessed by measuring the distance from the mandibular left first molar to the mandibular right first molar, the

anteroposterior (AP) distance from mandibular left first molar to central incisor; the mesiodistal (MD) and buccolingual (BL) dimension across the gingival shoulder of the simulated complete crown preparation and the occlusogingival (OG) measurement of the preparation from the gingival shoulder to the occlusal surface. The same five dimensions were measured on

working casts and die retrieved from impressions of the master model. Specimens were examined on a Zeiss stereomicroscope and pictures were taken using an Olympus Camedia C-5060 digital camera fitted on the microscope using a fixed magnification of x6.3.

**Table [2]:** Impression material tested: Morphometric measurements were done on an IBM compatible personal computer (PC). The image analysis software used was the “Image Tool for Windows version 3”. A graph paper was photographed using the same magnification of x6.3, for the purpose of calibrating the image analysis software, after the software is calibrated. The data obtained were then subjected to statistical analysis.

Product	Type	Working/Setting time (min:sec)	Batch no.	Manufacturer
Affinis perfect impressions	Polyvinyl Siloxane Rubber base material (Addition type) Putty Soft Fast base, Fast catalyst	Affinis fast regular body/ fast light body Mixing time (15 ml): 0:15 min Total working time: 1:15 min Oral setting time: 1:20 min	putty consistency ISO 4823, type 0 light consistency ISO 4823:2000	Coltene/Whaledent AG

#### Statistical analysis

Data were presented as mean and standard deviation (SD) values. ANOVA test was used to compare between master model and experimental models. Tukey's post-hoc test was used for pair-wise comparison between the means when ANOVA test is significant. Mann-Whiney U test was used to compare between the differences and percent relative changes from master model of the two groups. The significance level was set at  $P < 0.05$ . Statistical analysis was performed with SPSS 15.0® (Statistical Package for Scientific Studies) for Windows

### 3. Results

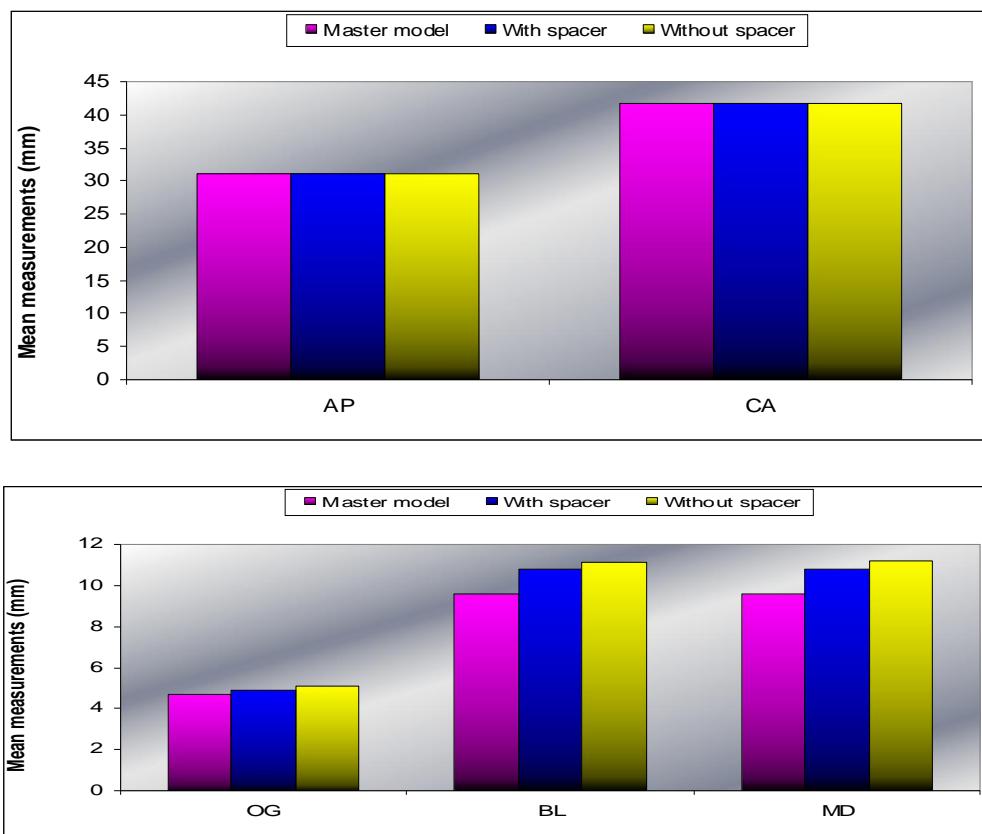
Comparison between cast and die measurements in the two techniques and master model

Dimension	Master model	With spacer		Without spacer		P-value		
		Mean	SD	Mean	SD			
	AP	31.1	0.1	31.1	0.1	31.1	0.2	0.966
Cast	CA	41.8	0.1	41.8	0.2	41.8	0.2	0.882
Die	OG	4.7 <sup>c</sup>	0.05	4.9 <sup>b</sup>	0.06	5.1 <sup>a</sup>	0.07	<0.001*
	BL	9.6 <sup>c</sup>	0.03	10.8 <sup>b</sup>	0.03	11.1 <sup>a</sup>	0.08	<0.001*
	MD	9.6 <sup>c</sup>	0.03	10.8 <sup>b</sup>	0.04	11.2 <sup>a</sup>	0.1	<0.001*

\*: Significant at  $P < 0.05$ , Means with different letters are statistically significantly different according to Tukey's test

As regards cast measurements, there was no statistically significant difference between double impression with spacer, without spacer and master model dimensions.

As regards die measurements in all dimensions, both techniques showed significant difference from master model measurements. Double impression without spacer showed statistically significantly higher mean measurement than double impression with spacer technique.



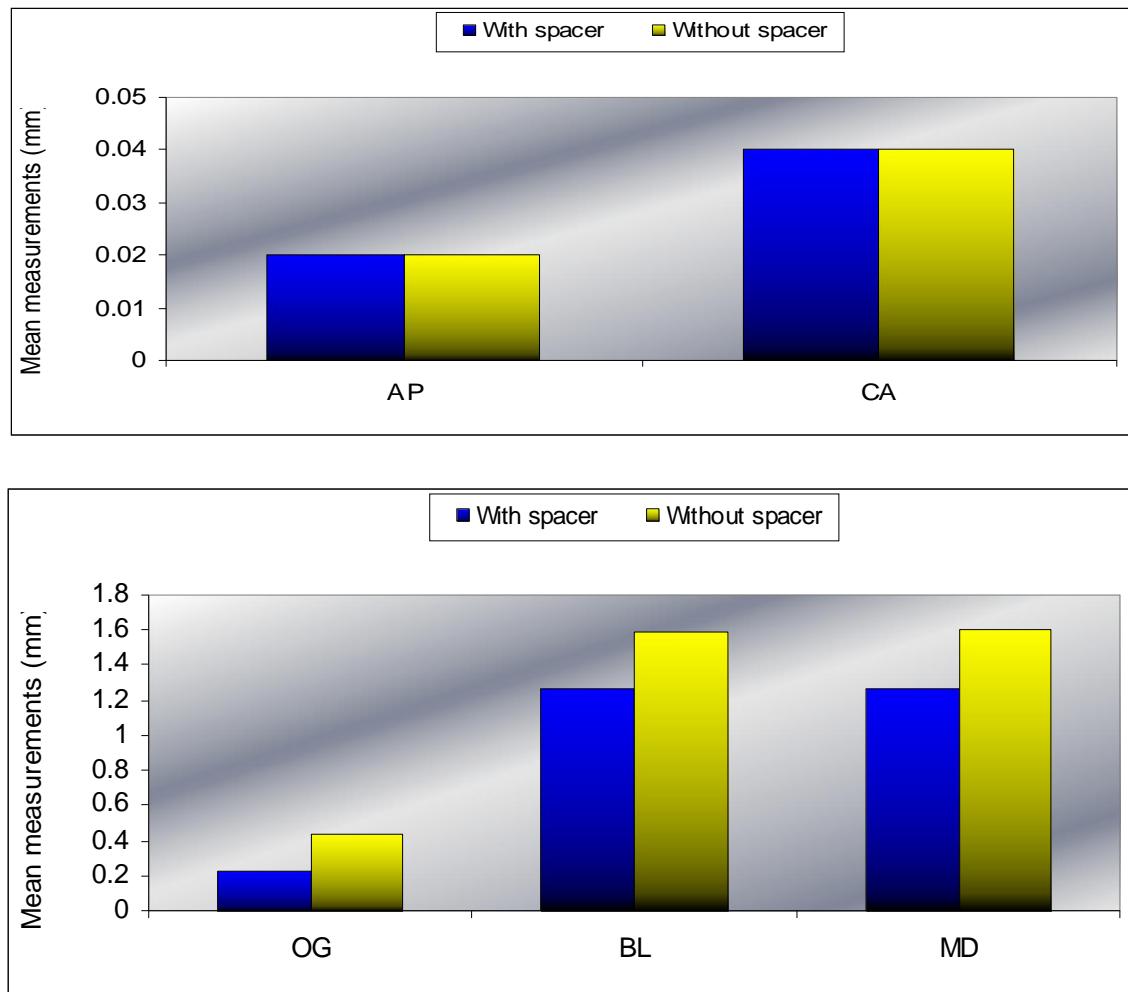
Comparison between differences from master model in cast and dies measurements

	Dimension	With spacer		Without spacer		P-value
		Mean	SD	Mean	SD	
Cast	AP	0.02	0.03	0.02	0.04	1.000
	CA	0.04	0.09	0.04	0.11	1.000
Die	OG	0.22	0.10	0.44	0.11	0.028*
	BL	1.26	0.05	1.59	0.06	0.009*
	MD	1.26	0.05	1.61	0.08	0.009*

\*: Significant at P < 0.05

As regards cast measurements, there was no statistically significant difference between double impression with spacer and double impression without spacer.

As regards die measurements in all dimensions, double impression without spacer showed statistically significantly higher mean difference than double impression with spacer technique.



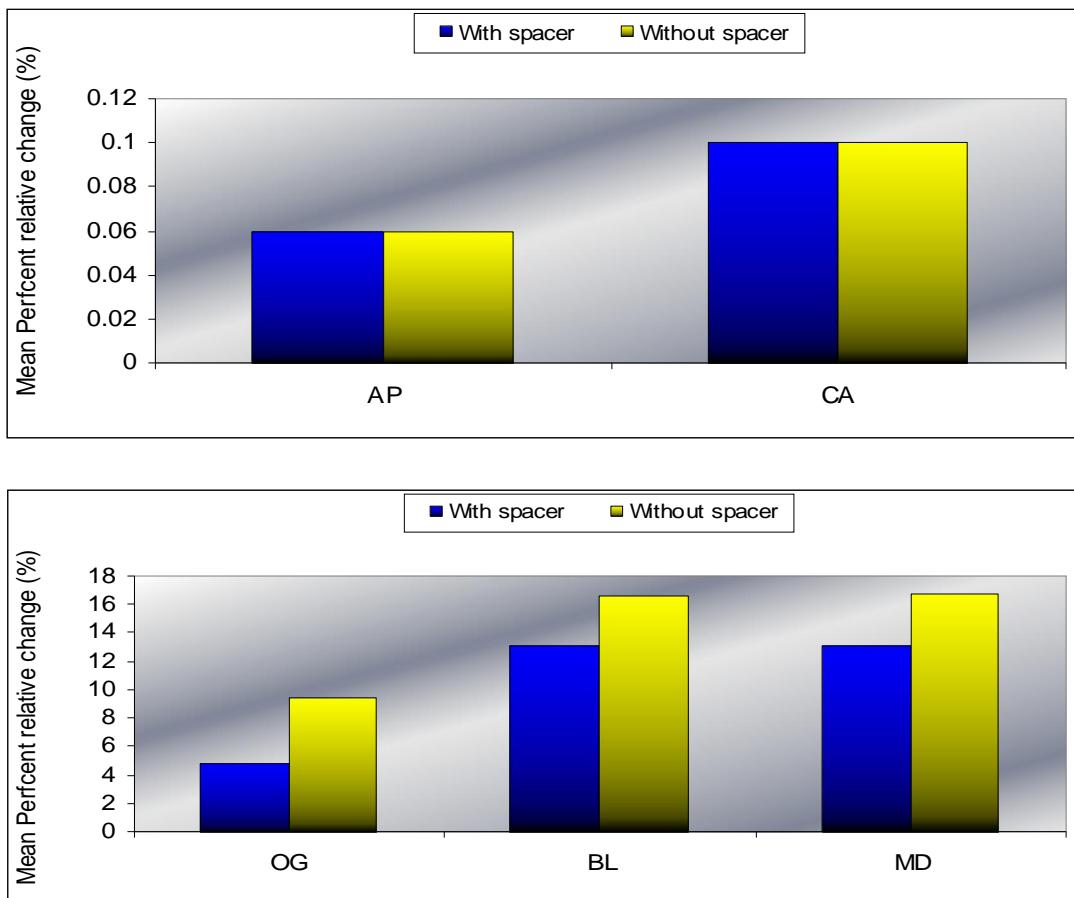
Comparison between percent relative changes from master model in cast and dies measurements

	Dimension	With spacer		Without spacer		P-value
		Mean	SD	Mean	SD	
Cast	AP	0.06	0.12	0.06	0.14	1.000
	CA	0.10	0.21	0.10	0.27	1.000
Die	OG	4.8	2.2	9.4	2.4	0.028*
	BL	13.1	0.5	16.6	0.6	0.009*
	MD	13.1	0.6	16.8	0.8	0.009*

\*: Significant at P < 0.05

As regards cast measurements, there was no statistically significant difference between double impression with spacer and double impression without spacer.

As regards die measurements in all dimensions, double impression without spacer showed statistically significantly higher mean percent relative changes than double impression with spacer technique.



There was no statistically significant difference between means percent relative change in the BL and MD dimensions which showed the statistically significant highest mean values. The means percent relative change in the OG dimension showed the statistically significant lowest mean value.

#### 4. Discussions

Distortion is a 3-dimensional problem that is inherent in all of the steps involved in fabricating an indirect dental restoration. Dimensional accuracy when making impressions is crucial to the quality of fixed prosthodontic treatment, and the impression technique is a critical factor affecting this accuracy<sup>21</sup>. As elastomeric impression materials have been reformulated to achieve a faster set, the accuracy of fast-setting elastomeric impression materials should be confirmed<sup>18</sup>. Accuracy of impression resulting from the 1-step putty-wash technique is controversial<sup>15</sup>. Some authors found that there was no difference in accuracy between techniques,<sup>11,12</sup> while others criticized several potential disadvantages with this

approach<sup>15</sup>. These disadvantages include lack of control of the bulk of wash material and the high risk of capturing portions of the prepared margin in putty material rather than lower viscosity material<sup>22</sup>. Most putty viscosity materials have inadequate fine detail reproduction for this purpose. In our study, it seems that this was the primary reason for the discrepancies in die dimensions resulted from double impression technique without spacer rather than double impression technique with spacer. An advantage of the 2-step double impression technique is that the impression of the teeth can be captured with the wash material<sup>11</sup>. The 2-step putty / light-body technique has been reported to be more accurate than the one-step putty/ light body technique<sup>11</sup>. With the 2-step technique, the impression with the light-body material is made after the putty has polymerized and contracted. Therefore, any further contraction of the light-body material results in minimal dimensional change<sup>13</sup>. This is in agreement with our study, where there was no statistically significant difference between the two techniques in accordance to cast dimensions. The 1-step putty/light-body technique

has also been criticized because of the uncontrolled bulk of the light-body material<sup>14</sup>. By diminishing the volume of the polymerizing material at each stage, the final contraction will also be reduced, and the accuracy of the impression can be improved<sup>23</sup>. Therefore, careful control of the bulk of the light-body impression material has been advocated because it affects the accuracies of the stone cast's<sup>24</sup>. The distortion of the die in a mesiodistal, buccolingual, and occlusogingival direction was investigated in this study. The machined stainless steel standard used in this investigation provided certain advantages in obtaining the measurements over that of a prepared plastic typodont tooth. The well-defined line angles of the stainless steel standard were clearly observed under the microscope, thereby reducing measurement error. The circular nature of the standard allowed observation of the relationship between the change in buccolingual and mesiodistal dimensions of the gypsum dies<sup>26</sup>. Dental elastomeric impression materials are subject to several factors that can result in dimensional changes<sup>18</sup>. For example, the process of polymerization, which involves cross-linking of the polymer chains, can result in a reduction of spatial volume<sup>5</sup>. Polymerization reactions have been shown to continue for a considerable period of time, beyond the achievement of what is considered a final clinical set, and continue after removal of the impression from the mouth<sup>5,6</sup>. Moreover, the lower viscosity of the material, the greater the contraction after polymerization<sup>12</sup>.

In the current study, the space resulted from contraction of the fast-setting putty material, was not enough to produce accurate detail reproduction by the light material. This ensures the necessity of using a spacer to provide enough room for the light-body to record fine details. The direction of dimensional change of impression materials has been reported to be dependent upon the bonding of the material to the impression tray<sup>8,25</sup>. Also; more rigid trays reduce the possibility of distortion in the impression<sup>26</sup>. In our study, a large mandibular disposable plastic impression tray was used for all impressions. This was a rigid, perforated tray with the ability to resist distortion expected during seating and removal of the tray. With a rigid tray and good adhesion to the tray; the material shrinks toward the tray, producing a larger die<sup>5</sup>. While when bonding to the tray may not be sufficient to constrain the material, shrinkage causes movement away from the tray and results in smaller dies. Eames et al<sup>25</sup> reported that the material may adhere to the adhesive of the tray, causing the negative image of the master die (the prepared tooth) to enlarge. In our study, the means percent relative changes in die measurements have also shown

statistically significant higher mean values than cast measurements. Nissan et al<sup>13</sup> studied the accuracy of three polyvinyl siloxane putty-wash impression techniques. When stone casts and the master model were compared, it was found that changes in the vertical dimension were greater than the horizontal. This phenomenon was attributed to the contraction of the impression toward the tray walls, making the stone dies wider in the horizontal aspect and shorter in the vertical one. In another study, Nissan et al<sup>14</sup> attributed the changes in the vertical dimensions (occlusogingival) to the same reason. It was concluded that wash thickness of 1 to 2 mm are most accurate for fabricating stone dies, when using polyvinyl siloxane impression materials with the 2-step putty/wash impression technique. Contrary, in the present study, there was no statistically significant difference between means percent relative change in the bucco-lingual and mesio-distal dimensions which showed the statistically significant highest mean values, while the means percent relative changes in the occluso-gingival dimension showed the statistically significant lowest mean values. This may be attributed to the fact that the amount of impression contraction on the expense of the horizontal aspect (mesio-distal and bucco-lingual), was not high enough to result in changes in the vertical (occlusogingival) direction. Hence, fast setting impression contraction has been greatly compensated for by the use of double step impression technique together with the use of a suitable spacer.

## 5. Conclusion

Within the conditions of this study, it was found that:

- Accuracy of fast-setting polyvinyl siloxane impression material was favorably affected with the use of spacer, as the space resulted from contraction of the putty material was not enough to produce accurate detail reproduction by the light material.
- The working dies; from the fast-setting polyvinyl siloxane impression material without spacer demonstrated an increase in (mesio-distal and bucco-lingual) dimensions, while for cast dimensions there was no difference between the two techniques.

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6/3/2010

# Kinetics and Thermodynamics of Oil Extraction from Jatropha Curcas in Aqueous Acidic Hexane Solutions

Sh. K. Amin, S. Hawash, G. El Diwani\*, and S. El Rafei

Chemical Engineering and Pilot Plant Department, National Research Center, Cairo, Egypt.

\*geldiwani@yahoo.com

**Abstract:** Jatropha oil curcas (JOC) extraction was performed in aqueous HCl, H<sub>2</sub>SO<sub>4</sub>, and H<sub>3</sub>PO<sub>4</sub> solutions with n-hexane (C<sub>6</sub>H<sub>14</sub>) at 30, 40, 50, and 60 °C using 10 gm of Jatropha seeds over 1 hours with 10 minutes sampling intervals. The optimum acid concentration was 15 % by weight for each acid, and the highest oil yield was obtained in the extraction procedure with n-hexane containing HCl. The extraction process was observed with regard to the percent oil yield versus time, and the reaction order was found to be first-order kinetics by the differential method. The activation energy for the oil extraction kinetics of Jatropha seeds with 15 % HCl was E<sub>a</sub> = 26.6763 kJ/mol, and the activation thermodynamic parameters at 60 °C were H = 23.908 kJ/mol, S = - 239.927 J/mol.K, and G = 103.803 kJ/mol. The enthalpy value was H = 0.1586 kJ/mol, and the other thermodynamic parameters at 60 °C were calculated to be S = 15.275 J/mol.K, and G = - 4.928 kJ/mol. [Journal of American Science. 2010;6(11):293-300]. (ISSN: 1545-1003).

**Key words:** kinetics, thermodynamics, oil extraction, Jatropha curcas.

## 1. Introduction

Jatropha is a genus of over 170 plants from the Euphorbiaceae family, native to the Central America but commonly found and utilized across most of the tropical and subtropical regions of the world. It has a yield per hectare of more than four times that of soybean and ten times that of corn <sup>(1)</sup>. Jatropha curcas is a wonder plant with a variety of applications and enormous economic potentials <sup>(2)</sup>.

Extracts from this species have been shown to have anti-tumor activity <sup>(3)</sup>, the leaves can be used as a remedy for malaria and high fever <sup>(4, 5)</sup>, the seeds can be used in treatment of constipation and the sap was found effective in accelerating wound healing procedure <sup>(4)</sup>. Moreover, this plant can be used as an ornamental plant, raw material for dye, potential feed stock, pesticide, soil enrichment manure and more importantly as an alternative for biodiesel production <sup>(6, 7)</sup>.

Oilseeds are extracted in two ways, by squeezing or mechanical pressing and with chemical solvents. Prior to the 1940's, mechanical pressing was the primary method used, but it had its limits in terms of oil recovery. By mechanical pressing, only 5–6 % of the oil was difficult to achieve <sup>(8)</sup>, and also pressing generates high temperatures which damage both oil and meal <sup>(8)</sup>.

Solid liquid extraction is a common and efficient technique in producing oil for biodiesel production. Solid liquid extraction, sometimes called

leaching, involves the transfer of a soluble fraction (the solute or leachant) from a solid material to a liquid solvent <sup>(9)</sup>. Solvent extraction was developed because it allows more complete extraction at lower temperatures. Extraction using supercritical fluid <sup>(10)</sup>, the oil produced has very high purity; however the operating and investment cost is high <sup>(10)</sup>. Solvent extraction has several advantages, it gives higher yield and less turbid oil than mechanical extraction, and relative low operating cost compared with supercritical fluid extraction <sup>(11)</sup>. For many years, commercial grade hexane was been the solvent of choice for the extraction of oil from oilseeds. Alternative hydrocarbon solvents for cottonseed extraction was recommended <sup>(12)</sup>, heptane and iso-hexane as potential replacements for hexane.

In the present investigation, oil extraction from Jatropha seeds in aqueous hydrochloric acid (HCl), sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) and ortho-phosphoric acid (H<sub>3</sub>PO<sub>4</sub>) solution with n-hexane (C<sub>6</sub>H<sub>14</sub>) was studied. The aim of the study was to find the optimum acid concentration and most effective acid from HCl, H<sub>2</sub>SO<sub>4</sub> and H<sub>3</sub>PO<sub>4</sub>, and, in physicochemical terms, to determine the kinetic and thermodynamic parameters of oil extraction from Jatropha seeds.

## 2. Materials and Experimental

### 2.1. Materials used:

### 2.1.1 Jatropha seeds:

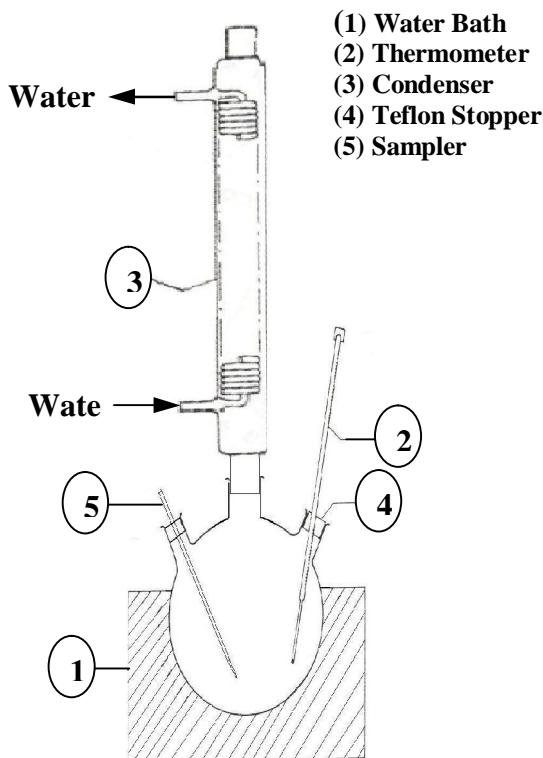
Jatropha seeds were purchased from local market "Ministry of Agriculture". For the extraction process, the seeds were shelled, and then ground to a mesh size of 40 (425  $\mu\text{m}$ ). The moisture content of the ground Jatropha seeds was 7.48 %.

### 2.1.2 Solvent acid solutions:

The normal hexane ( $C_6H_{14}$ ), hydrochloric acid (HCl), sulfuric acid ( $H_2SO_4$ ), and orthophosphoric acid ( $H_3PO_4$ ), were used to prepare aqueous acid solution as wt. 0 % (without acid only hexane with distilled water), 5 %, 10 %, 15 %, 20 %, 25 %, and 30 %.

### 2.2. Experimental Set-Up:

Figure (1) illustrates a schematic diagram of a bench scale extraction set-up which consists mainly of a three necked flask (250 mL) with a round bottom. The large neck in the middle of the flask was connected to a reflux condenser, a thermometer was placed in one of the two side necks, and the third neck was used for taken samples during the extraction process period. The flask was submerged in a temperature controlled water bath with magnetic stirrer.



**Fig. (1): Bench Scale Extraction Set-Up**

### 2.3. Procedure and Experimental Conditions:

#### 2.3.1. Laboratory Scale:

Ground Jatropha seeds (10 gm) was put in a three-necked flask (250 mL) with a round bottom containing 76 ml of hexane and 76 ml of acid solution (for each acid solution), and the following temperatures were used 30, 40, 50, and 60 °C. The total extraction time was 60 minutes. Miscella batch samples were collected in reweighed beakers at time intervals 10 minutes of extraction. The amount of oil extracted in each time interval was determined gravimetrically by measuring the weight of the residue after filtration and drying by recovering solvent.

#### 2.3.2. Bench Scale:

100 gm of ground Jatropha seeds were extracted using 760 ml of hexane and 760 ml of acid solution at 60 °C for 3 hours. Miscella obtained after filtration was collected in a separating funnel in which the upper clear layer is a mixture of oil and solvent. The amount of oil extracted was determined gravimetrically by weighing the oil after drying for solvent recovery.

## 3. Results and Discussion

### 3.1 Laboratory Scale Results:

#### 3.1.1 Effect of Acid's Types and Concentrations:

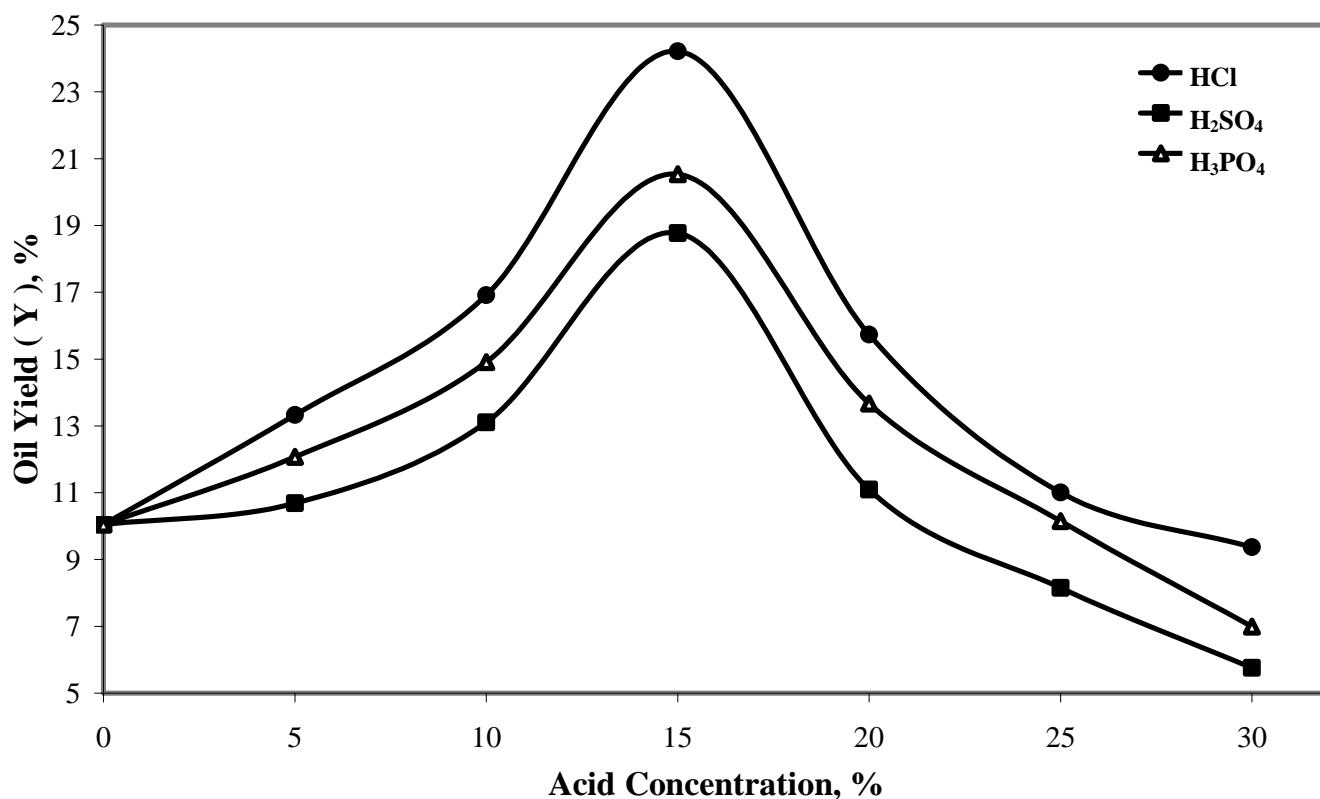
The percent oil yield values for the different acid solutions at 60 °C and 60 min time extraction are shown in figure (2) for oil extraction from Jatropha seeds. As seen in figure (2), the oil yields peaked (optimum value) at the 15 % concentration for each acid and decreased inversely to acid concentration after this optimum value. At this optimum acid value, it decreased the hexane penetration path lengths and resulted in an increase in the amount of oil extracted<sup>(13)</sup>.

Since the highest oil yield was obtained with HCl over  $H_3PO_4$  and  $H_2SO_4$ , then the most effective acid was HCl. The kinetic and thermodynamic parameters were calculated according to the values at a 15 % acid concentration. The percent oil yields for 15 % HCl at various temperatures are given in table (1) for Jatropha seed oil extraction.

From these results, it is obvious that maximum oil extracted reached 24.216 % of seeds at 60 °C, (i.e) the extracted oil is 85.569 % from the available oil which is 28.3 %, because the oil source was Jatropha trees only of two years old<sup>(14)</sup>.

**Table (1): Percent of JOC at various extraction temperatures for 15 % HCl containing n-hexane**

Time , min	Oil Yield ( % ), Y			
	30	40	50	60
10	12.578	14.211	16.112	16.948
20	13.263	15.148	17.201	18.201
30	13.976	16.113	18.438	19.552
40	14.782	17.145	19.713	21.048
50	15.578	18.321	21.172	22.536
60	16.481	19.518	22.602	24.216

**Fig. (2): Effect of Acid Concentration on Percent JOC ( Y ) at 60 °C and 60 min**

### 3.1.2 Extraction Kinetics:

A reaction rate equation for oil extraction from Jatropha seeds can be written as <sup>(13, 15)</sup>:

$$\frac{dY}{dt} = kY^n \quad (1)$$

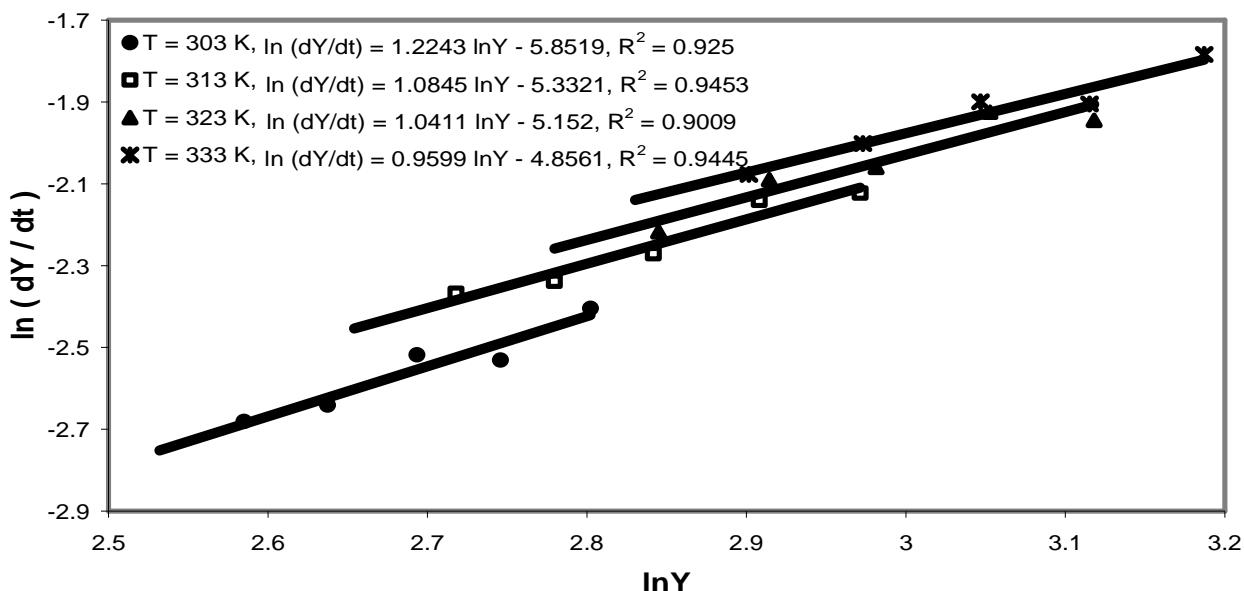
where Y is the percent oil yield, t is the time of extraction (min), k is the extraction constant, and n is the reaction order. Since the percent oil yield

increased in the course of time, the terms dY/dt have a positive sign <sup>(13)</sup>.

Using the values in table (1) and applying the Differential Method, plots of ln ( dY/dt ) versus ln Y for 15 % HCl were drawn and were found to be linear according to equation (1). A first-order kinetics was found from the values of n obtained, with average R<sup>2</sup> = 0.93, from the slopes of the straight lines in figure (3), and the reaction rate constants were calculated from the slopes (Table 2).

**Table (2): Values of the reaction rate constants for JOC extraction with 15 % HCl containing n-hexane at various temperatures**

T (°C)	k (min <sup>-1</sup> )
30	2.8744*10 <sup>-3</sup>
40	4.834*10 <sup>-3</sup>
50	5.7878*10 <sup>-3</sup>
60	7.781*10 <sup>-3</sup>

**Fig. (3): A Plot of [ ln (dY/dt) ] versus [ ln Y ] at Different Temperatures for JOC Extraction with 15 % HCl Containing n-Hexane**

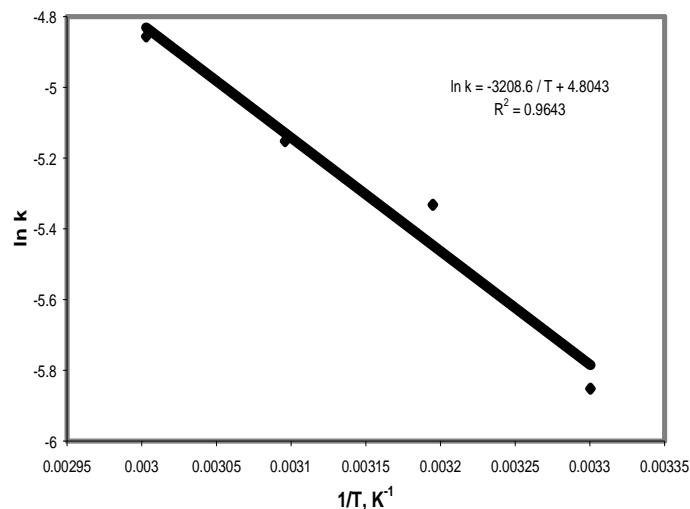
### 3.1.3 Calculation of Activation Energy:

The rate constant k increases with increasing temperature, and this trend is obvious in table (2). The changes can be described by the Arrhenius Equation (2, 13, 15):

$$k = Ae^{-Ea/RT} \quad (2)$$

where k is the reaction rate constant, A is the Arrhenius constant or frequency factor, Ea is the activation energy, R is the universal gas constant, and T is the absolute temperature.

A plot of ln k vs. 1/T gives a straight line whose slope represents the activation energy of extraction,  $-Ea/R$ , and whose intercept is the Arrhenius constant, ln A (Figure 4). Thus, the activation energy and the Arrhenius constant were calculated. These were  $Ea = 26.6763 \text{ kJ/mol}$  and  $A = 2.0339 \text{ s}^{-1}$ , respectively (2, 13, 15).



**Fig. (4): A Plot of [ ln k ] versus [ 1/T ] for JOC Extraction with 15 % HCl Containing n-Hexane**

### 3.1.4 Calculation of Activation Thermodynamic Parameters:

The activation thermodynamic parameters were calculated in the following equations according to the transition state theory<sup>(2, 13, 15)</sup>:

$$A = \frac{RT}{Nh} e^{\Delta S^\ddagger / R} \quad (3)$$

**Table (3): The activation thermodynamic parameters for JOC extraction with 15 % HCl containing n-hexane**

T, K	H , kJ/mol	S , J/mol.K	G , kJ/mol
303	24.157158	-239.1416839	96.61708823
313	24.074018	-239.4116427	99.00986217
323	23.990878	-239.6731108	101.4052928
333	23.907738	-239.9266061	103.8032978

### 3.1.5 Calculation of Thermodynamic Parameters:

Thermodynamic parameters (H, S, and G) for the extraction of Jatropha oil using n-hexane and 15 % HCl as solvents can be estimated using following equations<sup>(11, 13, 16)</sup>:

$$K = \frac{Y_T}{Y_u} \quad (6)$$

$$\ln K = -\frac{\Delta G}{R} \frac{1}{T} = -\frac{\Delta H}{R} \frac{1}{T} + \frac{\Delta S}{R} \quad (7)$$

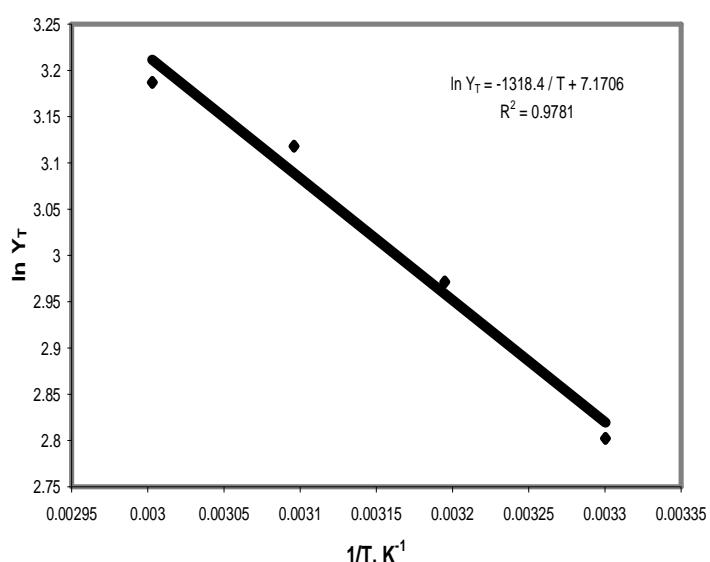
where K is the equilibrium constant, Y<sub>T</sub> is the percent oil yield at temperature T, Y<sub>u</sub> is the percent unextracted oil, H is the enthalpy change, S is the entropy change, and G is the free energy or Gibb's energy.

A plot of ln Y<sub>T</sub> vs. 1/T at 60 min, gives a straight line whose slope represents the enthalpy change of extraction, - H.R. Thus, the enthalpy change was calculated to be H = 0.1586 kJ/mol for Jatropha seed oil extraction with 15 % HCl (Figure 5). The H value obtained was indicating the physicochemical nature of the oil extraction process<sup>(11, 13, 16)</sup>.

$$\Delta H^\ddagger = Ea - RT \quad (4)$$

$$\Delta G^\ddagger = \Delta H^\ddagger - T\Delta S^\ddagger \quad (5)$$

where N is the Avogadro's constant, h is the Planck's constant, S is the activation entropy, H is the activation enthalpy, and G is the activation free energy or Gibb's energy. These activation thermodynamic parameters are shown in table (3) for each temperature.



**Fig. (5): A Plot of [ ln Y<sub>T</sub> ] versus [ 1/T ] for JOC Extraction with 15 % HCl Containing n-Hexane**

Other thermodynamic parameters (S and G) and the equilibrium constant values for Jatropha seed oil extraction with 15 % HCl at 60 min, are given in table (4) for each temperature.

**Table (4): The equilibrium constants (K) and the thermodynamic parameters ( S and G) for JOC extraction with 15 % HCl containing n-hexane**

T (K)	K	S (mol <sup>-1</sup> .K <sup>-1</sup> )	G (kJ/mol)
303	1.394449615	3.287756441	-0.837614202
313	2.222500569	7.146466889	-2.078268136
323	3.966654967	11.94700054	-3.700305173
333	5.929480901	15.27459769	-4.927865031

According to these results, the positive value of enthalpy indicates that the process is endothermic and requires energy during process. In addition, the negative value of G ( G < 0) at 60 °C indicates that there is a decrease in the free energy, that is, the extraction process of Jatropha oil using n-hexane and 15 % HCl at 60 °C is spontaneous process, which is in agreement with previous investigations <sup>(11, 13, 16)</sup>.

The reaction system initially consists of the ground Jatropha seed, aqueous acid solution and n-hexane, whereas the oil molecules are extracted from the Jatropha seeds during the extraction process, and

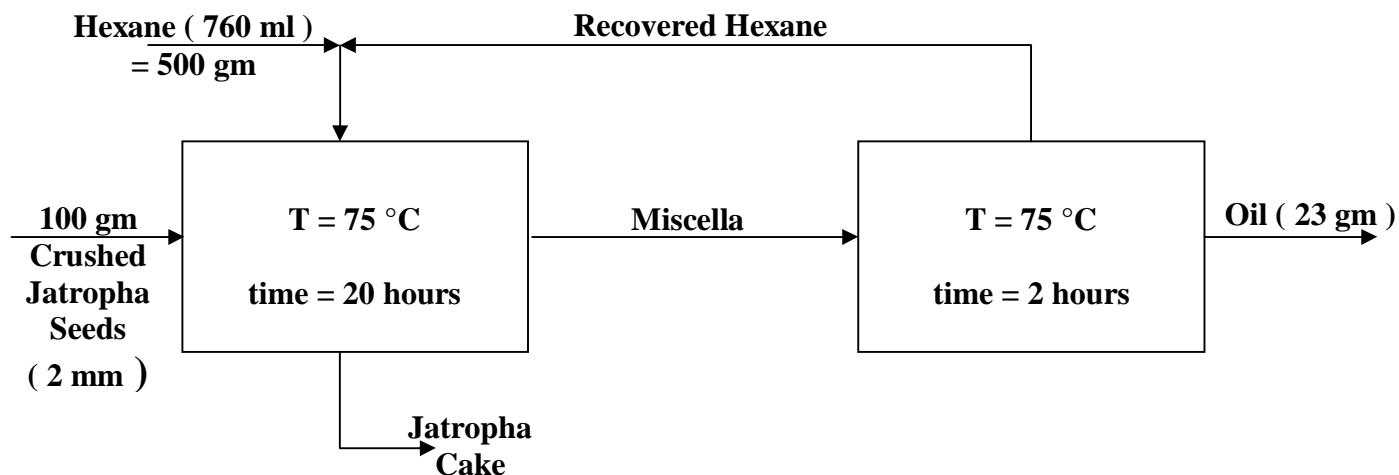
therefore, the entropy of the mixture increases in the course of the extraction, that is the positive value of entropy change ( S > 0) at 60 °C indicates that the process is irreversible <sup>(11, 13, 16)</sup>, while at temperatures less than 60 °C the reaction is reversible.

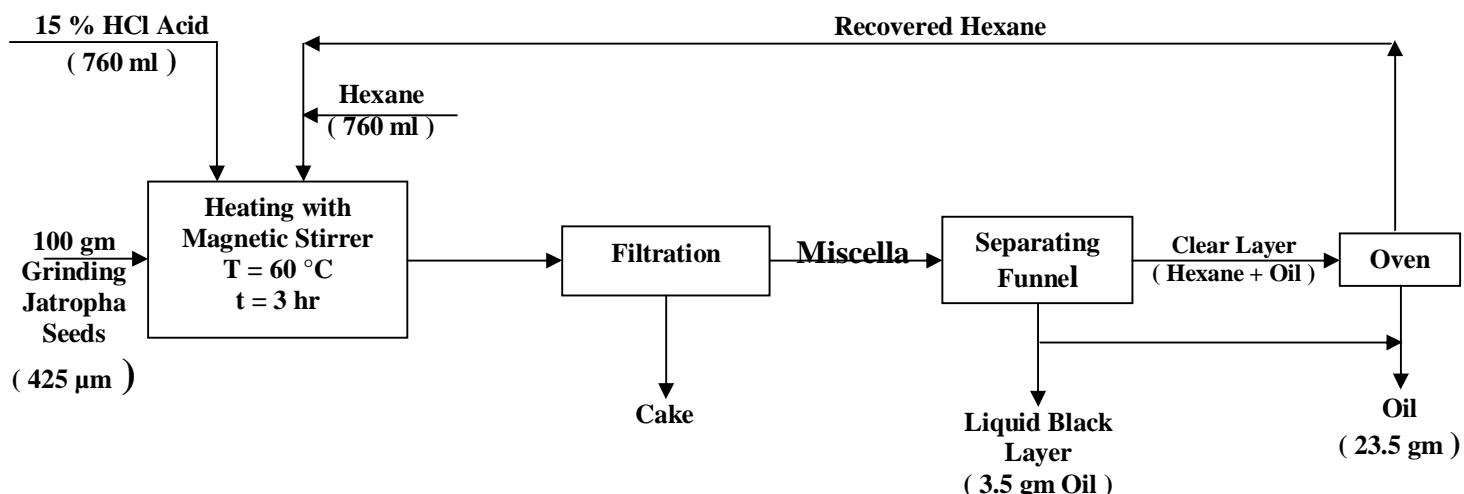
### 3.2. Bench Scale Results:

Flow sheets representing the mass balance at optimum conditions for acid extraction (Figure 6), compared with conventional solvent extraction (Figure 7), and mechanical pressing extraction (Figure 8). Table (5) illustrates differences in the three extraction procedures.

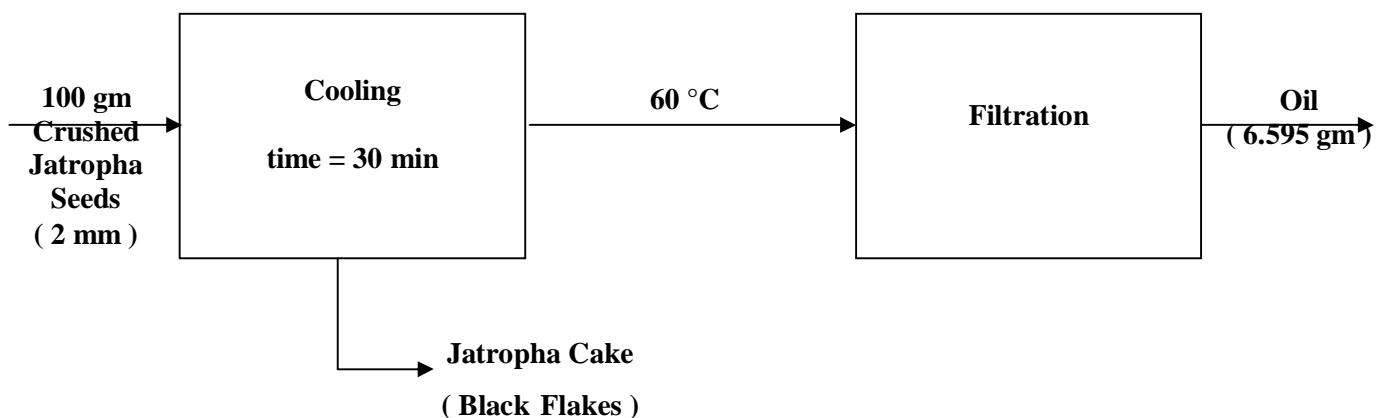
**Table (5): Comparison between different extraction procedures**

Extraction Procedure	Time of Extraction, ( hrs )	Temperature, ( °C )	Produced Oil, ( % )
Conventional hexane	20	75	23
Aqueous acidic hexane	3	60	23.5
Mechanical pressing	0.5	60	6.595

**Fig. (6): Solvent Extraction of Jatropha Curcas Oil**



**Fig. (7): Developed Oil Extraction from Jatropha Curcas Using Aqueous Acidic Hexane Solution**



**Fig. (8): Mechanical Pressing**

#### 4. Conclusion

It was found that Hydrochloric acid, HCl, was more effective and more suitable than  $H_2SO_4$  and  $H_3PO_4$  in the presence of n-hexane for oil extraction from Jatropha seeds. The optimum acid concentration was 15 %.

The Jatropha seed oil extraction process, using 15 % HCl containing n-hexane, has a first order kinetics.

The activation energy was  $E_a = 26.6763$  kJ/mol, and the activation thermodynamic parameters at 60 °C were  $H = 23.908$  kJ/mol,  $S = -239.927$  J/mol.K, and  $G = 103.803$  kJ/mol. The enthalpy value was  $H = 0.1586$  kJ/mol, and the other thermodynamic parameters at 60 °C were

calculated to be  $S = 15.275$  J/mol.K, and  $G = -4.928$  kJ/mol.

It also found that  $H$  is positive,  $S$  is positive, and  $G$  is negative indicating that this process are endothermic, irreversible, and spontaneous.

It is concluded from table (5) that extraction time with hexane in 15 % HCl is reduced to the seventh of that with hexane pure which corresponds to less energy required and in consecutively to less cost.

**Acknowledgment**

The authors acknowledge the financial support from STDF to which all rights are reserved. work.

**Corresponding author**

G. El Diwani\*  
 Chemical Engineering and Pilot Plant Department,  
 National Research Center, Cairo, Egypt.  
 \*geldiwani@yahoo.com

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5/29/2010

## Methodology for Selective Adsorption of Lithium Ions onto Polymeric Aluminium (III) Hydroxide

S. Hawash, E. Abd El Kader and G. El Diwani\*

Chemical Engineering and Pilot Plant Department, National Research Center, Cairo, Egypt.

[\\*geldiwani@yahoo.com](mailto:geldiwani@yahoo.com)

**Abstract:** The recovery of lithium as lithium aluminate from Egyptian bitterns was investigated. Studies were performed on synthetic  $\text{Li}^+$  solution and on three high – salinity end brines which contain  $\text{Li}^+$  of concentrations varying between 5.5- 19.5 ppm. Pretreatment with a mixture of  $\text{Na}_2\text{SO}_4$ -  $\text{Na}_2\text{CO}_3$  is achieved to precipitate  $\text{BaSO}_4$ ,  $\text{SrCO}_3$ ,  $\text{CaCO}_3$  and possibly  $\text{MgCO}_3$ . A co-precipitation method was employed using aluminum salt as ( $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ ). Lithium ion is adsorbed onto aluminum hydroxide, which is freshly produced by adding  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  and Na OH to the brines at  $\text{Al}^{3+} / \text{Li}^+$  molar ratio 5-7. Results obtained indicate that high  $\text{Li}^+$  adsorption was performed at pH = 6-7 for Alexandria-Arish and Emissal salines, even for small concentration of aluminum salt added. Also, Lithium ions uptake decreased with increasing adsorption temperature from 10°C to 30°C but over 30°C increase in temperature does not affect lithium uptake on  $\text{Al(OH)}_3$ , which proved that the process is physical adsorption. Equilibrium isotherms have been determined for the adsorption of  $\text{Li}^+$  onto  $\text{Al(OH)}_3$  at 30°C and pH= values (5 to 9), the maximum adsorption capacity of  $\text{Al(OH)}_3$  at 30°C and pH = 9 is 123 mg/gm. The results indicated that applied isotherms were shown to be "favorable" and were fitted with Langmuir and Freundlich isotherms.  $\text{Li}^+$  desorption from  $\text{Al(OH)}_3$  was investigated using hydrofluoric acid (HF) or sulphuric acid ( $\text{H}_2\text{SO}_4$ ) with different concentrations, and results obtained showed that HF is more efficient than  $\text{H}_2\text{SO}_4$  concerning  $\text{Li}^+$  desorption. From the obtained results, Li ion can be recovered successfully from bittern and saline solutions. [Journal of American Science. 2010;6(11):301-309]. (ISSN: 1545-1003).

**Keywords:** lithium; lithium aluminate; hydrofluoric acid (HF); sulphuric acid ( $\text{H}_2\text{SO}_4$ ); saline solution

### 1. Introduction

Lithium importance has been increasingly recognized being in wide range of industrial applications such as:

- Blanket material in fusion reactors.<sup>(1,2)</sup>
- Important ingredient in the production of organolithium compounds as an alloying addition to  $\text{Al}^{3+}$  and  $\text{Mg}^{2+}$  and as the anode in rechargeable lithium ion batteries due to its high electrochemical potential.<sup>(3,4)</sup>
- In heat transfer application because of its specific heat is the largest of any solid.
- Reducing agent in organic chemistry applications.
- In treatment of bipolar disorder.<sup>(3)</sup>
- Manufacturing certain kinds of glass and ceramic.

The world production of lithium amounts to 12,500 tons per year, the high unit price of the metal translates into annual sales of approximately \$ 1 billion.<sup>(5)</sup> In general, sources of hard water tend to be highest in  $\text{Li}^+$  concentrations, in sea water lithium is found at concentration of 11 ppm, and the presence of lithium in surface soils is fairly uniform and can vary between 1.2 ppm in light organic soils to 98 ppm in alluvial soils.<sup>(6)</sup> Bittern solutions have been subjected to several studies aiming extraction of other valuable salts.

However, there is no much studies on lithium ions, the present actual study was conceived to shed more light on lithium extraction.

There are many techniques to extract lithium from a lithium containing solutions. Main methods of lithium extraction are considered to be:

- Solvent extraction, or it is known as liquid-liquid extraction in which the separation of the components of a liquid mixture by treatment with a solvent in which one or more of the desired components is preferentially soluble.<sup>(7,8)</sup>
- Electro- deposition technique which can be used to obtain metals in very pure form, a dilute solution may be concentrated by evaporation and then placed in electrolytic cell. Also electro- chemical treatment consider to be rapid process and effective for certain metals.<sup>(9)</sup>
- Ion exchange method by which ions of a given species is displaced from an insoluble exchange material by ions of a different species in solution. Ion exchange is good mean for removal in a wide for heavy metals and dyes but the adsorbent requires regeneration or disposal.<sup>(9)</sup>
- Liquid membrane method which was introduced as an alternative separation technique to the liquid – liquid extraction and

- to the separation by means of solid polymer membrane.<sup>(10)</sup>
- Co- precipitation method which involves the conversion of soluble metal salts to insoluble salts by means of pH adjustment.<sup>(11)</sup>

This article investigation is trying to look into the possibility of lithium extraction by adsorption onto aluminum hydroxide which is the simplest, easier and most economical method for Li<sup>+</sup> adsorption. The selective coprecipitation of Li ions was vaguely explained as adsorption on alumina<sup>(12)</sup>. It is significant that no other common adsorbant expect alumina was found to be suitable for selective Li extraction. The study has also covered the experimental steps for Li<sup>+</sup> adsorption from synthetic solutions and studies the factors affecting the adsorption process such as : pH, temperature, Al<sup>3+</sup> / Li<sup>+</sup> molar ratio, stirring time and stirring rate. The adsorption isotherms constants of Li<sup>+</sup> onto polymeric Al(OH)<sub>3</sub> have been determined at different pH values. Optimum conditions obtained for Li<sup>+</sup> adsorption from synthetic solutions were applied to evaluate Li<sup>+</sup> extraction from local bitters: El-Nasr Salines in Alexandria-Arish and Emissal at Quaroon lake. The recovery of lithium from Egyptian brines is difficult due to their low concentrations<sup>(13)</sup> from 5.5ppm to 19.5 ppm and also due to the presence of high content of other salts.<sup>(13)</sup> The aim of this work is to evaluate the efficiency of different lithium concentration solutions recovery from Egyptian sources such as sea and lake water applying the co-precipitation procedure.

Also maximum adsorption capacity of Li<sup>+</sup> onto polymeric Al(OH)<sub>3</sub> was determined by the applicability of two mathematical models, Langmuir<sup>(14)</sup> and Freundlich<sup>(15)</sup> isotherms which were represented by the two following equations respectively :-

$$\begin{aligned} C_e/q_e &= 1 / K_{L+}(a_L / K_L) Ce \\ \text{Log } q_e &= \text{Log } K_f + (1/n) \text{ Log } Ce \end{aligned}$$

## 2. Materials and Experimental

### Experimental set-up

A bench scale apparatus is constructed to carry out the adsorption of lithium onto polymeric Aluminum III hydroxide. The apparatus consists mainly of a glass reactor (2L capacity) provided with electrical stirrer, its speed ranges from 0-2500 r.p.m. The whole reaction vessel was heated onto a hot plate and the temperature was measured using a thermometer dipped in the reaction mixture. Fig (1) represents the schematic diagram of the experimental apparatus.

### 2.2. Raw Materials:

The raw material used for bench scale experiments were synthetic solutions, natural brines and pure chemicals.

#### 2.2.1. Synthetic Solution

Standard lithium solution (1000 ppm Li<sup>+</sup>) from Li<sub>2</sub>CO<sub>3</sub> which was diluted to different Li<sup>+</sup> concentrations (10, 20, 30, ..., 100, 200 ppm) to form the synthetic solutions.

#### 2.2.2. Actual lithium solutions from El-Nasr company after salt production:

##### a) El-Max factory in Alexandria.

Residual brine left after the production of table salt is used for experimental work. The brine is of specific gravity 1.28 gm / cm<sup>3</sup> i.e. 31.72° Be.

##### b) El-Arish factory:

Residual brine left after the table salt production, which specific gravity is 1.23 gm/cm<sup>3</sup> i.e. 27.1° Be.

#### 2.2.3. Actual lithium solution from Emissal factory

A concentrated brine had been collected from "Emissal company" after the production of sodium sulphate, its specific gravity is 1.17gm/cm<sup>3</sup> i.e. 21.1° Be'.

Table (1) illustrates the concentrations of different ions and solids in the three used solutions used from three different locations in Egypt.

**Table (1) Concentrations of Different Ions in Actual Brines and Total Solids**

Element Conc. (gm/L)	El-Nasr (Alexandria El-Max)	El-Nasr (Arish El-Bardawel)	Emissal (Qaroun Lake)
Ca <sup>++</sup>	2.6	2	0.8
Mg <sup>++</sup>	64.39	29.7	15.8
Cl <sup>-</sup>	284	213	156.2
Li <sup>+</sup>	0.0195	0.0055	0.0088
T.S.	793.9	465.14	358.8

#### 2.2.4. Chemicals used

All chemicals used in experimental work are of chemically pure grade.

### 2.3. Procedure

About 70 experimental runs have been used to study the effects of reaction temperature and time, pH, Molar Al<sup>3+</sup> / Li<sup>+</sup> ratio and stirring rate on Li<sup>+</sup> adsorption. Synthetic solutions of different Li<sup>+</sup> concentrations were prepared in which aluminum chloride is added at a certain dose to adjust molar ratio of aluminum ions to Lithium ions from about 2.5 to 20. Different amounts of sodium hyd 3

are added dropwise to the reaction mixture to reach different pH values from 5 (to prevent use of large amount of NaOH) to 9, at different reaction temperature (from 10 to 70°C), so that to improve Li<sup>+</sup> adsorption on the precipitated Al(OH)<sub>3</sub> in alkaline medium.

The precipitated aluminum hydroxide after different reaction time (from 5 to 240 minutes) containing the adsorbed Lithium ions is filtered spontaneously, and the Lithium ions unadsorbed in the filtrate is determined by Atomic Adsorption Spectrophotometer to evaluate the efficiency of adsorption.

The real solutions containing Li salts from sea bitters collected from El-Nasr Salines factory, Emissal company, have been tested by the same procedure at optimum conditions obtained from synthetic solutions. Desorption step was also achieved using both hydrofluoric acid and sulfuric acids and desorption efficiency for different studied conditions were evaluated by measuring Li<sup>+</sup> concentration in the eluting solution.

To study the effect of pH on Lithium adsorption, diluted solutions with different pH solutions containing Li<sup>+</sup> of different alkalinity were checked. Experiments at pH ranging from 5-12 were conducted. Aluminum hydroxide is precipitated from acidic aluminum chloride solution (pH 3) by adding sodium hydroxide solution (1N). If base is added to an acidic solution, precipitation will occur because the solubility of Al(OH)<sub>3</sub> is very low between pH 4 and pH 9.5.<sup>(17)</sup> The precipitation of aluminum hydroxide is a dynamic process that depends on many parameters, e.g. stirring rate and base addition rate.

#### 2.4. Adsorption Isotherms

Adsorption isotherms were determined by dissolving a constant mass of AlCl<sub>3</sub>.6H<sub>2</sub>O(4.47g) in constant volume and different initial Li<sup>+</sup> ion concentrations (40,60,80,100,200 mg/L)

The equilibrium data from each experiment represents one point on the adsorption isotherm, where the difference between the initial concentration (C<sub>0</sub>) and the equilibrium concentration (C<sub>e</sub>) was used to compute the amount of Li<sup>+</sup> adsorbed (q<sub>e</sub>) from the solution as follows.

$$q_e = \frac{[C_0 - C_e] V}{M}$$

q<sub>e</sub> was plotted against C<sub>e</sub> to give isotherm curves. The linear plot of C<sub>e</sub> / q<sub>e</sub> vs C<sub>e</sub> at different pH values are used for testing the applicability of Langmuir and Frendlich isotherms.

#### 2.5. Lithium desorption from solid phase:

Recovery of Lithium at steady state during experiments conducted on saline brines under constant conditions of pH, initial Al<sup>3+</sup>/Li<sup>+</sup> MR, temperature, adsorption time and stirring rate were investigated according to the following steps<sup>(15)</sup>.

- a. Washing thoroughly with distilled water for 30 minutes (60ml).
- b. Leaching with sulphuric acid or hydrofluoric acid for another 30 minutes (40ml).
- c. After two steps of washing distilled water and leaching with acid solution of different concentrations (three different concentrations), Lithium content in filtrate was determined using atomic absorption technique.

The solid phase was analyzed to check the presence Al<sup>+</sup>, Li<sup>+</sup>, Ca<sup>++</sup>, Mg<sup>++</sup>, and Cl<sup>-</sup>.

### 3. Results and Discussion

#### 3.1. Effect of different operating conditions

The influence of pH, Al<sup>3+</sup> / Li<sup>+</sup> molar ratio, adsorption time, temperature, initial lithium concentration and stirring rate on lithium adsorption from both synthetic and real bittern solutions were investigated.

##### 3.1.1. Effect of adsorption time on lithium adsorption.

Figure(2) shows the dependence of lithium ion recovery on adsorption time intervals, from which it is observed that maximum lithium adsorption is reached after three hours. This result is in accordance with most of previous studies<sup>(16,18)</sup> but differs from other<sup>(19)</sup> which get best results after one hour.

##### 3.1.2. Effect of pH on Lithium adsorption

It was seen that the recovery lithium from synthetic solution is increased (up to about 88%) in parallel to increase in pH till pH= 9 as illustrated in figure(3). While for real brine solution containing Mg<sup>2+</sup>, Ca<sup>2+</sup>, Cl<sup>-</sup> besides other cations and anions, as mentioned before, maximum lithium adsorption were 80.1 % 64.5% and 69.4% at pH 6.5,7 and 6.9 respectively as shown in figure (4).

These results are in accordance to those obtained from previous studies<sup>(20)</sup> which were carried out on lithium adsorption from dead sea brine and end brine remaining after potash production while in other investigations increasing the pH from 3.5 to 10.8 improves the adsorption<sup>(15)</sup>

##### 3.1.3. Effect of temperature on Lithium adsorption

Results seen in figure(5) illustrated that, the higher the temperature the lower was the lithium yield. The highest yield (89.7%) was obtained at 10°C while above 30°C, the yield decreased.

Another factor increases this fact is that Lithium compound are unstable<sup>(20,24)</sup>. This fact assures that process of Li<sup>+</sup> removal at high temperatures (>30°C) is a physical adsorption process not chemical adsorption.

### 3.1.4. Effect of initial concentration on Lithium adsorption.

From experimental results showed in figure (6) it is obvious that: Lithium adsorption is more efficient with increasing initial lithium ion concentration in treated solutions and these results are in accordance with previous studies<sup>(25)</sup>.

### 3.1.5. Effect of stirring rate on percent lithium adsorption

Results of studying the effect of stirring rate on lithium adsorption are shown in figure (7) from which it is clear that : above stirring rate of 300 rpm there is no sensible increase in lithium adsorption efficiency.

### 3.1.6. Effect of Al<sup>3+</sup> / Li<sup>+</sup> molar ratio on percent Lithium adsorption.

A series of experiments at different Al<sup>3+</sup> / Li<sup>+</sup> molar ratios were carried on to study the effect of different molar ratios on Lithium yield, figure (8). It is observed that highest lithium adsorbed from synthetic solution (88.1%) was obtained at Al<sup>3+</sup>/Li<sup>+</sup> molar ratio of 5.1 while above and under this ratio, there is gradual decrease in lithium adsorption which can be explained: at higher molar ratio that aluminum hydroxide precipitate is transformed from g to crystalline form which decreases the adsorption. For real brine solutions, maximum lithium adsorption 80.1%, 64.5% and 69.4% were obtained at Al<sup>3+</sup> / Li<sup>+</sup> molar ratios of 5.3, 7 & 5.8 respectively, figure (8). These results are in agreement with the results obtained in the

literature<sup>(21)</sup>. In other situations lithium recovery requires larger Al<sup>3+</sup> / Li<sup>+</sup> molar ratios.

### 3.2. Adsorption Capacity

The capacity of Al(OH)<sub>3</sub> for Li<sup>+</sup> adsorption may be determined by plotting q<sub>e</sub> against C<sub>e</sub> at different pH values (5.4, 6.3, 7.4, 8.5 & 9) to give the isotherm curves. These isotherms are shown in figure (9).

The correlation coefficient (R) represents the standard deviation between real experimental Lithium ion concentration (x) values obtained by chemical analysis and the calculated values through the model (y), at the different number (n) of experiments, which is calculated by the following equation.

$$R = \frac{n \sum xy - (\sum x)(\sum y)}{\sqrt{n(\sum x^2)(\sum x)^2} \sqrt{n(\sum y^2) - (\sum y)^2}}$$

### 3.3. Analysis of Isotherm Data

Analysis of isotherms data is important to develop an equation which accurately represents the results and could be used for design purposes. In this study two of these isotherms are selected:

Langmuir and Freundlich isotherms.

#### 3.3.1. Langmuir Isotherm

A series of straight lines have been obtained by plotting C<sub>e</sub>/q<sub>e</sub> against C<sub>e</sub> for different pH, which indicates that the adsorption process confirms with Langmuir isotherm.

Langmuir parameters, K<sub>L</sub>, a<sub>L</sub> and K<sub>L</sub> / a<sub>L</sub> at different pH values have been calculated and tabulated in Table (2)

**Table(2): Parameters in the Langmuir adsorption model at different pH:**

Parameters pH	K <sub>L</sub> L/g	a <sub>L</sub> L/mg	q <sub>max</sub> mg/gm	R'	Correlation Coefficient
5.4	6.66	0.019	120	0.21	0.703
6.3	7	0.018	121.2	0.22	0.773
7.4	7.65	0.019	122.2	0.20	0.928
8.5	8.8	0.030	122.8	0.12	0.997
9	9	0.034	123.5	0.12	0.989

It is to be noted that K<sub>L</sub> increases with pH increase which means that adsorption of Li<sup>+</sup> is more favorable by increasing the alkali added to the solution.

Since value of R'(dimensionless constant separation factor) for Li – Al(OH)<sub>3</sub> system have been found between zero and 1, so this indicate that Li<sup>+</sup> – Al(OH)<sub>3</sub> system at different pH possesses favorable adsorption.

Lithium adsorption on polymeric Al(OH)<sub>3</sub> is a monolayer process because the results were fitted with Langmuir isotherm which is valid for monolayer adsorption

#### 3.3.2. Freundlich Isotherm

The experimental results have been plotted as log q<sub>e</sub> vs log C<sub>e</sub> where a series of straight lines have been obtained.

The Freundlich parameters  $K_f$  and  $n$ , for the adsorption of  $\text{Li}^+$  onto  $\text{Al(OH)}_3$  have been calculated

using the least square method and are illustrated in Table (3).

**Table (3): Freundlich adsorption model parameters at different pH.**

Parameter pH	$K_f$ (L/gm)	n	Correlation Coefficient
5.4	7.8	1.18	0.9602
6.3	9.4	1.18	0.974
7.4	9.4	1.23	0.979
8.5	10.2	1.28	0.982
9	11.14	1.28	0.9783

Values of Freundlich exponent,  $n$ , is greater than 1 indicating that  $\text{Li}^+$  is favorably adsorbed by  $\text{Al(OH)}_3$ .<sup>(24)</sup>

The Langmuir isotherm's correlation coefficient values ( $R^2$ ) for  $\text{Li}^+$  adsorption were ranged between 0.70 – 0.99 suggesting that Langmuir isotherm provides a good model for the process. According to Freundlich the ( $R^2$ ) values ranged between 0.96 – 0.98 which fit fairly well with the obtained experimental results. So regarding to the equilibrium isotherm's models applicability follows the order Freundlich > Langmuir.

### 3.4 Lithium desorption from solid phase.

Leaching of  $\text{Al}^{3+}$  precipitate using two different acids ( $\text{H}_2\text{SO}_4$  & HF) with different concentrations (0.05N, 0.1N, 0.2N&0.25N) was investigated. It was found that using different concentrations of  $\text{H}_2\text{SO}_4$  solution (0.05 , 0.1 , 0.2,0.25 N) gave us slight increase in  $\text{Li}^+$  recovery which means leaching of Al precipitate with  $\text{H}_2\text{SO}_4$  causing only a slight decrease in desorption of  $\text{Li}^+$  from the surface of  $\text{Al(OH)}_3$  as shown in table (4), because leaching of  $\text{Li}^+$  is also accompanied by leaching of  $\text{Al}^{3+}$  so that no complete separation is obtained.

**Table (4)  $\text{Li}^+$  and  $\text{AL}^{3+}$  contents in the solid phase after different – leaching processes**

Stage	El- Max Brine			
	$\text{Li}^+$ (mg)	% removal	$\text{Al}^{3+}$ (mg)	% removal
No washing	1.56	0	39.8	0
$\text{H}_2\text{O}$	1.46	6.41	37	7.04
0.05 N $\text{H}_2\text{SO}_4$	1.42	8.97	24.1	39.45
0.1 N $\text{H}_2\text{SO}_4$	1.25	19.87	24	39.7
0.2N $\text{H}_2\text{SO}_4$	0.23	85.26	1.9	95.23
0.25 N $\text{H}_2\text{SO}_4$	0.2	87.18	1.6	95.98

$\text{H}_2\text{SO}_4$  is reacted with  $\text{Al}^{3+}$  to form  $\text{Al}_2(\text{SO}_4)_3$  which is soluble in cold water<sup>(26)</sup>.

While using hydrofluoric acid (HF) as leaching agent, it was found that HF (0.2N) is more efficient than  $\text{H}_2\text{SO}_4$  in desorption from the solid phase, the results are illustrated in tables (5&6).

**Table (5) Decrease of  $\text{Li}^+$  from solid phase by different – leaching solutions**

Stage	El - Max		El- Bardawel		Qaroun	
	$\text{Li}^+$ mg	Removal %	$\text{Li}^+$ mg	Removal %	$\text{Li}^+$ mg	Removal %
No washing	1.527	-----	0.347	-----	0.604	-----
$\text{H}_2\text{O}$	1.444	5.44	0.305	12.10	0.521	13.74
0.1N HF	1.152	24.56	0.215	38.04	0.187	69.04
0.2 N HF	0.105	93.12	0.0068	98.04	0.0097	98.39
0.25N HF	0.104	93.19	0.0068	98.04	0.0097	98.39

**Table (6) Decrease of Al<sup>3+</sup> from solid phase by different – leaching solutions**

Stage	El - Max		El- Bardaweeel		Qaroun	
	Al <sup>3+</sup> mg	Removal %	Al <sup>3+</sup> mg	Removal %	Al <sup>3+</sup> mg	Removal %
No washing	39.646	-----	14.024	-----	18.340	-----
H <sub>2</sub> O	36.949	6.80	9.440	32.69	16.182	11.77
0.1N HF	32.094	19.05	8.630	38.46	10.249	44.12
0.2 N HF	29.667	25.17	6.743	51.92	8.091	55.88
0.25N HF	24.273	38.78	6.473	53.84	7.821	57.36

HF is reacted with Al<sup>3+</sup> to form two complexes, the first is Al<sub>2</sub>F<sub>6</sub>.7H<sub>2</sub>O which is insoluble<sup>(26)</sup> and AlF<sub>3</sub>.3H<sub>2</sub>O which is slightly soluble in water<sup>(26)</sup>. And Li<sup>+</sup> is leached from Li AlO<sub>2</sub> which was previously precipitated as LiOH.H<sub>2</sub>O which is completely soluble in water. So in this case leaching is more efficient, 93% of Li<sup>+</sup> is recovered in pure form, but still some is combined with Al<sup>3+</sup>.

#### 4. Conclusions

The promising results obtained for Li<sup>+</sup> adsorption from synthetic solution and representative samples obtained from local natural sources from El-Max, El-Bardaweeel and Qaroun lake, by studying the effects of different optimum conditions affecting Li<sup>+</sup> adsorption are summarized in the following points:-

- It is possible to extract lithium in three hours at temperature less than 30°C and pH slightly alkaline at a molar ratio of Al<sup>3+</sup> /Li<sup>+</sup> 5.1 which reached 89.7%,using moderate amount of aluminum chloride .
- Differences between obtained results for El-Max, El-Bardaweeel and Qaroun brine through the presented study are due to the difference in chemical composition of each brine. Chemical composition of each brine solution influences Li<sup>+</sup> adsorption rate, due to the changes at the surface of the precipitated phase. These changes lead to variations in electrostatic properties.
- Lithium extraction rate from El-Max was greater than that of El-Bardweel & Quaroun due to the difference in initial Li<sup>+</sup> concentration in each of them.
- Leaching of adsorbed Li<sup>+</sup> on Al(OH)<sub>3</sub> precipitate can be achieved successfully using Hydrofluoric acid 0.25N 93% as pure Li<sup>+</sup>.
- It has been demonstrated that hydrofluoric acid gave better results for Li<sup>+</sup> desorption from solid phase than sulphuric acid.
- Experimental results showed the possibility of minimizing concentration of elements, other than aluminum and lithium that remain in contact with the precipitate, by washing with water improved Li<sup>+</sup> desorption either using H<sub>2</sub>SO<sub>4</sub> or HF.

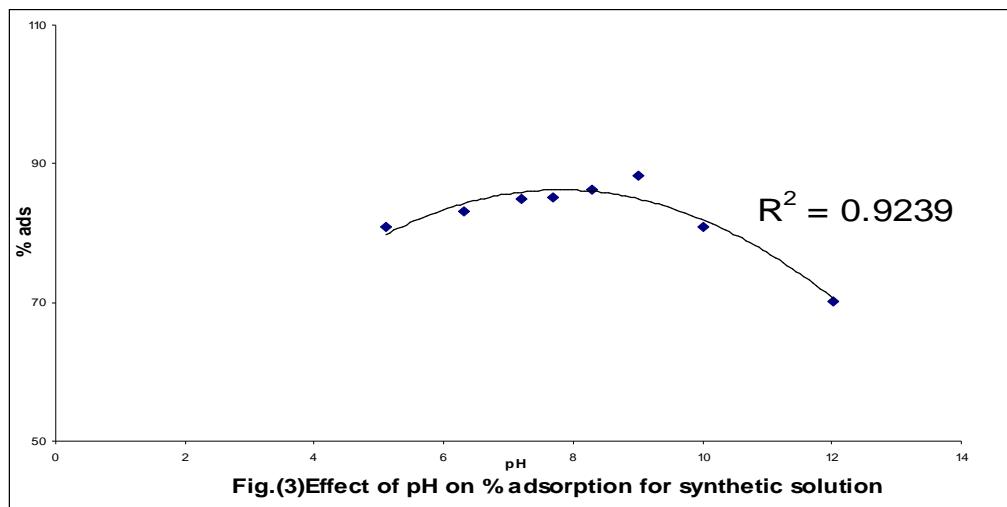
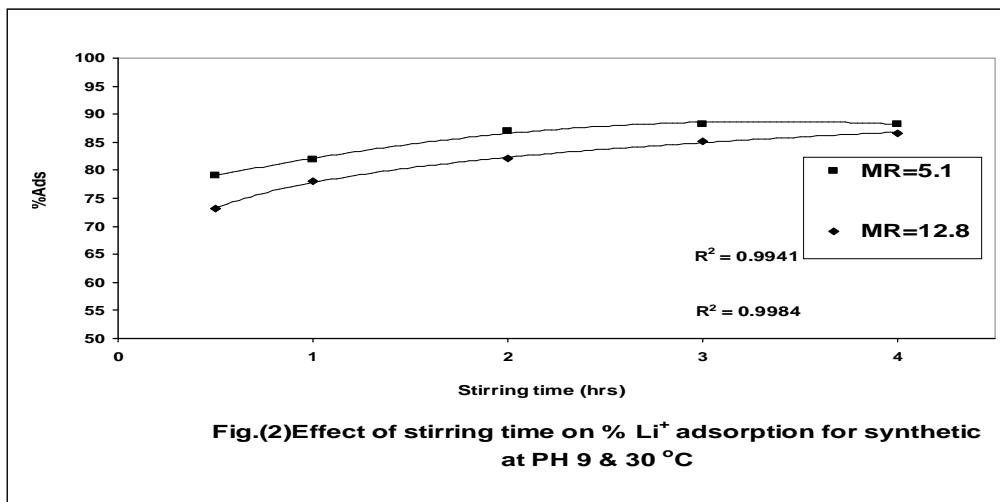
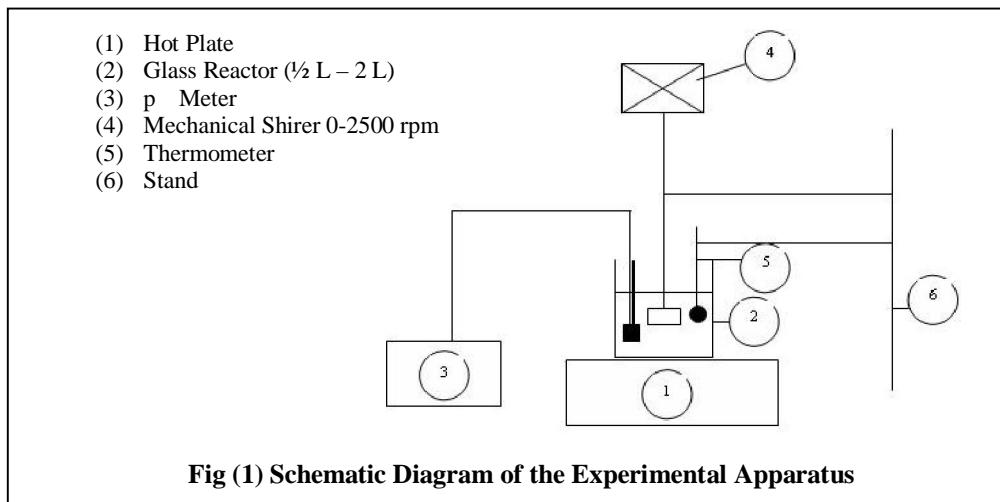
The values of equilibrium parameter (R) for Langmuir isotherm is less than 1 and the Freundlich exponent (n) is greater than 1 indicating that lithium is favorably adsorbed by Al(OH)<sub>3</sub>.

#### Corresponding author

G. El Diwani\*

Chemical Engineering and Pilot Plant Department,  
National Research Center, Cairo, Egypt.

\*geldiwani@yahoo.com



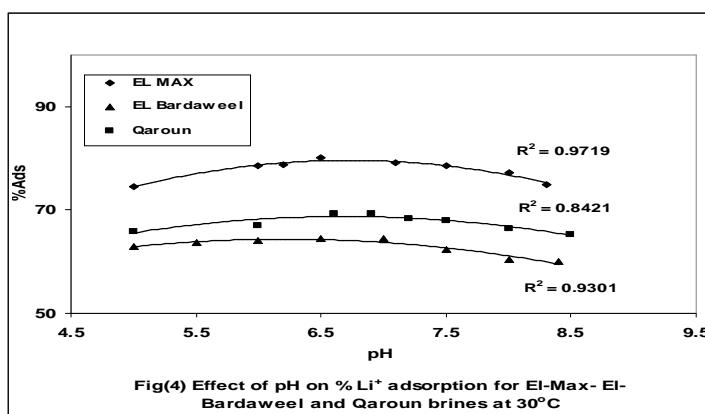


Fig.(4) Effect of pH on % Li<sup>+</sup> adsorption for El-Max- El-Bardaweeel and Qaroun brines at 30°C

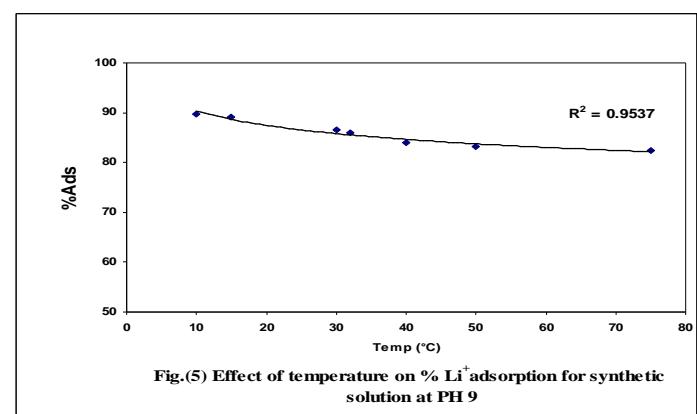


Fig.(5) Effect of temperature on % Li<sup>+</sup>adsorption for synthetic solution at PH 9

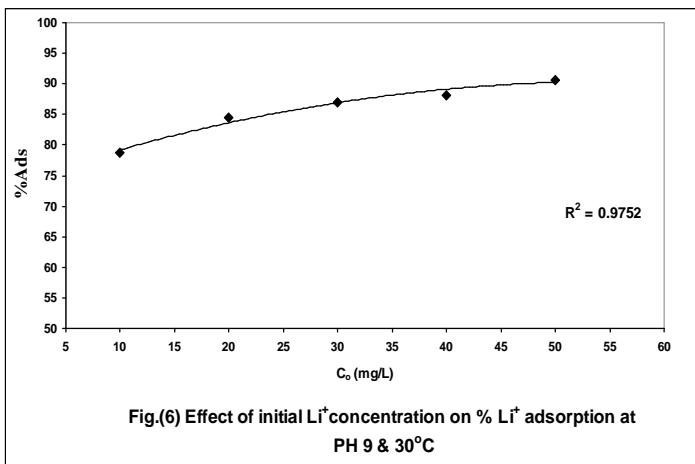


Fig.(6) Effect of initial Li<sup>+</sup>concentration on % Li<sup>+</sup> adsorption at PH 9 & 30°C

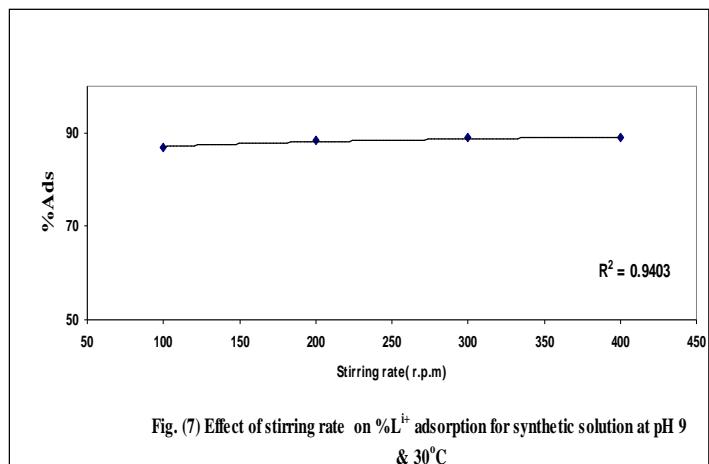


Fig. (7) Effect of stirring rate on %Li<sup>+</sup> adsorption for synthetic solution at pH 9 & 30°C

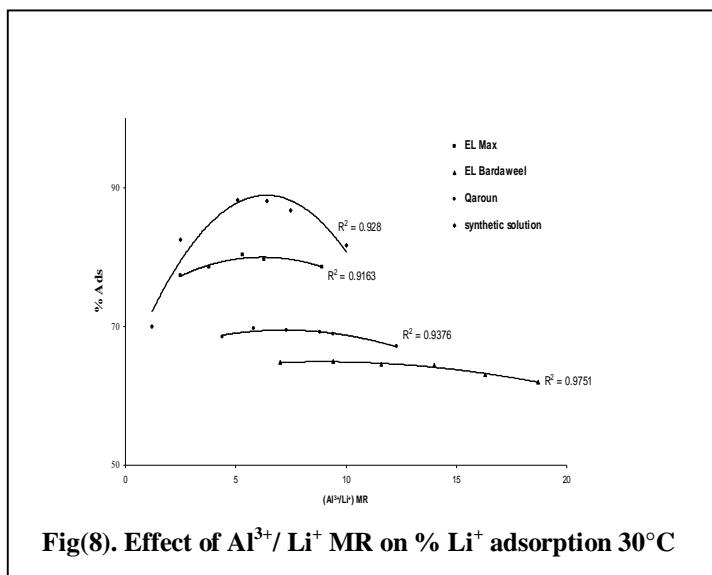
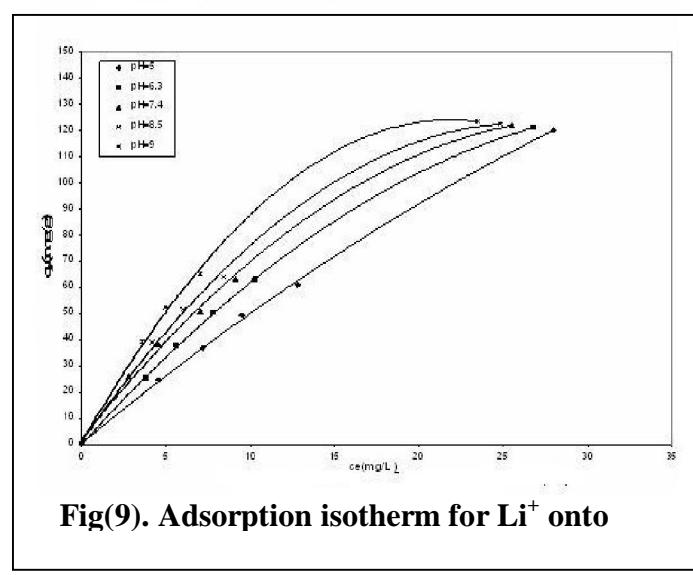


Fig.(8). Effect of Al<sup>3+</sup>/ Li<sup>+</sup> MR on % Li<sup>+</sup> adsorption 30°C



Fig(9). Adsorption isotherm for Li<sup>+</sup> onto

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## List of Abbreviations

- $a_L$  = equilibrium constant for Langmuir equation for adsorbate (L/mg).  
 $C_o$  = initial lithium concentration in the liquid phase (mg / L).  
 $C_e$  = equilibrium concentration of the solute in the liquid phase (mg /L).  
 $K_f$  = constant characteristic of Freundlich (L/ gm).  
 $K_L$  = Langmuir constant for adsorbant (L/ gm).  
 $M$  = mass of the solid material (gm).  
 $MR$  = molar ratio.  
 $n$  = Freundlich exponent.  
 $q_e$  = equilibrium concentration of the solute in the solid phase . (mg/gm)  
 $q_{max}$  =maximum concentration of the solute in the solid phase (mg/gm).  
 $rpm$  = revolutions per minute.  
 $R'$  = dimensionless separation factor for Langmuir isotherm.  
 $V$  = Volume of Solution (L)  
 $x$  = simple independent variable.  
 $y$  = simple dependent variable.

5/29/2010

# The Effects of Dietary Egyptian Propolis and Bee Pollen Supplementation against Toxicity if Sodium Fluoride in Rats

Fatma A. Khalil and Nora M. El-Sheikh

Biochemistry and Nutrition Department, Women's College, Ain Shams University, Cairo, Egypt.

**Abstract:** Propolis and bee pollen are substances produced by honey bees its components are strong antioxidant and free radical scavengers. The present study aimed to study the protective effects of propolis and bee pollen supplementation against toxicity of sodium fluoride in rats. After the end of experimental period, the rats sacrificed and biochemical analysis were carried out. The results showed that the administration of fluoride (F) alone causes significant increase of malondialdehyde (MDA) level and significant decrease of antioxidant system as erythrocyte superoxide dismutase (SOD) activity and reduced glutathione (GSH) levels in blood and brain. Also F causes significant increase alkaline phosphatase (ALP) activity, urea, creatinine, sodium and potassium levels. And significant decrease total protein, calcium, magnesium and phosphorus levels as compared to control group ( $P < 0.05$ ). Whereas administration of propolis or bee pollen with F led to significant decrease in MDA level and significant increase in SOD activity, GSH levels in blood and brain. And significant decrease ALP activity, urea, creatinine, sodium and potassium levels in serum. The propolis or bee pollen enhanced total protein, calcium, magnesium and phosphorus levels in serum as compared to F group alone.

In conclusion; supplementation of natural antioxidant (propolis or bee pollen) during Fluoride administration, facilitate reduction of the toxic effects and enhanced the antioxidant system, the levels of minerals is serum. [Journal of American Science. 2010;6(11):310-316]. (ISSN: 1545-1003).

**Keywords:** Propolis, bee pollen, sodium fluoride, rats, antioxidant system minerals.

## 1. Introduction

Fluoride (F) is highly electronegative anion with cumulative toxic effects, from prolonged ingestion that can lead to the pathogenesis known as fluorosis a condition especially persistent in third world countries, where populations have little choice as to the main source of their often times-F-contaminated drinking, other sources include private water supplies, dietary ingredients, dental products, industrial emissions, and/or occupational exposure, which can cause an individual's total F intake to exceed safe dose (Ozsvath, 2009).

Fluoride crosses the cell membrane very rapidly and distributed from the plasma to all tissue and organs (Bouaziz et al., 2006).

Propolis and bee pollen are natural substances collected by honey bees from buds and trees. Propolis a sticky substance that have bees manufacture by mixing their own waxes with resinous sap (Yoshimi et al., 2009). The main chemical classes found in propolis are flavanoids, phenolic and various aromatic compound. However, propolis contains many of B-complex vitamins, important mineral and trace elements, caffeic acid phenethyl ester (CAPE), an active component of propolis, exhibits antioxidant properties.

Nowadays propolis is used in many medical formulas and food supplements for improving health, preventing and treating infections, inflammatory

diseases and effects of toxic substances (Attalla and Ayman, 2008).

Bee pollen is rich in carotenoids, flavonoid and phytosterols. The exact profile varies depending on the plant sources and growing conditions, however, beta-carotene, beta sitosterol, isohammetin, kaempferol, lycopene, quercetin and rutin are consistently (Campos et al., 2003). The antioxidant activity of flavanoides present in propolis and bee pollen has been shown to be capable of scavenging free radical (Survswaran et al., 2007).

The aim of this study was to evaluate the antioxidant effects of dietary Egyptian propolis and bee pollen supplementation against toxicity of sodium fluoride in rats.

## 2. Materials and Methods

### Materials

Sodium fluoride (AR, BDH) was used as the source of fluoride. The sodium fluoride was added to the standard diet at 1 g/kg diet (Bellack and Schoube, 1968).

The propolis and bee pollen used in the present study originated from the hive in Cairo, Egypt. These samples were harvested in September 2009. Bee pollen was obtained as yellow granules, while propolis was derived in the form of yellow-brown powder.

**Experimental animals:**

Adult male albino rats weighing ( $130 \pm 13.9$  g) were kept in [12:12 h (light:dark) photo period] and temperature ( $22 \pm 0.5^\circ\text{C}$ ) controlled room maintained at constant relative humidity of 65-70% and fed standard diet and water *ad libitum*.

**Diet:**

The standard diet was prepared according to (Reeves et al., 1993).

**Experimental design:**

All animals fed on the standard diet and the animals were divided into 6 groups (10 animals in each group):

Group (1): Control rats (without any treatment).

Group (2): Rats administrated with sodium fluoride alone 1 g/kg diet (F groups).

Group (3): Rats administrated F and treated with propolis powder in diet 0.1%.

Group (4): Rats administrated F and treated with propolis powder in diet 0.2%.

Group (5): Rats administrated F and treated with bee pollen in diet 1%.

Group (6): Rats administrated F and treated with bee pollen in diet 2%.

After the end of the experimental period (42 days), all animals were fasted overnight and sacrificed. The two blood samples were collected from each animal, with and without anticoagulant for the following biochemical analysis. Reduced glutathione (GSH) was measured in blood and brain homogenate according to the method of Beutler et al. (1963). Malondialdehyde (MDA) level was measured in brain according to Satoh (1978).

Erythrocyte superoxide dismutase activity was determined in accordance with the method described by Sun et al. (1988). ALP activity in serum was determined by the method of Anon (1974), serum total protein level was determined by the method of Gornall et al. (1949). Serum creatinine and urea were determined by the methods of Bonsens and Taussky (1984) and Patton and Crouch (1977), respectively.

Serum sodium, potassium, calcium, magnesium and phosphorus were estimated by the colorimetric method of Berry et al. (1988), Sunderman and Sunderman (1958), Sarkar and

Chauvan (1967), Teitz (1983) and Drewes (1972), respectively.

**Statistical analysis:**

Results are expressed as mean  $\pm$  SD. The data were statistically analyzed following the one way analysis of variance [ANOVA, F test and least significant difference (L.S.D)] at ( $P < 0.05$ ) were carried out using SPSS version 11.5 (2002) SPSS Chicago / L, USA.

### 3. Results

From the results of Table (1) obtained it is evident that fluoride administration alone in group (2), caused significant increase of MDA level in brain and significant decrease in GSH levels in blood and brain, erythrocyte SOD activity as compared to control group ( $P < 0.05$ ). But the administration of propolis or bee pollen with fluoride significant decrease the MDA level in brain and significant increase GSH levels in blood and brain, erythrocyte SOD activity as compared to F group ( $P < 0.05$ ).

Table (2) shows that there was a significant increase in ALP activity, urea and creatinine. And significant decrease in total protein in F group as compared to control group ( $P < 0.05$ ). But the propolis or bee pollen enhanced the toxic effect of fluoride by a significant decrease in ALP activity, urea and creatinine. And significant increase in total protein in treated groups as compared to F group.

Table (3) and Figs. (1-5) shows the levels of serum cations in control group (G 1), fluoride group (G 2) and treated group (G 3 - G 6).

There was a significant increase in serum sodium and potassium levels and significant decrease in serum calcium, magnesium and phosphorus in F group as compared to control group ( $P < 0.05$ ). Whereas the administration of propolis and bee pollen improved the levels of cations in treated group as compared to F group ( $P < 0.05$ ).

**Table (1): Effects of propolis and bee pollen on lipid peroxide as (MDA) and antioxidant system in fluorotic rats.**

Parameters Groups	Brain MDA (nmol/ mg tissue)	Blood GSH (mg/dL)	Brain GSH (mg/g tissue)	Erythrocyte SOD activity (U/mL)
<b>Group (1) (control)</b>	d $0.70 \pm 0.11$	a $28.09 \pm 1.91$	b $14.99 \pm 0.99$	a $277.75 \pm 5.70$
<b>Group (2) (F group)</b>	a $3.08 \pm 0.23$	e $16.27 \pm 0.94$	e $8.04 \pm 0.27$	d $177.88 \pm 10.13$
<b>Group (3) (F + propolis 0.1%)</b>	e $0.51 \pm 0.08$	c $22.16 \pm 1.20$	c $13.25 \pm 0.88$	b $265.75 \pm 6.78$
<b>Group (4) (F + propolis 0.2%)</b>	b $1.10 \pm 0.12$	d $20.13 \pm 1.18$	d $9.75 \pm 0.46$	bc $260.25 \pm 4.68$
<b>Group (5) (F + bee pollen 1%)</b>	c $0.93 \pm 0.10$	a $27.70 \pm 2.13$	b $14.76 \pm 0.91$	a $279.38 \pm 10.15$
<b>Group (6) (F + bee pollen 2%)</b>	c $0.88 \pm 0.08$	b $25.38 \pm 1.07$	a $16.14 \pm 1.43$	b $270.75 \pm 7.98$
<b>L.S.D.</b>	0.135	1.49	0.91	7.93

Values are represented as mean  $\pm$  SD. 10 rats each group.

Same letters (a, b c, d) above each group indicate non significant difference between groups at ( $P < 0.05$ ) in same column.

Different letters in the same column indicate significant difference between groups.

**Table (2): Effects of propolis and bee pollen on serum ALP activity, total protein, urea and creatinine in fluorotic rats.**

Parameters Groups	ALP activity (IU/L)	Total protein (g/dL)	Urea (mg/dL)	Creatinine (mg/dL)
<b>Group (1) (control)</b>	e $167.56 \pm 5.23$	a $5.71 \pm 0.52$	b $33.93 \pm 2.96$	b $1.51 \pm 0.08$
<b>Group (2) (F group)</b>	a $323.13 \pm 11.63$	d $3.68 \pm 0.11$	a $63.57 \pm 6.20$	a $2.06 \pm 0.14$
<b>Group (3) (F + propolis 0.1%)</b>	c $258.00 \pm 11.14$	c $4.21 \pm 0.18$	b $36.68 \pm 3.40$	d $1.05 \pm 0.04$
<b>Group (4) (F + propolis 0.2%)</b>	b $269.75 \pm 7.74$	c $4.27 \pm 0.11$	b $34.68 \pm 2.61$	d $1.09 \pm 0.08$
<b>Group (5) (F + bee pollen 1%)</b>	bc $266.63 \pm 11.39$	c $4.36 \pm 0.10$	b $35.19 \pm 2.01$	c $1.22 \pm 0.10$
<b>Group (6) (F + bee pollen 2%)</b>	d $235.25 \pm 9.21$	b $4.83 \pm 0.15$	b $35.33 \pm 2.32$	d $1.08 \pm 0.07$
<b>L.S.D.</b>	9.76	0.25	3.64	0.09

Values are represented as mean  $\pm$  SD. 10 rats each group.

Same letters (a, b c, d) above each group indicate non significant difference between groups at ( $P < 0.05$ ) in same column.

Different letters in the same column indicate significant difference between groups.

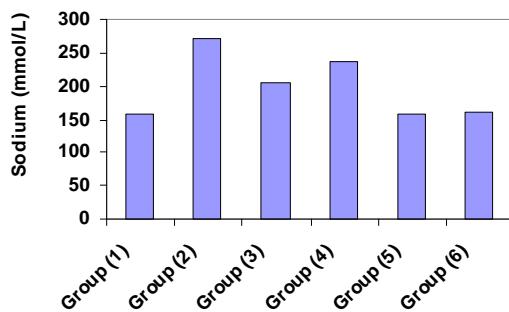
**Table (3): Effects of propolis and bee pollen on serum cations in fluorotic rats.**

<b>Parameters Groups</b>	<b>Sodium (mmol/L)</b>	<b>Potassium (mmol/L)</b>	<b>Calcium (mmol/L)</b>	<b>Magnesium (mmol/L)</b>	<b>Phosphorus (mmol/L)</b>
Group (1)	d 159.38±10.16	d 4.94 ± 1.12	a 3.48 ± 0.20	bc 1.41 ± 0.20	a 4.36 ± 0.11
Group (2)	a 272.75 ± 9.79	a 12.19 ± 1.40	d 2.34 ± 0.14	d 1.06 ± 0.08	d 3.01 ± 0.14
Group (3)	c 203.75±18.10	c 6.49 ± 0.30	b 3.09 ± 0.12	bc 1.49 ± 0.08	b 3.80 ± 0.09
Group (4)	b 237.38±11.65	b 8.05 ± 0.28	bc 2.84 ± 0.14	bc 1.49 ± 0.08	c 3.55 ± 0.16
Group (5)	d 158.13 ± 7.74	c 7.06 ± 0.61	b 2.98 ± 0.19	b 1.54 ± 0.05	a 4.28 ± 0.13
Group (6)	d 160.13 ± 9.82	b 8.33 ± 0.86	c 2.83 ± 0.09	a 1.66 ± 0.04	a 4.26 ± 0.14
L.S.D.	11.79	0.87	0.15	0.1	0.13

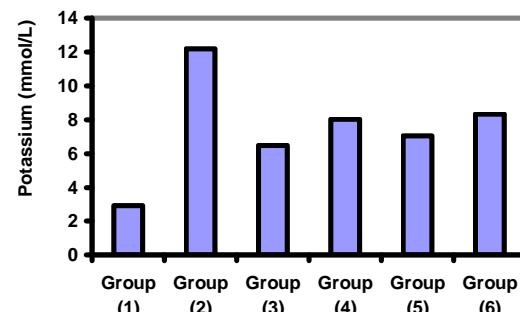
Values are represented as mean ± SD. 10 rats each group.

Same letters (a, b c, d) above each group indicate non significant difference between groups at ( $P < 0.05$ ) in same column.

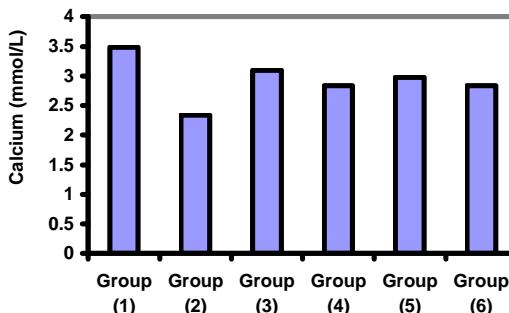
Different letters in the same column indicate significant difference between groups.



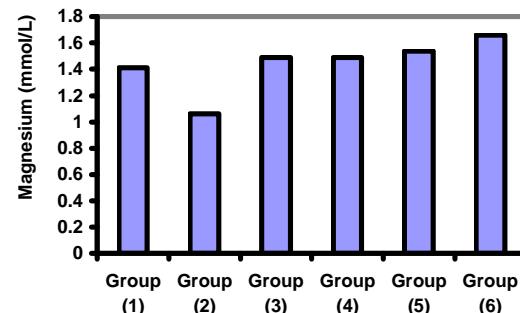
**Fig. (1): Effects of propolis and bee pollen on serum sodium in fluorotic rats.**



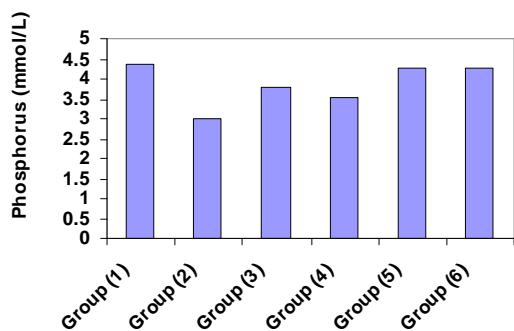
**Fig. (2): Effects of propolis and bee pollen on serum potassium in fluorotic rats.**



**Fig. (3): Effects of propolis and bee pollen on serum calcium in fluorotic rats.**



**Fig. (4): Effects of propolis and bee pollen on serum magnesium in fluorotic rats.**



**Fig. (5): Effects of propolis and bee pollen on serum phosphorus in fluorotic rats.**

#### 4. Discussion

Reactive oxygen species (ROS) play key roles in many physiologic and pathogenic processes. In fact, many ophthalmologic and neurodegenerative diseases seem to be mediated, at least in part, by oxidative stress (Finkel and Halbrook, 2000). The generation of free radicals constitute one of the underlying mechanisms of the fluoride intoxication (Birkner et al., 2000).

In the present study, the elevated level of MDA in brain and reduction of SOD activity and glutathione level in blood and brain in F group as compared to control group. Since the generation of free radicals also causes red blood cell damage occurs in tissues.

Chinoy and Shah (2004) have reported, fluoride can pass through in the blood brain barrier and accumulates in brain tissue and causes impaired antioxidant defense system. Furthermore, the other researchers have obtained similar results Kumari and Rao (1991) have reported an increase in MDA level in chronic fluoride intoxication.

In the present study, the results revealed that decrease in total protein and increase in ALP activity, urea and creatinine in F group as compared to control group. Fluoride is known to inhibit protein synthesis, mainly due to impairment of peptide chain initiation and by interfering with peptide chains on ribosomes (Michael et al., 1996).

Eraslan et al. (2007) have also reported ALP activity increase of the damage of hepatic cells and the obstruction of bile ducts. ALP is the marker enzyme of fluoride toxicosis and bone pathology increase in serum ALP activity in animals treated with fluoride has been reported (Shanthakumari and Subramanian, 2007), it may be due to fluoride induced cell injury in both osteoblast and osteocytes initiates a repair response. Birkner et al. (2000) have also reported increase in the serum urea level of rats

with acute fluoride intoxication. The administration of fluoride suggests failure of excretion in the kidney. In the present study, the F group showed the serum potassium and sodium levels increased significantly, calcium, magnesium and phosphorus decreased significantly as compared to control group. The results are similar to Chinoy et al. (1993) have reported that demonstrated rats with sodium fluoride, it may be due to change to alteration in adrenergic function. Fluoride interacts and alters the metabolism of calcium and magnesium, the decrease in serum calcium related to decrease of intestinal absorption of calcium by fluoride (Xin et al., 2006).

Propolis and bee pollen are opicultural products which are composed of nutritionally valuable substances and contain considerable amounts of polyphenol substances which may act as potent antioxidant (Teixeira et al., 2008). Flavonoids and phenolic acids are major classes of polyphenolic compounds, whose structure-antioxidant activity (Gardjeva et al., 2007). Mechanisms of antioxidant action may include suppression of ROS formation, removal or inactivation of oxygen reactive species and up-regulation or protection of antioxidant defenses (Montoro et al., 2005).

The results of the present study revealed that, administration of propolis or bee pollen with F led to significant decrease in MDA level in brain and significant increase in antioxidant system as SOD activity and GSH levels. The propolis or bee pollen significant decrease the ALP activity, urea, creatinine, potassium and sodium levels. Also propolis or bee pollen enhanced total protein, calcium, magnesium and phosphorus levels.

Caffeic acid phenethyl ester (CAPE) is an active component of propolis and has been used in traditional medicine to treat a number of diseases, CAPE treatment have been shown to protect tissues from ROS mediated oxidative stress and reduce lipid peroxidation in ischemia and toxic injuries. The antioxidant activity of CAPE is due to the presence of two hydroxyl groups in its structure (Sud'ina et al., 1993).

Twelve different flavonoids, pinocembrin, acacetin, chrysins, rutin, catechin, naringenin, galangin, luteolin, kaemferol, a pigenin, myricetin and quercetin, two phenolic acids, cinnamic and caffeic acid present in propolis (Volpi, 2004). Propolis contain acid derivatives such as benzoic-4-hydroxy benzoic which improves the digestive utilization of calcium, phosphorus and magnesium (Haro et al., 2000).

Propolis has an anabolic effect and bee pollens are rich in essential amino acids, protein, unsaturated fatty acids and also contains many

vitamins, minerals and trace elements which contribute to the health effects (Campos et al., 2003). Pollen is extremely rich in rutin and may have highest content of any source. Bee pollen has been shown to improve immune system and remove toxins from our bodies (Campos et al., 1997).

The recent investigations indicated that bee pollen contain significant amount of polyphenolic substances, mainly flavonoids. The polyphenols also have metal chelation properties and free radical scavenging activity (Abdella et al., 2009).

The conclusion of the present study suggests that the propolis or bee pollen its components, are strong antioxidants and free radical scavengers. Ameliorated the liver, kidney and brain from toxicity with sodium fluoride and enhanced the levels of minerals in serum.

#### Corresponding author

Fatma A. Khalil

Biochemistry and Nutrition Department, Women's College, Ain Shams University, Cairo, Egypt.

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6/1/2010

# Diet Selection, Feed Intake Capacity and Performance of Growing Female Camels: Effects of Type of Roughage and Level of Concentrates Offered

M.F.A. Farid, A.M. Abdel-Wahed, Safinaz M. Shawket\* and N.I. Hassan

Animal Nutrition Department, Desert Research Centre, Al-Matareya, Cairo, Egypt.  
drsafinazshawket@hotmail.com\*

**Abstract:** The feeding trials were intended to investigate diet selection, feed intake capacity (FIC) and animal performance when concentrates (corn grains and commercial concentrates mixture) and roughages (*Atriplex*, clover hay or rice straw) were fed ad lib free-choice in a cafeteria feeding system, and also the effect of restricting concentrates offered. The roughages were selected to represent different grazing conditions prevailing in arid rangelands. Eighteen growing she-camels were randomly allotted to three groups. Each group was assigned one of the three roughages offered ad lib for the duration of the whole experiment. Results indicated that type of roughage and concentrate levels, and their interaction, affected ( $P<0.05$ ) FIC and diet selection, and consequently live weight gain. Average total and roughage DMI were 78.9 and 16.1, 83.9 and 22.5, 96.4 and 33.4 g DM/day/Kg<sup>0.75</sup> for straw, hay and *Atriplex* groups, respectively. Irrespective of the roughage fed, growing camels consumed three-times as much corn grains as that from the cottonseed meal. Limiting concentrates offered to 75% or 50% of ad lib intake, decreased FIC, while the proportion of roughages in DMI increased significantly, total OMI and total protein decreased and crude fibres intake increased, more so in the straw fed camels. The *Atriplex* fed camels recorded the higher ADG, followed by the hay fed ones and the straw fed mates grew the least, 516, 429 and 240 g/d, respectively. Restricting the level of concentrates offered decreased significantly ( $P<0.05$ ) the ADG (691, 305 and 189 g/d in camels fed 100, 75 and 50% of ad lib concentrate intake. These results tend to indicate that growing camels having free choice to select their diets from both concentrates and roughages were capable of regulating their voluntary food intake predominantly through physiological mechanisms to satisfy energy requirements. This was true for the *Atriplex* and hay groups but not for the straw group or when concentrates offered was limited. [Journal of American Science. 2010;6(11):317-326]. (ISSN: 1545-1003).

**Key words:** camels, diet selection, feed intake capacity, weight gain

## 1. Introduction

Camels are famed for their peculiar adaptation to the harsh conditions prevailing in arid zones. They possess distinct behavioral, physiological and nutritional adaptive mechanisms that enable them withstand extreme direct and indirect environmental stresses (Schmidt-Nielsen 1964, Farid et al. 1979, 1997, Gauthier-Pilters 1979, Gauthier-Pilters and Dagg 1981). These animals, like other herbivores grazing arid rangelands, are seasonally challenged with feed and water deficiencies, both in quantity and quality.

Earlier studies indicated that yearling and older camels can be maintained and even fattened on clover hay, grain straws or native halophytes (e.g. *Atriplex* sp. and *Acacia saligna*) supplemented with different levels of grains as energy sources (Shawket 1999, Shawket and Ahmed 2001, Shawket et al. 2005). Similar experiments on pregnant and lactating

camels were conducted (Shawket et al. 2009). Results were encouraging in terms of the overall performance realized. It seems that performance was largely dependent both on the type of roughage constituting the basal diet and the concentrate supplement. Differences between species, camels vs. sheep or goats, were also recorded (Farid et al. 1979, Farid et al. 1997). Camels were found to out-perform other species in utilizing nutritionally lower quality or imbalanced diets. However, differences between species were minimal when receiving adequate and better balanced diets.

The present investigation was intended to study, in growing female dromedaries, feed intake capacity, diet selection and live weight changes in relation to the type of roughage offered and the effect thereon of concentrate supplementation. The roughages used were

intended to represent different grazing conditions typically prevailing in arid rangelands. Free choice cafeteria feeding was employed to facilitate the study of diet selection and to assess the animals' feed intake capacity. Results are discussed in relation to the practice of supplementary feeding of grazing herbivores in arid rangelands, and in the light of factors affecting voluntary food intake.

## 2. Materials and Methods

### 2.1. Animals and management

Eighteen healthy growing she-camels were used in the present experiment to study diet selection and feed intake capacity, as well as their live weight changes under simulated grazing conditions and the effect of supplementary feeding. They were 24-30 months old and their live body weight averaged  $393.1 \pm 27.02$  kg (range: 349.5-445.0 kg). Animals were housed individually in shaded floor pens for the duration of the experiment. They were weighed every two weeks after overnight fast and on two consecutive days. Their average daily gain was calculated for each period.

### 2.2. Feeds and feeding

The different grazing conditions prevailing in arid rangelands were represented by the three roughages used. Those were Egyptian clover hay to represent optimum grazing conditions, rice straw to represent dry season grazing and *Atriplex halimus* to represent arid rangelands dominated by halophytes. The concentrates used were corn grains and cottonseed meal selected as the commonly used energy and protein supplements, respectively. Roughage feeding was ad lib throughout and offered twice daily at 8:00 and 16:00 hours. Refusals, if any, were weighed the following morning and daily intake was recorded on dry matter basis. Concentrates, when fed ad lib, were restricted to only 10 hours daily, from 8:00 to 18:00 hours daily, in an attempt to control anticipated excessive soluble carbohydrates intake and possible adverse effects on rumen function and feed utilization (Farid et al. 2005a, 2005b). Water was made available free choice once daily at the morning feeding time. The proximate composition of feed ingredients is presented in Table 1.

**Table 1. Proximate composition of feed ingredients, % DM basis.**

Proximate constituents	Egyptian Clover hay	Rice Straw	Atriplex <i>halimus</i> <sup>2</sup>	Corn grains	Cottonseed meal <sup>1</sup>
Dry matter	86.08	87.43	34.98	86.65	90.88
Ash	13.35	21.68	25.01	1.71	24.73
Organic matter	86.85	78.32	74.99	98.29	75.27
Total (crude) protein	14.26	4.55	11.70	10.76	15.84
Crude fibres	34.23	28.86	28.62	3.77	19.30
Ether extract	4.40	2.52	2.94	3.92	10.86
N-free extract	33.76	42.39	31.37	79.84	29.27

1. Un-decorticated, heat treated and mechanically pressed CSM, produced in a traditional mill,  
2. Leaves and succulent branches typically consumed by grazing animals,

### 2.3. Cafeteria feeding trials

The feeding trials were intended to investigate diet selection and feed intake capacity when roughages and concentrates were fed ad lib, and the effect on roughage intake and animal performance when concentrates were restricted. In order to achieve this goal, a free-choice cafeteria feeding system was used. Feeders were divided to allow for the separate offering of feed ingredients and hence the recording of daily intake from each.

The animals were randomly allotted to three groups, six animals each. Each group was assigned one of

the three roughages offered ad lib for the duration of the whole experiment.

The feeding trials lasted 100 days in four consecutive periods. The first period (14 days) was intended to gradually introduce the animals to full free-choice roughage and concentrate intake. This was followed by a second period (33 days) where individual intakes were recorded daily when all roughage and concentrate ingredients were fed ad lib. During the third period (22 days) roughages were fed ad

lib but concentrates offered were reduced to 75% of average ad lib intake recorded during the second period. Concentrates offered were further limited to only 50% of ad lib during the fourth period (31 days). Daily intake from each ingredient was recorded separately for each animal throughout the experiment.

#### 2.4. Analytical procedures and calculations

Determination of the proximate composition of feeds (Table 1) was carried out according to official procedures (A.O.A.C. 1990). Nutrients intake were calculated from feed ingredients intake recorded daily and their determined organic matter, total protein and crude fibres contents.

#### 2.5. Statistical analysis

Main effects and interactions were evaluated using the GLM repeated-measures analysis of variance procedures of the NCSS statistical package (Hintze, 2007). The type of roughage and concentrate levels were the independent variables, and concentrate levels were repeated within roughages. Duncan's multiple-range test was applied to the means of the main effects and to the two-way interactions (Duncan, 1955).

### 3. Results

#### 3.1. Dry matter intake

Average daily feed intake data for the 100-day experimental period are illustrated in Figure 1. During the two-week adaptation period, as the amount of concentrates offered increased roughage intake decreased but total DM intake increased. Thereafter, and during the following 33 days when all roughage and concentrate ingredients were offered free-choice, grain intake continued to increase whereas cottonseed meal and roughages decreased, and total DMI decreased as well. This lasted for about two weeks only. Grains intake then tended to decrease and cottonseed meal and the roughage increased but only slightly. Total DMI exhibited a gradually decreasing trend especially in the straw-fed group. Similar patterns of probable rhythmic responses were observed earlier in camels, sheep and goats (Farid et al. 1997).

Limiting the concentrates offered to 75% and 50% of ad lib intake in the hay and straw fed groups increased roughage intake but total DMI decreased. However, in the atriplex-fed group the increase in roughage intake was substantial and total DMI was not practically different from that when concentrates offered were not limited.

#### 3.2. Diet selection and feed intake capacity

When both roughages and concentrates were

offered free-choice, total DMI was regarded as the animal's most favorable feed intake capacity (FIC). The results (Table 2) indicated that the type of roughage and concentrate levels, and their interaction, significantly affected FIC and diet selection. Camels offered the rice straw consumed the least total and roughage DM (78.9 and 16.1 g DM /day/kg<sup>0.73</sup>, respectively), and the roughage proportion in the consumed diet was low (20.6%). When fed the better quality clover hay, on the other hand, camels consumed more total and roughage DM (83.9 and 22.5 g DM /day/kg<sup>0.73</sup>, respectively), and the roughage proportion in the consumed diet increased to (26.9%). Noteworthy, feeding the native saltbush *Atriplex halimus* promoted better DMI, both total and from the roughage (96.4 and 33.4 g DM /day/kg<sup>0.73</sup>, respectively), and the roughage proportion in the consumed diet was practically optimum at 34.7%.

Irrespective of the roughage fed, camels consumed, on average, three-times as much corn grains as that from the cottonseed meal, 47.2 vs. 15.1 g DM /day/kg<sup>0.73</sup>, respectively. Between roughages, Atriplex promoted more grain and less meal intake than hay, and straw was intermediate. This might indicate that grains as a source of available carbohydrates were preferred when total and soluble nitrogen contents were higher in the roughage.

#### 3.3. Effects of limiting concentrate intake on total and roughage intake

On average, when concentrates offered were limited to 75% and 50% of respective ad lib intake in the three roughage groups (Table 2), roughage DMI as well as the roughage proportion in total DMI increased significantly, whereas total DMI decreased. The atriplex-fed camels appeared at advantage when concentrate feeding was limited. When fed the 50% concentrate supplements their total DMI amounted to 100.0 g/day/kg<sup>0.73</sup>, and a high 71% of which came from the roughage. Their straw-fed mates, on the other hand, were only able to consume half as much total DMI (56.2 g/day/kg<sup>0.73</sup>), only half of it came from straw (48.8%). Although clover hay is considered good dry roughage, results from the hay-fed camels were intermediate but more like that from the straw-fed mates. Their total DMI was 64.2 g/day/kg<sup>0.73</sup> and hay comprised a satisfactory 55.4% of it.

#### 3.4. Nutrients intake

Organic matter (OM), total protein (TP) and crude fibres (CF) intakes were calculated

from the proximate composition of ingredients and the ingredients' DM intake (Table 3). On average, restricting concentrate feeding to 75% and 50% of ad lib intake decreased organic matter and total protein intake and increased crude fibres intake per unit metabolic size, kg<sup>0.73</sup>. Between the three roughage groups, and irrespective of the level of concentrate offered, the atriplex-fed camels consumed significantly more of the three nutrients, OM, TP and CF, than camels in the other two roughage groups. The straw-fed camels consumed the least and those fed hay were intermediate.

The decrease in total OM intake as concentrates offered were restricted to 50% ad lib

amounted to only 4.4% in the atriplex-fed camels, whereas it was 24.6% and 32.1% in the hay- and straw-fed camels, respectively.

Total protein (TP) intake increased slightly (3.5%) in camels fed the atriplex, but decreased 20.1% and 43.2% in their hay- and straw-fed mates when concentrates offered were limited to 50% of ad lib. On the other hand, crude fibres intake increased in all three roughage groups, more so (61.3%) in the atriplex-fed camels which experienced the least decrease in total OM intake. The increased CF intake amounted to 13.6% and 7.7% in the hay- and straw-fed camels, respectively.

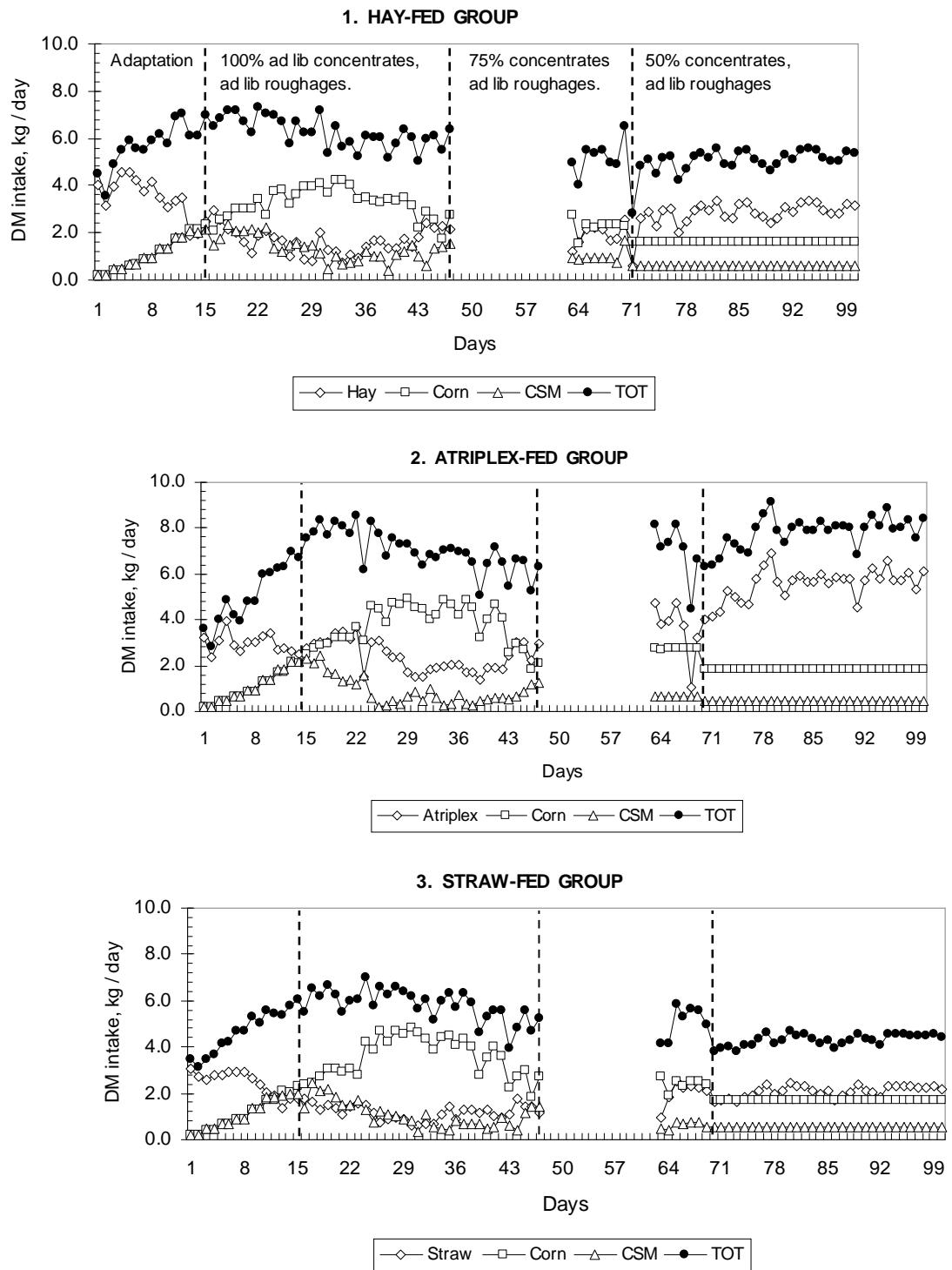
**Table 2. Diet selection and feed intake capacity of growing female dromedaries**

Roughage feeding groups [R]	Concentrate feeding level [C]			R-means	SEM ±	F-test <sup>1</sup> (P = )
	100	75	50			
<b>Total DM intake, g DM/d/kg<sup>0.73</sup></b>						
Hay	83.89 <sup>c</sup>	63.56 <sup>b</sup>	64.18 <sup>b</sup>	<b>70.54<sup>A</sup></b>	1.227	R 0.000
Atriplex	96.39 <sup>d</sup>	89.28 <sup>c</sup>	99.98 <sup>d</sup>	<b>95.22<sup>B</sup></b>		C 0.000
Straw	78.92 <sup>c</sup>	66.41 <sup>b</sup>	56.16 <sup>a</sup>	<b>67.17<sup>A</sup></b>		RC 0.000
<b>C-means</b>	<b>86.40<sup>B</sup></b>	<b>73.09<sup>A</sup></b>	<b>73.44<sup>A</sup></b>			
<b>Roughage intake, g DM/d/kg<sup>0.73</sup></b>						
Hay	22.53 <sup>b</sup>	23.27 <sup>b</sup>	35.54 <sup>d</sup>	<b>27.11<sup>B</sup></b>	0.652	R 0.000
Atriplex	33.37 <sup>d</sup>	49.75 <sup>e</sup>	71.18 <sup>f</sup>	<b>51.47<sup>C</sup></b>		C 0.000
Straw	16.11 <sup>a</sup>	26.52 <sup>c</sup>	27.38 <sup>c</sup>	<b>23.34<sup>A</sup></b>		RC 0.000
<b>C-means</b>	<b>24.04<sup>A</sup></b>	<b>33.18<sup>B</sup></b>	<b>44.70<sup>C</sup></b>			
<b>Roughage, % in DMI</b>						
Hay	26.87 <sup>a</sup>	36.76 <sup>b</sup>	55.37 <sup>e</sup>	<b>39.66<sup>B</sup></b>	0.694	R 0.000
Atriplex	34.72 <sup>b</sup>	55.76 <sup>e</sup>	71.24 <sup>f</sup>	<b>53.91<sup>C</sup></b>		C 0.000
Straw	20.57 <sup>a</sup>	40.06 <sup>c</sup>	48.77 <sup>d</sup>	<b>36.47<sup>A</sup></b>		RC 0.000
<b>C-means</b>	<b>27.39<sup>A</sup></b>	<b>44.19<sup>B</sup></b>	<b>58.46<sup>C</sup></b>			
<b>Corn grains intake, g DM/d/kg<sup>0.73</sup></b>						
Hay	43.09 <sup>bc</sup>	28.85 <sup>b</sup>	20.21 <sup>a</sup>	<b>30.71<sup>A</sup></b>	1.025	R 0.088
Atriplex	50.82 <sup>c</sup>	31.94 <sup>b</sup>	23.29 <sup>a</sup>	<b>35.35<sup>B</sup></b>		C 0.000
Straw	47.84 <sup>c</sup>	31.52 <sup>b</sup>	21.89 <sup>a</sup>	<b>33.75<sup>B</sup></b>		RC 0.157
<b>C-means</b>	<b>47.25<sup>C</sup></b>	<b>30.77<sup>B</sup></b>	<b>21.80<sup>A</sup></b>			
<b>Cottonseed meal intake, g DM/d/kg<sup>0.73</sup></b>						
Hay	18.27 <sup>e</sup>	11.45 <sup>c</sup>	8.43 <sup>b</sup>	<b>12.72<sup>C</sup></b>	0.332	R 0.000
Atriplex	12.10 <sup>c</sup>	7.59 <sup>b</sup>	5.51 <sup>a</sup>	<b>8.40<sup>A</sup></b>		C 0.000
Straw	14.97 <sup>d</sup>	8.38 <sup>b</sup>	6.89 <sup>ab</sup>	<b>10.08<sup>B</sup></b>		RC 0.000
<b>C-means</b>	<b>15.11<sup>C</sup></b>	<b>9.14<sup>B</sup></b>	<b>6.94<sup>A</sup></b>			

1. Repeated-measures ANOVA results, only *p* values for main effects and the two-way interaction are shown, adjusted total df = 53,

A-C Means within a main effect, C-means or R-means, not sharing a superscript were significantly (*P*<0.05) different according to Duncan's multiple range test.

a-f Duncan's multiple range test was performed on the nine means from the RC interaction, and for each parameter, means within a parameter not sharing a superscript were significantly (*P*<0.05) different.



**Figure 1.** Average daily dry matter intake (kg/day) from individual feed ingredients during periods of free-choice and restricted concentrates supplementation and the effect of the type of roughage.

**Table 3. Average daily weight changes and nutrients intake**

Roughage feeding groups [R]	Concentrate feeding level [C]			R-means	SEM ±	F-test <sup>1</sup> (P = )
	100	75	50			
<b>Average daily gain, g/d</b>						
Hay group	744.7 <sup>b</sup>	297.7 <sup>ab</sup>	244.5 <sup>ab</sup>	<b>428.9<sup>A</sup></b>	148.67	R 0.248
Atriplex group	735.8 <sup>b</sup>	523.7 <sup>ab</sup>	289.0 <sup>ab</sup>	<b>516.2<sup>A</sup></b>		C 0.001
Straw group	594.7 <sup>ab</sup>	95.2 <sup>a</sup>	32.2 <sup>a</sup>	<b>240.3<sup>A</sup></b>		RC 0.889
C-means	<b>691.4<sup>B</sup></b>	<b>305.5<sup>A</sup></b>	<b>188.6<sup>A</sup></b>			
<b>OM intake<sup>2</sup>, g DM/d/kg<sup>0.73</sup></b>						
Hay	75.67 <sup>cd</sup>	57.18 <sup>b</sup>	57.07 <sup>b</sup>	<b>63.31<sup>A</sup></b>	1.143	R 0.000
Atriplex	84.16 <sup>d</sup>	74.42 <sup>cd</sup>	80.42 <sup>d</sup>	<b>79.66<sup>B</sup></b>		C 0.000
Straw	70.91 <sup>c</sup>	58.05 <sup>b</sup>	48.15 <sup>a</sup>	<b>59.04<sup>A</sup></b>		RC 0.000
C-means	<b>76.91<sup>B</sup></b>	<b>63.22<sup>A</sup></b>	<b>61.88<sup>A</sup></b>			
<b>TP intake<sup>2</sup>, g DM/d/kg<sup>0.73</sup></b>						
Hay	10.74 <sup>de</sup>	8.24 <sup>c</sup>	8.58 <sup>c</sup>	<b>9.19<sup>B</sup></b>	0.141	R 0.000
Atriplex	11.30 <sup>de</sup>	10.46 <sup>d</sup>	11.71 <sup>e</sup>	<b>11.16<sup>C</sup></b>		C 0.000
Straw	8.25 <sup>c</sup>	5.93 <sup>b</sup>	4.69 <sup>a</sup>	<b>6.29<sup>A</sup></b>		RC 0.000
C-means	<b>10.10<sup>B</sup></b>	<b>8.21<sup>A</sup></b>	<b>8.33<sup>A</sup></b>			
<b>CF intake<sup>2</sup>, g DM/d/kg<sup>0.73</sup></b>						
Hay	12.86 <sup>c</sup>	11.26 <sup>b</sup>	14.55 <sup>d</sup>	<b>12.89<sup>B</sup></b>	0.205	R 0.000
Atriplex	13.83 <sup>cd</sup>	16.91 <sup>e</sup>	22.31 <sup>f</sup>	<b>17.68<sup>C</sup></b>		C 0.000
Straw	9.34 <sup>a</sup>	10.46 <sup>ab</sup>	10.06 <sup>a</sup>	<b>9.95<sup>A</sup></b>		RC 0.000
C-means	<b>12.01<sup>A</sup></b>	<b>12.88<sup>B</sup></b>	<b>15.64<sup>C</sup></b>			

1. Repeated-measures ANOVA results, only *p* values for main effects and the two-way interaction are shown, adjusted total df = 53,  
 2. Calculated from the proximate composition of ingredients (Table 1) and ingredients' DM intake (Table 2),  
 A-C Means within a main effect, C-means or R-means, not sharing a superscript were significantly (*P*<0.05) different according to Duncan's multiple range test.  
 a-f Duncan's multiple range test was performed on the nine means from the RC interaction, and for each parameter, means within a parameter not sharing a superscript were significantly (*P*<0.05) different.

### 3.5. Animal performance

Data on average daily weight gain (ADG) of the growing female dromedaries in response to changing the type of roughage and the level of concentrate supplementation are presented in Table 3. Overall, between and within variation were great as indicated by the substantial pooled SEM value. However, the lack of statistical significance of differences between roughage means should not mask the biological significance of the observed results.

On average, the atriplex-fed animals gained the most, followed by the hay-fed ones and the straw-fed mates grew the least. Observed ADG values were 516, 429 and 240 g/d, respectively. The effect of the level of concentrate feeding on ADG was also evident. It amounted, on average, to 691, 305 and 189 g/d in camels fed 100, 75 and 50 percent of ad lib concentrate intake. The roughage-concentrate interaction was not significant.

As indicated above, decreasing the level of concentrate supplementation decreased organic matter and total protein intake and increased that of crude fibres. Atriplex feeding promoted better intake of all three nutrients as compared to hay-feeding, whereas straw-feeding resulted in the least intake. These are in accord with observed effects on performance.

### 4. Discussion

Ruminant herbivores are characterized by their multi-compartmented stomach. This anatomical structure and the microorganisms inhabiting the rumen-reticulum allow for longer retention of ingested food and the anaerobic microbial digestion of cellulosic material, and hence the production of volatile fatty acids and the synthesis of microbial protein, all are for the benefit of the host animal. The camel is a

pseudo-ruminant. It also has a multi-compartmented stomach but the two compartments, omasum and abomasum, are fused together into one 'tubiform' compartment. Nevertheless, it is functionally and metabolically a ruminant herbivore.

Voluntary food intake and the selection between foods when offered free choice for ad lib feeding are important issues of continued interest. High levels of voluntary intake are required for efficient production, in general, because the more the animal eats the more it produces. Selection between foods is how the animal attempts to acquire a 'balanced' diet to supply essential nutrients commensurate with its basic needs for maintenance and potential production, and including continued fat deposition in the adult. Grazing herbivores have been known to select diets that are richer in nutrients and lower in toxins than the average composition of available vegetation (Fontenot and Blaser, 1965, Arnold, 1970).

In natural environments the availability and composition of foods vary both spatially and temporally. In arid rangelands there are distinct grazing and dry seasons. In addition, animals also graze halophytes dominant in certain areas and depressions. Under these conditions grazing ruminants, camels included, are challenged with seasonal shortage of food and water, both in quantity and quality. Supplementary feeding is then not an uncommon practice. Properly practiced, and in addition to economic considerations and the fact that achieving the full production potential may not be the realistic objective under certain conditions, the following, among other things, need to receive appropriate consideration: (a) supplementation should be an addition to what the animal is able to acquire from the available pasture and not to replace it, (b) to compliment and balance nutrients needed by the animal but deficient in the pasture, (c) does not restrict the mobility of the animal on the range, (d) does not increase the need for free drinking water usually in short supply under these conditions even when the animals are adapted to intermittent water intake and (e) expected total intake not to exceed the animal's expected feed intake capacity, otherwise the objectives and benefits of supplementation would not be realized.

The present experiment addressed some of these topics as related to the supplementary feeding of growing she camels in arid rangelands. The roughages used were chosen to represent different arid grazing conditions. They were clover hay to represent optimum quality grazing, straw to represent dry season grazing and atriplex to represent grazing in areas dominated by halophytes. The results are foreseen to be of value when attempting to develop appropriate supplementary feeding system for camels inhabiting

such environments.

Although not experimentally demonstrated in the present study, it is acknowledged that the three roughages used and the liberal concentrate ingredients offered imposed different sets of factors known to affect the regulation of voluntary food intake of the animals. In all three roughage groups, liberal intake of corn grains especially when offered ad lib might have increased rumen acidity and osmolality and intensified the effect of propionate in the liver and impair fiber digestion (Hoover 1986, Nsahlai and Umunna 1996 and Faverdin 1999). Long term adjustment to liberal corn feeding was observed as of about day 30 and beyond (Fig. 1) where corn grains intake started to decrease, roughage and cottonseed meal intake increased and total DMI decreased.

In the atriplex fed animals, the high content of sodium chloride and soluble proteins might have resulted in increased rumen osmolality and ammonia concentration, both known to negatively affect VFI (Kyriazakis and Oldham 1993 and Stevens et al. 2004). However, since fresh drinking water was freely available, along with that available from the lush plant, it seems that the anticipated negative effect on VFI was counter-balanced. Similar results were reported by Konig (1993) and Alicata et al. (2002). In the straw fed group, its poor digestibility, longer retention time in the GIT and low nutritive value (Cianci et al. 2004) did limit the VFI and adversely affected animal performance.

Ad lib feeding of concentrates along with ad lib roughages resulted in similar intakes of total concentrates, the sum of corn grains and cottonseed meal, amounting to 61-63 g DM/d/kg<sup>0.73</sup> irrespective of the roughage offered and the different ratio of grains to meal. Roughage intake differed, however. It was greatest in the atriplex group, least in the straw group and the hay group was intermediate: 33.4, 16.1 and 22.5 g DM/d/kg<sup>0.73</sup>. This was reflected upon total DM intake, i.e. the feed intake capacity, and the proportion of the roughage in total DMI.

Maximum feed intake capacity at free choice ad lib feeding amounted to 84, 96 and 79 g DM/d/kg<sup>0.73</sup> for camels fed hay, atriplex and straw, respectively. The present VFI capacity values of hay and straw were close to those indicated by Farid et al. (1997), being 87 and 79 g DM/Kg<sup>0.75</sup> for adult camels fed free choice ad lib concentrate mixture and barley grains with either clover hay or rice straw, respectively. When concentrates offered were only 50% of ad lib (28 g DM/d/kg<sup>0.73</sup>), and although roughage intake increased some, feed

intake capacity (total DMI) decreased to 64 and 56 g DM/d/kg<sup>0.73</sup>, in camels fed hay and straw, respectively. Noteworthy, it was not affected in the *triplex* fed camels where it amounted to 100 g DM/d/kg<sup>0.73</sup>. This may be due to the positive response of camels to *triplex* feeding which is attributed to two principal factors (Shawket et al. 2010). First, camels appear to need more salt, probably more than other herbivores, which is in higher proportion in this plant. This fact was demonstrated previously by Chamberlain (1989) that camel requires six to eight times the amount of salt required by other livestock, and camels without regular access to salty feed require about 140 g of salt per day. So, these findings explain the higher ( $P<0.05$ ) intake of DM, OM, TP and CF when camels fed *triplex* in comparison to their mates fed either hay or straw. Second, in comparison to bovines, camel saliva contain a varying content of high molecular weight mucin-glycoprotein (MGP) that confers protection to the mucosa of the digestive tract from mechanical injuries and fixes the plant tannins preventing their negative effects on protein metabolism in the rumen (Schmidt-Witty et al. 1994). In addition, *triplex* being a lush green plant was more palatable and preferred by camels in comparison to the dry long clover hay.

When concentrates offered were limited, dry matter intake from roughages increased to 35.5, 71.2 and 27.4 g DM/d/kg<sup>0.73</sup> in hay, *triplex* and straw fed animals, respectively, an increase of 58, 113 and 70 percent. Reflecting upon the concept of supplementary feeding, these percentages represent reduction of pasture intake when concentrate supplementation increase from about 28 to 63 g DM/d/kg<sup>0.73</sup>. The same trend, it was found by Jakhmola and Roy (1992) when growing camels were fed ad lib on a local Indian roughage (moth chara) with three levels of protein concentrate supplements (HPN, MPN and LPN). When concentrate supplementation increased from 0, to 18 and 28 g DM/d/Kg<sup>0.75</sup>, roughage intake decreased significantly by 22% and 12% in the HPN group less than that of MPN and LPN, respectively. The authors indicated that this decrease may be due to changes in the rumen fermentation pattern. Whereas feeding low levels of concentrates stimulated cellulolytic fermentation in the rumen, high levels of concentrates tend to change fermentation pattern from typical cellulolytic to amylolytic. Thus rumen retention time of roughage in the ad lib grains treatments as well as in the HNP group of Jakhmola and Roy (1992) might have been increased and hence, reducing the intake of roughages. In comparison, Shawket and Ahmed (2001) reported that intake of adult camels from *Atriplex nummularia*, which is nutritionally superior to *A. halimus*, increased from 53.7 g DM/d/kg<sup>0.75</sup> when fed alone to 69.6 g DM/d/kg<sup>0.75</sup> when a supplement of corn grains was offered to supply only 40% of the ME

required for maintenance (Farid 1995). The corn grains supplement amounted to 13.5 g DM/d/kg<sup>0.75</sup>, equal to 21% of ad lib concentrate supplement realized in the present experiment. The present result of feed preference by camels is supported by the finding of Rutagwanda et al. (1990) that camels are superior to other species in selecting plants and feeds of better quality. It was concluded that animals have developed mechanisms, behavioral and else, that allow them to recognize foods on the basis of their nutritional as well as other properties (Kyriazakis et al., 1999).

Decreasing the level of concentrate supplementation to 75% and 50% decreased the ADG by 56% and 73%, respectively less than that of ad lib concentrate supplementation. The same trend was reported by Jakhmola and Roy (1992), for growing camels. It is noticeable that *triplex*-feeding with ad lib concentrate supplementation or when limited to 75% and 50% of ad lib, recorded the highest DMI capacity in comparison to hay or straw-feeding. These reflected the camel preference to salty roughages as discussed above. Irrespective of the level of concentrate offered, *triplex* feeding promoted better intake of all three nutrients, OM, TP and CF, as compared to hay or straw-feeding resulting in the highest observed ADG. Similar results were reported earlier by Shawket et al. (2010) who indicated that growing camels fed *triplex* supplemented with crushed barley grains (100% of their growth energy requirements, Wardeh and Farid 1990) recorded higher ( $P<0.05$ ) ADG than their mates fed hay, 560 g/d vs. 421 g/d, respectively.

## 5. Implications

Supplementation during optimum grazing season is practiced only if the pasture is over-stocked, animal movement is restricted or nutrient requirements are high during demanding physiological or productive states. On the other hand, supplementary feeding during the dry season or when grazing halophytes is practiced to rectify deficiencies and imbalances of nutrients in the available pasture, frequently at or below the maintenance level of feeding to minimize losses in life and condition.

Developing supplementary feeding schemes for camels, like other ruminant herbivores, require knowledge of their requirements especially at maintenance (Farid et al. 1990, Farid, 1995). These were translated into provisional requirements for different physiological and productive states (Wardeh and Farid 1990). Secondly, it was essential to evaluate in growing she camels, the present

experiment, diet selection and the maximum feed intake capacity under ad lib cafeteria feeding conditions, and the effect thereon of restricting the concentrate supplements, while using three roughages to simulate the different arid grazing conditions indicated above. The study under these controlled conditions was not expected to deviate much from natural conditions. Kyriazakis et al. (1999) stated that even under partly artificial conditions the diet selected will follow from the general adaptive nature of the animal's feeding behavior.

The present results provide preliminary guidelines to the supplementary feeding of camels under different grazing conditions. Of paramount importance, the camel's preference for concentrates and the reduction of their intake from roughages, i.e. the pasture, when supplements are abundant. The results are supported by the findings of Yacout and El-Badawy (2001) and taking into consideration the fact that responses of ruminants to energy and protein intake are not independent (Blaxter 1975). However, it seems that camels are more sensitive to energy than protein deficiency and supplementation and they can utilize poor quality roughages if forced to use exclusively on it (Holler et al. 1986, Lechner and Englehardt 1989).

#### **Corresponding author**

Safinaz M. Shawket

Animal Nutrition Department, Desert Research Centre, Al-Matareya, Cairo, Egypt.  
drsafinazshawket@hotmail.com

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6/1/2010

# Application of Proposed Distribution Network Planning Rules on Fast Developing Countries

Salem M. Elkhodary<sup>1</sup>, and M. Khafagy<sup>2</sup>

<sup>1</sup> Faculty of Engineering, Ain Shams University,, Cairo, Egypt,<sup>2</sup> Saudi Electricity Company, KSA.

**Abstract:** With the ever increasing need to electric energy and the fast development of loads in many countries especially in the fast developing ones such as the GULF countries, the load growth as well as the forecasted loads, are highly increased depending on new and arising factors and conditions. In turn, Electricity Companies build rapidly generating plants, transmission and distribution networks to meet the rapid load demand. Usually, power system expansion follows the load growth which may exist at random locations. This adds to the absence of prior proper planning, especially medium and long term planning, resulting in network configurations that do not match with optimum siting and sizing planning rules. Operation of such networks faces several problems that may sacrifice the power quality. Thus, proper planning of new networks, expansion or rehabilitation of existing ones should be based on most accurate and proper planning rules. This calls for the investigation of a new exact cost function for optimum sizing and siting of network substations, and hence the H.V. feeds (incoming) and the M.V. distribution (outgoing) feeders. Therefore, this paper presents a newly proposed methodology that takes into consideration the capital costs of all electrical components, losses in these components, operation and maintenance costs. The inflation rate can be also taken into consideration. This methodology gives important results, which conclude that the optimum distance between substations and hence the optimum number of substations, greatly depends on different factors that were not taken into consideration before, for example : the kWh price, cost of the HV incoming feeders (66-110 kV feeders) besides the cost of the MV outgoing feeders (6.6-22 kV feeders), cost of the distribution substations (MV/LV), cost of losses in transformers, cost of losses in all feeders, incoming and outgoing, Operation and maintenance costs ....etc. [Journal of American Science. 2010;6(11):327-]. (ISSN: 1545-1003).

**Keywords:** Cost function. Objective function., Distribution network planning. Optimum siting and sizing of substation

## 1- Introduction:

On the application of the proposed cost function to re-plan, expand and rehabilitate the network of Jeddah City, as an example of a fast developing network, the obtained results in this paper present the most important conclusion that the today number of substations in a fast developing network can be kept optimally the same without increase till year 2023, but only needs the increase of the substation capacity, and the enforcement of the H.V. and M.V. lines, according to the forecasted loads and the given rate of load growth.

In the planning of transmission, sub-transmission and distribution networks of an electric power system, cost plays a major role and is a main deciding factor. From the technical point of view, there are always several alternative designs that can satisfactorily fulfill the objectives of the system, namely; continuity, voltage level and power losses. Then, the cost will be the deciding factor on differentiating between the best technically selected plans [1], hence deciding the optimum sites and sizes of the network elements, especially the network substations.

Therefore, since early of the second half of the 20<sup>th</sup> century, investigations of cost functions supporting

the appropriate system plans have been attempted [1-11]. In this respect, authors considered only the capital costs of the network elements, believed to be most effective, and ignored those thoughts to be non-effective. This rendered most of the previously proposed cost functions to be approximate. This becomes very clear when dealing with fast growing loads in fast growing countries and with heavy load densities, where, on application, can lead to misleading plans. Therefore, this paper has been devoted to propose an accurate cost function that takes all cost factors into consideration. Comparing with the results obtained using previous approximate formulae, important results are obtained.

## 2- Review of Previous Related Investigations :

Ponnavaienko [2] proposed an optimal planning solution aimed at optimizing the substation feed area, load carrying limit of the feeders and the conductor size for feeders. Pannavaienko considered that the substation cost is a function of the substation size and the number of feeder bays provided at the substation.

From the point of view of energy conservation, Swedan [3] suggested that the system network, which is required to be built in such a way that the power distributed to the load center can take place at minimum cost, may be achieved by evaluating an

objective function in terms of the annual energy losses costs.

Considering that the best distribution system planning could be achieved using the best size and location of substations, EBASCO [4] proposed that such achievement could be realized by finding the least annual cost of the sum of the fixed, charges on substations and feeders. Operation, maintenance and losses costs were neglected.

Since the formula presented by EBASCO [4] is believed to be commonly used for optimum siting and sizing of network substations, however being approximate, it is worthy to present here in some details, and to be compared with the present proposed cost function.

Increasing the number of substations for a given load density tends to increase total cost. However, increasing the number of substations reduces the cost of feeders. Clearly then, the least total annual cost is a function of substation and feeder costs, capacity of feeder and load density.

The total cost of substations and feeders/ unit area is :

$$C_T = \text{feeders Cost/ Unit Area (km}^2\text{)} + s/s \text{ Cost} \\ = \left( a + \frac{bS}{2} \right) \frac{D}{kVA_f} + \frac{c}{S^2} + dD \quad (1)$$

Minimizing the above equation with respect to S results in the optimum substation size and site (represented by the distance between substations S) as follows:

$$S = 1.59 \left[ \frac{kVA_f \cdot c}{bD} \right]^{0.333} \text{ km} \quad (2)$$

and the corresponding optimum substation size would be :

$$kVA_s = S^2 D = 2.52 D^{0.333} \left[ \frac{kVA_f \cdot c}{b} \right]^{0.666} \quad (3)$$

Where :

**D** is the Load density in kVA per square kilometer ( $\text{km}^2$ ).

**kVA<sub>s</sub>** is the substation kVA capacity.

**kVA<sub>f</sub>** is the feeder kVA capacity.

**S** is the distance in km between substations.

**a** is the fixed charges on feeder equipment and regulators in dollars per year.

**b** is the cost of feeders in US\$ per km.

**c** is the part of substation cost not proportional to substation capacity.

**d** is the cost per kVA of the substation capacity required to carry the load in the area  $S^2$ .

**C<sub>T</sub>** is the total cost of substations and feeders per square km.

K.S. Hindi et al [5,6], Harley et al [7] and Adler et al [8] confined their investigations to problems related with low voltage networks, namely to radial layout of a distribution network [5,6], replacement of the transformers (dynamic design) with the growth of the load demands [7] and to a model focusing on the treatment of residential and light commercial service areas with time-varying load characteristics, including customer load profile changes, per customer load growth and service area population growth. Clearly, such investigations for related low voltage plans cannot be of effective use for HV/MV distribution network planning, where optimum siting and sizing of substations and hence HV incoming and MV outgoing feeders are the main objective.

Further, M. Kaplan, and A. Braunstein, [9] presented a contribution to the determination of the optimum site for substations. The method enables to limit the number of possible solutions and by using grapho-analytical methods to home on the optimum solution. The optimum site for a substation is the location which will result in minimum construction and operation costs.

Furthermore, G.L. Thompson, and D.L. Wall, [10] formulated a distribution planning model which considers existing and potential substation locations, their capacities and cost, together with the primary feeder network represented by small area demand locations to represent non-uniform loads, and feeder segments having variable distribution costs and limited capacities.

Ibraheim et al [11] presented an economic comparison between two suggested technical alternatives for an integrated network (composite distribution system comprising primary substations, distributions points, primary feeders connecting the distribution points, distribution transformers (kiosks) and feeder sections tying these kiosks (normally in the form of loops)). All such costs have been added by computation for various alternative plans of a distribution district. The most economical variant was recommended. To fulfill this, extensive data was necessary to be provided, arranged in tables and laborious computations were carried out, other than using a simple objective function.

Thus, it is thought that anyone of the preceding formulae has selected only several factors thought to be the effective ones, but ignored other factors that may be of cost effectiveness in planning. This called for the present investigation. The proposed cost function presented in this paper has taken into consideration even every minor factor of the many that have been ignored in the previous proposed cost functions, such as; the kWh price to account properly for the cost of losses, the main fixed charges on substations and feeders, the operation and maintenance costs, losses

cost in all system elements, substation cost and the substation loading.

### 3- Proposed Cost Function:

In the present work it has been aimed to investigate a cost function that considers all affecting factors, including capital cost, costs of losses, operation and maintenance costs with only basic data required, where none of the factors, even having slight effect, is neglected such as done before [e.g. 2,3,4].

Since the loads of distribution networks in fast developing countries are characterized by high densities and high growth rates, distribution substations are fed by elevated voltages e.g. up to 110 kV, such as in Saudi Arabia networks, where the distribution substations are 110/13.8 kV substations. This means that the distribution networks in such countries comprises the 110 kV feeding network. This agrees with same generally accepted classifications of networks voltages. This calls to consider the cost of the substations incoming feeders. Consequently, in the presently suggested formula, all of the following factors are considered for optimizing the locations and capacities of the substations and hence, the incoming and outgoing feeders lengths and sizes defining the basic network plan. Thus, the taken factors are :

- Cost of the HV incoming feeders (66-110 kV feeders).
- Cost of the MV outgoing feeders (6.6-22 kV feeders).
- Cost of the HV/MV substations equipment (part of substation cost depending on its capacity).
- Cost of the HV/MV substation land, civil work, public works ...etc (fixed part of substation cost).
- Cost of losses in transformers.
- Cost of losses in all feeders, incoming and outgoing.
- Operation and maintenance costs ....etc.

The following symbols will be used to determine the relationship of the above factors in deriving the equation of the total cost.

**D** is the load density in kVA/km<sup>2</sup>.

**kVA<sub>s</sub>** is the substation MVA capacity.

**kVA<sub>fi</sub>** is the incoming feeder kVA capacity.

**kVA<sub>fo</sub>** is the outgoing feeder kVA capacity.

**S** is the distance in km between substations.

**a<sub>i</sub>** is the fixed charges on incoming feeders equipment and regulators (cable ends, feeder cells, ....etc.).

**a<sub>o</sub>** is the fixed charges on outgoing feeders equipment and regulators.

**b<sub>i</sub>** is the cost of incoming feeder/km.

**b<sub>o</sub>** is the cost of outgoing feeder/km.

<b>c</b>	is the part of substation cost not proportional to substation capacity (land, civil work, building, ....etc.).
<b>d</b>	is the cost per kVA of the capacity required to carry the load in the area S2
<b>n<sub>fi</sub></b>	is the number of incoming feeders required/ km2.
<b>n<sub>fo</sub></b>	is the number of outgoing feeders required/ km2.
<b>C<sub>s</sub></b>	is the substation cost / km2.
<b>C<sub>TC</sub></b>	is the total construction costs or expenses / km2.
<b>x</b>	is the ratio between the value of power losses at full load of the substation transformer and its rating.
<b>I<sub>f</sub></b>	is the loss factor of a substation transformer.
<b>C<sub>tr</sub></b>	is the cost of losses in substation transformers/ unit area.
<b>I<sub>i</sub></b>	is the rated current of incoming HV feeder.
<b>I<sub>o</sub></b>	is the rated current of outgoing MV feeder.
<b>C<sub>o&amp;m</sub></b>	is the expenses of substations operation and maintenance/ unit area.

To investigate the total costs and hence the cost function that is optimized to get the optimum spacing between substations and hence their sizes and feeder lengths, Using One Square km As a Unit Area, calculations have been carried out as follows :

#### 3-1- Cost of incoming and outgoing feeders [12]:

No. of incoming feeders/ substation is normally 4 to 6 as a substation is usually fed from two or three different sources via double circuit lines or cables. The most common practice, a HV/MV substation is fed from two double circuit feeders.

So, the No. of incoming feeders per substation is equal to 4.

$$n_{fi} \text{ is the No. of incoming HV feeders/km}^2 = \frac{4}{S^2}$$

$$\text{Cost / incoming feeder is given by : } a_i + b_i \frac{S}{2}$$

Cost of incoming feeders to HV/MV substations / Unit area = n<sub>fi</sub> \* Cost / incoming feeder

$$= \frac{4}{S^2} \left( a_i + b_i \frac{S}{2} \right) \quad (4)$$

Similarly, the cost of outgoing feeders required/km<sup>2</sup>

$$= n_{fo} \left( a_o + b_o \frac{S}{2} \right) = \frac{D}{kVA_{fo}} \left( a_o + b_o \frac{S}{2} \right) \quad (5)$$

#### 3-3- Cost of substations:

The cost of a substation comprises that part of cost that does not depend on the substation capacity (c) which includes the land, the building and civil work and as such in addition to the part that depends on the substation capacity which is given by d\*kVA<sub>s</sub>.

Where :

**d** is the cost/ kVA of the capacity required to carry the load in the area  $S^2$ .

**kVA<sub>s</sub>** is the substation capacity or size=  $S^2 \cdot D$

Using one square km as a unit area for calculations.

$$C_s = \text{substation cost/ km}^2 = \frac{c}{S^2} + dD \quad (6)$$

### 3-3- Cost of losses [12]:

#### 3-3-1- Losses in substation transformers [12]:

Let the ratio between the value of power losses of the substation transformer and its rating be x, where x, i.e.

$$x = (1 - )$$

where :

is the transformer efficiency percentage/100.

Transformer losses in a substation at full load=

$$x(kVA)_s$$

and transformer losses in substations/ unit area ( $\text{km}^2$ )

$$= \frac{x(kVA)_s}{S^2}$$

Then, the cost of losses in substation transformers per unit area is equal to:

$$\frac{x(kVA)_s}{S^2} * \text{transformer operation age} * \text{loss factor (lf)} * \text{PF} * \text{cost of energy/kW}H$$

where the loss factor is the ratio between the averaged value of power losses of a transformer and its rating which depends on its load curve.

$$\text{Loss Factor (lf)} = \sum_{\text{hour}1}^{\text{Hour}24} \frac{(kVA_{actual})^2}{(kVA_{rating})^2} / 24$$

∴ Cost of losses in substation transformers/  $\text{km}^2$  is :

$$\frac{x(kVA)_s}{S^2} * 25 (\text{years}) * 8760 (\text{hours/ year}) * \text{loss factor (lf)} * \text{PF} * \frac{k_s (kVA)_s}{S^2} / \text{kW}H$$

Where :

$$k_s = x * 25 * 8760 * \text{loss factor (lf)} * \text{PF} * \frac{\$/kW}{\$/\text{kW}H} \quad (7)$$

$x = 1\% = 0.01$

Cost of losses in substation transformers/  $\text{km}^2$  ( $C_{tr}$ ) is :

$$\frac{k_s (kVA)_s}{S^2} = \frac{k_s * S^2 D}{S^2} = k_s D \quad (8)$$

### 3-3-2- losses in H.V. incoming and outgoing feeders [12]:

Similarly, cost of losses in incoming feeders = Losses cost = loss/ feeder at rated current \* No. of incoming feeders/ unit area \* loss factor \* life duration

in hours \* cost/ kWh

$$= \frac{I_i^2 (R_i / \text{km})}{1000} * \frac{S}{2} * \frac{4}{S^2} * 25 * 8760 * lf * \frac{\$/kW}{\$/\text{kW}H}$$

$$= \frac{I_i^2 (R_i / \text{km})}{1000} * \frac{2}{S} * 25 * 8760 * lf * \frac{\$/kW}{\$/\text{kW}H} \quad (9)$$

Also, energy losses in the MV outgoing feeders and their cost over the life time of cables (assumed 25 year)/ unit area can be calculated as follows [12] :

Energy Losses cost in MV outgoing feeder = loss/ feeder at rated current \* No. of outgoing feeders/ unit area \* loss factor \* life duration in hours \* cost/ kWh =

$$= \frac{I_o^2 (R_o / \text{km})}{1000} * \frac{S}{2} * n_{fo} * 25 * 8760 * lf * \frac{\$/kW}{\$/\text{kW}H}$$

$$= \frac{I_o^2 (R_o / \text{km})}{1000} * \frac{S}{2} * \frac{D}{(kVA)_{fo}} * 25 * 8760 * lf * \frac{\$/kW}{\$/\text{kW}H} \quad (10)$$

### 3-4- Operation and maintenance costs :

The expenses of operation and maintenance of substation equipment may considerably affect the cost function and hence the optimization of the substations sites and sizes. In an independent study of the operation and maintenance expenses of a substation, the authors could conclude that these costs over the substation life add to approximately the total substation capital (construction) costs [12].

Therefore, the cost of operation and maintenance/ unit area can be estimated as:

$$C_{o\&m} = \frac{s / s \text{ cost}}{S^2} \quad (11)$$

### 3-5- Suggested exact cost function:

From the above detailed analysis of the costs of a system including substations, incoming feeders, outgoing feeders, system energy losses, operation and maintenance costs, the believed most exact cost function suggested in the present work can be formulated by the additions of costs given in equations (4) to (11) as follows :

$$C_T = \left( \frac{c}{S^2} + dD \right) + \frac{4}{S^2} \left( a_i + b_i \frac{S}{2} \right) + \frac{D}{(kVA)_{fo}} \left( a_o + b_o \frac{S}{2} \right) + (12)$$

$$k_s D + k_f \frac{2I_i^2 R_i / km}{1000 S} + k_f \frac{2I_o^2 R_o S}{2000} * \frac{D}{(kVA)_{fo}} + \frac{s / s \cos t}{S^2}$$

Where :

$$k_s = 25 * 8760 * lf * PF * \$ / kWh$$

$$= 2.19 \times 10^5 * lf * PF * \$ / kWh$$

and

$$k_f = 25 * 8760 * lf * \$ / kWh$$

$$= 2.19 \times 10^5 * lf * \$ / kWh$$

In order to minimize the total cost :  $\frac{dC_T}{dS} = 0$

$$\frac{dC_T}{dS} = \frac{-2c}{S^3} + 0 - \frac{8a_i}{S^3} - \frac{4b_i}{2S^2} + 0 + \frac{b_o D}{2(kVA)_{fo}} + 0$$

$$- \frac{2k_f I_i^2 R_i / km}{1000 S^2} + k_f \frac{I_o^2 R_o}{2000 (kVA)_{fo}} - \frac{2x(s / s \cos t)}{S^3} = 0$$

$$\frac{dC_T}{dS} = \frac{-1}{S^3} (2c + 8a_i + 2 * (s / s \cos t))$$

$$- \frac{1}{S^2} \left( 2b_i + \frac{2k_f I_i^2 R_i / km}{1000} \right) + \left( \frac{b_o D}{2(kVA)_{fo}} + k_f \frac{I_o^2 R_o}{2000 (kVA)_{fo}} \right) = 0$$

Multiply by  $S^3$

$$S^3 \left( \frac{b_o D}{2(kVA)_{fo}} + k_f \frac{I_o^2 R_o}{2000 (kVA)_{fo}} - \frac{D}{(kVA)_{fo}} \right) - S \left( 2b_i + \frac{2k_f I_i^2 R_i / km}{1000} \right) - (2c + 8a_i + 2 * (s / s \cos t)) = 0$$

or

$$\alpha S^3 - \beta S - \gamma = 0 \quad (13)$$

Where :

$$\alpha = \frac{b_o D}{2(kVA)_{fo}} + \frac{k_f I_o^2 R_o}{2000 (kVA)_{fo}} - \frac{D}{(kVA)_{fo}}$$

$$\beta = 2b_i + \frac{2k_f I_i^2 R_i}{1000}$$

$$\gamma = 2c + 8a_i + 2 * (s / s \cos t)$$

Solving equation (13) for S gives the optimum distance between substations and feeders length for minimum cost.

Then, the optimum substation size is given by

$$(kVA)_s = S^2 D$$

Solving equ. (13); S and (kVA)<sub>s</sub> are obtained :

#### 4- Sample Results of Application of the Proposed Cost Function to the Planning of an Actual Network In A Fast Growing Country And Analysis of Results:

In order to verify the benefits from using the proposed cost function, calculations have been carried out to obtain the optimum sites and sizes of the 110/13.8 kV substations and incoming and outgoing feeders of Jeddah 110/13.8 kV networks, for the sake of optimum networks planning and/or rehabilitation. Calculations were also carried out for the same network using the simplified approximate cost function [4] commonly used, for comparison, where high economical and technical benefits will be shown by using the proposed cost function in the present work.

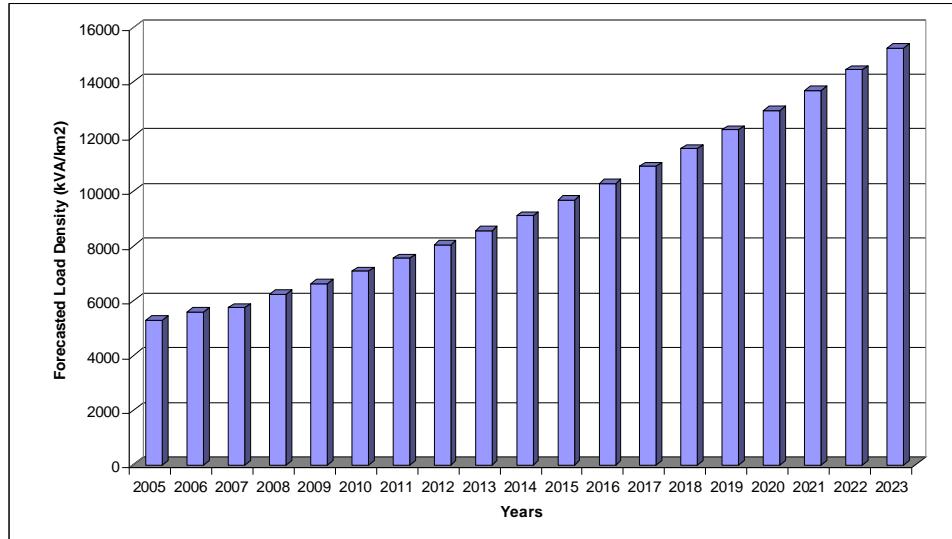
Load forecast for Jeddah area based on a new approach for load forecast in fast developing countries is presented in the authors' paper [13]. Considering the forecasted load density over the years, as presented in Figure (1), sample results are presented in figure (2) for the variation of the optimum distance (S) between substations with the years under the given otherwise conditions. These are compared with the results obtained by the approximate formula. Fig. (2), shows that the optimum distance between substations that decrease with the years (as the load and load density increase, Fig. (1)), is larger when using the investigated (proposed) cost function than using the approximate one, while, the number of required substations is less. Thus, it is required to use less number of substations but with larger capacities. This is an excellent advantage in fast growing countries since at the beginning, substations are built everywhere to meet the loads at various locations. Just increasing the capacities of the existing substations will meet the future loads (up to year 2023 in the studied case). This will save high costs of building new substations (as given by the approximate formula) where the cost of land becomes excessively high as well as saving new routes for the feeding (HV incoming) feeders and the distribution (MV outgoing) feeders.

Further, Figs. (3) presents the same as in Figs. (2), but increasing the cost of energy \$/kWh. Figures (2), (3) indicate that as the cost of electric energy/kWh increases i.e. as the costs of feeders losses increase, it is appropriate to increase the number of substations i.e., decrease the distance between substations. The losses of feeders here play an effective role.

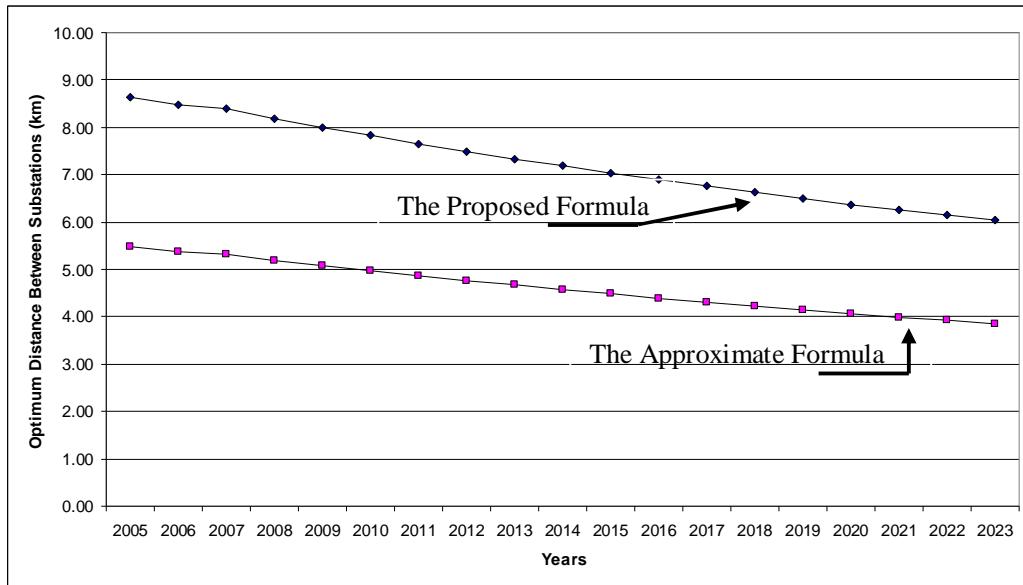
Furthermore, expressive results are shown in Fig. (4), where the calculated optimum distances between substations are presented when varying the fixed part of the substation cost (c) using the proposed cost function compared with the approximate one, under the given conditions. Figure shows clear dependence of the optimum distance between substations on the fixed part of the substation cost.

More and above Figure (5), shows the comparison between the calculated distance between substations dependence on the kWh prices (0.05, 0.08, 0.11, 0.2, 0.27 \$), When  $I_o = 300$  Amp,  $I_i = 600$  Amp, and  $c = 6.7$  M \$, with the use of the newly investigated (proposed) methodology compared with the approximate one. It can be seen that the increase of the

kWh price decreases the distance between substations with the use of the exact proposed formula while no effect is seen when using the approximate formula. This is quite clear as being due to ignoring the losses costs in the approximate formula.



**Fig. 1:** The forecasted load density for Jeddah area.



**Fig. 2:** The calculated distance between substations dependence on the different calculated methodologies, When the  $I_o = 200$  Amp,  $I_i = 400$  Amp, the kWh price is 0.05 \$, and the fixed part of substation cost ( $c$ ) is 4M \$.

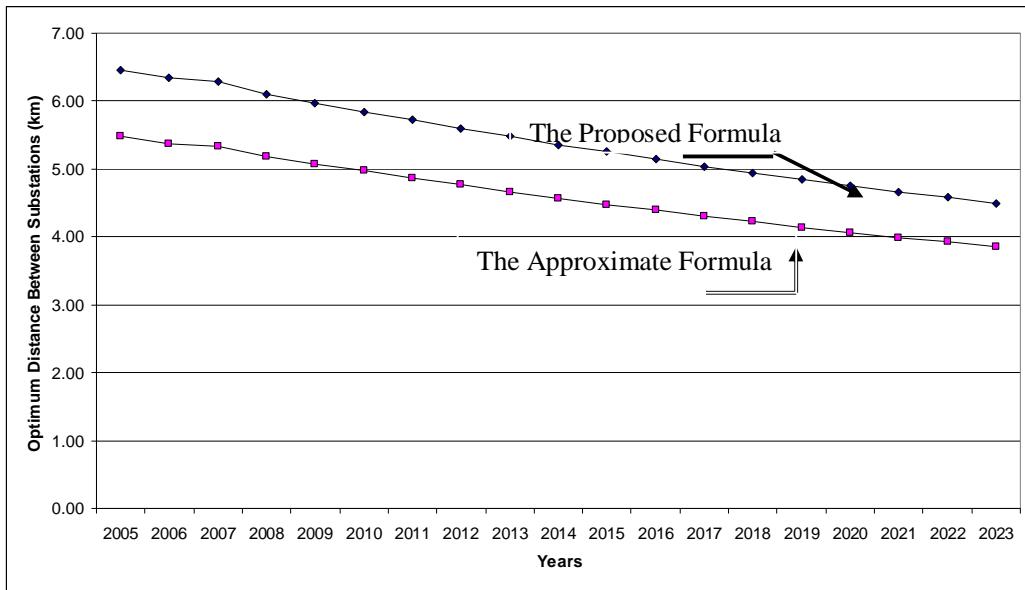


Fig. 3: The calculated distance between substations dependence on the different calculated methodologies, When the  $I_o = 200$  Amp,  $I_i = 400$  Amp, the kWh price is 0.27 \$, and the fixed part of the substation cost ( $c$ ) is 4M \$.

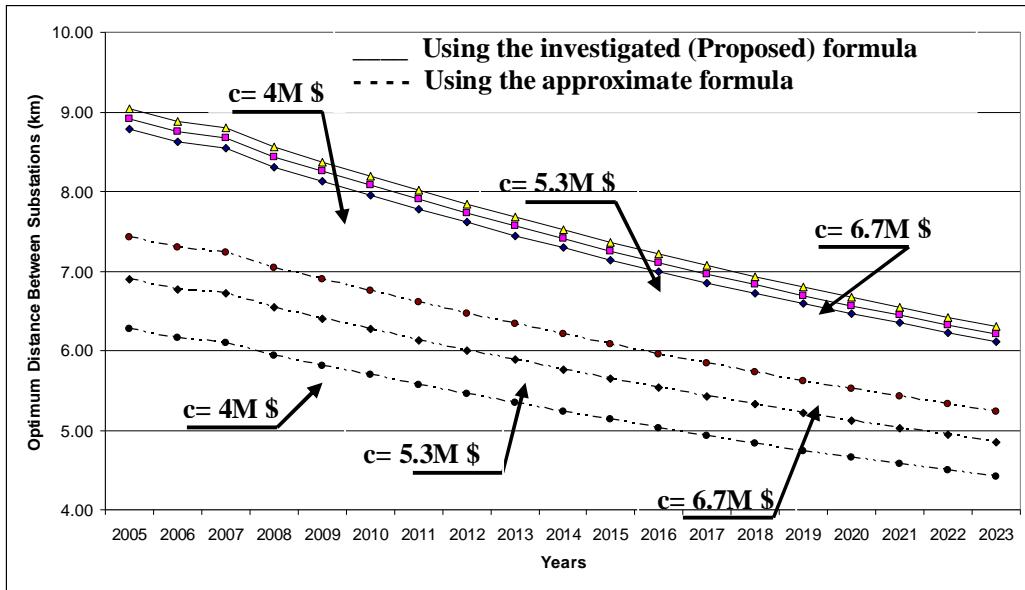
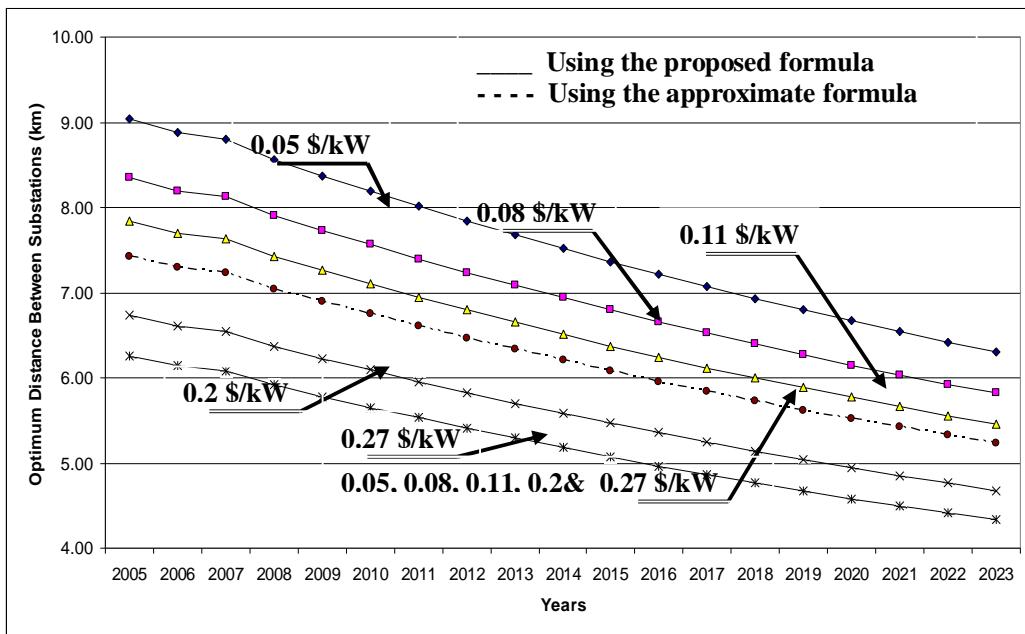


Fig. 4, Comparison between the calculated distance between substations dependence on the fixed part of substation cost ( $c = 4, 5.3, 6.7$  M \$), When the  $I_o = 300$  Amp,  $I_i = 600$  Amp, the kWh price is 0.05 \$, using the proposed cost function compared with the approximate one.



**Fig. 5, Comparison between the calculated distance between substations dependence on the kWh prices (0.05, 0.08, 0.11, 0.2, 0.27 \$/kWh),**  
When the  $I_o = 300$  Amp, and the fixed part of substation cost  $c = 6.7$  M \$, using the proposed cost function compared with those using the approximate formula.

### 5. Conclusions and Recommendations:

The main conclusions of this paper can be summarized as follows:

- 1- The approximate cost functions commonly used nowadays may lead to misleading results for good network planning. The use of different methodologies will result in different distances between substations and hence different number of substations.
- 2- The proposed cost function in the present work yields realistic results, compatible with the best of experience in network planning, where the centers of loads are well served. It does take the costs of all the electrical components into consideration, their losses and their operation and maintenance costs.
- 3- For fast developing countries, where the loads are rapidly increasing and the load densities are high, the high voltage of the substations between 66 kV and 110 kV, actually and effectively serves as distribution voltage, as the high number of substations will consequently serve the load

centers directly.

- 4- An important and vital recommendation for the rehabilitation and development of the 110/13.8 kV networks in fast developing countries, such as Jeddah Region Network, is to increase the capacity of the existing 110/13.8 kV substations, strengthen the 110 kV network by more 110 kV circuits between the substation to meet the growing loads, other than increasing the number of substations accompanied with so many difficulties, thus saving much cost of land and other costs.
- 5- The increase of the kWh cost results in decreasing the distance between substations (increasing the number of substations). The higher the substation cost the higher is the distance between substations.

### Corresponding Author

Salem M. Elkhodary  
Ain Shams University, Faculty of Engineering, Cairo,  
Egypt

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5/29/2010

## New Proposed Method of Damping Temporary Overvoltages on Power System Interconnections

Salem M. Elkhodary<sup>1</sup>, and Ali S. Abd El-Munem<sup>2</sup>

<sup>1</sup> Faculty of Engineering, Ain Shams University , <sup>2</sup>Egyptian Electricity Holding Company, Cairo, Egypt

**Abstract:** The interconnection between countries links different networks. These interconnections may be exposed to several disturbances. These disturbances (such as transient and temporary overvoltages phenomena, faults ...etc.) threaten the interconnection security and reliability. This paper presents actual field measurements of transient and temporary overvoltages appearing on the Egyptian – Libyan system interconnection as an example. These overvoltages were recorded for different cases of operation. These cases were modeled and simulated using the most recent version of Alternative Transient Program (ATP) computer package to compare the results of the computational method with the actual field measurements. The comparison between the ATP output results and the actual field measurements were found less than  $\pm 4\%$ . Within the research activities of the Egyptian Electricity Holding Company (EEHC) temporary overvoltage phenomena on the Egyptian – Libyan interconnection network were detected. EEHC carried field measurements of the temporary overvoltage by using a special transient mobile test laboratory. This detected temporary overvoltage was due to the generated reactive power along the line on switching, in spite of this leading reactive power was compensated by connecting number of reactors at different nodes. The economical aspect has been taken into consideration to reduce the number of reactors to the network, which showed the best effect on damping the temporary overvoltage. This paper, thus, presents a proposed technique to damp the temporary overvoltage and keep the system voltage within the permissible limits by estimating the optimum number and location of reactors that must be connected to the network. [Journal of American Science. 2010;6(11):336-342]. (ISSN: 1545-1003).

**Keywords:** interconnection; networks; disturbances; Alternative Transient Program (ATP); Egyptian Electricity Holding Company (EEHC)

### 1. Introduction

Due to the very fast progress and development in the different sectors of the electrical power system all over the world, the electrical interconnection between neighboring countries have been an important goal for most countries. These interconnections are exposed to many disturbances that threaten their security, such as overvoltage phenomena and earth faults ...etc.

As the Egyptian unified electrical network interconnected with the Jordan electrical network through 500/400 kV interconnection system, overvoltage studies were carried out. The overvoltage studies decided to use shunt reactors at different locations to compensate the generated reactive power of the long transmission line from Cairo to Naqab [1]. This study was based on the field measurements and EMTP output results.

Adding shunt reactors are used on many high voltage transmission lines as a means of shunt compensation to improve the performance of the line. They have the additional advantage of reducing the energization surge magnitudes. This is accomplished mainly by the reduction in temporary overvoltage [2]. All these effort were done to keep the voltage profile within the permissible level to protect the electrical equipments in the network.

Therefore, when the Egyptian – Libyan power systems were interconnected, the appearing disturbance should have been studied and treated. This paper is, thus, devoted to this objective.

### 2. The Egyptian–Libyan Interconnection Configuration:

The Egyptian – Libyan electrical interconnection network, of 613 km from Borg Al-arab in Egypt to Tobrouk in Libya, consists of double circuit transmission line of 220 kV passing through a number of 220/66 kV sub-stations (S/S), as shown in Fig.(1).

This transmission line consists of different sectors between sub-stations. These sectors have the following lengths:

**Table (1)**

Sector No.	From	To	Distance (km)
1	Borg Al-Arab	Omayed	35
2	Omayed	Matrouh	215
3	Matrouh	Saloum	198
4	Saloum	Tobrouk	165

The numbers of reactors already connected to the sub-stations are as follows:

**Table (2)**

No	S/S	No. of reactors	Reactor rating (MVAR )	Connected to
1	Borg Al-Arab	-----	-----	-----
2	Omayed	-----	-----	-----
3	Matrouh	2	25	Bus Bar
4	Saloum	2	25	Transmission line (Saloum-Matrouh)
5	Tobruk	2	25	Bus Bar

The reactors are not switchable; they are solidly connected to the bus bar or to the transmission line.

The Thevenin equivalent circuit of the Egyptian and Libyan Unified electrical power network, and the different sub-stations were calculated and simulated in Fig. (2).

### 3. Field Measurements and ATP Results:

Due to the long length of the Egyptian – Libyan electrical interconnection network (613 km) and the light loading nature of the sub-stations along the line, a leading reactive power is generated along the line. This leading reactive power distorts the voltage profile along the line.

The field measurements require advanced technology with high sensitivity and accuracy. A mobile test laboratory for overvoltage measurements in EEHC has been used for this purpose. This laboratory includes PC programmable sequence controller, computer assisted measuring and data acquisition system for registration and analysis of the test results [3].

Field measurements were carried out by the EEHC to measure the transient and temporary overvoltages for five different cases. These cases are as follows:

- 1- Disconnecting Tobrok 220/66 kV s/s bus coupler with two reactors connected to its bus bar.
- 2- Disconnecting Tobrok 220/66 kV s/s bus coupler with no reactors connected to its bus bar.
- 3- Disconnecting Saloum 220/66 kV s/s bus coupler.
- 4- Disconnecting Matrouh 220/66 kV s/s bus coupler.
- 5- Disconnecting Omayed 220/66 kV s/s bus coupler.

The field measurements record that the most severe case was (Case 5). Case 5 shows unacceptable temporary overvoltage due to switching off the interconnection network from Omayed 220/66 kV substation. The network configuration shown in Fig. (1). This case was modeled, simulated in Fig. (2) and studied by the ATP program.

Table (3) shows the system voltage before disconnection, the maximum transient overvoltage, the temporary overvoltage and the error percentage between actual field measurements and output results of ATP.

Table (3) shows the measured voltage at different sub-stations, there voltages are as follows:

- Saloum sub-station records a maximum transient overvoltage of 1.17 p.u. and temporary overvoltage of 1.12 p.u.
- The maximum transient overvoltage at Matrouh sub-station reaches 1.25 p.u. damped to 1.2 p.u. as temporary overvoltage.
- While Omayed sub-station measuring points records 1.25 p.u. for maximum transient overvoltage and temporary overvoltage.

The average error between the actual filed measurements and output results of ATP for the system voltage before disconnection was  $\pm 1.79\%$ , while for the maximum transient overvoltage the error was  $\pm 3.26\%$  and for the temporary overvoltage the error percentage was  $\pm 3.91\%$ . This emphasizes the reasonable accuracy, in such cases, of the computations of ATP output results compared to the field measurements.

### 4. Effect of Adding Reactors:

A sensitive node analysis was done to find the more sensitive bus for reactive power compensation. In this bus a number of reactors were added one by one. A 220 kV reactor of capacity 25 MVAR was added to the most sensitive nodes. The sensitive node analysis shows that Saloum, Matrouh and Omayed sub-stations were the most sensitive buses.

Optimization studies were done to find the optimum number of reactors at sensitive buses. The output results of the optimization studies are shown in Fig. (3, 4 and 5). These figures show the system voltage, the maximum transient overvoltage and the temporary overvoltage with the number of reactors. The maximum and minimum permissible limits ( $\pm 10\%$  of the nominal voltage) assign the optimum number of reactors that gives the most acceptable temporary overvoltage. [4]

**Table (3). The percentage error between the field measurements and ATP computation with the disconnection of 220kv bus coupler at Omayed 220/66kV S/S**

No.	Measuring point	Phase	System Voltage Before Disconnect (kV)			Max. Transient O.V. (kV)			Ovvoltage (kV)		
			Field measurements	ATP calculation	Error %	Field measurement	ATP calculation	Error %	Field measurements	ATP calculation	Error %
1	Matrouh - Saloum [I]	R	224	228	-1.79	264	267	-1.14	258	266	-3.10
		T	226	228	-0.88	275	267	2.91	262	266	-1.53
2	Matrouh - Saloum [II]	R	226	228	-0.88	264	267	-1.14	261	266	-1.92
3	Bus Bar [I]	R	227	228	-0.44	264	267	-1.14	263	266	-1.14
4	Bus Bar [II]	R	227	228	-0.44	263	267	-1.52	263	266	-1.14
5	Matrouh - Omayed [I]	T	225	228	-1.33	276	267	3.26	260	266	-2.31
6	Matrouh - Omayed [II]	R	227	228	-0.44	260	267	-2.69	262	266	-1.53
		T	224	228	-1.79	274	267	2.55	256	266	-3.91
7	Saloum- Tobrok [I]	T	224	227	-1.34	255	252	1.18	245	251	-2.45
8	Saloum- Tobrok [II]	T	225	227	-0.89	257	252	1.95	245	251	-2.45
9	Saloum- Matrouh [I]	T	224	227	-1.34	258	252	2.33	246	251	-2.03
10	Omayed - Matrouh [I]	R	225	224	0.44	274	277	-1.09	270	275	-1.85
		S	225	224	0.44	276	277	-0.36	276	275	0.36
11	Omayed - Matrouh [II]	S	226	224	0.88	275	277	-0.73	275	275	0.00
			average error	±1.79			average error	±3.26		average error	±3.91

Fig (3) shows that the optimum number of reactors at Saloum sub-station will be 2 reactors of 25 MVAR (already exist)

Fig (4) shows that the optimum number of reactors at Matrouh sub-station will be 4 reactors of 25 MVAR (2 reactors already exist and 2 reactors are to be necessarily added to damp the temporary overvoltage at this bus)

Fig (5) shows that the optimum number of reactors at Omayed sub-station will be 2 reactors of 25 MVAR (no reactors connected to this bus and 2 reactors are to be added to damp the temporary overvoltage at this bus). Adding 3 reactors will give better results for the voltage profile, but from the economical point of view adding 2 reactors is good enough.

The output results of the ATP program shown in Figs. (6, 7, 8, 9 and 10). These figures show the system voltage before disconnection, maximum transient overvoltage and temporary overvoltage after disconnection along the line (at Sloum, Matrouh and Omayed sub-stations).

Fig (6) (**solution 1**) comprises the implementation of the output results from fig (4) at Matrouh sub-station by adding 4 reactors by capacity 25 MVAR (adding 2 extra reactors to the already existing reactors). This figure shows that the temporary overvoltage was reduced from 246 kV to 232.58kV at Saloum sub-station. This means that adding 2 extra reactors reduced the temporary overvoltage by 5.46 %. This figure also shows that the temporary overvoltage was reduced from 263kV to 237.23 kV at Matrouh sub-station. This means that the temporary overvoltage was reduced by 9.8 %. This figure also shows that the temporary overvoltage was reduced from 276 kV to 245.32kV at Omayed sub-station with a reduction percentage of the temporary overvoltage by 11.12 %. Solution (1) is still considered to result in overvoltage values are over the maximum permissible limits at Omayed sub-station.

Figs. (7 and 8) were the implementation of the output results from Fig (5). Fig (7) (**solution 2**) shows the effect of adding 2 reactors of capacity 25 MVAR at Omayed sub-station. This

will reduce the temporary overvoltage from 246 kV to 232.13 kV at Saloum sub-station with reduction percentage of 5.64 %. While the temporary overvoltage at Matrouh sub-station was reduced from 263 kV to 236.52 kV which means a reduction percentage of 10.07 %. Where, at Omayed sub-station temporary overvoltage decreased from 276 kV to 235.35 kV, this means that the reduction percentage at Omayed S/S is 14.73 %.

On the other hand, Fig (8) (**solution 3**) shows the best performance of voltage profile along the interconnection line due to adding 3 reactors of 25 MVAR at Omayed sub-station. There is no fluctuation between the system voltage before disconnection, transient overvoltage and temporary overvoltage. At Saloum sub-station the temporary overvoltage reached to 224.62 kV with reduction percentage of 8.69 %. While at Matrouh sub-station temporary overvoltage reached to 224.99 kV. This means that the reduction percentage is 14.45 %. At Omayed sub-station the temporary overvoltage decreased to 219.63 kV. This means that the reduction percentage 20.42 %.

Fig (9) (**solution 4**) shows the effect of adding 4 reactors at Matrouh sub-station and 2 reactors at Omayed sub-station of capacity 25 MVAR. Solution (4) reduces the temporary overvoltage from 246 kV to 217.33 kV at Saloum sub-station. This means that the reduction percentage is 11.65 %. While the temporary overvoltage at Matrouh reduced from 263 kV to 213.8 kV which means a reduction percentage of 18.71 %. At Omayed S/S temporary overvoltage decreased from 276 kV to 212.74 kV, which means reduction percentage of 22.92 %.

Fig (10) (**solution 5**) shows the effect of adding 4 reactors at Matrouh sub-station and 3 reactors at Omayed sub-station of capacity 25 MVAR. Solution (5) reduces the temporary overvoltage from 246 kV to 211.15 kV at Saloum sub-station with reduction percentage 14.17 %. While the temporary overvoltage at Matrouh reduced from 263 kV to 204.29 kV which means a reduction percentage of 22.32 %. At Omayed temporary overvoltage decreased from 276 kV to 199.51 kV by reduction percentage 27.71 %.

The output results of the ATP for the five proposed solutions, which clarify the calculated temporary overvoltages (T.O.V) and the reduction percentage (%) from the actual field measurements at Saloum S/S (246 kV), Matrouh S/S (263 kV) and Omayed S/S (276 kV) are shown in table (4).

**Table (4)**

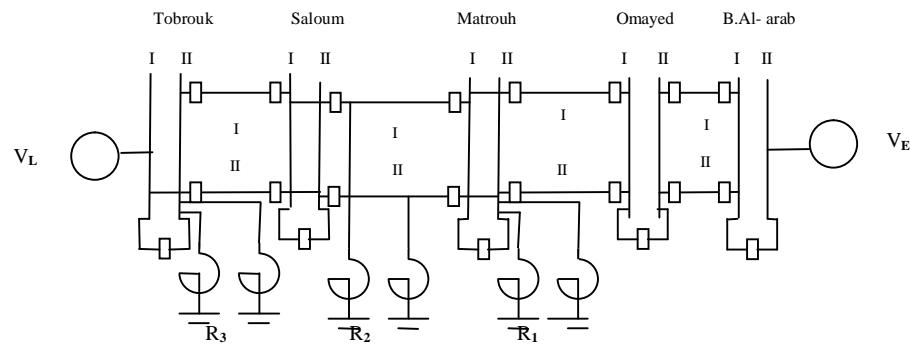
Proposed solution	Saloum		Matouh		Omayed	
	T.O.V	%	T.O.V	%	T.O.V	%
Sol. 1	232.58	5.46	237.23	9.8	245.32	11.12
Sol. 2	232.13	5.64	236.52	10.07	235.35	14.73
Sol. 3	224.62	8.69	224.99	14.45	219.63	20.42
Sol. 4	217.33	11.65	213.8	18.71	212.74	22.92
Sol. 5	211.15	14.17	204.29	22.32	199.51	27.71

#### 5. Conclusions and Recommendations:

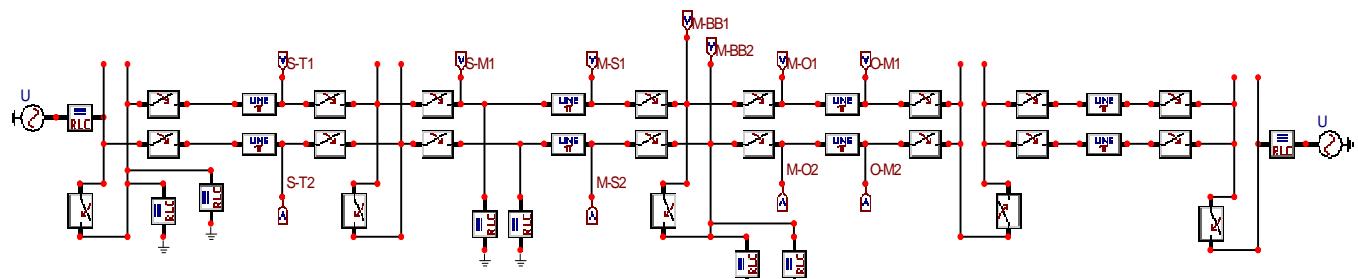
- 1) Interconnection between various countries power systems can suffer from various disturbing problems, especially temporary overvoltage during operation due to the differing nature of the systems and loading conditions.
- 2) The performance of systems interconnection could be predicted by computations as well as by field measurements with a reasonable degree of accuracy.
- 3) Suggestion are presented to improve the performance of the interconnected systems, for example, which can be extended to other systems interconnections.
- 4) The sensitive node analysis helps to find the most effective bus for the reactive power compensation.
- 5) This paper shows that adding a certain number of reactors at a certain sensitive bus greatly reduces the maximum transient overvoltage, temporary overvoltage and improves the system voltage profile. This protects the electrical equipment in the network and secures the system reliability and prevents system outage.
- 6) Optimization technique was done to find the most optimum number of reactors at the sensitive bus. The optimum number of reactors was regulated by economical considerations. The economical considerations show that the most effective and economical solution were solutions (2 and 3) as detailed in section (4).

#### Corresponding author

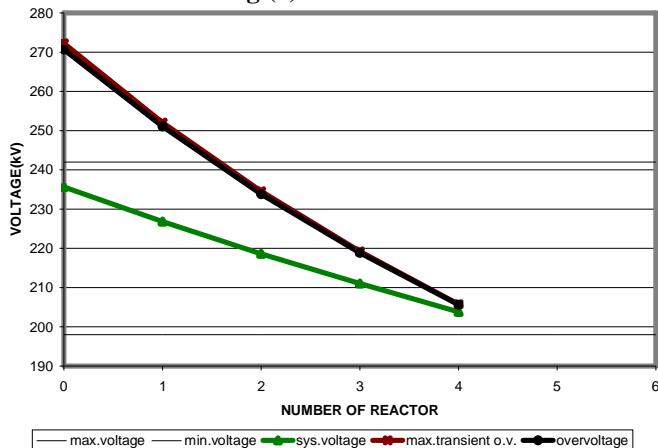
Salem M. Elkhodary  
Ain Shams University, Faculty of Engineering,  
Cairo, Egypt



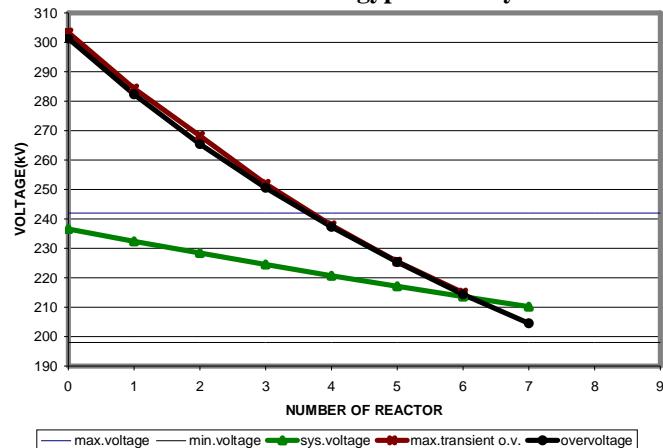
**Fig (1) The network configuration when switching off Omayed bus coupler**



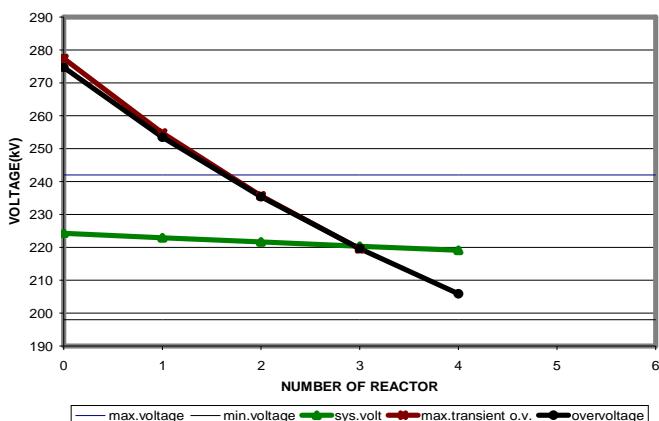
**Fig (2) ATP simulation of the electrical interconnection network between Egypt and Libya**



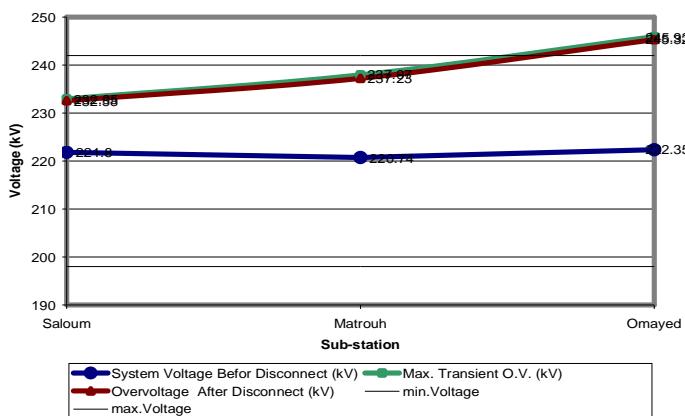
**Fig (3) Number of reactor against voltage profile at Saloum**



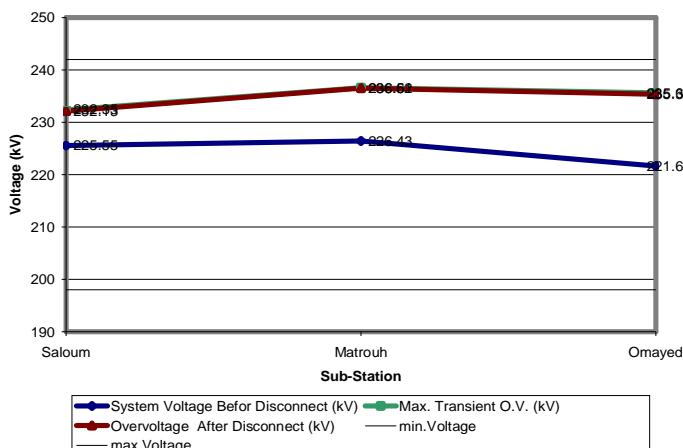
**Fig (4) Number of reactor against voltage profile at Matrouh**



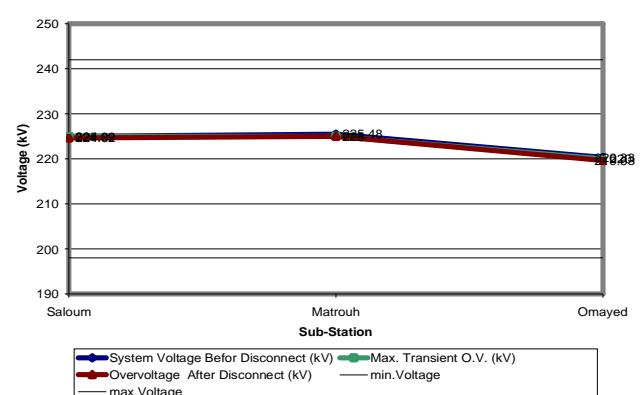
**Fig (5) Number of reactor against voltage profile at Omayed**



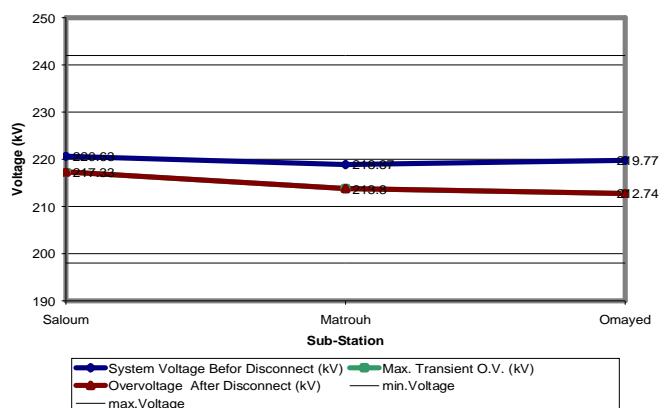
**Fig (6) Voltage profile along the interconnection electrical network between Libya and Egypt after adding 4 reactors at Matrouh S/S**



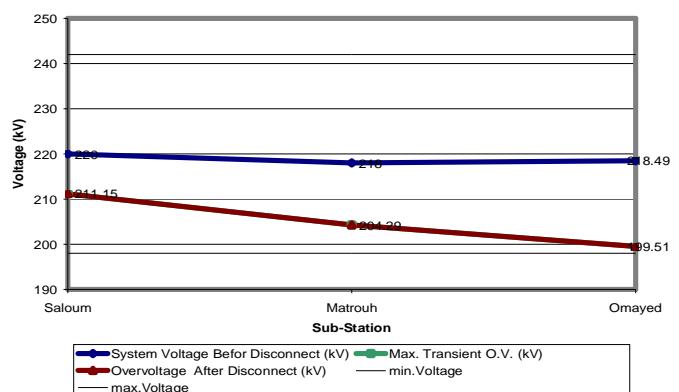
**Fig (7) Voltage profile along the interconnection electrical network between Libya and Egypt after adding 2 reactors at Omayed S/S**



**Fig (8) Voltage profile along the interconnection electrical network between Libya and Egypt after adding 3 reactors at Omayed S/S**



**(9) Voltage profile along the interconnection electrical network between Libya and Egypt after adding 4 reactors at Matrouh S/S and 2 reactors at Omayed S/S**



**Fig (10) Voltage profile along the interconnection electrical network between Libya and Egypt after adding 4 reactors at Matrouh S/S and 3 reactors at Omayed S/S**

**6. References:**

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6/1/2010

## Evaluation of *Corynebacterium variabile* Sh42 as a degrader for different poly aromatic compounds

Nour Sh. El-Gendy<sup>1\*</sup>, Yasser M. Moustafa<sup>1</sup>, Salem A. Habib<sup>2</sup>, Sherif Ali<sup>1</sup>

<sup>1</sup>Egyptian Petroleum Research Institute, Cairo, P.O. 11727, Egypt.

<sup>2</sup>Mansoura University, Faculty of Science, Damietta, Egypt.

**Abstract:** *Corynebacterium variabile* sp. Sh42 is used to investigate the biodegradation potentials and metabolic pathways of different poly aromatic compounds (PACs) in batch flasks. Effects of PACs size, molecular weight, alkylation and their presence individually or in mixture on biodegradation potentials of Sh42 were studied; Naphthalene (Nap) as a model compound for di-aromatic ring; Anthracene (Ant) and Phenanthrene (Phe) as model compounds for tri-aromatic ring; while Pyrene (Pyr) as a model compound for four-aromatic ring compounds were used as representatives for different PAHs. Dibenzothiophene (DBT), 4-methyldibenzothiophene (4-MDBT) and 4,6-dimethyldibenzothiophene (4,6-DMDBT) were taken as representative models for PASHs compounds. While, 2-hydroxybiphenyl (2-HBP) and 2, 2'-bhydroxybiphenyl (2, 2'-BHPB) were taken as models for phenolic compounds. The experimental results show that biodegradation rate decrease with increase ring size, alkylation's group within homologous series and Sh42 has the highest capability to biodegradation of toxic phenolic compounds either in single (BD% ≈ 90%) or mixed substrates cultures (BD% ≈ 48%). To ensure detoxification and mineralization of these toxic PACs; metabolic pathways of representative model compounds (Pyr, DBT and 2,2'-BHPB) were elucidated by GC/MS analysis which confirmed that, Sh42 completely metabolized all representative compounds to CO<sub>2</sub> and H<sub>2</sub>O. [Journal of American Science. 2010;6(11):343-356]. (ISSN: 1545-1003).

**Keywords:** Polynuclear aromatic compounds, Biodegradation, Metabolic pathways.

### 1. Introduction

Contamination by poly aromatic compounds (PACs); i.e. polynuclear aromatic hydrocarbons (PAHs) and poly aromatic sulfur heterocycles (PASHs) are of great environmental concern because of their toxic, mutagenic and carcinogenic properties (Hirano et al., 2004 and Perugini et al., 2007). These PAHs and PASHs are actually persistent compounds in the environment. Nevertheless, various bacteria and fungi are reported for their ability to degrade different PAHs and PASHs (Cheung and Kinkle, 2001; El-Gendy, 2006; Valentin et al., 2007; El-Gendy and Abo-State, 2008 and Seo et al., 2009).

Phenol and phenolic compounds are hazardous pollutants that can be found in waste waters from oil refineries, petrochemical plants, coal gasification plants, coking plants and dyes industry (Martíková et al., 2009). Phenolic compounds are known to affect microbial growth and degradation activities even in low concentrations (Vincenza and Liliana, 2007).

Dibenzothiophene (DBT) which is a typical PASH in crude oil is used as a model compound for biodesulfurization and biodegradation studies of

PASHs (Chen et al., 2008). Biodegradation of DBT can be classified into three independent categories, among which; complete mineralization, Kodama and the sulfur-specific cleavage, i.e. 4S

pathway, which is the most extensively studied (Xu et al., 2006). In "4S" pathway, DBT is first oxidized to DBT sulfoxide, then DBT sulfone and finally to sulfinate, followed by hydrolytic cleavage to free sulfur product, 2-HBP or 2, 2'-BHPB and subsequently release of sulfite or sulfate (Chen et al., 2008). 2-HBP and 2,2'-BHPB were reported to be furtherly metabolized to benzoic acid and salicylic acid, respectively in complete mineralization pathway (Kohler et al., 1988 and El-Gendy, 2004). Previously mentioned hydroxylated biphenyls are reported to be also produced through the bio-transformation reactions precede via hydroxylation of the aromatic rings in biodegradation of different studied PACs (Kohler et al., 1993 and Sondossi et al., 2004). Some previous studies have indicated that the final metabolite 2-HBP or 2,2'-BHPB of DBT biodegradation via 4S pathway could inhibit the microbial growth and DBT biodegradation (Lee et al., 1995 and Chen et al., 2008). Consequently, knowledge of microbial metabolism and environmental fate of these compounds is desired because they are by-products which have been identified as contaminants in almost every component of the global ecosystem and because they constitute a severe environmental hazard because of their high toxicity. Thus obtaining of microbial isolates with the ability to utilize several PACs is of

great interest from the stand point of understanding the principles of PACs utilization and their use in bioremediation technologies.

It is rather unusual for environments to be polluted by a single poly aromatic compound (PACs) (Tang et al., 2005; Seo et al., 2009 and Wei et al., 2009).

Therefore, the aim of this study is to investigate the potentiality of *Corynebacterium variabilis* sp. Sh42 to metabolize different PACs as single or mixture substrates; Naphthalene (Nap) as a model compound for di-aromatic ring; Anthracene (Ant) and Phenanthrene (Phe) as model compounds for tri-aromatic ring; while Pyrene (Pyr) as a model compound for four-aromatic ring compounds were used as representatives for different PAHs. Dibenzothiophene (DBT), 4-methyldibenzothiophene (4-MDBT) and 4,6-dimethyldibenzothiophene (4,6-DMDBT) were taken as representative models for PASHs compounds. While, 2-hydroxybiphenyl (2-HBP) and 2,2'-bhydroxybiphenyl (2,2'-BHPB) were taken as models for phenolic compounds. Also, elucidation of the possible metabolic pathways for a model compound representative to each PACs group has been done to ensure the metabolism of these toxic compounds to non-toxic ones.

## 2. Material and Methods

### Chemical reagents

All 9 PACs and other chemical reagents employed in this study were of analytical grade and purchased from Sigma Chemical Company, USA.

Acetonitrile (Ace) and Water (W) used for HPLC analysis were of HPLC grade and purchased from Aldrich.

### Microorganism

*Corynebacterium variabilis* sp. Sh42 was isolated from hydrocarbon polluted waster sample collected from El-Lessan Area of Damietta River Nile Branch in Egypt during year 2008 for its ability to degrade and tolerate high concentration of 2, 2'-BHPB.

### Media:

#### Tryptone glucose yeast extracts (TGY)

This media was used for preparation of inocula and monitoring of total viable count, TCFU (cells/mL) and was prepared according to Benson (1994).

#### Basal salts medium (BSM)

This media was used for studying BD capabilities of *C. variabilis* sp. Sh42 and was prepared according to Piddington et al. (1995).

### Analytical tools:

Bacterial growth was monitored by count the total viable colony (cells/mL) on TGY-agar plates.

### High Performance Liquid Chromatographic (HPLC) analysis

Liquid-liquid extraction for quantitative analysis of residual PACs was carried out by using ethyl acetate as the extractant. After the extraction, ethyl acetate layer was analyzed using HPLC model Waters 600E equipped with a UV detector model Waters 2487 (set at 254 nm) and C18 reversed phase column (4.6x250 mm, 300 $\text{\AA}$ , 5 $\mu$ ). The mobile phase was Ace: W (40:60 v/v), and the flow rate was 1 mL/minute, injection volume 2  $\mu$ L. Standard curves were established for each of the studied PACs from 5 to 1000 mg/L.

### Gas Chromatography/Mass Spectroscopy (GC/MS) analysis

GC/MS Perkin Elmer Clarus 500 with mass-selective detector was used to monitor the BD of mixture of PACs; the tested mixtures of nine poly aromatic compounds (PACs) were analyzed using GC/MS (system-1) to quantify percent removal. Identification of expected pathway for metabolism of different studied PACs (phenolic, PAHs and PASHs compounds) by *C. variabilis* sp. Sh42 GC/MS (system-2) was used (Table 1).

### Preparation of samples

For GC/MS analysis, ethyl-acetate extracts of the studied mixture were dehydrated over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated by evaporation at 60 °C before injection.

The GC/MS was equipped with HP-1 column, polymethyl siloxane (capillary 60 m x 0.32 mm x I.D. 0.25  $\mu$ m film thickness), mass-selective detector with ionization mode, E.I. ev 70, source temperature 250 °C; the carrier gas was helium with flow rate 1.5 mL/minute. Sample size was 1  $\mu$ L.

### Preparation of inoculum

Cells were incubated at 30 °C in TGY broth medium for 24 hours in a shaking incubator (150 rpm). Cells were harvested by centrifugation at 5000 rpm for 15 minutes and then washed three times with BSM then re-suspended in BSM free of any C-source to be used as inoculum.

**Table (1):** Programmable methods

programmable method	System (1)	System (2)
Injector temperature	Start from 60 °C held (0.1 minute) to 250 °C at fixed rate 200 °C/minute. Split (1:50)	Start from 80 °C held (0.1 minute) to 250 °C at fixed rate 200 °C/minute. Split (1:50)
Oven temperature	Start from 60 °C held for 1 minute, followed by 7 °C/minute ramp rate to 200 °C held to 5 minute, followed by 4 °C/minute ramp rate to a final temperature 280 °C which was held to another 15 minutes.	Start from 50 °C held for 1 minute, followed by 8 °C/minute ramp rate to a final temperature 280 °C which was held to another 15 minutes.
Run Time	50 minutes	60 minutes
	Standard calibration for mixture of the studied 9 PACs was established for their identification and quantification.	Possible structure assignments of metabolites were confirmed using NIST library and/or from the available literature data.

Identification of biodegradation pathway of different PACs using *C. variabilis* sp. Sh42

This was done using GC/MS analysis system (2) of the ethyl acetate extract of different PACs compounds; Pyrene as a model for PAHs; DBT as a model for PASHs and 2,2'-BHPB as a model for phenolic compounds after incubation at 30 °C for 10 days in shaking incubator (150 rpm).

Study of the biodegradation capabilities of *Corynebacterium Variabilis* sp. Sh42 on different individual PACs

This was used for studying the ability of Sh42 to utilize different PACs as sole source of carbon and energy for growth. Washed cells were inoculated into BSM that contained 1000 mg/L of each of the studied PACs and mixture of all. The growth and concentration of PACs were monitored at prescribed time intervals of (1- 4 weeks). Changes in pH values were also recorded. All steps were done in duplicates.

### 3. Results and discussion

Biodegradation capabilities of *Corynebacterium Variabilis* sp. Sh42 on different PACs:

Losses due to abiotic processes were calculated; recorded average ranged between ≈ 6%, 4% and 2.6 for phenolic, PAHs and PASHs compounds after 4 weeks, respectively. Any observed loss exceeding these values in the inoculated flasks can be attributed to biodegradation processes.

The experimental results show that pH decrease with incubation period up to 3 weeks for all cultures but increase with further incubation period. This decrease in pH might be due to the formation of acidic metabolites from biodegradation of these PACs in the cultures while the increase of pH again might be due to the further degradation of the acidic metabolites or due to the production of intermediates

that might increase the pH.

Generally, Figures (1 and 2) show that the maximum growth and biodegradation (BD) potential were observed after 2 weeks for all representative model compounds of PACs in single substrate cultures, it was observed that there was no difference in growth and BD% within further incubation period. While in mixed substrates cultures, it showed good increasing growth and BD% throughout the whole incubation period reaching its maximum at the end of incubation period.

This observation is in agreement with other reports where, the removal of PACs was directly related to the cell density and growth potential. Hong et al. (2008) reported that; the removal of Phe and Flu in mixture by *Nitzschia* sp. increase by increasing its cell density in the medium.

It was observed that, the average rate of biodegradation can be ranked in the following decreasing order; phenolic > PAHs > PASHs in either single or mixed substrates cultures at the end of incubation period. This might be attributed to the enrichment of *C. variabilis* sp. Sh42 on phenolic compounds showing higher BD efficiency and high resistance of hetero-PAH (PASHs) to biodegradation which have the lowest aqueous solubility.

It is obvious that phenolic compounds and Nap show nearly similar and highest BD% in single substrate cultures with average BD% ≈ 90% after 4 weeks. This might be due to the high capability of Sh42 to utilize phenolic compounds as it was enriched and isolated on 2, 2'-BHPB in addition to the high evaporation and assimilation rate of Nap than other PACs compounds or due to the higher specificity of Sh42 towards Nap biodegradation. While in mixed substrates culture only Nap show the highest biodegradation efficiency (BD% of 100% after 1 week of incubation). This might be due to the

high capabilities of Sh42 to utilize Nap over phenolic compounds or the mixture might have enhanced the metabolic rate of Nap over phenolic compounds.

Also, the BD rate of 2,2'-BHPB is higher than that of 2-HBP (Figures 1 and 2) in both single and mixed substrate cultures which might be due to the enrichment and isolation of Sh42 on 2,2'-BHPB, therefore, it would have higher capabilities towards 2,2'-BHPB biodegradation than 2-HBP, the higher toxicity of 2-HBP than 2,2'-BHPB or the difference in activity of enzymatic system required for biodegradation of 2,2'-BHPB and 2-HBP. Hiraoka et al. (2002) reported that; monohydroxylated biphenyl (2-HBP or 3-HBP) inhibited cell division of biphenyl biodegrader strain *Comamonas testosteroni* TK102, but the effect was not observed with 2,3-dihydroxybiphenyl which confirm that 2-HBP was more toxic than 2,2'-BHPB.

Generally, Figures (1 and 2) show that, the biodegradation efficiency for all tested PAHs compounds follow the same trend in either single or mixed substrate cultures which can be ranked in the following decreasing order; Nap > Ant ≈ Phe > Pyr. This observation is in agreement with other reports where low molecular weights PAHs are more biodegradable than high molecular weights PAHs. The same trend was observed for PASHs compounds; where the biodegradation efficiency for all tested PASHs compounds can be ranked in the following decreasing order; DBT > 4-MDBT > 4,6-DMDBT.

Hong et al. (2008) reported that; the molecular weight, water solubility and lipophobicity of a compound would affect its bioaccumulation and degradation by microorganisms. Yu et al. (2005) reported that; PAHs with low molecular weights such as 2-rings, naphthalene and 3- rings, phenanthrene and anthracene are more susceptible to bacterial degradation and more extensive than high molecular weights PAHs having more than 3-rings, pyrene.

On the other hand, by comparing the results in Tables (2 and 3) and represented in Figure (3) it is observed that; there are high significant differences in biodegradation efficiencies of all tested 9 PACs in single and mixed substrates cultures. Where, BD% for each substrate in a mixture is lower than that in single substrate culture except for Nap which shows no significant difference.

The average BD% recorded ≈ 90%, 70% and 64% for phenolic, PAHs and PASHs in single culture after 4 weeks, respectively while in mixed substrate culture the BD% reached ≈ 48%, 42% and 31%, respectively. This might be due to one or all of the following reasons;

First, the interactions between PACs in mixture might influence biodegradation rate and led

to negative or positive effect on its biodegradation efficiency and this agrees with Stringfellow and Attken (1995); Yuan et al. (2000); Johnsen et al. (2002) and Wei et al. (2009). Wei et al. (2009) reported that, there were inhibitory effects from interactions between the three PAHs mixture (Phe, Pyr and Flu) on *Mycobacterium* sp. MEBIC 5140 which led to a negative effect on the biodegradation of PAHs.

Second, different studies in culture media have shown that hetero-PAHs (NSO-PAHs) can have a significant inhibiting effect on the biodegradation of PAHs and mono aromatic hydrocarbons (Arcangeli and Arvin, 1995; Dyreborg et al., 1996 and Lantz et al., 1997). Meyer and Steinhart (2000) reported that; degradation of two- to five-ring PAHs was inhibited by the presence of hetero-PAHs, whereas degradation of just some hetero-PAHs was inhibited by the presence of PAHs.

Third, there may be another reason for the decrease in biodegradation of different studied PACs in a mixture reported by Bastiaens (1998) and Herwijnen et al. (2003); the toxic effect caused by produced metabolites during biodegradation processes.

Fourth, the order of biodegradability of single substrates in complex mixtures is determined by their polarity and bioavailability. Consequently, as the Molecular weight of PAHs and number of alkyl group of PASHs increase the aqueous solubility and bioavailability decrease and therefore exhibits a protective function against biodegradation.

There is no observed significant difference between growth potential for *C. variabilis* sp. Sh42 in single or mixed substrate cultures of PACs, this might indicate that mixtures of PACs may not have any significant effect on cell growth but might have a significant effect on the biodegradation enzymatic system for *C. variabilis* sp. Sh42. Where, the negative effects might be due to competitive inhibition of multiple substrates or other means, retarding the degradation of one substrate in the presence of another. Similar or identical enzyme systems may catalyze the degradation of compound(s) which are structurally similar (Bauer and Capone, 1988).

Hong et al. (2008) reported that, when two or more PACs are present together, one PAC has the capacity to influence the rate and extent of biodegradation of the other.

Seo et al. (2009) reported that, alkylated and hetero PACs are more resistant to be biodegraded than the parent PACs where, alkyl- and hetero-PACs are among common substituted PACs and have substantial toxicities. The ability of PACs dioxygenase to remove the substitutions is currently

the subject of debate and probably requires additional steps to be removed. Also, their presence may inhibit proper orientation and accessibility of the PACs into dioxygenases.

In general, our results are in agreement with other reports where, the rate of polycyclic aromatic compound biodegradation in the mixed substrates culture decreases with increasing ring size and within a homologous series, decreases with increasing alkylation (Neff, 1979; Douglas et al., 1994; Elmendorf et al., 1994).

Fedorak and Westlake (1983) and (1984) reported that; microbial degradation of organic sulfur compounds in Prudhoe Bay crude oil revealed that the order of susceptibility of the sulfur heterocycles in homologous series was; DBT > C1-DBTs > C2-DBTs > C3-DBTs.

Nagata et al. (1978) reported the degradation of crude oil by *Corynebacterium* sp. isolated from sea water in the harbor of Kobe showing good biodegradation capabilities on diaromatic and polyaromatic hydrocarbons than monoaromatic ones.

In general, *C. variabilis* sp. Sh42 expressed the highest ability to utilize PACs mixture especially the toxic phenolic compounds and because of its metabolic versatility, this bacterium has been thought to be a potential candidate for bioremediation of PACs-contaminated areas.

#### Metabolic pathway study:

##### Study of 2, 2'-BHP metabolic pathway using *Corynebacterium variabilis* sp. Sh42

Data obtained from GC/MS analysis system (2) of ethyl-acetate extracte of 2, 2'-BHP cultures with *Corynebacterium variabilis* sp. Sh42 suggesting the biodegradation pathway illusterated in Figure (4) which is a meta-cleavage pathway. This pathway is similar to that reported by Kohler et al. (1993) and Sondossi et al. (2004) for biodegradation of 2,2'-BHP with *Pseudomonas* sp. Strain HB1 and *Comamonas testosterone* B-356, respectively. The obtained suggested pathway in this study suggesting that aromatic ring of 2, 2'-BHP is degraded via a site-specific monooxygenase that hydroxylates aromatic compounds at the C-3 position where there is a hydroxyl group at C-2 and alkyl or phenyl rest at C-1. Interestingly, 2, 2', 3-trihydroxybiphenyl also serves as a substrate for the monooxygenase activity producing 2, 2', 3, 3'-tetrahydroxybiphenyl. Therefore, the monooxygenase also hydroxylates the C-3' postion of 2, 2', 3- trihydroxybiphenyl. This finding provides additional evidence for the previously suggested relaxed specificity of the monooxygenase with respect to the molecular rest at

the C-1 position of the aromatic backbone structure reported by Kohler et al. (1988).

There was a yellow colouration observed in phenolic cultures inoculated with Sh42 which might be due to the production of 2,2',3,3'-tetrahydroxybiphenyl.

Kohler et al. (1993) reported the formation of yellow meta-cleavage compounds from 2,2',3-trihydroxybiphenyl (2-hydroxy-6-(2-hydroxyphenyl)-6-oxo-2,4-hexadienoic acid) and 2,2',3,3'-tetrahydroxybiphenyl (2-hydroxy-6-(2,3-dihydroxyphenyl)-6-oxo-2,4-hexadienoic acid) which did not remain stable in an aqueous solution. They also reported the formation of yellow meta-celavage metabolite 2-hydroxymuconic semialdehyde from catechol produced from salicylate monooxygenases of salicylic acid produced through the biodegradation of 2,2',3-trihydroxybiphenyl which was produced from monooxygenase of 2,2'-biphenol.

The proposed pathway for the metabolism of 2,2'-biphenol presented in Figure (4) indicate that the first intermediate; 2,2',3-trihydroxybiphenyl, may be metabolized via two different routes. On one hand, it serves as a substrate to the extradiol ring cleavage dioxygenase, and on the other hand, it can be turned over by the monooxygenase.

The conversion of produced catechol and pyrogallol to 2-hydroxymuconic semialdehyde and 2-hydroxymuconic acid, respectively; indicate that the extradiol ring cleavage dioxygenase activity from strain Sh42 is a broad-spectrum meta-cleavage dioxygenase because it is able to turn over various 2,2',3-trihydroxy- and 2,2',3,3'-tetrahydroxybiphenyl, catechol and pyrogallol.

##### Study of DBT metabolic pathway using *Corynebacterium variabilis* sp. Sh42

Data obtained from GC/MS analysis system (2) of ethyl acetate extracte of DBT cultures inoculated with *Corynebacterium variabilis* sp. Sh42 suggest the biodegradation pathway illusterated in Figure (5) which is complete mineralization pathway. Where DBT was first oxidized through 4S-pathway to DBT-sulfoxide, DBT-sulfone then to 2'-HBP-2-sulfonic acid and 2'-HBP-2-sulfonic acid which leads to the production of 2-HBP and 2,2'-BHP, respectively and which were furtherly degraded through meta-cleavage pathway as illustrated before. 2-HBP can be also degraded by dioxygenation of vicinal ortho-meta carbons of the un-substituted ring producing 2,2'-BHP and then 2,2',3-trihydroxybiphenyl which is furtherly degraded through meta-cleavage pathway as discussed before.

Sondossi et al. (2004) reported that *Comamonas testosterone* B-356 is able to metabolize

monohydroxybiphenyls through the biphenyl catabolic pathway leading to the production of benzoic acid and 2-hydroxypentanoate.

Omori et al. (1992) reported that, the soil isolate, *Corynebacterium* sp. strain SY1, utilized DBT and a wide range of organic and inorganic sulfur compounds as sole source of sulfur, such as DBT sulfone, dimethyl sulfide, dimethyl sulfoxide, dimethyl sulfone, CS<sub>2</sub>, FeS<sub>2</sub> and even elemental sulfur. Strain SY1; metabolize DBT to DBT sulfoxide and DBT sulfone and 2-HBP, which subsequently nitrate to produce at least two different hydroxynitobiphenyls during cultivation.

Also, Constanti et al. (1996) reported that, *Corynebacterium* sp. MC401 and *Corynebacterium* sp. MC402 isolated from a coal mine area by enrichment culture with DBT. Both cultures were able to use DBT, DBTO<sub>2</sub> as sole source of sulfur for growth. These compounds were metabolized to 2-HBP and sulfate.

#### Study of Pyrene metabolic pathway using *Corynebacterium variabilis* sp. Sh42

Data obtained from GC/MS analysis system (2) of ethyl acetate extracte of Pyrene cultures inoculated with *Corynebacterium variabilis* sp. Sh42 suggest the proposed Pyrene biodegradation pathway illustrated in Figure (6) which also involve phenanthrene, naphthalene and o-phthalate degradation pathways.

This pathway was similar to that proposed by Liang et al. (2006) for biodegradation of Pyrene by *Mycobacterium* sp. Strain KMS. According to the literature review, at least 15 enzymes are involved in the degradation of Pyrene and the o-phthalate degradation from phenanthrene, with some enzymes being common to the degradation of both PAHs. Pyrene is first oxidized in the K region by a dioxygenase to form cis-4, 5-pyrene-dihydrodiol, which is rearomatized to form 4, 5-dihydroxy-pyrene by dihydrodiol dehydrogenase. 4, 5-Dihydroxy-pyrene is subsequently cleaved to yield phenanthrene-4, 5-dicarboxylic acid by intradiol dioxygenase, followed by loss of a carboxyl group by decarboxylase and 4-phenanthroic acid is formed. Oxidation of 4-phenanthroic acid by ring-hydroxylating dioxygenase produces 3,4-phenanthrene dihydrodiol-4-carboxylic acid, which is further transformed to 3,4-dihydroxyphenanthrene by dehydrogenase/decarboxylase. Once 3,4-dihydroxyphenanthrene is formed, it enters the phenanthrene degradation pathway (Krivobok et al., 2003). Where it is metabolized to 1-hydroxy-2-naphthoic acid and then mineralized through two different pathways (Figure 6), in one pathway, 1-hydroxy-2-naphthoic acid is oxidized to 1, 2-

dihydroxy naphthalene, which is furtherly metabolized via salicylic acid. In the other pathway (Figure 6), 1-hydroxy-2-naphthoic acid undergoes ring cleavage and furtherly metabolize via o-phthalic acid and protocatichic acid. Similar pathway was reported by Pinyakong et al. (2000) for biodegradation of Phenanthrene by *Sphingomonas* sp. P2. It has been demonstrated that a common set of enzymes is responsible for the conversion of Phe to 1-hydroxy-2-naphthoic acid as well as that of naphthalene to salicylic acid (Yang et al., 1994).

Due to the known reported toxicity of pyrene-4,5-dione, it is important to identify this metabolite and determine its fate during Pyrene metabolism. It was observed as an end product in some gram negative cultures (Kazunga and Aitken, 2000) and may result in an increase in toxicity during in situ bioremediation (Guthrie et al., 2003).

Identification of Pyrene-4, 5-dione in the GC/MS chromatogram of the studied culture *Corynebacterium variabilis* sp. Sh42 might indicate the presence of dioxygenase gene in the obtained bacterial isolate *Corynebacterium variabilis* sp. Sh42. As according to Khan et al. (2001), Pyrene-4,5-dione was identified to be Pyrene metabolite in the phagemid clone My6-pBK-CMV, which contain a dioxygenase gene when it was incubated with Pyrene.

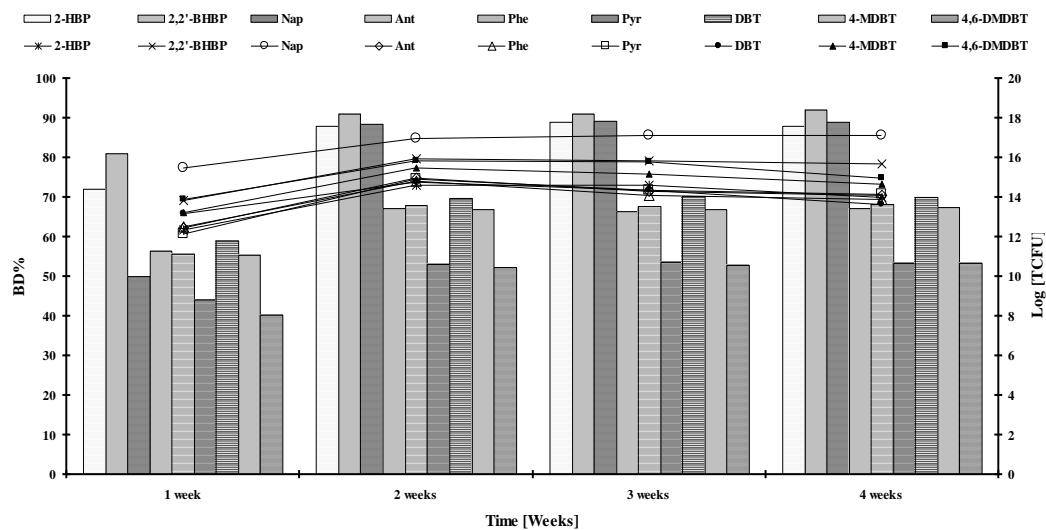
The quinone, Pyrene-4, 5-dione can be also formed following the non-enzymatic autoxidation of 4, 5-dihydroxypyrene.

Pyrene-4,5-dione may be reduced back to 4,5-dihydroxypyrene by quinone reductase (PQR), as reported for *Mycobacterium* sp.( Kim et al., 2004 and Liang et al., 2006). This might explain the abundance of 4, 5-dihydroxypyrene and pyrene-4, 5-dione peaks, respectively in the GC/MS chromatogram. The presence of the quinone, pyrene-4,5-dione might explain the observed pink coloration occurred in the cultures.

#### 4. Conclusion

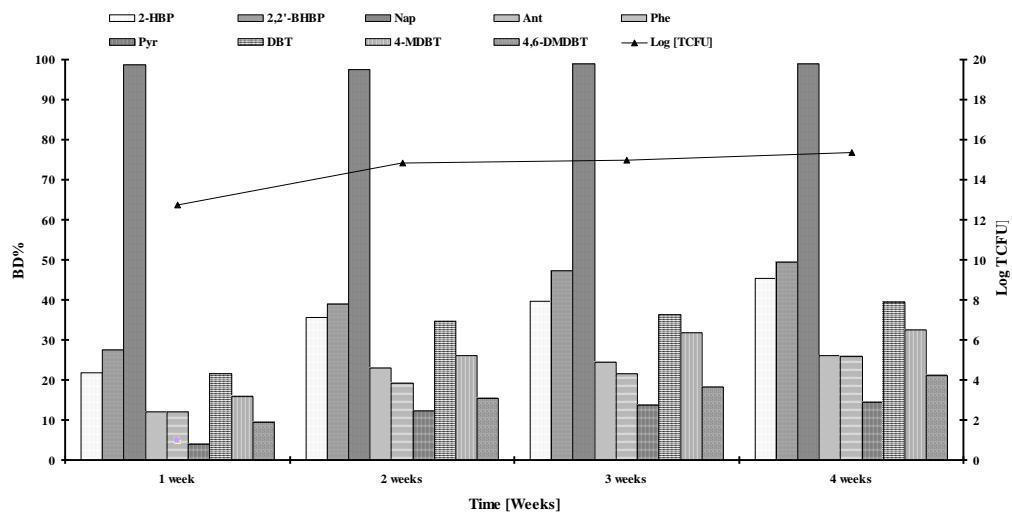
*Corynebacterium variabilis* Sh42 isolated from El-Lessen Area of Damietta River Nile Branch in Egypt has a high capability to metabolism of different PACs (phenolic, PAHs and PASHs) and utilizes them as a carbon and energy source for its growth either in single or mixed substrates cultures. While the biodegradation rate decreased in mixed substrate culture than single substrate cultures for all PACs, this might attribute to inhibition effect of metabolic enzymatic system. Also, BD% decrease with increasing ring size, within homologous series and BD potentials can be ranked in the following decreasing order; phenolic > PAHs > PASHs. In addition, *C. variabilis* Sh42 has capability to completely metabolism of 2,2'-BHP through meta-

cleavage pathway, DBT through 4S pathway and pyrene which also involve phenanthrene, naphthalene and o-phthalate metabolic pathways.



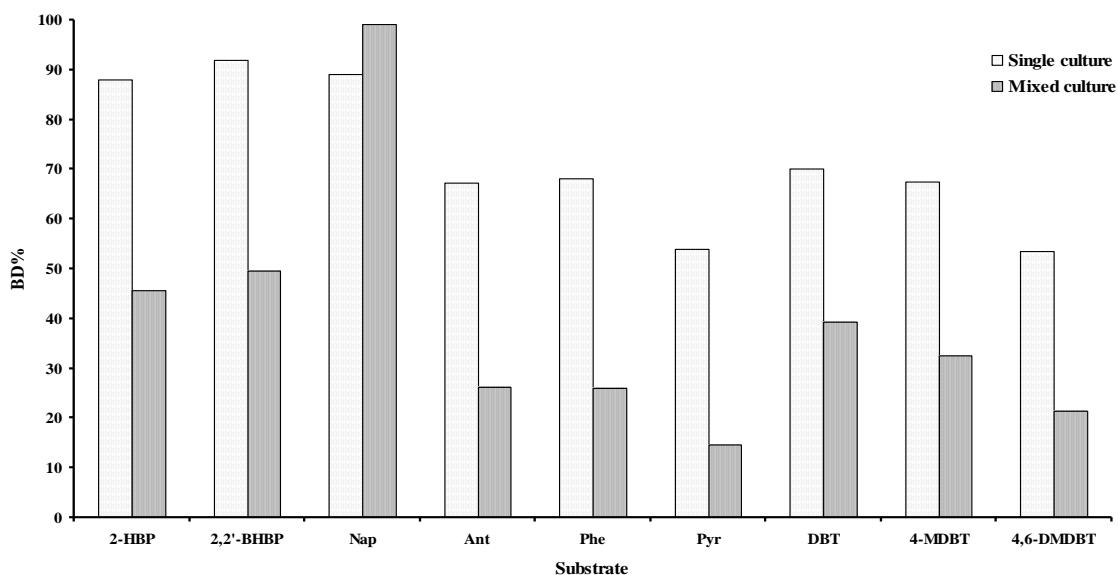
**Legend:** columns represented BD% while lines represented growth potentials.

**Figure (1):** Effect of different incubation periods on growth potential and PACs BD efficiency of *Corynebacterium variabilis* sp. Sh42 in single substrate cultures.



**Legend:** columns represented BD% while lines represented growth potentials.

**Figure (2):** Effect of different incubation periods on growth potential and PACs BD efficiency of *Corynebacterium variabilis* sp. Sh42 in mixed substrate cultures.



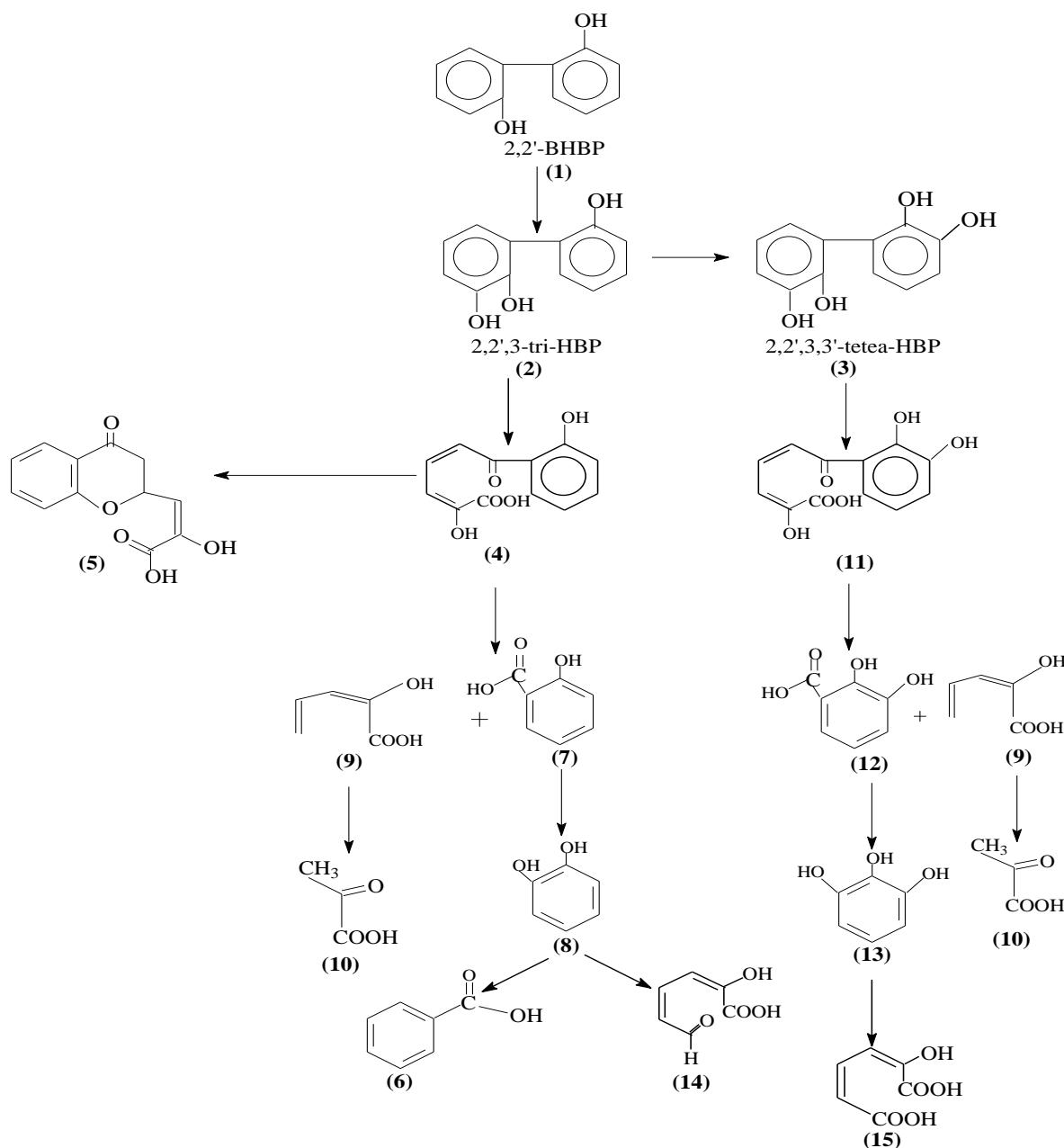
**Figure (3):** Comparison between biodegradation efficiencies of different PACs in single and mixed substrate cultures.

**Table (2):** The biodegradation percent (BD %) of different PACs compounds in single and mixed substrate cultures after different incubation periods.

Period [weeks]	1 week		2 weeks		3 weeks		4 weeks	
	BD%							
Culture	Single	Mixed	Single	Mixed	Single	Mixed	Single	Mixed
2-HBP	72	21.9	88	35.8	89	39.7	88	45.6
2,2'-BHPB	81	27.7	91	39.1	91	47.3	92	49.6
Nap	49.9	97.5	88.4	98.9	89	99.1	89	99.2
Ant	56.3	12.1	65.5	23.2	67	24.5	67.3	26.2
Phe	55.7	12.2	67	19.4	67.8	21.8	68.1	25.9
Pyr	44	4.1	53	12.3	53	13.9	54	14.7
DBT	58.9	21.8	69.7	34.7	70	36.3	70	39.4
4-MDBT	55.4	16	66.8	26.3	67	32	67.4	32.6
4,6-DMDBT	40.3	9.5	52.2	15.4	53	18.2	53.5	21.3

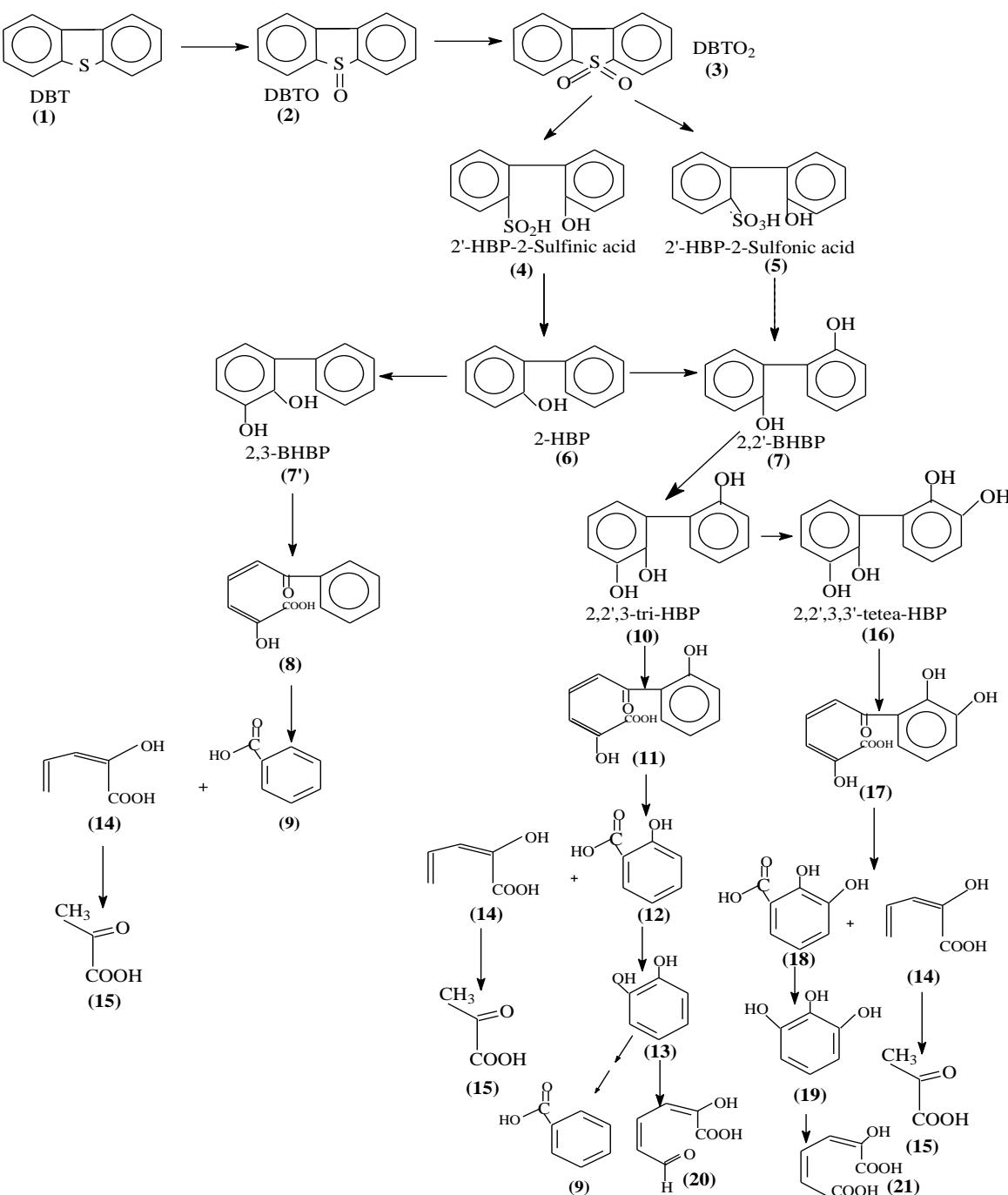
**Table (3):** The average biodegradation percent (average BD %) of different PACs groups in single and mixed substrate cultures after different incubation periods.

Compounds	Phenolic		PAHs		PASHs	
	BD Average%					
Time [weeks]	Single	Mixed	Single	Mixed	Single	Mixed
1 week	76.5	24.83	51.5	29.31	51.53	15.73
2 weeks	89	37.41	69.15	38.11	62.9	25.47
3 weeks	90	43.48	69.3	39.83	63.37	28.84
4 weeks	90	47.61	69.48	41.47	63.6	31.09



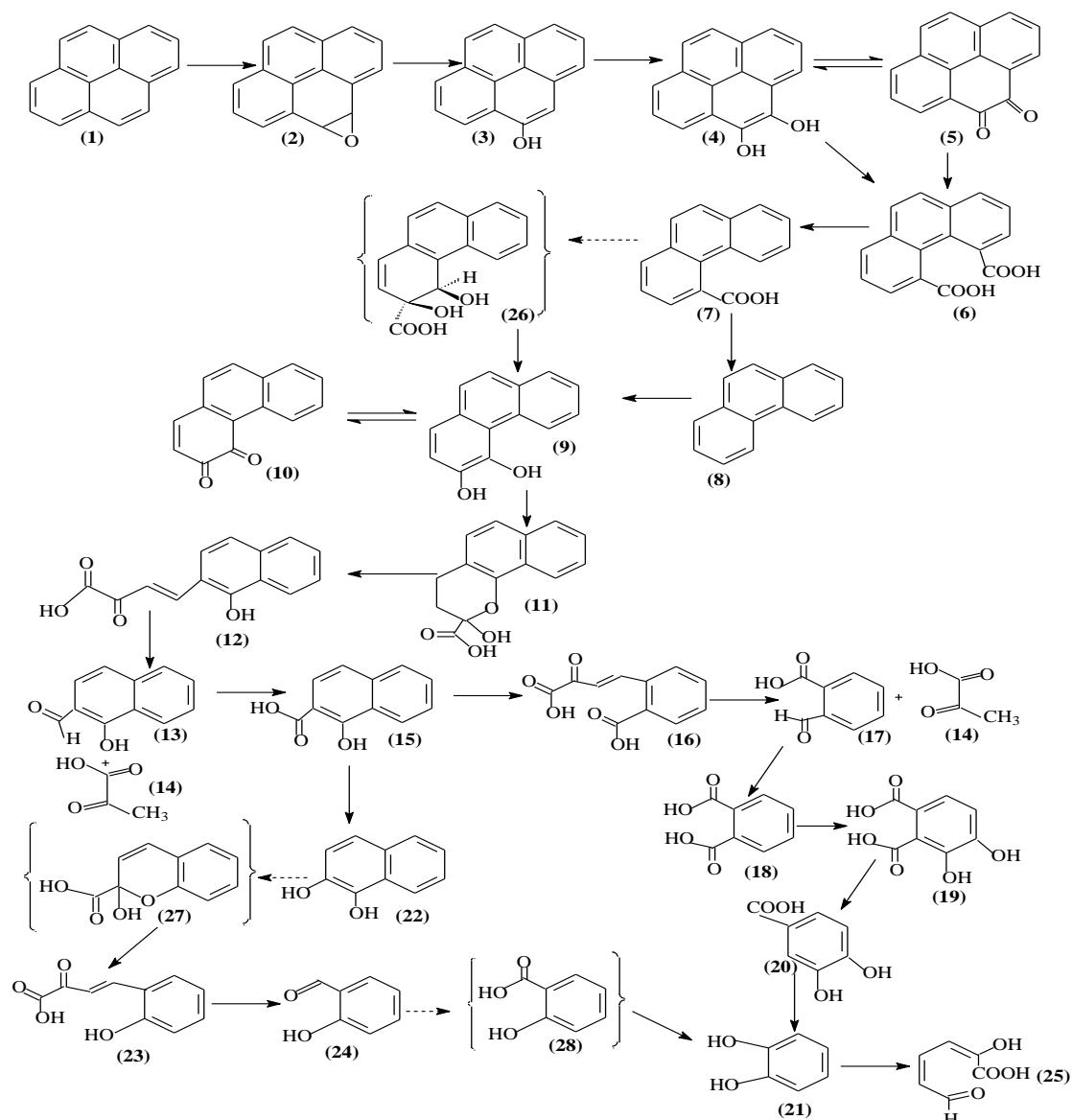
**Legend:** **1**, 2,2'-biphenol; **2**, 2,2',3-trihydroxybiphenyl; **3**, 2,2',3,3'-tetrahydroxybiphenyl; **4**, 2-hydroxy-6-(2-hydroxyphenyl)-6-oxo-2,4-hexadienoic acid; **5**, 3-(chroman-4-on-2-yl)pyruvate; **6**, benzoic acid; **7**, salicylic acid; **8**, catechol; **9**, 2-hydroxy-2,4-pentadienoic acid; **10**, pyruvic acid; **11**, 2-hydroxy-6-(2,3-dihydroxyphenyl)-6-oxo-2,4-hexadienoic acid; **12**, 2,3-dihydroxybenzoic acid; **13**, pyrogallol; **14**, 2-hydroxymuconic semialdehyde; **15**, 2-hydroxymuconic acid.

**Figure (4):** Proposed pathway for the metabolism of 2,2'-biphenol by *Corynebacterium variabilis* sp. Sh42.



**Legend:** **1**, dibenzothiophene (DBT); **2**, DBT-sulfoxide; **3**, DBT-sulfone; **4**, 2-hydroxybiphenyl sulfenic acid; **5**, 2-hydroxybiphenyl sulfuric acid; **6**, 2-hydroxybiphenyl; **7**, 2,2'-bihydroxybiphenyl; **7'**, 2,3-bihydroxybiphenyl; **8**, 2-hydroxy-6-oxo-phenylhexa-2,4-dienoic acid; **9**, benzoic acid; **10**, 2,2',3-trihydroxybiphenyl; **11**, 2-hydroxy-6-(2-hydroxyphenyl)-6-oxo-2,4-hexadienoic acid; **12**, salicylic acid; **13**, catechol; **14**, 2-hydroxy-2,4-pentadienoic acid; **15**, pyruvic acid; **16**, 2,2',3,3'-tetrahydroxybiphenyl; **17**, 2-hydroxy-6-(2,3-dihydroxyphenyl)-6-oxo-2,4-hexadienoic acid; **18**, 2,3-dihydroxybenzoic acid; **19**, pyrogallol; **20**, 2-hydroxymuconic semialdehyde; **21**, 2-hydroxymuconic acid.

**Figure (5):** Proposed pathway for the metabolism of dibenzothiophene by *Corynebacterium variabilis* sp. Sh42.



**Legend:** 1, Pyrene; 2, Pyrene-4,5-oxide; 3, Pyrene-5-ol; 4, Pyrene-4,5-diol; 5, Pyrene-4,5-dione; 6, Phenanthrene-4,5-dicarboxylic acid; 7, Phenanthrene-4-carboxylic acid; 8, Phenanthrene; 9, 3,4-dihydroxyphenanthrene; 10, Phenanthrene-3,4-dione; 11, 2-hydroxy-2H-benzo[h]chromene-2-carboxylic acid; 12, *Trans*-4-(1'-hydroxynaphth-2'-y1)-2-oxobut-3-enoic acid; 13, 1-hydroxy-2-naphthaldehyde; 14, Pyruvic acid; 15, 1-hydroxy-2-naphthoic acid; 16, *Trans*-2'-carboxybenzalpyruvic acid; 17, 2-carboxybenzaldehyde; 18, Phthalic acid; 19, 3,4-dihydroxyphthalic acid; 20, 3,4-dihydroxybenzoic acid; 21, Catechol; 22, 1,2-dihydroxynaphthalene; 23, *Trans*-o-hydroxybenzylidenepyruvic acid (tHBPA); 24, Salicylaldehyde; 25, 2-hydroxymuconic semialdehyde; 26, 3,4-dihydroxy-3,4-dihydro-phenanthrene-4-carboxylic acid; 27, 2-hydroxychromene-2-carboxylate (HCCA); 28, Salicylic acid.

**Figure (6):** Proposed pathway for the metabolism of Pyrene by *Corynebacterium variabilis* sp. Sh42.

#### Corresponding Author:

Dr. Nour Sh. El-Gendy

Fax No.: (+202) 22747433

E-mail: [nourepri@yahoo.com](mailto:nourepri@yahoo.com)

**Abbreviation**

2,2'- 2-HBP	2,2'-bihydroxybiphenyl
4,6- 4-MDBT	4,6-dimethyldibenzothiophene 4-methyldibenzothiophene
Ace	Acetonitrile
Ant	Anthracene
BD	Biodegradation
BSM	Basal salt medium
DBT	Dibenzothiophene
Flu	Fluoranthene
Nap	Naphthalene
PACs	Poly aromatic compounds
PAHs	Poly aromatic hydrocarbons
PASHs	Poly aromatic sulfur heterocyclic
Phe	Phenanthrene
Pyr	Pyrene
TCFU	Total colony forming unit
TGY	Tryptone glucose yeast extract
W	Water

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6/1/2010

# In vitro assessment of gastrointestinal viability of potentially probiotic Lactobacilli

Kawther, EL-Shafei, N.F.Tawfik, Nadia, M.A.Dabiza, O.M.Sharaf, and B.A.Effat  
Dairy Science Department, National Research Center Dokki, Cairo, Egypt.

**Abstract:** The objectives of this study were to assess the potential of four probiotic lactobacillus strains, *Lactobacillus bulgaricus*, *Lactobacillus johnsonii* B-2178, *Lactobacillus gasseri* B-14168 and *Lactobacillus salivarius* B-1950 in human upper gastrointestinal tract in vitro and evaluate the effect of milk proteins addition on viability of these strains in simulated gastric juices and in yoghurt during storage for 15 days at 4°C. The viability of lactobacilli strains in simulated gastric transit conditions (pH 2.0, pH 3.0 and pH 4.0) gastric juices with or without milk proteins singly or in combination with starch was tested. All the treatments were determined with three replicates. The simulated gastric transit tolerance of *L. johnsonii*, *L. gasseri* and *L. salivarius* strains was pH-dependent and correspondingly showed lower viability at pH 2.0 after 180 min compared with pH 3.0 and pH 4.0. The addition of milk proteins singly or in combination with starch enhanced the survival of probiotic lactobacilli strains in simulated gastric juices different tested pH values. Results showed that addition of milk proteins in combination with starch improved the viability of *L. johnsonii* B-2178, *L. gasseri* B-14168 and *L. salivarius* B-1950 in yoghurt during storage. Sensory evaluation showed that yoghurt fortified with milk proteins plus starch recorded the highest score for and overall acceptability than the other treatments. However, yoghurt manufactured with *L. johnsonii* and *L. gasseri* and fortified with sodium caseinate plus starch showed the highest organoleptic score. It is suggested that the yoghurt of acceptable quality and high total probiotic bacterial count during storage can be made from milk supplemented with 0.5% (w/v) starch plus 0.5% (w/v) sodium caseinate. [Journal of American Science. 2010;6(11):357-367. (ISSN: 1545-1003)].

**Keywords:** Probiotics, Gastric tolerance, *L. johnsonii*, *L. gasseri*, *L. salivarius*

## 1. Introduction

In the last decades consumer demands in the field of food production has changed considerably. Consumers more and more believe that foods contribute directly to their health (Qiang *et al*, 2009). Today foods are not intended to only satisfy hunger and to provide necessary nutrients for humans but to prevent nutrition-related diseases and improve physical and mental well-being of consumers (Siro *et al*, 2008). In this regard, functional foods play an outstanding role. The increasing demand on such foods can be explained by the increasing cost of healthcare, the steady increase in life expectancy, and desire of old people for improved quality of their later years (Kotilainen *et al*, 2006). In addition, functional dairy products offer requirements, benefits to health that are strengthened by the addition of probiotics as well as by certain types of soluble fibers known as prebiotics.

Probiotics for human consumption, generally either lactobacilli or bifidobacteria, are of increasing interest due to the growing evidence of health benefits associated with their use. Probiotic bacteria that are delivered through food systems have to firstly survive during the transit through the upper gastrointestinal tract, and then persist in the gut to provide beneficial effects for the host (Huang and Adams 2004). In order to be used as potential probiotics, lactobacillus strains need to be screened

for their capacity of transit tolerance to the upper gastrointestinal tract conditions.

The low pH of the stomach and the antimicrobial action of pepsin are known to provide an effective barrier against entry of bacteria into the intestinal tract (Holzapfel *et al*, 1998). The pH of the stomach could be as low pH 1.5 or as high as pH 6.0 or above after food intake (Jonhson, 1977), but generally ranges from pH 2.5 to pH 3.5 (Holzapfel *et al*, 1998). There are no agreed rules for the screening of acid tolerance of potential probiotic strains. A range of pH values, from pH 1.0 to pH 5.0 has been used to screen in vitro acid tolerance of *Lactobacillus* and *Bifidobacterium* (Chung *et al*, 1999 and Zarate *et al*, 2000).

Food and food ingredients have been shown to protect probiotic bacteria from acid conditions and enhance gastric survival. Milk has been reported to increase the viability of acid sensitive *Lactobacillus* and *Bifidobacterium* strains during simulated gastric tract transit (Huang and Adams, 2004). The protective effect may be due to the increase of gut pH after milk addition. Maize starch granules at pH 3.5 have also been found to increase the viability of the more sensitive *Bifidobacterium* strains (Wang *et al*, 1999). Currently, orally ingested probiotic bacteria for humans are mainly prepared in conjunction with dairy products (Huang and Adams, 2004).

Ice cream, yoghurt and cheese have been found to be carriers of probiotic organisms (Madureira *et al*, 2005); another potential food vector is whey cheese- on which, unfortunately very little (if any) research has been performed to date. Whey is the aqueous portion of milk that obtained following acid-or rennet -driven coagulation (i.e. precipitation of caseins) in cheesemaking and is still disposed of in significant overall volumes, especially by small dairy industries, to public sewage systems (Pintado *et al*, 2001).

Yoghurts fortified with casein based ingredients (SMP, Na-caseinate or Ca-caseinate) showed an increase in firmness (or viscosity) and a reduction in syneresis compared with unfortified yoghurt (Amatayakul *et al*, 2006). On the other hand, there were no consistent trends between the physical characteristics of yoghurts and the addition of whey protein-based ingredients. Therefore, the objectives of this study were to test the viability of four strains of lactobacilli in simulated gastric transit conditions (PH 2.0, pH 3.0 and pH 4.0 gastric juices). In addition, the effects of milk proteins in combination with starch on viability of probiotic lactobacilli in simulated gastric juices and in yoghurt during storage were determined.

## 2. Material and Methods

### Bacterial Strains:

Three *Lactobacillus* strains were obtained from Northern Regional Research Lab., Illinois, USA (NRRL). These organisms are *Lactobacillus johnsonii* B-2178, *Lactobacillus gasseri* B-14168 and *Lactobacillus salivarius* B-1950. All strains had previously been shown to possess properties required of probiotic microorganisms including bile salt tolerance, tolerance to low pH values and antagonistic activity (Amin *et al*, 2002). Additionally, *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were provided from Dairy Microbiology Lab., National Research Centre, Dokki, Cairo, Egypt.

### Preparation of simulated gastric juice:

Simulated gastric juice was prepared fresh daily as described by Huang and Adams (2004). It prepared by suspending pepsin (obtained from Sigma, BDH Chemicals Ltd., Poole, England) (1:1000, ICN) in sterile saline (0.5% w/v) to a final concentration of 3g<sup>-1</sup> and adjusting the pH to 2.0, 3.0 and 4.0 with concentrated HCl or sterile 0.1mol l<sup>-1</sup> NaOH using a pH meter (Model 8417N, Hanna Instrument, Singapore). Effect of different components in simulated gastric juice on viability of lactobacilli:

Sodium caseinate (Listowel, Co. Kerry, Ireland) whey protein (El-Masserin Milk products Co., Egypt) and Hi-maize starch (National Starch, Melbourne, Victoria, Australia) were used in this investigation. The solutions of these components were prepared fresh

daily by suspending each singly in sterile saline (0.5% w/v) at a concentration of 1g<sup>-1</sup>.

In order to analyze the effects of various components of simulated gastric juice on viability of lactobacilli, an aliquot (0.2ml) of each washed cell suspension was transferred to a 2.0 ml capacity screw-cap tube and then mixed with 0.3ml of NaCl (0.5% w/v) and 1.0ml of simulated gastric (pH 2.0, pH 3.0 or pH 4.0) and this treatment served as a control. Solutions of various components were replaced the sterile saline addition and added as follows:

- 1- Treatment I: Sodium caseinate at 1% concentration.
- 2- Treatment II: Whey protein at 1% concentration.
- 3-Treatment III: Sodium caseinate and starch (each, at 0.5% concentration).
- 4- Treatment (IV): Whey protein and starch (each, at 0.5% concentration).

These mixtures were then vortexed at maximum setting for 10s and incubated at 37°C. When screening gastric transit tolerance, aliquots of 0.1 ml were removed after 0, 60, 120 and 180 min for determination the viability of lactobacilli. Viability was assessed in three repeat experiments.

Viability of probiotics in yoghurt supplemented with prebiotics Yoghurt was manufactured in triplicate according to (Donkor *et al*, 2007) from standardized fresh cow's milk (3% fat). Milk was divided into three main portions. The first portion was applied as a control (C), without addition of prebiotics. The other two yoghurt base was supplemented with 0.5% (w/v) starch (I) and then whey protein (TI) or sodium caseinate (TII)(each,0.5 w/v) were individually incorporated.

Each mix and control were then pasteurized at 85°C /30min and cooled to approximately 40°C. Then, each portion was divided into three equal portions. Starters were added as follows:

- 1-Treatment *S.thermophilus*, *L. bulgaricus* and *L.johnsonii* B-2178 (TI<sub>1</sub>&TII<sub>1</sub> C<sub>1</sub>).
- 2-Treatment (TI<sub>2</sub> &TII<sub>2</sub> &C<sub>2</sub>) *S.thermophilus*, *L.bulgaricus* and *L. gasseri* B-14168.
- 3-Treatment (TI<sub>3</sub>&TII<sub>3</sub>&C<sub>3</sub>) *S.thermophilus*, *L. bulgaricus* and *L.salivarius* B-1950.

All starters were added at level of 3%. Inoculated yoghurt bases were then poured into 70ml sterile plastic cups and incubated at 42°C until pH reached 4.5, followed by cooling to 4°C and storing for two weeks.

### Analytical procedures:

Yoghurt was sampled when fresh and after 15days of storage.

### Microbiological analysis:

Viability of lactobacillus strains was monitored after production (zero time) and 15days of storage. To

this end, 10g portions of duplicate yoghurt samples were blended with 90ml of simulated gastric juice prepared as described above and enumerated after incubation at 37°C for 2h, reflecting the time spent by food in the stomach. Then samples were submitted to serial dilutions of peptone water. Viability of *L. johnsonii*, *L. gasseri* and *L. salivarius* were determined on MRS-raffinose agar (Abd El-Khalek *et al.*, 2004), MRS agar (Salem *et al.*, 2006) and MRS mannitol agar (Salem *et al.*, 2007) respectively. The plate's incubation was done at 37°C for 72h, in an anaerobic environment (BBL Gas Pak Becton Dickinson, Cockeysville MA, USA) for all lactobacilli.

#### Sensory evaluation of yoghurts:

Sensory evaluation of yoghurts was carried out when fresh and after 15 days of storage at 4°C. A panel consisting of 20 members evaluated the yoghurt samples presented in cooled cups in individual booths at room temperature. Samples were evaluated for flavour (50 points), body and texture (40 points) and appearance (10 points) according to (Abd El-Khalek *et al.*, 2004).

### 3. Results

Comparative survival of probiotic lactobacilli in simulated gastric juice containing protective nutrients:

The effects of simulated gastric juice containing protective materials in vitro on viability of tested probiotic lactobacilli strains are presented in Figs (1, 2, 3, &4).

In general, each strain showed lower viability in simulated gastric juice either in control or containing protective materials at pH 2.0 than in simulated gastric juice with pH 3.0 or pH 4.0.

When the simulated gastric juice was at pH 2.0, all the strains showed progressive reduction in viability during 180 min of simulated gastric transit, especially *L. bulgaricus*, which lost total viability after 180 min of simulated gastric transit in control (Fig.1.a.).

When the simulated gastric juice was at pH 3.0, *L. salivarius* had the highest survival rate over the 180 min of exposure to simulated gastric juice for control treatments. While the poorest survivor was *L. bulgaricus*, whose concentration declined to undetectable levels after 180 min of exposure (Fig 1.b).

When the simulated gastric juice was at pH 4.0, all of the tested four strains retained the same level of viability during 180 min of simulated gastric juice

transit in the absence of a protective matrix, such as sodium caseinate, whey protein or starch (Fig.1c.).

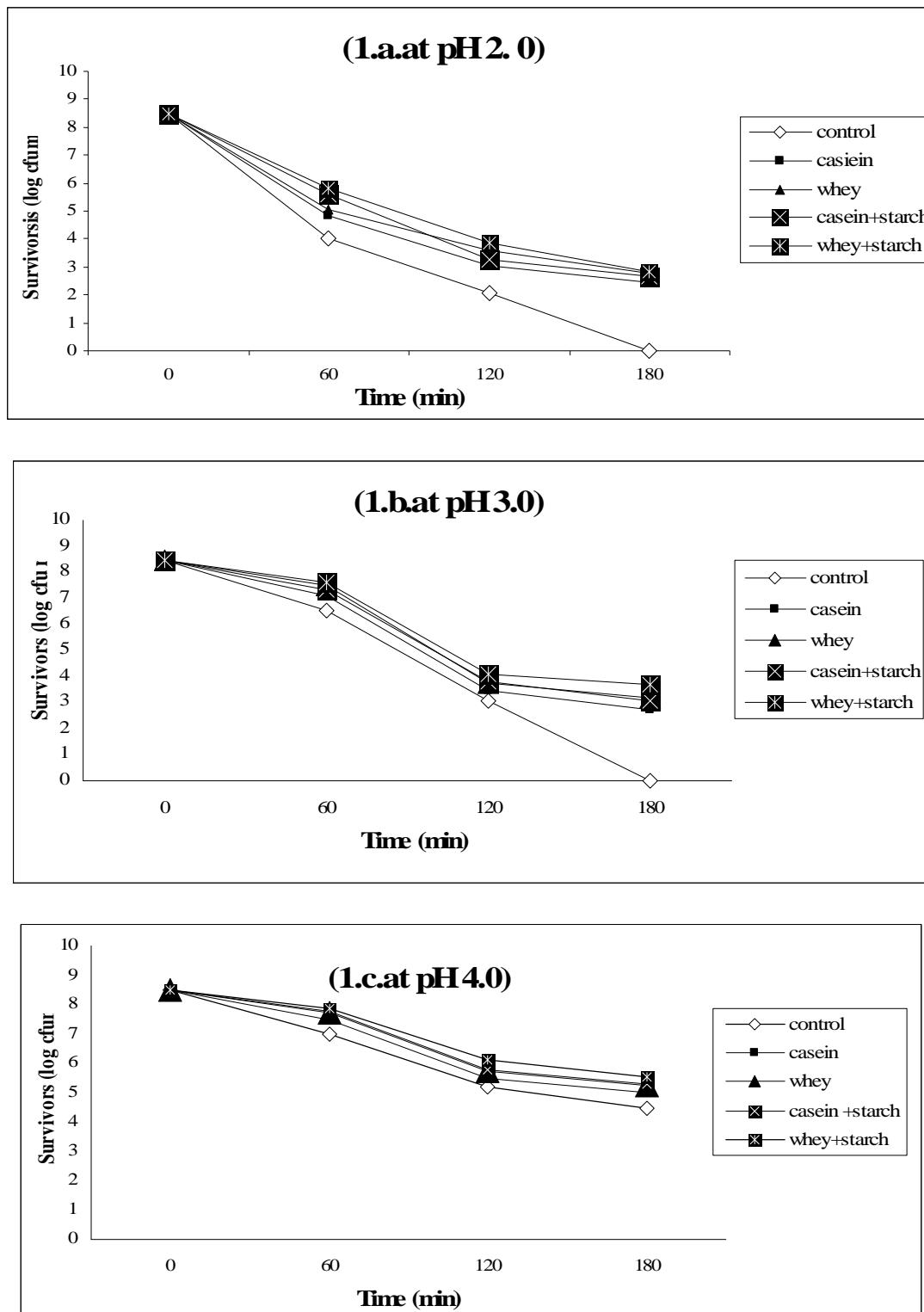
The effect of sodium caseinate and whey protein addition, singly and in combination with starch on viability during simulated gastric transit with pH 2.0, 3.0 and 4.0 simulated gastric juice is presented in Figs(1,2,3 and 4). In general, sodium caseinate and whey protein addition improved simulated gastric transit tolerance. In this regard, all tested strains exhibited complete tolerance to simulated gastric transit in the presence of sodium caseinate or whey protein singly and in combination with starch. The results showed that the greatest survival effect attributable to sodium caseinate plus starch occurred in *L. salivarius* followed by *L. johnsonii* at pH 4.0.

The strain of *L. bulgaricus* had a poor survival during 180 min at pH 2.0 and 3.0.

The intrinsic resistance to acid of *L. bulgaricus* is poor (Conway *et al.*, 1987 and Charteris, *et al.*, 1998). In this study, intrinsic resistance to gastric transit tolerance was observed to be rare probiotic property among the strains examined and to be influenced by the presence of milk proteins (sodium caseinate and whey protein, singly and in combination with starch).

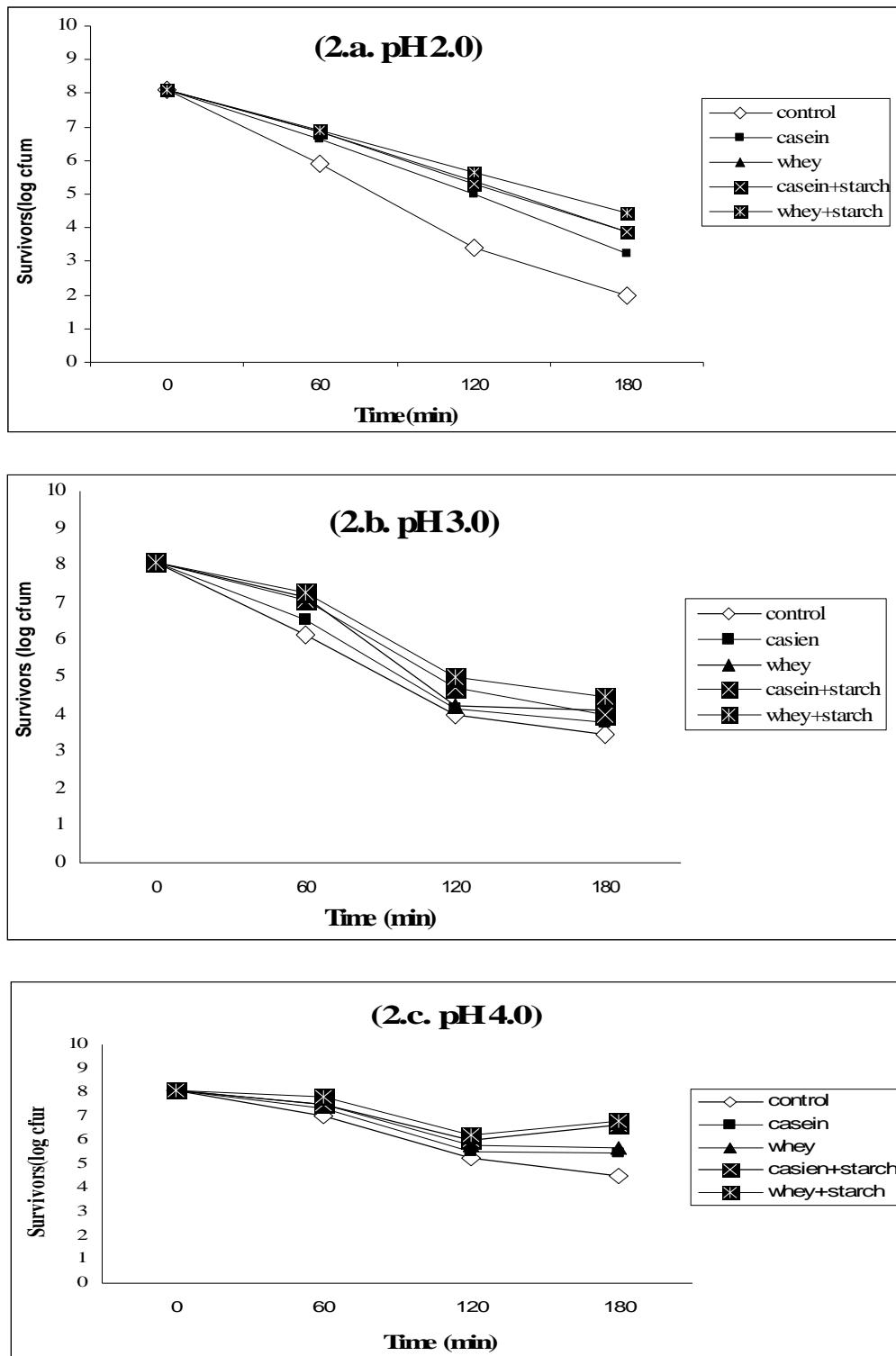
Although pH could be used as, a suitable direct measure for selection of probiotic strains, most probiotics are consumed in food products. The presence of food and food ingredients has been reported to improve viability of microorganisms during gastric transit (Huang and Adams, 2004). The suggested mechanism for the beneficial effect of food ingredients is the pH increase of the gastric contents resulting from the addition of the food (Zarate *et al.*, 2000). In the current study, the presence of milk proteins, singly and in combination with starch at pH 2.0 and pH 3.0, exerted a major effect on the gastric tolerance of some strains but not others. In this regard, *L. johnsonii*, *L. gasseri* and *L. salivarius* were capable of undiminished survival during simulated gastric transit in the presence of sodium caseinate, whey protein and their combination with starch. These data indicate that some strains of lactobacillus species may survive passage through the human stomach, particularly when ingested with milk products or milk protein-based foodstuffs.

Survival of lactic acid bacteria in human gastric juice adjusted to low pH has been previously shown to be enhanced by the addition sodium caseinate, whey protein and skim milk (Conway *et al.*, 1987 and Charteris *et al.*, 1998).



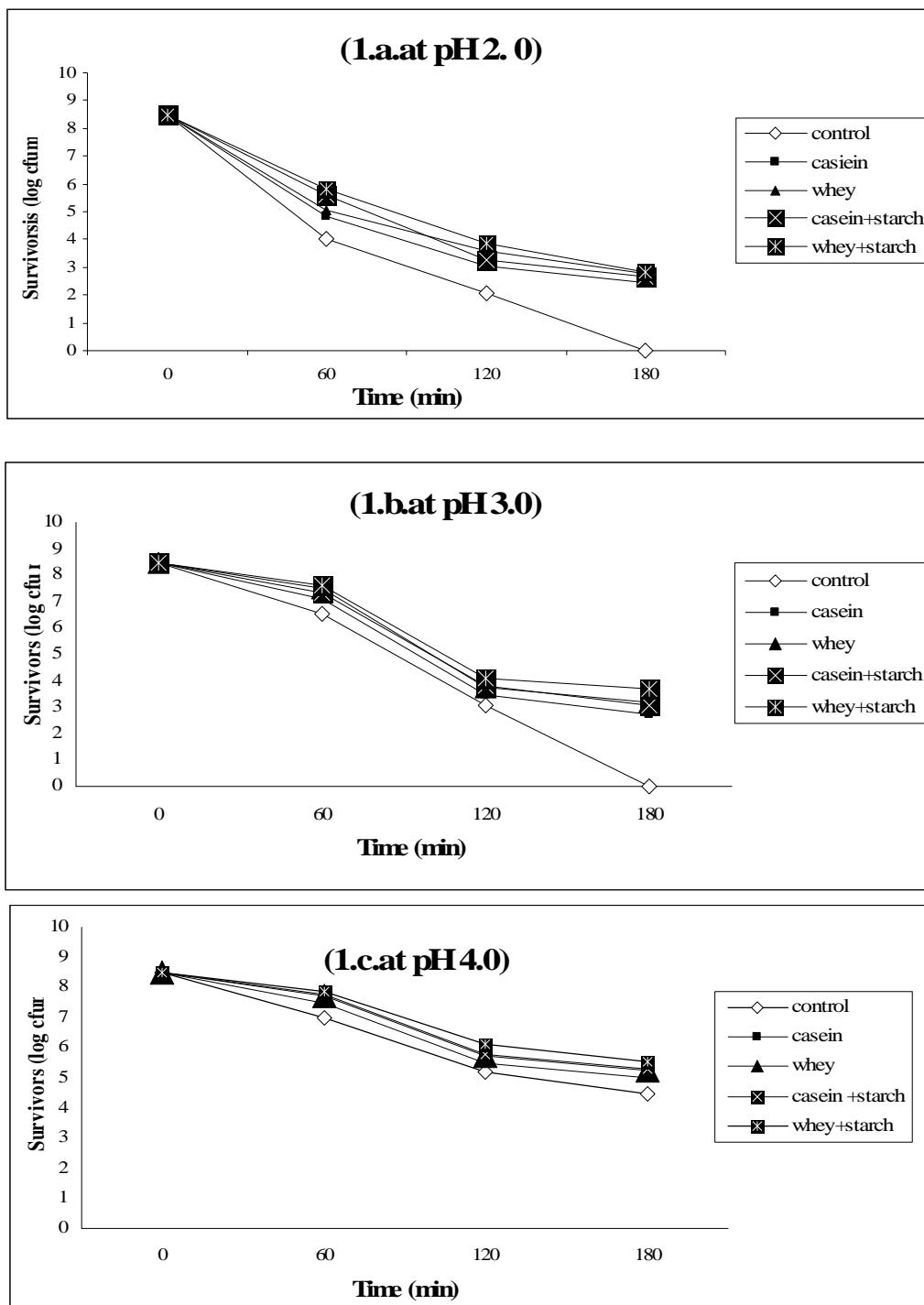
The data are the means of triplicate experiment

**Fig(1):** Survival of *L.bulgaricus* in simulated gastric juice containing sodium caseinate, whey Protein, (sodium caseinate and starch) and (whey protein and starch) at pH 2.0, pH 3.0 and pH 4.0.



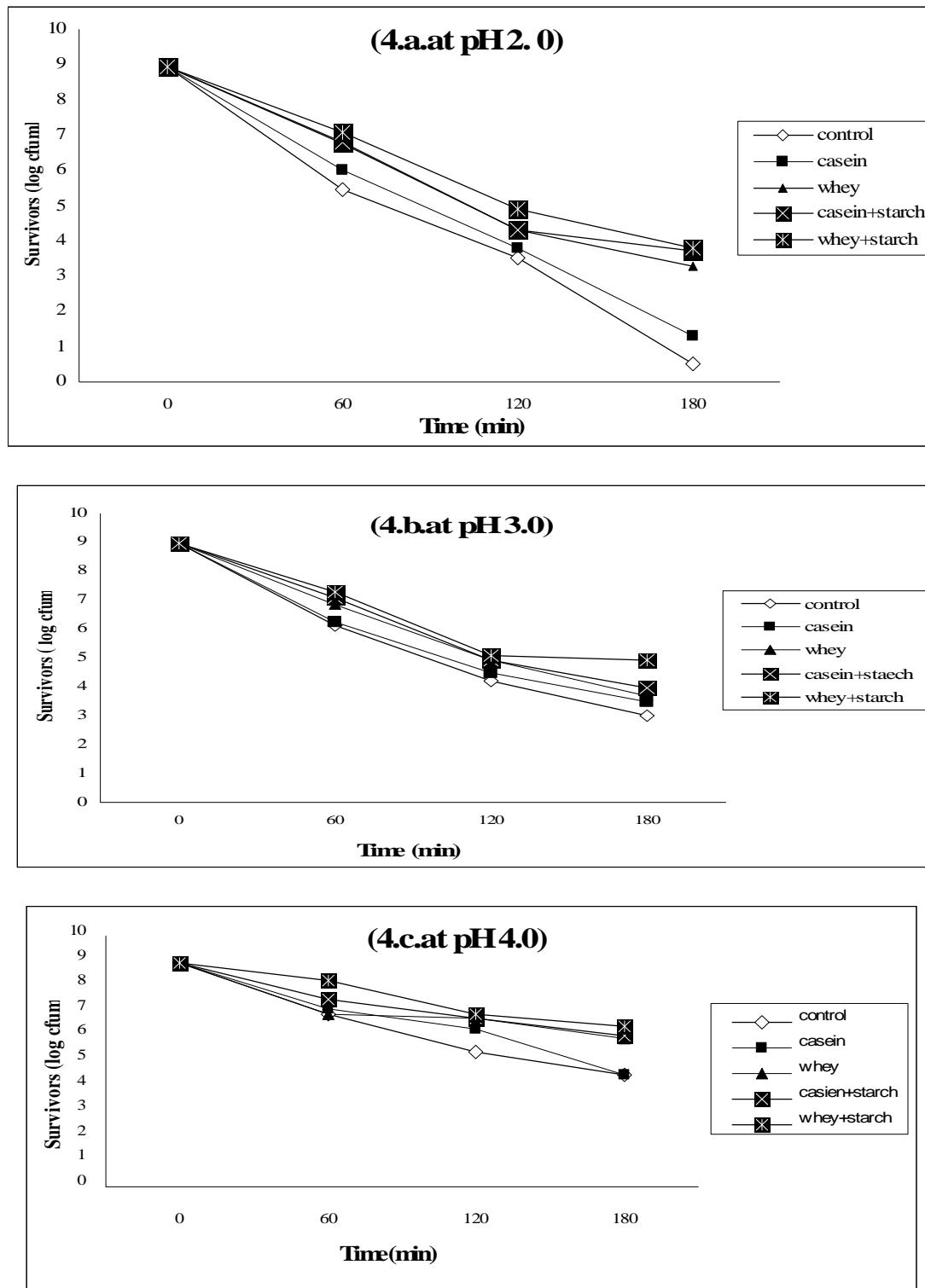
The data are the means of triplicate experiment

**Fig(2):** Survival of *L.johnsonii* in simulated gastric juice containing sodium caseinate, whey Protein, (sodium caseinate and starch) and (whey protein and starch) at pH 2.0,pH 3.0 and pH 4.0.



The data are the means of triplicate experiment

**Fig(3):** Survival of *L.gasseri* in simulated gastric juice containing sodium caseinate and whey (sodium caseinate and starch) and (whey protein and starch) at pH 2.0,pH 3.0 and pH 4.0.



The data are the means of triplicate experiment

**Fig(4):** Survival of *L. salivarius* in simulated gastric juice containing sodium caseinate, whey Protein, (sodium caseinate and starch) and (whey protein and starch) at pH 2.0,pH 3.0 and pH 4.0.

Viability of probiotic lactobacillus strains in yoghurt supplemented with prebiotics :

The viability of the three strains *L. johnsonii*, *L. gasseri* and *L. salivarius* in yoghurt containing milk proteins (whey protein or sodium caseinate) in combination with starch stored at a refrigerator ( $\sim 4^{\circ}\text{C}$ ) for two week is shown in Table (1). In general, the viability of all three strains decreased during storage. However, the viability was in many cases higher than that of the control, without protective materials. On an average, best viability was observed with whey protein plus starch.

The highest viability of 84.04% was recorded for *L. johnsonii* with whey protein plus starch. Overall, sodium caseinate plus starch was the least effective in maintaining viability, with average viabilities of 60.6%, 52.27 and 59.37%. The lowest

viability was recorded by *L. gasseri* with an average viability of 52.27 %.

The control samples containing no protective materials had average survival rate of 34.32%, 22.69 % and 40% for *L. johnsonii*, *L. gasseri* and *L. salivarius*, respectively. Sodium caseinate and whey protein plus Hi-maize starch were only helpful in improving viability of probiotic organisms in yoghurt during storage (Table 1).The improved viability is possibly due to prebiotics providing extra solids, which tend to protect cells from injury (Capela *et al*, 2006).

Viability of lactobacilli is affected because of several factors including acid produced during fermentation, oxygen content in the product and oxygen permeation through the packaging material (Desai *et al*, 2004).

**Table (1):** Viability of Lactobacillus strains in yoghurt supplemented with milk proteins plus starch during storageat  $4^{\circ}\text{C}$  for days.

Lactobacillus strains	Reading interval	Control	Whey + starch	Casein +starch
		Count cfu/m		
<i>L. johnsonii</i>	Zero time	$6.7 \times 10^8$	$9.4 \times 10^8$	$8.6 \times 10^8$
	15day	$23 \times 10^6$	$7.9 \times 10^8$	$5.2 \times 10^8$
	% viability	34.32	84.04	60.6
<i>L. gasseri</i>	Zero time	$2.6 \times 10^8$	$5.7 \times 10^8$	$4.4 \times 10^8$
	15 days	$5.9 \times 10^7$	$3.3 \times 10^8$	$10^8$
	% viability	22.7	57.9	52.27
<i>L. salivarius</i> ,	Zero time	$4.5 \times 10^8$	$6.3 \times 10^8$	$6.4 \times 10^8$
	15 days	$1.8 \times 10^7$	$4.2 \times 10^8$	$3.8 \times 10^8$
	% viability	40.0	66.66	59.37

% of viability = (count after 15 days cfu/g / zero time count cfu/g  $\times 100$ ).

**Table (2):** Organoleptic properties of probiotic yoghurt containing starch in combination with whey or casein throughout storage course on refrigerator.

Properties	Storage period (days)	Yoghurt treatments								
		C <sub>1</sub>	TI <sub>1</sub>	TII <sub>1</sub>	C <sub>2</sub>	TI <sub>2</sub>	TII <sub>2</sub>	C <sub>3</sub>	TI <sub>3</sub>	TII <sub>3</sub>
Flavor(50)	0	49	49	49	49	49	49	48	48	48
	15	48	48	48	48	48	48	46	46	46
Body&texture (40)	0	36	37	37	38	38	37	35	36	36
	15	37	38	38	38	38	38	30	30	30
Appearance (10)	0	9	9	9	9	9	9	7	7	7
	15	8	8	8	8	8	8	6	6	6
Total (100)	0	94	95	95	96	96	95	90	91	91
	15	93	94	94	94	94	94	82	82	82

C<sub>1</sub>: Control with *S. thermophilus*, *L.bulgaricus* and *L. johnsonii*.

TI<sub>1</sub>: *S. thermophilus*, *L.bulgaricus* and *L. johnsoni* containing starch and whey protein (each, 0.5% w/v).

TII<sub>1</sub>: *S. thermophilus*, *L.bulgaricus* and *L. johnsonii* containing starch and sodium caseinate (each 0.5% w/v).

C<sub>2</sub>: Control with *S. thermophilus*, *L.bulgaricus* and *L. gasseri* B-14168.

TI<sub>2</sub>: *S. thermophilus*, *L.bulgaricus* and *L. gasseri* B-14168 containing starch& whey protein (each 0.5% w/v).

TII<sub>2</sub>: *S. thermophilus*, *L.bulgaricus* and *L. gasseri* B-14168 containing starch & sodium caseinate (each 0.5% w/v).

C<sub>3</sub>: Control with *S. thermophilus*, *L.bulgaricus* and *L.salivarius* B-1950.

TI<sub>3</sub>: *S. thermophilus*, *L.bulgaricus* and *L. salivarius* B-1950 containing starch& whey protein (each 0.5% w/v).

TII<sub>3</sub>: *S. thermophilus*, *L.bulgaricus* and *L. salivarius* B-1950 containing starch & sodium caseinate (each 0.5% w/v).

### Sensory evaluation

In recent years, per capita consumption of yoghurt has increased drastically because many consumers associate yoghurt with good health (Hekmat and Reid 2006). Yoghurt is characterized as a fermented milk product with a refreshing flavor, a smooth viscous gel and a slight sour taste (Bodyfelt *et al.*, 1988). These sensory properties offer quality control criteria, and therefore, yoghurt should be evaluated for appearance, flavor, texture and overall quality.

Data presented in Table (2) show that the addition of starch in combination with whey or casein did not alter the mean appearance score of all samples of probiotic yoghurt either when fresh or after 15 days of storage. There were no flavor differences among T<sub>I1</sub>,T<sub>I1</sub>,T<sub>I2</sub>,T<sub>I2</sub> and T<sub>I3</sub>,T<sub>I3</sub> Table (2).These results indicate that the addition of *L. johnsonii*, *L. gasseri* and *L. salivarius* did not affect the flavor of the yoghurt. Yoghurt flavor is influenced by the presence of lactic acid and other flavoring compounds produced by culture bacteria during fermentation process.

The three probiotic strains did not inhibit the standard yoghurt cultures or overtly contribute to acid production from conversion of lactose to lactic acid.

The addition of starch in combination with casein did not alter texture of T<sub>4</sub> and T<sub>5</sub> in comparison to other treatments and control samples. The texture of yoghurt is affected by the rate of acid production during the fermentation process. Also, the heating processes of the mix at 85°C for 30 minutes affect the texture of the yoghurt. Heating the mix denatures whey protein, increases the water holding capacity of milk protein and reduces syneresis in yoghurt. Panelists rated the texture of T<sub>4</sub> and T<sub>5</sub> samples higher than other treatments.

This current study has demonstrated that although the viability of lactobacilli is affected by pH 2.0, most of the tested strains survived well at pH 3.0 and pH 4.0.

Furthermore, survival of lactobacilli in simulated gastric juice at pH 2.0 is enhanced by the addition of milk proteins singly or in combination with starch. Moreover, this study indicates that there are potential benefits of adding starch (0.5% w/v) plus sodium caseinate (0.5%w/v) to milk-based media aimed to preparing probiotics

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6/1/2010

## Extraction of oil from canola seeds with supercritical carbon dioxide: Experimental and Modeling

Soroush Zarinabadi<sup>1\*</sup>, Riyaz Kharrat<sup>2</sup>, Ali Vaziri Yazdi<sup>3</sup>

1, 3-Islamic Azad University- Science & Research Branch – Tehran, Iran

2-Petroleum University of Technology - Tehran, Iran

[avy123@behta.com](mailto:avy123@behta.com) , [3-2-kharrat@put.ac.ir](mailto:3-2-kharrat@put.ac.ir) , [1-zarinabadi@yahoo.com](mailto:1-zarinabadi@yahoo.com)

**ABSTRACT:** In this work extraction oil from canola (*Brassica Napus*) seed with supercritical CO<sub>2</sub> extraction at pressure of 1500 to 2750 Psi , temperature of 308 to 333 k, and particles size 0.08 to 0.2 mm in flow rate 5 Lit/hr was investigated in a bench scale apparatus, The extraction was modeled by the sovova extended lack's model. The fluid phase mass transfer coefficient (k<sub>f</sub>), solid phase mass transfer coefficient (k<sub>s</sub>), and hardly accessible solute (x<sub>k</sub>) were a just able parameter of Models. The broken and intact cells model fit the experimental data, quite well, showing the applicability of the model to the supercritical extraction system studied here. [Journal of American Science. 2010;6(11):368-373. (ISSN: 1545-1003)].

**Keywords:** supercritical fluid extraction, canola oil, mathematical modeling, sovova model

### Introduction

Supercritical carbon dioxide (SC-CO<sub>2</sub>) extraction of oil from seed is alternative process to solvent extraction, hydro distillation and steam distillation because of certain advantage of SC-CO<sub>2</sub> with low critical temperature. The extraction of vegetable oils using supercritical carbon dioxide has been studied as a potential alternative to the current industrial process of expeller pressing, prepress solvent extraction and straight liquid solvent extraction. Hexane is widely used as solvent in the processing of vegetables oils; however, the limitation of this extraction method development of alternative extraction procedures such as supercritical fluid extraction with carbon dioxide. This solvent has advantages such as nontoxicity, non explosive, non flammability, low cost, availability and ease of removed from extract oils. Compared to the liquid extraction the investment costs are higher but operating costs are lower due to simple solvent regeneration. SFE of seed oil has been studied by several works (Brunner, 1985; Goodarznia, & Bikini, 1998). Mathematical models used for extraction of solute from natural matrixes are classified as (1) empirical models (Esquivel, 1999; McHugh & Krakens, 1986), (2) models based on heat transfer analogue (Mgyesy, 1993; Nayyar, 1992), (3) models based on differential mass balance (Nolting, 1988; Papamichail et al., 2000; Reversion, 1993), since the scale up of the equipment and the evaluation of the cost of a process cannot be done without mass transfer rate data in a convenient form.

### Material and methods

For taking the extract oil out of Canola seeds by supercritical fluid, a thermodynamic machine is required to extract the high pressure. The designed method of SCFE has been presented by different researchers such as Van Leer, Paulaititis, Kurnik, Hollow, Red Krukonis, Eckert and Johnson, and Praunits(10, 11, 12). This pilot can be used for separation and extraction of oil out of canola seeds by SCFE laboratory pilot used in this research. As it can be seen in the FIG 1, this system can function in static and dynamic states. In this system two specially designed Transfer Vessel are used provide system pressure by Nitrogen gas. The possibility of establishing of flow of Co<sub>2</sub> gas in the machine in two separate, different directions by fixing the existing valve in the machine, are the characteristics of the system. The extraction vessels are made of stainless steel like other parts of the system. It also resists against 10000 Psi pressure. The container has a side glass made of silicon material, and can withstand high pressure, so seeing the contents of internal compartment, and the process of formation of fluids by machine is made possible. The ability of the designed pump for rotating the supercritical fluid within the system is another unique feature of this machine comparing to other devices. the mechanical part this pump are designed and made manually and has the ability of two phase fluid in thermal range up to 100 degrees Celsius and with Flow Rate between 2 liters to 8 liters regardless of creation of Cavitations in the system, can be tuned by operator. This device uses air bath system to provide temperature. The designed air bath is able to provide temperature of 100 degrees Celsius uniformly.

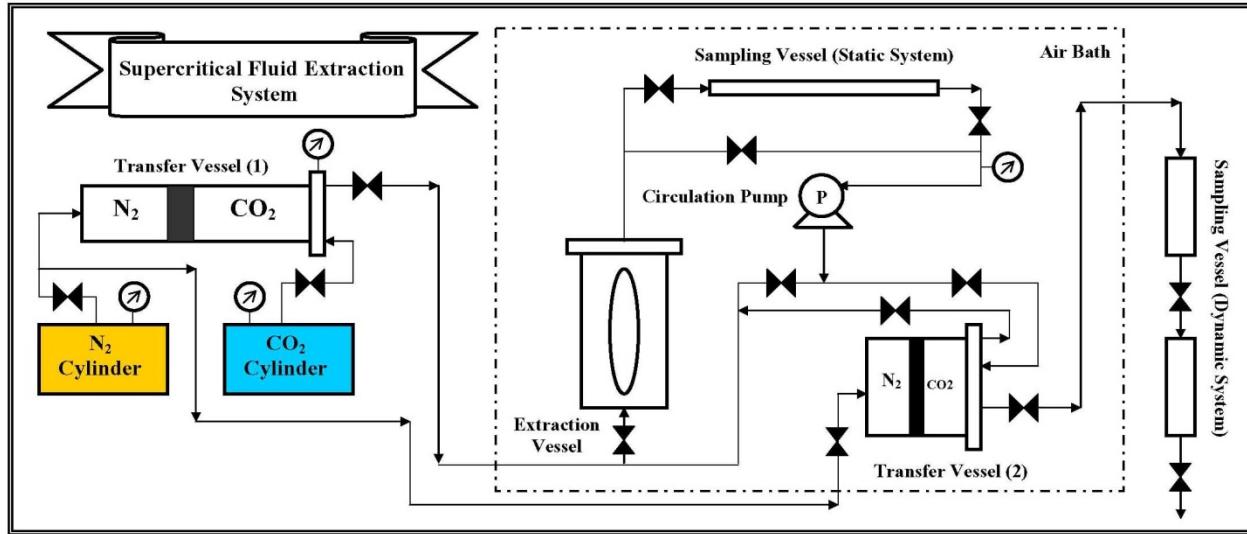


Fig1. Schematic diagram of the Supercritical Fluid Extraction System (SCFE) for Extraction of oil from canola seed with supercritical carbon dioxide

### Mathematical and Modeling

The broken and intact cells model was employed for correlating the experimental data (Papamichail et al., 2000; Reversion, 1993). This model based on differential mass balance equation in a fixed bed. Assuming plug flow and negligible axial dispersion. The pressure and temperature and bed void fraction ( $\varepsilon$ ) are constant during the extraction in the bed, the solute accumulation in the solvent is negligible. In this model the extraction process is divided into three periods. During the first period extraction precedes a constant rate determined by the oils solubility in sc-co<sub>2</sub>. The second period is transition period, in these periods easily accessible solute is completely depleted at the extractor's entrance the diffusion mechanism starts. In the last period mass transfer occurs only by the diffusion in the bed and inside the solid substratum particles. Sovova (1994) obtained analytically the equation for the overall extraction curve (Eq. (1)).

$$e = m_{extr} = \begin{cases} qy_r [1 - \exp(-z)] & q < q_m \\ y_r [q - q_m \exp(z_w - z)] & q_m \leq q < q_n \\ x_0 - \frac{y_r}{w} \ln \left\{ 1 + \left[ \exp \left( \frac{wx_0}{y_r} \right) - 1 \right] \exp [w(q_m - q)] \frac{x_k}{x_0} \right\} & q \geq q_n \end{cases} \quad (1)$$

Where:

$$q_n = q_m + \frac{1}{w} \ln \frac{x_k + (x_0 - x_k) \exp \left( \frac{wx_0}{y_r} \right)}{x_0}$$

$$\frac{z_w}{z} = \frac{y_r}{wx_0} \ln \frac{x_0 \exp [w(q - q_m)] - x_k}{(x_0 - x_k)}$$

$$q_m = \frac{(x_0 - x_k)}{y_r z}$$

Parameter z and w are directly proportional to the fluid phase and to the solid-phase mass transfer coefficients respectively and are given below:

$$z = k_f a_0 \rho / [q(1-\varepsilon) \rho_s]$$

$$w = k_s a_0 / [q(1-\varepsilon)]$$

In the present work, the parameters,  $k_f$ ,  $k_s$ ,  $x_k$ , and  $y_r$  were adjustable and determined by minimizing the errors:

$$\text{AAD (\%)} = \frac{1}{n} \sum_{i=1}^n \frac{|y_{\text{exp}} - y_{\text{cal}}|}{y_{\text{exp}}} \times 100$$

## Results and discussion

The operational conditions for each experiment are given in Table 1. The measured yield of oil extraction (Y) is defined by the following equation:

$$\text{Yield of oil extraction (\%)} = \frac{\text{Mass of oil extracted}}{\text{Mass of oil in seeds}} \times 100$$

Table 1. Yield of oil extracted under different extraction process conditions

Run	Pressure (Psi)	Temperature (K)	particle size (mm)	Yield (gr/gr)
1	1500	308	0.2	20.14
2	1750	318	0.18	34.32
3	2000	323	0.16	48.25
4	2250	308	0.14	55.09
5	2250	328	0.12	58.45
6	2500	318	0.1	58.25
7	2750	323	0.08	57.81

### Effect of pressure

Fig. 2 shows the effect of pressure on yield at temperature of 328 K and particle size 0.12 mm. with pressure increase, Co<sub>2</sub> density increases significantly, due to the increase of the solubility of the oil components. A faster extraction rate at low extraction times was observed when pressure increases. The volume mass transfer coefficient in the fluid phase,  $k_f$ , decreased with pressure increased. Consequently the mass transfer resistance increases contrast the mass transfer coefficient in the solid phase  $k_s$ , increases as pressure increase and the resistance in the solid decreases (Table 2).

### Effect of temperature

As it is shown in Fig. 3 temperature increases, the extraction rate decreases, the solubility of oil directly affects the extraction rate and it is controlled by a balance between the SC-Co<sub>2</sub> density and the oil vapor pressure. The overall mass transfer coefficient in the fluid phase increased with increase temperature. In contrast the overall mass transfer coefficient in the solid phase increased with temperature at 2250 Psi. (Table 2)

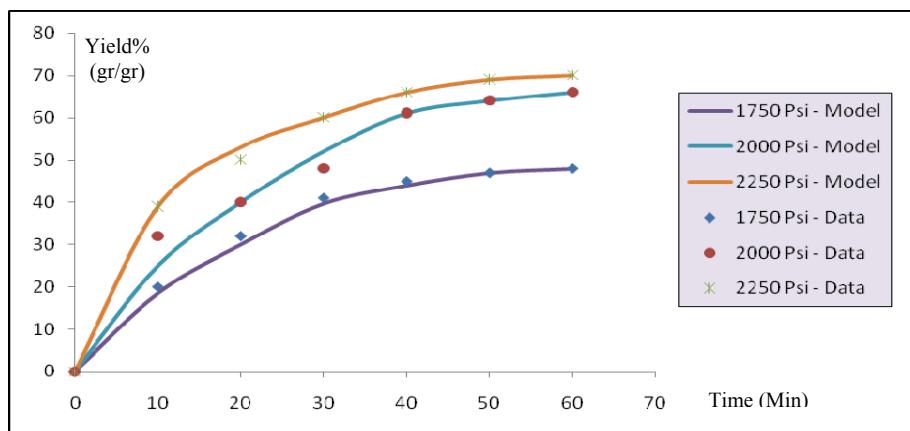


Fig 2. Effect of pressure on the extraction yield of oil at T= 328 K and dp= 0.12 mm

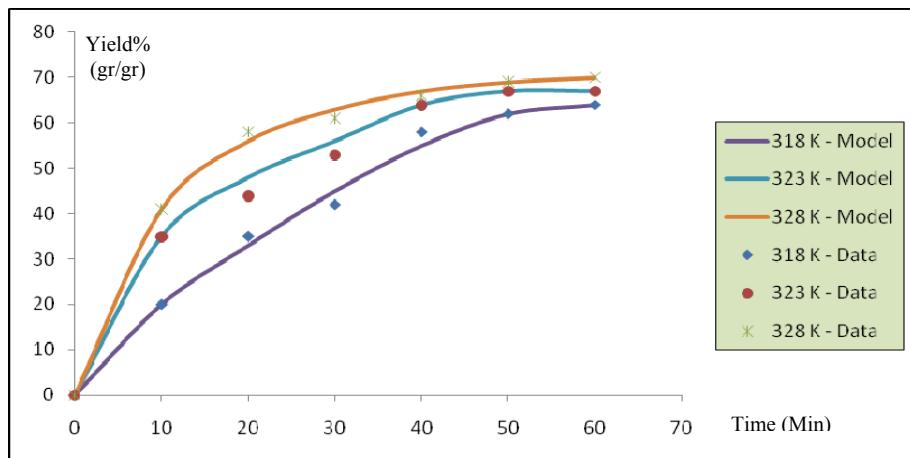


Fig 3. Effect of temperature on the extraction yield of oil at P= 2250 Psi and dp= 0.12 mm

Table 2. Calculated values of parameters and AAD (%) in BIC model.

Run	X <sub>0</sub>	Y <sub>P</sub>	X <sub>H</sub>	k <sub>PA</sub>	k <sub>ea</sub> *10 <sup>5</sup>	% AAD
1	0.54	0.00974	0.463	0.0079	3.01	5.03
2	0.54	0.00583	0.462	0.008	0.104	7.1
3	0.54	0.003	0.5	0.0155	1.157	5.43
4	0.54	0.00967	0.47	0.0056	1.58	3.85
5	0.54	0.00948	0.472	0.00486	1.356	4.01
6	0.54	0.00953	0.4805	0.00756	1.597	5.25
7	0.54	0.00298	0.52	0.0159	1.52	4.87

## Effect of particle size

At 2250 Psi pressure and 328 K, the yield increases with decreasing particle size (Fig 4), grinding of canola before extraction not only increase the interfacial area but also releases oil from the broken cells, results indicated that extraction rate is high if the oil is released on the surface of particle, and it is comparable very slow if it is embedded in the kernel particles. More ever, after milling the diffusion paths in the solid matrix become shorter results in a smaller intra particles resistance to diffusion. But production of very small particles could produce bed caking with formation of channels along the bed in which supercritical fluid can preferentially flow, thus reducing the extraction efficiency in the fluid phase,  $k_f a$ , and solid phase,  $k_s a$ , increased (Table 2).

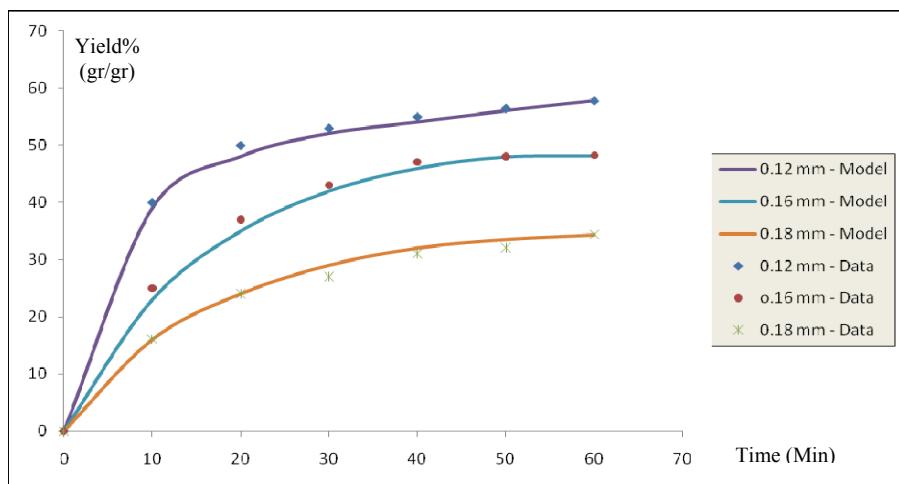


Fig 4. Effect of particle size on the extraction yield of oil at P= 2250 Psi and T= 328 K

## Conclusion:

The results indicated that the extraction curves in a plot of extraction yield versus time are significantly affected by the extraction pressure and particle size. But temperature has a slight effect on the extraction curves. Model of sovova represented canola oil extraction well. A theoretical model based on the evidence that part of the oil is freely available to the solvent after pre-treatment of the seeds was successfully used to fit the experimental extraction curves.

## Nomenclature

- a specific interfacial area ( $\text{m}^2/\text{m}^3$ )
- e Mass of extract ( $\text{kg}$ )
- $k_f a$  fluid-phase mass transfer coefficient ( $\text{s}^{-1}$ )
- $k_s a$  solid-phase mass transfer coefficient ( $\text{s}^{-1}$ )
- n number of data point
- q Mass of solvent ( $\text{kg}$ )
- $q_m$  q value at start of the extraction of difficult Accessible oil ( $\text{kg}/\text{kg}$ )
- $q_n$  q value at end of the extraction of easily Accessible ( $\text{kg}/\text{kg}$ )
- $q'$  specific flow rate ( $\text{kg}/\text{s}$ )
- W parameter of solid-phase mass transfer
- $\rho_s$  density of solid phase

$\rho$  density of solvent ( $\text{kg}/\text{m}^3$ )

$\varepsilon$  bed voidage

Z parameter of fluid phase mass transfer

$y_r$  oil solubility in the supercritical fluid

$x_k$  hardly accessible solute ( $\text{g/g}$ )

$x_0$  initial oil concentration in the solid ( $\text{g/g}$ )

## Corresponding author:

Soroush Zarinabadi –

Ph.D student of chemical engineering –

Islamic Azad University

Tehran Science & Research Branch

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8/21/2010

# Biochemical Significance of Proinflammatory Cytokines in Psoriasis vulgaris among Egyptian Patients

Halla M. Ragab\*, Nabilah Abd El Maksoud\* and Mohamed M. Farid Roaiah\*\*

\*Department of Biochemistry, Genetic Engineering and Biotechnology Division, National Research Centre, Dokki, Giza, Egypt. \*\* Dermatology & Andrology and S. T. D.S, Kasr El Aini Hospital, Cairo university.

## Abstract

**Background:** Psoriasis has been characterized by hyperproliferation accompanied by acanthosis and aberrant differentiation of keratinocytes. Several growth factors and cytokines, are assumed to be important. Recent studies indicate that various cytokines including tumor necrosis factor -  $\alpha$  ( TNF -  $\alpha$  ), IL - 2R and IL - 6 play an essential role in the induction and maintenance of psoriatic lesion.

**Objectives:** To analyse relevant inflammatory mediators in the serum of patients with active psoriasis ( Psoriasis vulgaris ) of mild-to-moderate and severe psoriasis compared to healthy controls.

**Patients / Methods:** Forty psoriasis patients were recruited from the dermatology outpatient clinic of Cairo University Hospital. Patients body mass index ( BMI ), waist circumference and psoriasis area and severity index ( PASI ) were recorded. Fasting serum samples were obtained on enrolment. All the patients did not receive any treatment ( locally or systemically ), for at least four weeks before enrolment. Age, sex and ( BMI ) matched with forty healthy controls were also recruited. Serum TNF -  $\alpha$ , IL - 2R and IL - 6 levels were estimated using an Enzyme-Linked Immunosorbent Assay ( ELISA ) technique. The patients group were subdivided to two groups according to the diseases severity, PASI , into, mild-to-moderate psoriasis group and severe psoriasis group.

**Results:** Serum TNF -  $\alpha$ , IL - 2R and IL - 6 were all statistically significant elevated in the patients group compared to healthy controls (  $p < 0.05$  ). Also they were all statistically significant increased in severe psoriasis compared to mild-to-moderate psoriasis (  $p < 0.05$  ).

**Conclusions:** These data support the view that serum TNF -  $\alpha$ , IL - 2R and IL - 6 are involved in the pathogenesis of psoriasis, possibly by induction and maintenance of psoriatic lesion. We recommend a use of an array of these cytokines as a useful follow-up marker for monitoring of psoriatic patients and optimizing therapeutic strategies. Also we suggest the study of antisense therapy using the antibody of these cytokines in psoriatic patients.

[Journal of American Science 2010;6(11):374-380]. (ISSN: 1545-1003).

**Keywords:** Psoriasis vulgaris, Cytokines, TNF -  $\alpha$ , IL - 2R and IL - 6.

## 1. Introduction

Psoriasis is relatively common, chronic, inflammatory and hyperproliferative skin disease that may appear at any age and affect any part of the skin. It affects 1.4 % to 2.0 % of the population and comprises 2.6% of skin related visits to primary care physicians, or between 0.3% and 1.6% of all visits to family physicians. It is a very troublesome disease with a high economic impact ( **Ulrich and Kristian 2009** ). The disease often persists for life, and the patient has an increased risk of cardiovascular diseases and their complications. One out of five patients develops psoriatic arthritis. The clinical picture of psoriasis is highly variable with regard to lesional characteristics and the severity of disease ( **Batya et al. 2010** ).

Psoriasis vulgaris is a multifactorial heritable disease characterized by severe inflammation resulting in poorly differentiated, hyperproliferative keratinocytes. It is including genetic background, environmental factors, and vascular and immune system disturbances. Current research is dominated by the hypothesis that an immunological disorder with inflammatory reaction, mediated through T-

lymphocytes, plays a key role in the pathogenesis of psoriasis ( **Nograles et al. 2010** ).

The characteristic histological features of the disease are epidermal hyperproliferation and infiltration of both dermis and epidermis by inflammatory cells including neutrophils, lymphocytes, macrophages and mast cells. Interactions between infiltrating T cells and skin resident cells (keratinocytes, fibroblasts, endothelial cells) are often mediated by the synthesis and release of different proinflammatory cytokines ( **Krueger and Ellis 2005** ).

Recently, much attention has been directed towards the influence of cytokines in psoriasis, as they play an important role in inflammatory diseases. In addition, a number of studies have suggested that various cytokines released by keratinocytes and inflammatory leucocytes could contribute to the induction or persistence of the inflammatory processes in psoriasis; however, the precise mechanism of their involvement in psoriasis remains unclear. Few studies have been reported on serum cytokine levels that may be expected to alter if they are involved in the pathogenesis of psoriasis ( **Kristina and Krueger 2009** ).

Although the cytokine mediated response is an essential part of the natural protective mechanism, excessive production of pro-inflammatory cytokines, or production of cytokines in the wrong biological context are associated with the pathology in a wide range of diseases including psoriasis. At the present time, one of the main areas of research in the psoriasis field concerns the role of cytokines in the pathogenesis of this disease. Different cytokines play a part in sustaining the two main characteristics of a psoriatic lesion; keratinocyte hyperproliferation and inflammation (**Stephen and Gelfand 2008**).

Interleukin - 2 ( IL - 2 ), interleukin - 6 ( IL - 6 ), and tumor necrosis factor alpha ( TNF -  $\alpha$  ) are the hallmark cytokines in a psoriatic cytokine network. Several investigators have suggested the possible use of TNF -  $\alpha$  , IL - 6 , IL - 8 and soluble interleukin - 2 receptor ( IL - 2R ) as markers of disease severity in psoriasis. The interleukin - 2 receptor ( IL - 2R ) is a heterotrimeric protein expressed on the surface of certain immune cells, such as lymphocytes, that binds and responds to a cytokine called interleukin 2 (**Hidetoshi et al. 2009** ).

Recent studies indicate that various cytokines including tumor necrosis factor alpha ( TNF -  $\alpha$  ) play an essential role in the induction and maintenance of psoriatic lesion. TNF -  $\alpha$  is a 17 - k D polypeptide that plays a central role in the regulation of innate immune responses. It is involved in stimulating the production of inflammatory cytokines, inducing the expression of cell surface adhesion molecules, enhancing the phagocytic/bactericidal properties of macrophages, and activating apoptotic pathways. TNF -  $\alpha$  is produced by a wide variety of cells, ranging from lymphocytes and monocytes, to keratinocytes, mast cells and antigen presenting cells in the skin. It is believed to contribute to the pathogenesis of psoriasis through its ability to both promote immune cell trafficking to the skin and induce keratinocyte proliferation (**Gottlieb et al. 2003** ).

Overexpression of IL - 6 has been implicated in the pathology of numerous autoimmune and chronic inflammatory diseases, including psoriasis (**Kristina and Krueger 2009** ). Cytokine IL - 6 is a multifunctional immunoinflammatory mediator with a MW of 25 to 30 kDa protein (**Kawano et al. 1988** ). It is produced by a number of different cell types including keratinocytes (**Kupper et al. 1989** ) and leukocytes (**Baumann et al. 1984** ). It also stimulates the proliferation of human keratinocytes in culture (**Krueger et al. 1991** ) and this proliferative effects are suggested to be mediated indirectly via the epidermal growth factor/transforming growth factor alpha receptor (**Elder et al. 1992** ). Thus IL - 6 has been speculated to play an important role in the pathogenesis of psoriasis, and in fact, its enhanced expression was demonstrated in the psoriatic lesional skin (**Ohta et al. 1991** ), together with the reports of its increased production by monocytes and of its

elevated circulating levels (**Neuner et al. 1991** ). Overexpression of IL - 6 has been implicated in the pathology of numerous autoimmune and chronic inflammatory diseases, including psoriasis (**Wojciech et al. 2008** ).

## 2. Aim of the work

This study aimed to evaluate the association of a panel of some proinflammatory cytokines (TNF -  $\alpha$  , IL - 6 , and soluble interleukin - 2 receptor ( IL - 2R ), in the serum of patients with active psoriasis ( Psoriasis vulgaris ) and compare them to healthy controls.

Also, to investigate which would be an attractive, patient-independent, and observer-independent marker of disease severity. And, to determine the use of these cytokines as markers of disease severity in patients of mild – to - moderate and severe psoriasis.

## 3. Material and Methods

### 3.1. Subjects

This study comprised forty consecutive patients of psoriasis were recruited from the dermatology outpatient clinic of Cairo University Hospital. All the patients were subjected to detailed examination including the elicitation of dermatological and psychiatric complaints. The diagnosis was made clinically, based on the presence of characteristic plaque-type psoriatic lesions. All the patients were asked to provide socio-demographic data, medical history, and family history. Other questions included the duration of disease, age of onset of the disease, any treatment taken and use of psychotropic drugs. Dermatological examination, hairs, mucosal involvement and nail changes were recorded. The patients group were subclassified to two groups according to the diseases severity, severity index ( PASI ) into, mild-to-moderate psoriasis group and severe psoriasis group. Forty healthy age and sex matched volunteers with no family history of psoriasis were included in the study as a control group. The purpose and nature of the study were explained to all subjects. All included subjects have consented to be enrolled in this study.

### 3.2. Exclusion Criteria

Obese subjects with history of acute or chronic infections, liver disease, renal disease, recent history of cardiovascular disorder, hypertension, neurological disease, or diabetes mellitus were excluded from the study. Moreover, patients who had received oral or topical antipsoriatic therapy within four weeks were not included in the study.

### 3.3 Methods

#### 3.3.1. Clinical Assessment

Disease severity was monitored by assessing the psoriasis area and severity index ( PASI ). It includes assessment and recording of erythema, infiltration, desquamation and extent of the disease ( area % ) by using numerical rating of 0 - 4 for each of the parameter:

0 for absent; 1 for slight (light pink, rare scales, no elevation with area involvement < 10 % ); 2 for moderate ( light red, poorly defined scales, slight elevation with area involvement 10 - 30 %); 3 for severe ( red, defined scales, moderate elevation with area involvement 30 - 50 % ); and 4 for very severe (very red, heavy scales, marked elevation with area involvement 50 - 70 % ). Accordingly, mild-moderate psoriasis and severe psoriasis were defined as PASI < 15 and PASI >15, respectively.

#### 3.3.2. Blood Sampling

Blood samples (10 ml) were collected from patients and control subjects in serum separator vacutainers ( BD Vacutainer Systems, Plymouth, UK). Sera were separated and immediately stored at - 80° C until analysis.

#### 3.3.3. Laboratory Investigations

Major laboratory parameters, including blood sedimentation rate, liver and renal function tests, blood cell counts; random blood sugar, were evaluated at the same time points for all participants to exclude any organic disease or inflammation.

#### 3.3.4. Serum Cytokines Measurements

The quantitative determination of TNF -  $\alpha$ , IL - 2R and IL - 6 levels were conducted by an Enzyme-Linked Immunosorbant Assay ( ELISA ) technique, using a commercial available kit. Every sample was run in duplicate, measurements differed by less than 10 %, and the mean value was calculated and used for statistical analysis.

**Assessment of plasma TNF-alpha :** Analysis was performed by TNF- alpha ELISA Kit, Diaclone research, (URS) - France (Catalog Number 850.090.096) .

**Assessment of human sIL-2R: Human sIL-2R** levels were measured using commercially available kit (ELISA) based on the sandwich principle, manufactured by T-Cell diagnostics (Endogen Inc., Cambridge, CA). The human sIL-2R concentrations were determined from the standard curve after being run concurrently with the standards .

**Assessment of plasma IL-6:** Analysis was performed using commercially available kit (IL-6 ELISA Kit), Diaclone Research, (URS), - France (Catalog Number 850.030.096).

The minimum detectable dose of IL-6 is less than 2pg/ml. Intra and Inter - Assay coefficients of

variation of the assay were 0.83-3.86% and 1.89-5.84%.

#### 3.3.5. Statistical analysis

All data were coded and entered using the program statistical package for social sciences (SPSS) version 15 under windows XP. Descriptive data was summarized using mean, standard deviation (SD). Linear regression analysis was done to test for significant predictors for psoriasis severity as measured by PASI score. P values < 0.05 were considered statistically significant.

### 4. Results

#### 4. 1. Clinical Data

Forty patients with psoriasis vulgaris were included in this study. Twenty of the patients had mild to moderate psoriasis (PASI <15), while the other twenty had severe psoriasis (PASI > 15). Of the forty patients, 26 were females (65%) and 14 were males (35%). Their age ranged between 18 – 62 years. The mean age and standard deviation (SD) was  $38.50 \pm 12.83$  years. The duration of the disease ranged between 4 months to 180 months, with a mean  $\pm$  SD  $57.05 \pm 54.08$ . The PASI score for clinical assessment ranged between 3.5 - 28.5, the mean  $\pm$  SD was  $14.61 \pm 6.6$ . The control group included 29 females (74.5%) and 11 males (25.5%). Their age ranged between 18 - 54 years with mean  $\pm$  SD  $35.70 \pm 9.09$ . Controls were age and sex matched.

#### 4. 2. Estimation of serum cytokines levels by ELISA technique

The demographic, clinical and biochemical data of the studied subjects are showed in Table ( 1, 2 ).

In this study we compare the serum levels of TNF -  $\alpha$ , IL - 2R and IL - 6 between forty psoriasis patients and forty age- and sex-matched healthy controls from the Egyptian population. All the patients were untreated, both locally and systemically, for at least four weeks before enrolment. It was also ensured that control subjects had no medication during the 4 weeks before blood sampling.

The mean value ( mean  $\pm$  SD ) of serum TNF -  $\alpha$  level estimated in patients with mild to moderate psoriasis was (  $121.24 \pm 59.35$  pg/ml ) and controls (  $30.85 \pm 25.45$  pg / ml ). A statistically significant difference was found in the serum TNF-  $\alpha$  level between patients and controls (  $P < 0.05$  ). However, when patients were evaluated according to disease severity, serum TNF -  $\alpha$  level was significantly higher in patients with severe psoriasis (  $173.23 \pm 70.45$  pg/ml ) than patients with mild to moderate psoriasis and controls (  $p < 0.05$  ) ( fig.1 ).

The mean value (mean  $\pm$  SD) of serum s IL - 2R level estimated in patients with mild to moderate psoriasis was (  $355.32 \pm 104.21$  pg/ml ) and controls (  $144.65 \pm 69.44$  pg / ml). A statistically significant difference was found in the serum s IL - 2R level between patients and controls (  $P < 0.05$  ). However,

when patients were evaluated according to disease severity, serum s IL - 2R level was significantly higher in patients with severe psoriasis ( $475.45 \pm 111.45$  pg/ml) than patients with mild to moderate psoriasis and controls ( $p < 0.05$ ) (fig.2).

The mean value (mean  $\pm$  SD) of serum IL - 6 level estimated in patients with mild to moderate psoriasis was ( $15.24 \pm 8.58$  pg/ml) and controls ( $5.99 \pm 1.34$  pg/ml). A statistically significant difference

was found in the serum IL - 6 levels between patients and controls ( $P < 0.05$ ). However, when patients were evaluated according to disease severity, serum IL - 6 level was significantly higher in patients with severe psoriasis

( $33.76 \pm 11.34$  pg/ml) than patients with mild to moderate psoriasis and controls ( $p < 0.05$ ) (fig.3).

**Table (1): The demographic data of the studied subjects**

	Control	Psoriasis patients
<b>Number</b>	40	40
<b>Age ( years )</b>		
<b>Range</b>	18 – 54	18 – 62
<b>mean <math>\pm</math> SD</b>	$35.7 \pm 9.09$	$38.5 \pm 12.83$
<b>Sex</b>		
<b>Male</b>	11 ( 25.5 % )	14 ( 35 % )
<b>Female</b>	29 ( 74.5 % )	26 ( 65 % )
<b>Duration of illness (months)</b>		
<b>mean duration <math>\pm</math> SD</b>		4 – 180 $57.05 \pm 54.08$
<b>PASI</b>		
<b>Range</b>		3.5 – 28.5
<b>Mean <math>\pm</math> SD</b>		$14.61 \pm 6.6$

**(Table 2): Serum levels of the studied cytokines in patients with Psoriasis vulgaris compared to healthy controls ( mean  $\pm$  SD )**

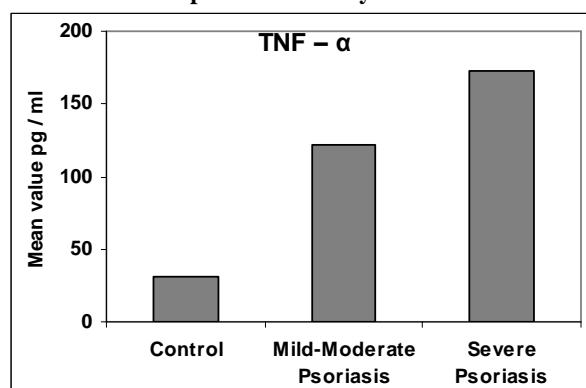
Cytokines	Mild to Moderate Psoriasis ( n = 20 )	Severe Psoriasis ( n = 20 )	Control ( n = 40 )
TNF – $\alpha$ ( pg / ml )	$121.24 \pm 59.35$	$173.23 \pm 70.45$	$30.85 \pm 25.45$
P value	*	*	
	**	**	
s IL - 2R ( pg / ml )	$355.32 \pm 104.21$	$475.45 \pm 111.45$	$144.65 \pm 69.44$
P value	*	*	
	**	**	
IL - 6 ( pg / ml )	$15.24 \pm 8.58$	$33.76 \pm 11.34$	$5.99 \pm 1.34$
P value	*	*	
	**	**	

Values are mean  $\pm$  SD (pg/ml of serum).

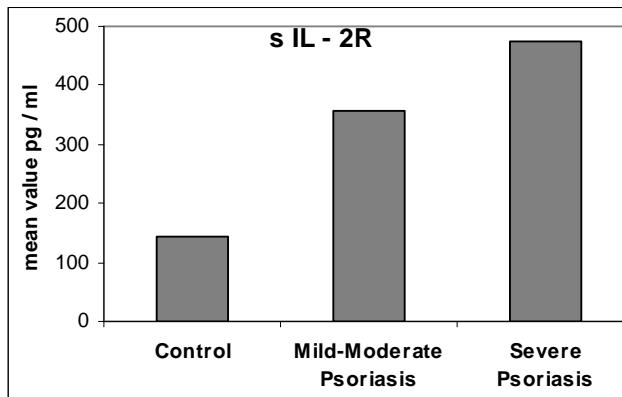
\*P: compared to control

\*\*P: comparison between mild to moderate psoriasis and severe Psoriasis significant ( $P < 0.05$ ) ( t - test ).

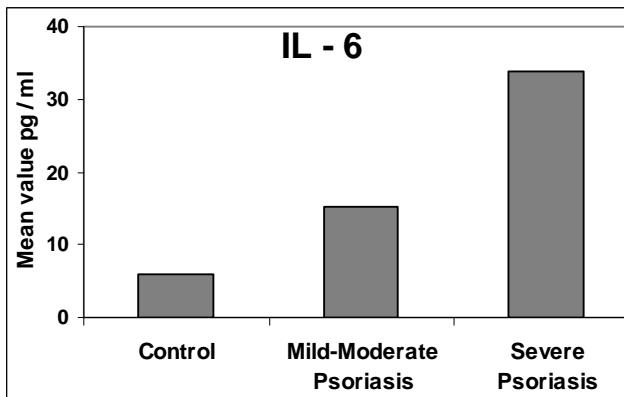
**( Fig. 1 ) : Serum levels of TNF –  $\alpha$  in patients with psoriasis vulgaris compared to healthy controls**



( Fig. 2 ) : Serum levels of s IL - 2R in patients with psoriasis vulgaris compared to healthy controls



( Fig. 3 ) : Serum levels of IL - 6 in patients with psoriasis vulgaris compared to healthy controls



## 5. Discussion

Psoriasis is a common inflammatory disease of the skin and joints. Its aetiology remains unknown, however, it has been linked to complex interactions between predisposing genes and the environment. The pathophysiology of psoriasis is characterized by epidermal hyperproliferation, enhanced antigen presentation, T helper ( Th - 1 ) cytokine production, T cell expansion, and angiogenesis. Tremendous advances in understanding of this disorder has led to the development of novel therapeutics and the FDA approval of more systemic agents for its treatment in the last 5 years than in the previous 50 years combined. Improved understanding of the pathogenesis of psoriasis has led to epidemiologic studies that have contributed towards further characterizing its natural history ( **Stephen and Gelfand 2008** ). In this study we focused on the impact of serum levels of proinflammatory cytokines (TNF -  $\alpha$ , IL - 2R and IL - 6 ) in psoriasis vulgaris In

Egyptian patients which are of major clinical relevance to the clinician.

**Sagawa et al. ( 1993 )** pointed out that TNF -  $\alpha$  in combination with other cytokines like IL-6 may be highly injurious due to complex interactions between these cytokines, suggesting a rationale for monitoring of multiple cytokines in the sera of psoriatic patients. Moreover, the cytokine assay results may vary due to the clinical stage and type of disease, methods used for cytokines detection and their sensitivities, lesion activity, interferences due to different drugs used, demographic differences in the patient groups, and the effect of concomitant pathologies.

We evaluated the association of serum levels of some proinflammatory cytokines *in vivo* and their correlation with severity of psoriasis. The serum levels of cytokines levels were determined with the use of the ELISA method. All mean values of patients were significantly higher than those of controls. There was a significant relation between serum levels of TNF -  $\alpha$ , IL - 2R and IL - 6 and the severity of the disease.

The clinical severity and activity of psoriasis, and those measurements of serum levels of these cytokines may be objective parameters for the disease severity.

In an earlier studies that was performed by **Ameglio et al., in 1994**, they shown that there was an observed significant reduction in IL - 6, IL - 8, IL - 2R and TNF -  $\alpha$  levels following effective therapies in psoriasis patients. Also, **Deeva et al. in 2010** had investigated in their study patients affected by very severe forms of psoriasis and they were characterized by increased plasma levels of IL - 4, IL - 6, MCP - 1, VEGF. Also, in mild to moderate psoriasis patients, they had showed higher levels of IL - 4, IL - 6, IL - 10, and IL - 13 when compared to healthy controls.

In our results the increment of the investigated cytokines, showed a significant increase in severe psoriasis than in mild-to-moderate ones which are not in agreement to the results obtained by **Deeva et al. in 2010** who found that there is no correlation between psoriasis severity assessed by PASI ( Psoriasis Area and Severity Index ) and levels of these mediators.

Our results are in agreement with earlier studies demonstrated by **Mohammad in 2005**, who showed a significant increase in levels of serum IL-6, IL-8, IL-2R and TNF-  $\alpha$  in Saudi psoriasis patients as compared with healthy controls. And he has been suggested that proinflammatory cytokines not only play a fundamental role in the worsening of the disease or activating its pathogenetic mechanisms, but are also directly related to the clinical symptoms and disease evolution after effective therapy.

**Eiko et al. in 2006** had shown that psoriatic lesions showed elevated mRNA expression for type 1 cytokines ( IFN - gamma, IL - 2, and TNF-  $\alpha$  ), compared with lesion-free psoriatic skin and normal skin, without a significant component of type 2 cytokines ( IL - 4, IL - 5, and IL - 10 ) which confirm our results in the increment of the studied serum cytokines .

Also, similar to our results **Ozer et al. in 2005** have demonstrated that, serum TNF -[alpha], IFN -[gamma], IL - 6, IL - 8, IL - 12, and IL - 18 levels were significantly higher in active psoriatic patients than in controls. Furthermore, high levels of these parameters have been correlated with the clinical severity and activity of psoriasis, and they concluded that measurements of serum levels of these cytokines may be objective parameters for the disease severity.

in Japanese patients with psoriasis, **Takahashi et al. in 2009** have shown that serum levels of tumour necrosis factor ( TNF )-alpha, interferon ( IFN ) - gamma, interleukin IL-2, IL - 6, IL - 7, IL - 8, IL - 12, IL - 17, IL - 18 and vascular endothelial growth factor ( VEGF ) were significantly increased in patients with

psoriasis compared with those of healthy controls. And, increased serum levels of these cytokines were correlated with PASI. Furthermore, these cytokine levels were decreased after psoriasis treatment.

As we gain further insight into the immunopathogenesis of psoriasis, we hope it will provide the basis for the development of safer, more efficacious, and more durable therapeutics in the future. Given its enormous toll on patient health and quality of life, steps should be taken to prevent or decrease the risk of psoriasis associated comorbidities.

## 6. Conclusion

Psoriasis is a common chronic relapsing and remitting papulosquamous skin disease that may appear at any age and affect any part of the skin. The systemic overexpression of a variety of proinflammatory cytokines such as TNF -  $\alpha$ , IL - 2 and IL - 6 have been evaluated in this study. We found that these cytokines are significantly elevated in patients suffering from psoriasis when compared to control. With increase of the severity of the disease, these cytokines are significantly elevated in severe psoriasis patients than in mild to moderate one which is attributed to the role of these cytokines in the pathogenesis and progress of psoriasis and their elevation is responsible for the development, maintenance and resolution of psoriatic lesions.

We suggest that, a use of an array of these cytokines may be considered as a useful follow-up marker for monitoring of psoriatic patients and optimizing therapeutic strategies. However, detailed time-course studies on sequential analysis of these cytokines in relation to disease severity and / or treatment modalities are warranted to ascertain its real application. Finally, we recommend the study of the effectiveness of use of antisense therapy using the antibody of these cytokines in psoriatic patients, in particular anti - TNF therapy.

## Corresponding author:

Halla M. Ragab

\*Department of Biochemistry, Genetic Engineering and Biotechnology Division, National Research Centre, Dokki, Cairo, Egypt.  
E-mail: hmragab@yahoo.com

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# Antihepatotoxic Effect of *Eruca Sativa* Extracts on Alcohol Induced Liver Injury in Rats

Jihan Hussein<sup>1</sup>, Azza Salah<sup>2</sup>, Fatma Oraby<sup>1</sup>, Amany Nour El-Deen<sup>2</sup> and Zakarya El-Khayat<sup>1</sup>

<sup>1</sup> Medical Biochemistry Department, National Research Center, Dokki, Giza, 12311, Egypt

<sup>2</sup> Biochemistry Department, Faculty of Science, Ain Shams University, 12311, Egypt

[jihan\\_husein@yahoo.com](mailto:jihan_husein@yahoo.com)

**Abstract:** Food derived antioxidants have a strong potential for long term use as chemopreventive agents in disease states involving oxidative stress, such as hepatitis and alcoholic liver diseases. This study aimed to investigate the effect of different extracts of *Eruca Sativa* in ethanol induced liver injury in rats. Eighty eight male albino rats were divided into 3 main groups included control, prophylactic and treated groups using different extracts of *Eruca sativa*. Serum liver functions tests, lipid profile and oxidants/antioxidants profile were estimated. The results showed that *Eruca sativa* extracts improved liver functions, Lipid profile and antioxidants parameters. We concluded that, *Eruca sativa* extracts may exert their prophylactic and treatment role against oxidative stress produced by ethanol by increasing/maintaining the levels of antioxidant molecules and antioxidant enzymes. [Journal of American Science 2010;6(11):381-389]. (ISSN: 1545-1003).

**Key words:** *Eruca sativa*, Ethanol, Liver, Ethanolic extract, Antioxidants, Oxidative stress.

## 1. Introduction

Liver is the first organ to metabolize all foreign compounds and hence it is susceptible to many different diseases (Sakar et al., 2005). Alcohol administration is one of the most common causes of chronic liver disease in the world and it was found that alcohol affects the liver, through not only nutritional disturbances but also its direct toxicity, because its predominant metabolism in the liver is associated with oxidation-reduction changes and oxidative stress (Lieber, 2004). The body's natural defenses against free radicals (e.g. antioxidants) are inhibited by alcohol consumption resulting in the increasing of liver damage (Augustyniak et al., 2005).

There has been a great deal of interest in the role of complementary and alternative medicines for the treatment of various acute and chronic diseases. Several hundreds of plants have been examined for use in a wide variety of liver disorders including *Eruca sativa* (Family: *Cruciferae*) that modulate oxidative stress due to its antioxidant properties. Fresh *Eruca sativa* has a characteristic pungent flavor that is thought to be related to the presence of glucosinolates and their breakdown products, e.g: isothiocyanates (Bennett et al., 2006) which have several biological activities including anticarcinogenic, antifungal, antibacterial and antioxidant effects (Kim et al., 2004).

Alam et al., (2007) indicated that *Eruca sativa* seeds and leaves possessed a potent free radical scavenging antioxidants and protected against oxidative damage by increasing /maintaining the levels of antioxidant molecules and antioxidant enzymes.

Thus, the aim of this study is to evaluate the prophylactic and treatment effects of petroleum ether extract of *Eruca sativa* seeds (oil) and ethanolic extracts of both seeds and leaves on alcohol induced hepatotoxicity in rats.

## 2. Materials and Methods

Male albino rats weighting 160-180 g and mice of both sexes weighting 25-28 g were purchased from the animal house of National Research Center (NRC), Giza, Egypt and *Eruca sativa* (seeds and leaves) was purchased from the local market.

Ethanolic and petroleum ether extracts were prepared according to the method of Harborne (Harborne, 1988).

LD<sub>50</sub> of ethanolic extracts of *Eruca sativa* leaves and seeds were determined according to Behrens & Karber (1970).

The guidelines of the ethical care and treatment of the animals followed the regulations of the ethical committee of NRC.

Eighty eight healthy male albino rats were used in this study and randomly divided into 3 main groups , the 1<sup>st</sup> group is the control group and included 1-Normal control rats received saline 2-Ethanol group: normal rats received oral dose of 20% (v/v) ethanol 5ml/100g body weight daily for four weeks .Oil control group: normal rats received oral dose of *Eruca sativa* oil (0.06 ml / kg B.wt / day) for twelve weeks .Seed control group: normal rats received oral dose of *Eruca sativa* seeds ethanol extract (0.5 g/Kg B.wt /day) for twelve weeks (according to LD<sub>50</sub>).Leaf control group: normal rats received oral dose of *Eruca sativa* leaves ethanol extract (0.5 g/Kg B.wt /day) for twelve weeks (according to LD<sub>50</sub>).

The 2<sup>nd</sup> group is the Prophylactic groups and included 1- Oil prophylactic group: rats received oral dose of *Eruca sativa* oil (0.06 ml/Kg B.wt / day) together with 20% ethanol (5ml/100g B.wt./day) for four weeks 2- Seed prophylactic group: rats received oral dose of *Eruca sativa* seeds ethanol extract (0.5 g/Kg B.wt / day) together with 20% ethanol (5ml/100g B.wt./day) for four weeks. 3- Leaf prophylactic group: rats received daily oral dose of *Eruca sativa* leaves ethanol extract (0.5 g/Kg B.wt / day) together with 20% ethanol (5ml/100g B.wt./day) for four weeks.

The 3<sup>rd</sup> group is the treated group ad included 1- Oil treated group: rats received oral dose of 20% ethanol (5ml/100g B.wt./day) for four weeks followed by *Eruca sativa* oil (0.06ml/Kg B.wt / day) for twelve weeks.2-Seed treated group: rats received oral dose of 20% ethanol (5ml/100g B.wt./day)for four weeks followed by *Eruca sativa* seeds ethanol extract (0.5 g/Kg B.wt / day) for twelve weeks.3-Leaf treated group: rats received oral dose of 20% ethanol (5ml/100g B.wt./day) for four weeks followed by *Eruca sativa* leaves ethanol extract (0.5 g/Kg B.wt / day) for twelve weeks.

At the end of the experimental period, animals were kept fasting, subjected to light ether anaesthesia, blood was collected from retro orbital venous plexus and sera were separated by centrifugation and kept at -20°C until used.

Serum Aspartate amino transferase (AST) and Alanine amino transferase (ALT) were assayed by using commercial kits purchased from BioMed Diagnostics.  $\gamma$  glutamile transferase ( $\gamma$ GT) was estimated kinetically by using Linear Laboratories kit. Serum total protein (TP) and serum albumin (Alb) were estimated by Centronic GmbH-Germany kit . Lipid profile was determined by assaying serum total cholesterol (TC), triglycerides (TG) and High

Density Lipoprotein Cholesterol (HDL-C) using commercial kits purchased from BioMed Diagnostics. Lipid according to the method of Uchimaya and Mihara (1978). Total antioxidants (TA) were measured kinetically using commercial kits purchased from Biodiagnostic. Nitric oxide (NO) was determined by the method of Miranda et al., (2001). Superoxid dismutase activity was determined according to Ming Sun and Zigman (1978).

Data were analyzed by one way analysis of variance (ANOVA) followed by LSD test. Results were expressed as mean  $\pm$ S.E, p-values <0.05 were regarded as statistically significant.

### 3. Results

Administration of *Eruca sativa* extracts had no effect on the all studied parameters compared to control group indicating its safe administration (Table 1).

The mean values of serum liver enzymes ALT, AST and  $\gamma$ GT were significantly increased, while the mean values of TP, Alb and A/G ratio were significantly decreased in ethanol group compared to control group. Oil, seeds and leaves prophylactic groups and seeds treated group showed a significant decrease in the mean values of serum ALT, AST and  $\gamma$ GT and a significant increase in TP, Alb and A/G ratio compared to ethanol group (Table 2).

The mean values of serum lipid profile TC, TG and Low Density Lipoprotein Cholesterol (LDL-C) were significantly increased, while the mean values of HDL-C and HDL-C/LDL-C ratio were significantly decreased in ethanol group compared to control group. Oil, seeds and leaves prophylactic groups and seeds treated group showed a significant decrease in the mean values of serum TC, TG and LDL-C and a significant increase in HDL-C and HDL-C /LDL-C ratio compared to ethanol group (Table 3).

The mean values of serum (TBARS) and NO were significantly increased, while the mean values of serum SOD and TA were significantly decreased in ethanol group compared to control group. All prophylactic groups showed a significant decrease in the mean values of serum TBARS and NO and a significant increase in SOD and TA compared to ethanol group but seeds treated group showed a significant decrease in NO only (Table 4).

**Table (1): Effect of *Eruca sativa* extracts in all studied parameters**

Groups	Liver function tests						
	AST (U/l)	ALT (U/l)	γ GT (U/l)	T.P (g/dl)	Alb (g/dl)	Glob.(g/dl)	A/G ratio
Control							
Mean	57.50	34.75	2.88	6.75	3.75	3.00	1.27
±S.E.	0.87	1.44	0.40	0.11	0.06	0.14	0.07
Oil control							
Mean	55.63	33.88	2.63	6.88	3.88	3.00	1.31
±S.E.	0.98	1.33	0.46	0.06	0.07	0.10	0.06
%Change from control	-3.25	-2.50	-8.68	1.93	3.47	0.00	3.15
Seeds control							
Mean	60.13	34.75	2.88	6.76	3.88	2.88	1.35
±S.E.	0.79	0.67	0.44	0.06	0.06	0.09	0.06
%Change from control	4.57	0.00	0.00	0.15	3.47	-3.67	6.30
Leaves control							
Mean	56.38	34.13	2.75	6.63	3.76	2.86	1.32
±S.E.	0.56	0.69	0.49	0.07	0.05	0.06	0.04
%Change from control	-1.95	-1.78	-4.51	-1.78	0.27	-4.67	3.94
Lipid profile							
Groups	T.C (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	HDL-C/LDL-C ratio		
Control							
Mean	112.38	94.88	41.75	51.65	0.81		
±S.E.	0.68	0.74	0.99	1.17	0.04		
Oil control							
Mean	114.13	94.13	43.13	52.18	0.83		
±S.E.	0.44	0.52	0.81	0.59	0.02		
%Change from control	1.56	-0.79	3.31	1.03	2.47		
Seeds control							
Mean	113.37	96.50	41.00	53.08	0.77		
±S.E.	1.07	0.78	0.46	0.90	0.01		
%Change from control	0.89	1.71	-1.80	2.77	-4.94		
Leaves control							
Mean	111.25	93.50	42.38	50.18	0.85		
±S.E.	0.41	0.68	0.98	1.33	0.04		
%Change from control	-1.01	-1.98	1.51	-2.91	4.94		
Oxidants/antioxidants profile							
Groups	TBARS (μmol/l)	SOD (U/ml)	TA (mmol/l)	NO (μmol/l)			
Control							
Mean	0.33	0.66	1.30	18.25			
±S.E.	0.04	0.05	0.05	1.10			
Oil control							
Mean	0.35	0.71	1.41	18.13			
±S.E.	0.03	0.04	0.05	0.58			
%Change from control	6.06	7.58	8.46	-0.66			
Seeds control							
Mean	0.36	0.70	1.38	18.13			
±S.E.	0.03	0.05	0.06	0.58			
%Change from control	9.09	6.06	6.15	-0.66			
Leaves control							
Mean	0.30	0.73	1.54	18.00			
±S.E.	0.05	0.04	0.05	0.53			
%Change from control	-9.09	10.61	18.46	-1.37			

Values are given as mean ± S.E. for 8 rats in each group.

**Table (2): Liver function tests in prophylactic and treated groups**

Groups	Control	Ethanol	Prophylactic			Treated		
			Oil	Seeds	Leaves	Oil	Seeds	Leaves
<b>AST (U/l)</b>								
Mean	57.50	83.13 <sup>a</sup>	75.00 <sup>a,b#</sup>	72.38 <sup>a,b♦#</sup>	68.50 <sup>a,b*#</sup>	83.00 <sup>a</sup>	79.0 <sup>a,b</sup>	82.00 <sup>a</sup>
±S.E.	0.87	1.02	0.71	0.62	0.87	0.57	0.46	0.50
%Change from control	0.00	44.56	30.43	25.88	19.13	44.35	37.39	42.61
%Change from ethanol	--	0.00	-9.78	-12.93	-17.60	-0.16	-4.97	-1.36
<b>ALT (U/l)</b>								
Mean	34.75	51.38 <sup>a</sup>	47.25 <sup>a,b</sup>	44.75 <sup>a,b#</sup>	41.38 <sup>a,b*#</sup>	50.00 <sup>a</sup>	47.63 <sup>a,b</sup>	51.00 <sup>a</sup>
±S.E.	1.44	1.19	0.65	0.59	1.41	0.46	0.46	0.33
%Change from control	0.00	47.84	35.97	28.78	19.08	43.88	37.06	46.76
%Change from ethanol	--	0.00	-8.04	-12.90	-19.46	-2.69	-7.30	-0.74
<b>γ GT (U/l)</b>								
Mean	2.88	11.63 <sup>a</sup>	8.88 <sup>a,b</sup>	8.00 <sup>a,b</sup>	6.38 <sup>a,b*#</sup>	11.13 <sup>a</sup>	9.50 <sup>a,b</sup>	11.63 <sup>a</sup>
±S.E.	0.40	0.50	0.52	0.46	0.37	0.40	0.57	0.50
%Change from control	0.00	303.82	208.33	178.78	121.53	286.46	229.86	303.82
%Change from ethanol	--	0.00	-23.65	-31.21	-45.14	-4.30	-18.31	0.00
<b>T.P (g/dl)</b>								
Mean	6.75	5.63 <sup>a</sup>	5.90 <sup>a,b</sup>	6.00 <sup>a,b#</sup>	6.30 <sup>a,b*#</sup>	5.50 <sup>a</sup>	5.80 <sup>a</sup>	5.60 <sup>a</sup>
±S.E.	0.11	0.06	0.06	0.06	0.03	0.05	0.05	0.05
%Change from control	0.00	-16.59	-12.59	-11.11	-6.67	-18.52	-14.07	-17.04
%Change from ethanol	--	0.00	4.80	6.67	11.90	-2.31	3.02	-0.53
<b>Alb (g/dl)</b>								
Mean	3.75	2.50 <sup>a</sup>	2.93 <sup>a,b</sup>	3.13 <sup>a,b*#</sup>	3.40 <sup>a,b*#</sup>	2.50 <sup>a</sup>	2.80 <sup>a,b</sup>	2.38 <sup>a</sup>
±S.E.	0.06	0.05	0.06	0.06	0.07	0.05	0.05	0.04
%Change from control	0.00	-33.33	-21.87	-16.35	-9.33	-33.33	-25.33	-36.53
%Change from ethanol	--	0.00	17.20	25.20	36.00	0.00	12.00	-4.80
<b>Glob. (g/dl)</b>								
Mean	3.00	3.13	2.98	2.88	2.90	3.00	3.00	3.23
±S.E.	0.14	0.09	0.07	0.08	0.07	0.07	0.06	0.05
%Change from control	0.00	4.33	-0.67	-4.00	-3.33	0.00	0.00	7.67
%Change from ethanol	--	0.00	-4.79	-7.99	-7.35	-4.15	-4.15	3.19
<b>A/G ratio</b>								
Mean	1.27	0.81 <sup>a</sup>	1.00 <sup>a,b</sup>	1.10 <sup>b#</sup>	1.18 <sup>b*#</sup>	0.84 <sup>a</sup>	0.94 <sup>a,b</sup>	0.74 <sup>a</sup>
±S.E.	0.07	0.04	0.04	0.05	0.05	0.03	0.03	0.02
%Change from control	0.00	-36.22	-22.05	-13.39	-7.09	-33.36	-25.98	-41.73
%Change from ethanol	--	0.00	23.46	35.80	45.68	3.70	16.05	-8.64

Values are given as mean ± S.E. for 8 rats in each group.

a: Significant difference at P<0.05 compared to control group.

b: Significant difference at P<0.05 compared to ethanol group.

\*:Significant difference at p<0.05 compared to seed and oil prophylactic groups.

♦:Significant difference at p<0.05compred to oil prophylactic group.

#:Significant difference at p<0.05compred to seed treated group.

**Table (3): Lipid profile in prophylactic and treated groups**

Groups	Control Ethanol		Prophylactic			Treated		
			Oil	Seeds	Leaves	Oil	Seeds	Leaves
TC (mg/dl)								
Mean	112.38	144.87 <sup>a</sup>	130.75 <sup>a,b#</sup>	126.00 <sup>a,b*#</sup>	122.00 <sup>a,b*#</sup>	144.00 <sup>a</sup>	135.00 <sup>a,b</sup>	143.00 <sup>a</sup>
±S.E.	0.68	0.69	0.53	0.71	0.60	0.46	0.71	0.71
%Change from control	0.00	28.92	16.35	12.12	8.56	28.14	20.13	27.25
%Change from ethanol	--	0.00	-9.75	-13.03	-15.79	-0.61	-6.82	-1.30
TG (mg/dl)								
Mean	94.88	131.5 <sup>a</sup>	126.00 <sup>a,b#</sup>	122.0 <sup>a,b*#</sup>	117.63 <sup>a,b*#</sup>	130.0 <sup>a</sup>	128.00 <sup>a,b</sup>	131.00 <sup>a</sup>
±S.E.	0.74	0.80	0.46	0.53	0.94	0.46	0.46	0.46
%Change from control	0.00	38.60	31.75	28.58	23.98	37.02	34.91	38.07
%Change from ethanol	--	0.00	-4.94	-7.22	-10.55	-1.14	-2.66	-0.38
HDL-C (mg/dl)								
Mean	41.75	32.63 <sup>a</sup>	36.25 <sup>a,b</sup>	37.00 <sup>a,b</sup>	38.00 <sup>a,b</sup>	32.13 <sup>a</sup>	36.00 <sup>a,b</sup>	32.00 <sup>a</sup>
±S.E.	0.99	0.86	0.59	0.60	0.89	0.91	0.46	0.91
%Change from control	0.00	-21.84	-13.17	-11.38	-8.98	-23.05	-13.77	-23.35
%Change from ethanol	--	0.00	11.09	13.39	16.46	-1.55	10.33	-1.93
LDL-C (mg/dl)								
Mean	51.65	85.95 <sup>a</sup>	69.50 <sup>a,b#</sup>	64.77 <sup>a,b*#</sup>	60.48 <sup>a,b*#</sup>	85.88 <sup>a</sup>	73.40 <sup>a,b</sup>	84.80 <sup>a</sup>
±S.E.	1.17	0.73	0.79	0.97	0.49	1.03	0.62	1.37
%Change from control	0.00	66.41	34.56	25.07	17.10	66.34	46.91	64.18
%Change from ethanol	--	0.00	-19.14	-24.84	-29.63	0.00	-11.68	-1.34
HDL-C/LDL-C ratio								
Mean	0.81	0.38 <sup>a</sup>	0.52 <sup>a,b</sup>	0.57 <sup>a,b*#</sup>	0.63 <sup>a,b*#</sup>	0.38 <sup>a</sup>	0.49 <sup>a,b</sup>	0.38 <sup>a</sup>
±S.E.	0.04	0.01	0.01	0.02	0.02	0.01	0.01	0.02
%Change from control	0.00	-53.09	-35.80	-29.63	-22.22	-53.09	-40.74	-53.09
%Change from ethanol	---	0.00	36.84	50.00	65.79	0.00	26.32	0.00

Values are given as mean ± S.E. for 8 rats in each group.

a: Significant difference at P<0.05 compared to control group.

b: Significant difference at P<0.05 compared to ethanol group.

\*:Significant difference at p<0.05 compared to seed and oil prophylactic groups.

♦:Significant difference at p<0.05compred to oil prophylactic group.

#:Significant difference at p<0.05compred to seed treated group.

**Table (4): Serum Oxidants/antioxidants profile in prophylactic and treated groups**

Groups	Control	Ethanol	Prophylactic			Treated		
					Oil	Seeds	Leaves	
TBARS (μmol/l)								
Mean	0.33	0.90 <sup>a</sup>	0.70 <sup>a,b</sup>	0.60 <sup>a,b#</sup>	0.44 <sup>b*</sup> #	0.90 <sup>a</sup>	0.80 <sup>a</sup>	0.90 <sup>a</sup>
±S.E.	0.04	0.06	0.05	0.04	0.04	0.05	0.05	0.03
%Change from control	0.00	172.73	112.12	81.82	33.33	172.73	142.42	172.73
%Change from ethanol	--	0.00	-22.22	-33.33	-51.11	0.00	-11.11	0.00
SOD(U/ml)								
Mean	0.66	0.30 <sup>a</sup>	0.46 <sup>a,b</sup>	0.50 <sup>a,b</sup>	0.55 <sup>b*</sup> #	0.35 <sup>a</sup>	0.40 <sup>a</sup>	0.30 <sup>a</sup>
±S.E.	0.05	0.03	0.04	0.05	0.04	0.03	0.03	0.05
%Change from control	0.00	-54.55	-30.30	-24.24	-16.67	-46.97	-39.39	-54.55
%Change from ethanol	--	0.00	53.33	66.67	83.33	16.67	33.33	0.00
TA (mmol/l)								
Mean	1.30	0.40 <sup>a</sup>	0.60 <sup>a,b</sup>	0.80 <sup>a,b*#</sup>	1.10 <sup>a,b*#</sup>	0.40 <sup>a</sup>	0.50 <sup>a</sup>	0.40 <sup>a</sup>
±S.E.	0.05	0.03	0.04	0.05	0.05	0.03	0.03	0.03
%Change from control	0.00	-69.23	-53.85	-38.46	-15.38	-69.23	-61.54	-69.23
%Change from ethanol	--	0.00	50.00	100.00	175.00	0.00	25.00	0.00

NO( $\mu\text{mol/l}$ )									
Mean	18.25	46.00 <sup>a</sup>	34.00 <sup>a,b#</sup>	30.00 <sup>a,b♦#</sup>	25.00 <sup>a,b*#</sup>	46.00 <sup>a</sup>	39.00 <sup>a,b</sup>	45.00 <sup>a</sup>	
$\pm\text{S.E.}$	1.10	0.46	0.46	0.46	0.63	0.53	0.57	0.38	
%Change from control	0.00	152.05	86.30	64.38	36.99	152.05	113.70	146.58	
%Change from ethanol	--	0.00	-26.09	-34.78	-45.65	0.00	-15.22	-2.17	

Values are given as mean  $\pm$  S.E. for 8 rats in each group.

a: Significant difference at  $P<0.05$  compared to control group.

b: Significant difference at  $P<0.05$  compared to ethanol group.

\*:Significant difference at  $p<0.05$  compared to seed and oil prophylactic groups.

♦:Significant difference at  $p<0.05$ compred to oil prophylactic group.

#:Significant difference at  $p<0.05$ compred to seed treated group.

#### 4. Discussion

In this study, alcohol intake increased the mean values of liver enzymes (ALT, AST and  $\gamma$  GT). These results were in agreement with Rajakrishnan and Menon (2001) who indicated that exposure of hepatocytes to ethanol alters the membrane structure and functions by increasing the leakage of enzymes into the circulation. Also, Das et al., (2005) reported that excess alcohol consumption has been linked with altered liver metabolism and liver damage, with leakage of cytoplasmic liver enzyme  $\gamma$ GT into blood.

In all prophylactic groups and *Eruca sativa* seeds ethanolic extracts treated group, liver enzymes (AST, ALT and  $\gamma$ GT) were significantly decreased compared to ethanol group. These results were in agreement with El-Nattat and El-Kady (2007) who indicated that administration of rocket caused improving in AST, ALT and  $\gamma$ GT activities in male rabbits, which may be due to the high content of sulfur in *Eruca sativa* that works as a cleansing of body wastes, clearing congestion like sinusitis and assisting liver and immune function.

In the present study, there was a significant decrease in serum total proteins, albumin and A/G ratio in ethanol group. These results were in agreement with Ahmed et al (2002) who found a decrease in serum total proteins and albumin in ethanol-administered rats and he suggested that was due to the decrease in the functional ability of liver in ethanol-administered rats. Also, the decrease in A/G ratio is a predictor of a bad out come and poor health.

In the current study, serum total proteins, albumin and A/G ratio were significantly increased in all prophylactic groups and also in treated group of *Eruca sativa* seeds ethanolic extracts compared to ethanol group. In the same line, El-Missiry and El-Gindy (2000) indicated the ability of *Eruca sativa* oil to stimulate the regeneration of hepatic tissue which increased protein synthesis in damaged liver and improved the functional statuses of the liver cells.

Several studies demonstrated that alcohol intake is associated with changes in serum lipid concentrations and whole-body lipid balance (Siler et al., 1999). In the present study, there was a significant increase in the mean values of serum TC, TG and LDL-cholesterol and a significant decrease in the mean values of serum HDL-C and HDL-C / LDL-C ratio in ethanol group. These results were in agreement with kumar et al. (2002) who reported that ethanol blocks fat oxidation and favors fat accumulation. The accumulation of fat in liver acts as a stimulus for the secretion of lipoproteins into the blood stream and the development of hyperlipidemia.

In prophylactic groups, *Eruca sativa* significantly decreased serum cholesterol, triglycerides and low density lipoprotein cholesterol levels while the mean values of high density lipoprotein cholesterol and HDL-C/LDL-C ratio were significantly increased. These results were in agreement with El-Gengaihi et al., (2004) who reported that *Eruca sativa* induced a marked decrease in different lipid parameters values.

It was found that the inflammatory reactions and oxidative stress play a major role in alcohol hepatotoxicity (Albano et al., 2002). In this investigation, there was a significant increase in serum MDA concentration in ethanol treated rats; these results were in agreement with Saravanan et al., ( 2006) who observed a significant increase in MDA concentration in ethanol-treated rats and he suggested that reactive oxygen intermediates, generated during the metabolism of ethanol, these free radicals attack the polyunsaturated fatty acids in membranes and organelles to produce lipid peroxides leading to decrease in the membrane permeability, and ultimately cellular necrosis and death.

Free radicals are involved in various human diseases that can possibly be prevented by antioxidants (Chatterjee et al., 2005). Exposure of living organisms to a constant generation of reactive oxygen species (ROS) resulting in the development of antioxidative defense systems which protect cells and tissues against their harmful effects. The

efficiency of enzymatic and non-enzymatic antioxidative systems could be detected by the determination of single components of this system or by so-called total antioxidant capacity (TAC) (Kankofer et al., 2005) In the present study, a significant decrease in serum total antioxidants in ethanol treated rats was observed. In agreement, Masalkar et al., (2005) found a decrease in antioxidant status in alcoholic patients and showed that increased generation of free radicals and deficiencies of dietary antioxidants can be important etiological factor in alcoholic liver disease.

In this study, NO level was significantly elevated in alcohol -treated rats. In agreement, Li et al., (2004) showed an elevation of NO level with the increased volume of alcohol infusion.

In the present study, the activity of superoxide dismutase was significantly decreased in ethanol -treated rats. In agreement, Puntarula et al., (1999) reported that superoxide dismutase and other antioxidative enzymes may be inactivated by ethanol.

All prophylactic groups showed a significant decrease in malondialdehyde (MDA) and nitric oxide (NO) levels and a significant increase in total antioxidants levels and superoxide dismutase activity. El-Gindy & El-missiry (2000) indicated that oil of *Eruca sativa* seed extract (ESS) induced an increase in hepatic GSH content which might enhance the GSH/GSSG ratio and decrease hepatic lipid peroxidation and hence aldehydic concentration. Parallel to these events, hepatic SOD activity was increased in rats supplemented with ESS. That is may be due to the fact that *Eruca sativa* seeds possess a potent free radical scavenging, antioxidants activities.

From the current study we noticed that, *Eruca sativa* leaves extract is consider the best hepatoprotective extract in prophylactic groups and *Eruca sativa* seeds extract is consider the best treated extract in treated groups. That's may be related to the fact that, *Eruca sativa* seeds ethanolic extract have a potent antioxidant activity and protect against ethanol induced hepatotoxicity. Several studies on phytochemical analysis of *Eruca sativa* seeds has shown the presence of many compounds to which antioxidant activity may be ascribed, these include glucosinolate, flavonoids (Quercetin,Kaempferol and isohamnetin),Carotenoids, Vitamine C (Barillari et al., 2005). The main compound that exerts antioxidant activity in *Eruca sativa* seeds extract is glucoerucin, unlike other glucosinolates (GLS) (e.g. glucoraphanin, the bio-precursor of sulforaphane), glucoerucin (GER) possesses good direct as well as indirect antioxidant activity (Alam et al., 2007). The antioxidant activity of glucoerucin, the bio-precursor of erucin (ERN) implicates free radical scavenging activity and an ability to induce phase II

metabolizing enzymes (e.g. glutathione transferases, GSTs; NAD-(P) H: Quinone reductase (QR),epoxide hydrolase and heme oxygenase)which are important in the detoxification of electrophiles. Reactions of glucoerucin and erucin with free radicals (hydroperoxides) produce glucoraphanin (GRP) and sulphoraphane, respectively (Barillari et al., 2005).

In contrast, Perocco et al., ( 2006) reported that glucoraphanin only slightly affects glutathione-S-trasferase, which was the selected marker of phase II detoxifying enzymes, and also found that, GRP powerfully induces phase -I bioactivating enzymes(e.g.CYP1A which activates polycyclic aromatic hydrocarbons,CYP3A1 that activates aflatoxin and CYP2E1 activates ethanol). CYP2E1 metabolizes and activates many toxicological substrates, including ethanol, to more toxic products and it generates superoxide anion radical ( $O_2^-$ ) and hydrogen peroxide ( $H_2O_2$ ). This effect on the redox status of the liver can cause activation of Kupffer cells and subsequently, hepatic cells, and thus contributing to the generation of alcoholic liver disease (Kessova and Cederbaum, 2003).

So, this extract (ethanolic extract of seeds) induced the beneficial effect in treatment study and not in prophylactic one. On the other hand, Bennett et al. (2002) indicated that leaves of *Eruca sativa* contain 4-mercaptopbutyl GL as the major GL among nine, while GER is present only in small amounts. In addition Kim et al., (2004) isolated 4-(B-D-Glucopyranosyldisulfanyl) butyl a new glucosinolate from leaves of rocket and reported that this new glucosinolate has antioxidant activity in vitro..Rocket seeds and sprouts contain glucoerucin(GER) as main glucosinolates ,in large amounts in comparison to leaves (mature plants which contain 4-mercaptopbutyl GL in large amount beside 4-(B-D-Glucopyranosyldisulfanyl) butyl and a small amount of GER ( Weckerle et al., 2001).From these results we concluded that, *Eruca sativa* ethanolic extract of leaves was better than ethanolic extract of seeds and petroleum ether extract of seed (oil) in the prophylactic study. Since in prophylactic study glucoraphanine(produced by glucoerucin) which found in *Eruca sativa* seeds activates phase 1 enzyme (CYP2E1) which in turn activates ethanol metabolism to produce free radicals and more toxic products, despite giving a beneficial effect in treatment study. Regarding the present study it could be concluded that *Eruca sativa* extracts possessed both prophylactic and therapeutic effects against experimentally induced liver injury in rats. However, the prophylactic role of these extracts was more potent than their treatment capacity.

**Acknowledgements:**

Authors are grateful to the National Research Center, Giza, Egypt for unlimited help and support to carry out this work.

**Corresponding Author:**

Dr. Jihan Seid Hussein  
Ass.Prof. of Medical Biochemistry  
Medical Biochemistry Department  
National Research Center  
Giza,Egypt.  
E-mail: [jihan\\_husein@yahoo.com](mailto:jihan_husein@yahoo.com)  
Mobile: 012 217 43 49

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8/2/2010

# In vivo and in vitro studies on *Thevetia* Species Growing in Egypt

## I: Isolation, Identification, and Quantification of cardiac glycosides in in vivo and in vitro cultures of immature seeds.

**Taha H. S. <sup>1\*</sup>, Farag H.S. <sup>2</sup>, Shams A. K. <sup>2</sup>, Abdel-Azim S.N. <sup>2</sup>, Hanna G. A. <sup>3</sup> Ewais E. E. <sup>4</sup> and Seif El-Nasr M. M. <sup>2</sup>**

<sup>1</sup> Plant Biotechnology Department, National Research Centre, Dokki, Giza, Egypt.

<sup>2</sup> Phytochemistry Department, National Research Centre, Dokki, Giza, Egypt.

<sup>3</sup> Chemistry of Natural Compounds Department, National Research Centre, Dokki, Giza, Egypt.

<sup>4</sup> Botany and Microbiology Department, Faculty of Science, Al-Azhar University ,Cairo, Egypt

*Corresponding author* [hussein.taha2@yahoo.com](mailto:hussein.taha2@yahoo.com)

**ABSTRACT:** *In vivo* and *in vitro* extracted cardiac glycosides of immature seeds (IS) cultures of *Thevetia nerifolia* Jussieu. and *T. thevetioides* Kunth. were chemically identified. Calli were grown on modified Murashige & Skoog (MS) medium supplemented with 1mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) +3mg/l kinetin (Kin). The content of cardiac glycosides in IS cultures of *T. nerifolia* and *T. thevetioides* were monitored by HPLC. Two major compounds were detected and isolated from IS extracts i.e. digitoxigenin and thevetin B. The different structures of the *in vivo* and *in vitro* isolated compounds were verified by means of MS and NMR spectral analysis, as well as those compounds were identified and determined using HPLC technique. [Journal of American Science 2010;6(11):390-395]. (ISSN: 1545-1003).

**Key words:** Cardiac glycosides, callus, *Thevetia spp.*, HPLC, MS medium, immature seed cultures

### INTRODUCTION

*Thevetia nerifolia* Juss. and *T. thevetioides* Kunth. belonging to Apocynaceae family, which are commonly known as the dogbane or oleander family (Omino and Kokwaro, 1993). Apocynaceae plants are distributed in tropical America and the West Indies and widely grown in various parts of the world for ornamental purpose (Rizk and Al-Nowaihi, 1989; Sabira *et al.* 1993). It has been used in folk medicine as a purgative, emetics, and a remedy for intermittent fever (Githens, 1948). Moreover, It is having cardiotonic activity (Aleshkina and Berezhinskaya, 1962), anticancer (Cardellina *et al.* 1993), neuroprotection against ischemic stroke, as well as insecticidal properties (James *et al.* 2006). The toxicity of *Thevetia* species attributed to their cardenolide content in their tissues (Goncalves *et al.* 2003; Gaillard *et al.* 2004; Gaillard *et al.* 2004). Cardiac glycosides Cerberin (monoacetylnerifolin), ruvoside and perusitin were isolated from the seeds of *Thevetia peruviana* (Siddiqui *et al.* 1992). Abe *et al.*, (1994) isolated sixteen cardenolide glycosides and one pregnane glycoside from the frozen fresh leaves of *Thevetia nerifolia*. Siddiqui *et al* (1992) isolated nerifoside from the fresh uncrushed leaves of *Thevetia nerifolia*, in addition to four triterpenes; oleanolic acid, ursolic acid,  $\alpha$ -amyrin acetate and  $\beta$ -amyrin acetate. Also, cerberoside, 2-O-acetylcerberoside, nerifolin, thevetin A&B, peruvoside, digitoxigenin were isolated (Balsam and

Kufner 1971; Seitz and Riphahn 1975; Said, 1985; Decosterd *et al.* 1994). The triterpenes, sterol, Fatty acids and flavonoid constituents of *Thevetia peruviana* fruit pericarp and flowers were also studied (Qazi *et al.* 1973; Rao *et al.* 1975; Schum-Obasi *et al.* 1990).

*In vitro* culture technology has been proven to be effective in some cases for the production of secondary metabolites such as taxol (Oksman-Caldentey and Inzé 2004). Moreover, Dantas *et al.* (1994) studied six strain of *T. nerifolia* cell suspension cultures for cardenolides production. Even after two years of subculture, cardenolides proved to be present in all these strains. The cardenolides content varied from one strain to another according to the nature of the original explants. Furthermore, Lopes *et al.* (2001) reported that some compounds found in the intact plant could accumulate in cultured cells. Cardenolides of *T. nerifolia*, were accumulated in cultured cells during one year of cultivation.

In the present work, cardiac glycoside in seed cultures of *T. nerifolia* and *T. thevetioides* were identified and compared qualitatively and quantitatively with those isolated previously from *in vivo* seeds of the same plants.

### MATERIALS AND METHODS

#### *General procedures*

<sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD) and <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD) were recorded on a JEOL LA500 MHz, Germany spectrometer with TMS as an internal standard. The FAB-MS spectrum was taken on a JEOL JMS\_AX500 mass spectrometer MS-FAB09A positive. HPLC was carried out on Agilent a series 1100 interface with stationary phase (RP18), injection volume (10 µl), oven temperature (25°C), diode array detector (254 nm), flow rate (1ml / min) and mobile phase: MeOH/H<sub>2</sub>O (1:1) under gradient conditions. Column chromatography was carried out on silica gel 60 (Merck; 230 - 400 mesh). TLC: pre-coated silica gel 60F<sub>254</sub> plates (Merck); CC: silica gel type 60 (Merck). MS: Murashige and Skoog medium (Duchefa Biochemie The Netherlands).

#### Plant materials

Immature seeds (IS) of *T. nerifolia* Juss. and *T. thevetioides* Kunth were collected from Al-Orman garden, Giza, Egypt in March 2006. They were identified by Prof. Dr. K. H. El-Batanouny, Botany Department, Faculty of Science, Cairo University. Voucher specimens are deposited at the Herbarium of NRC, Dokki, Cairo, Egypt.

#### Authentic compounds

The reference of cardiac glycosides (peruvoside and nerifolin) were purchased from Sigma Chemicals Co., St. Louis Mo. USA. Thevetin B and digitoxigenin were separated and identified throughout this work.

#### Initiation of IS calli cultures

Calli cultures of *T. nerifolia* and *T. thevetioides* derived IS were performed as described by Taha *et al.* (2010).

#### Extraction and isolation of cardiac glycosides

In vitro derived calli of *T. nerifolia* and *T. thevetioides* and *in vivo* IS were lyophilized and powdered. Then they were percolated in methanol (3x3L) at room temperature for 24 hrs and filtered. The percolation was repeated three times and the methanolic extracts for each plant were combined and evaporated *in vacuo* at 45 °C. The dry crude extract was defatted with petroleum ether. The residue was dissolved in MeOH/H<sub>2</sub>O (1:1) and extracted with chloroform. The chloroform extracts were combined together, dehydrated and the solvent distilled of *in vacuo* at 45 °C to give total cardenolides (Fried and Sherma, 1994 and Abdel-Azim *et al.*, 1996).

#### Identification of the isolated compounds

All isolated compounds were identified by FAB-MS, <sup>1</sup>H-NMR and <sup>13</sup>C NMR spectroscopy. Furthermore, the determination of cardiac glycosides was carried out using co-chromatography TLC and HPLC techniques with samples of reference compounds.

#### HPLC analysis

One gram each of air-dried powdered of *in vivo* and *in vitro* derived calli of IS of *T. nerifolia* and *T. thevetioides* was accurately weighed. Each sample was extracted with 50 ml 70% MeOH till exhaustion. The obtained methanolic extract was evaporated till dryness. The residue was re-dissolved in 1 mL methanol and filtered. The filtrate was used for quantitative determination of the isolated compounds using HPLC (10 µL was injected). Which, then they were performed on RP18 column using water (A) and methanol (B) as solvents and detected at the wave length of 220 nm. The following gradient was employed: 10% B for 25 minutes, 100% B within 30 minutes, then isocratic elution at 20% B for 20 minutes. Standard curves of authentic compounds and calculations of unknown amounts of cardiac glycosides in calli and regenerated culture samples were done using routine protocols as described by Scott (1996).

#### Statistical analysis of data

All experiments were statistically analyzed using the F-test according to Steel and Torrie (1960). ANOVA was determined and the LSD was calculated at P=0.05. The data presented are the means of five replicates ± standard error (SE).

## RESULTS AND DISCUSSION

#### Isolation and structure elucidation

The crude cardenolides extract (5 g) were applied onto the top of a silica gel column 50x5 cm. Elution was carried out using CHCl<sub>3</sub> followed by gradual increasing of the proportions of MeOH till 100% MeOH. The course of the chromatographic fractions (100 ml each) was monitored by silica gel for TLC and developed using solvent system CHCl<sub>3</sub>: MeOH (8:2). The chromatoplates were visualized using Kedde's reagent (Wagner and Bladt 1995), which give violet colour with cardiac glycosides. Similar fractions were combined and concentrated to dryness under reduced pressure. Thevetin B (compound I) was isolated in a pure form *T. nerifolia* and having R<sub>f</sub> values 0.12. Also digitoxigenin (compound II) was isolated from *T. thevetioides* and having R<sub>f</sub> values 0.81.

### Compound (I)

Compound I was identified as thevetin B (**Fig. 1**) by comparing its spectroscopic measurements with that published with Rodrigo *et al.* (2005). Its FAB-MS (positive mode) spectrum showed a molecular ion peak at  $m/z$  858 (calcd. 858.96), which is corresponding to the molecular formula  $C_{42}H_{66}O_{18}$ .  $^1H$ -NMR spectrum ( $CD_3OD$ ), showed two methyl protons at  $\delta$  0.8 and 0.9 corresponding to  $C_{18}$  and  $C_{19}$ , respectively. Signals due to the cardenolides ring were identified at  $\delta$  5.00 ( $H_{21\alpha}$ ), 4.8 ( $H_{21\beta}$ ) and 5.8 ( $H_{22}$ ). In addition to the signal detected at  $\delta$  2.7, which is corresponding to  $H_{17}$ . The three anomeric protons of the three sugar units were identified at  $\delta$  4.3, 4.5 and 5.11, respectively. The oxygenated methines were also identified at  $\delta$  3.54 for  $H_3$  and  $\delta$  3.54 for C-3'-OMe.

### Compound (II)

Compound II was identified as digitoxigenin (**Fig. 1**) by comparing its spectroscopic measurements with that published with Rodrigo *et al.* (2005). Its FAB-MS (positive mode) showed a fragment ion peak at  $m/z$  374.25 (calcd 374.51), which is corresponding to the molecular formula  $C_{23}H_{34}O_3$ .  $^1H$ -NMR spectrum ( $CD_3OD$ ), showed two methyl signals at  $\delta$  1.05 and 0.99 corresponding to  $H_{18}$  and  $H_{19}$ , respectively. Signals due to the cardenolides ring were identified at  $\delta$  6.15 ( $H_{22}$ ), 5.06 ( $H_{21\alpha}$ ) and 4.67 ( $H_{21\beta}$ ), in addition to  $H_{17}$  at  $\delta$  2.84.  $^{13}C$ -NMR spectrum ( $CD_3OD$ ), showed an ester carbonyl at  $\delta$  172.1 ( $C_{23}$ ) and the vinylic carbons were identified at  $\delta$  117.7 ( $C_{22}$ ) and 172.0 ( $C_{20}$ ). Additional oxygenated carbons were observed for  $C_{14}$  ( $\delta$  86.0) and  $C_{21}$  ( $\delta$  75.8). Also, the two methyl carbons were identified at  $\delta$  16.4 ( $C_{18}$ ) and  $\delta$  24.1 ( $C_{19}$ ).

The results obtained are in agreement with those reported by Mahran *et al.* (1971), who isolated thevetin B from seeds of *T. nerifolia*, and Perez *et al.* (1993), who isolated digitoxigenin from seeds of *T. thevetioides*.

### Initiation of IS calli cultures

The highest value of IS calli of *T. nerifolia* and *T. thevetioides* (Fig. 2 A and B) was recorded with MS+ 1mg/l 2,4-D + 3mg/l Kin as described by Taha *et al.* (2010).

## Qualitative and quantitative determination of cardenolides

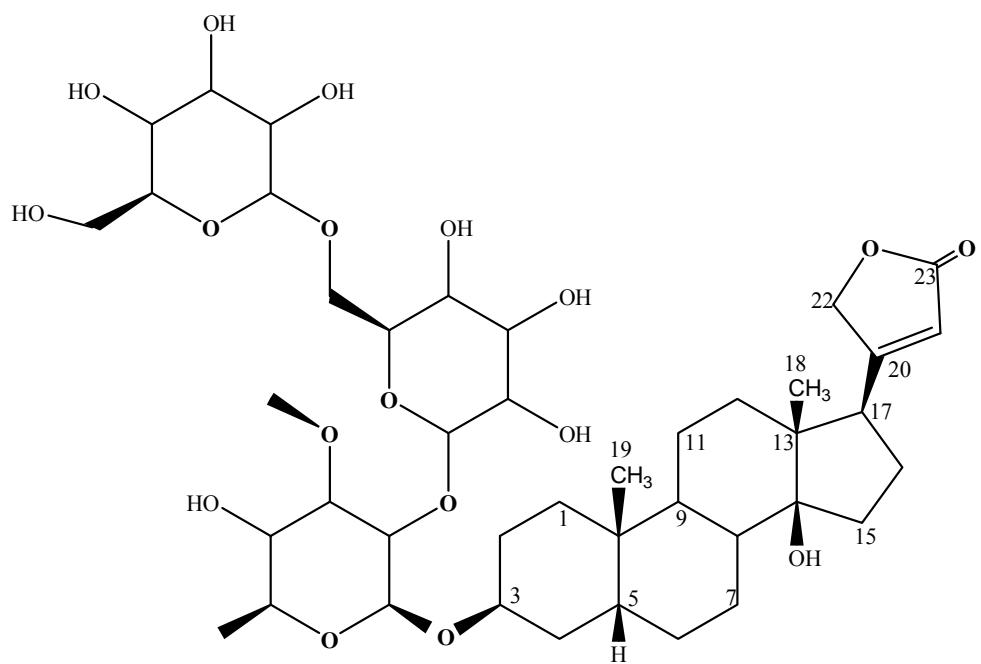
### 1. Qualitative determination

TLC chromatoplates as a preliminary screening to check the presence of the examined cardenolids compounds in calli and regenerated shootlet extracts. The chloroform extracts were compared with authentic samples using system solvent chloform/methanol (8:2) and visualized with Kedde's reagent. The spots corresponding to  $R_f$  values 0.12, 0.60, 0.67 and 0.81 were referred to thevetin B, nerifolin, peruvoside and, digitoxigenin, respectively. The four compounds were detected in all the examined calli and regenerated shootlet extracts.

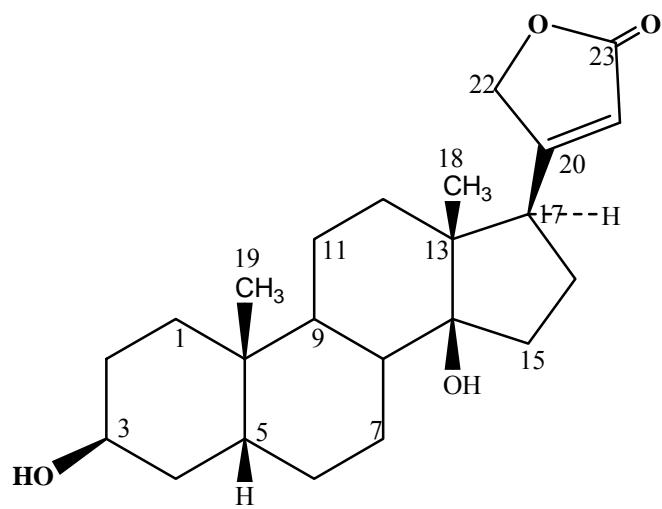
### 2. Quantitative determination (HPLC technique)

As shown in Table 1 thevetin B, digitoxigenin, peruvoside and nerifolin *in vivo* and *in vitro* seeds cultures of *T. nerifolia* and *T. thevetioides* were illustrated in (Table 1). The amount of thevetin B, digitoxigenin, peruvoside and nerifolin of *in vivo* seeds of *T. nerifolia* plant were 0.19, 0.11, 0.39 and 0.25 mg/g DW, respectively. On the other hand, the amount of these compounds of *in vivo* seeds of *T. thevetioides* plant were 0.99, 0.15, 0.24 and 0.39 mg/g DW, respectively. The highest concentration of thevetin B (85 mg/g DW) and digitoxigenin (0.028 mg/g DW) was recorded in *T. nerifolia* IS calli cultures and that of peruvoside (0.017 mg/g DW) and nerifolin (0.032 mg/g DW) was found in *T. thevetioides*.

Concerning, the accumulation of cardenolides in *T. nerifolia* calli cultures and in close of our obtained results Dantas *et al.* 1994 and Lopes *et al.* 2001 reported that some compounds of cardenolides were accumulated in cultured cells of *T. nerifolia*. Morevor, some studies by Fett-Netto *et al.* (1992) reported that 2,4-D and Kin combination were more effective in accumulation of secondary metabolites *viz.* taxol and related taxanes in cell suspension of *Taxus* species. It is interesting to note that the technique used in this experiment, together with other reported techniques e.g. callus and cell suspension cultures (Stuhlemmer *et al.* 1993) can be offered alternative sources for large scale production of cardiac glycosides.



**Fig. 1** Thevetin B = (Compound I )



**Fig. 2** Digitoxigenin = (Compound II)

**Table 1: Concentrations of the cardiac glycosides (mg/g DW)**

Extract	Thevetin B	Digitoxigenin	Peruvoside	Neriifolin
In vivo seeds*	0.19	0.12	0.39	0.25
In vivo seeds**	0.99	0.15	0.24	0.39
IS calli cultures*	0.085	0.028	0.013	0.019
IS calli cultures **	0.047	0.027	0.017	0.032

where:

Thevetin B ( $R_t = 9.48$ ), digitoxigenin ( $R_t = 11.01$ ), peruvoside ( $R_t = 11.32$ ) and neriifolin ( $R_t = 12.72$ ).

(\*) *T. nerifolia*; (\*\*) *T. thevetioides*

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## Comparative Analysis Of Resource Use Efficiency In Rice Production Systems In Abia State Of Nigeria

Nwaru, J. C. and O. R. Iheke

Department of Agricultural Economics  
Michael Okpara University of Agriculture, Umudike  
PMB 7267 Umuahia, Abia State, Nigeria  
E-mail: [nwaruj@yahoo.com](mailto:nwaruj@yahoo.com)

**ABSTRACT:** Arresting the observed low productivity and continued decline in the output of rice especially in the face of rising population and the concomitant escalating increases demand has been a lingering socioeconomic problem. Continued increase in rice production through a number of options including expansion into high potential areas especially the inland valleys has been proposed. This study was designed to examine resource use efficiency in rice production systems in Abia State of Nigeria. Primary data collected from a sample of 142 farmers consisting of 46 inland valley, 41 upland and 55 swamp rice farmers were analysed by the ordinary least squares multiple regression analysis and analysis of variance (ANOVA). Results indicate that the upland rice farmers are technically more efficient than the swamp and inland rice farmers and that there is no difference in technical efficiency between the swamp and inland rice farmers. None of the farmer groups achieved absolute allocative efficiency. The upland rice farmers achieved least allocative efficiency ( $W_{ij}$  is farther from unity), underutilized all farm resources ( $W_{ij} > 1$ ) while both the inland valley and the swamp rice farmers under utilized farmland, other inputs and capital and over utilised ( $W_{ij} < 1$ ) family labour and hired labour. There was no significant difference in the mean output of rice from the production systems; upland, inland valley and swamp while each operated in region one on the production surface indicating that overall, resource levels could be increased to achieve higher levels of productivity in each system. Economic policies and programmes that could encourage the reallocation and if possible the redistribution of farm production inputs for increased farm productivity and efficiency were recommended. [Journal of American Science 2010;6(11):396-408]. (ISSN: 1545-1003).

**Key words:** Resource use efficiency, rice production systems, Nigeria.

### INTRODUCTION

The struggle for food is desperate for the 240 million people of West Africa: one of every three of who is a Nigerian (WARDA, 2002). Nigeria has experienced rapid growth in per capita rice consumption during the last three decades from 5Kg in the 1960s, 11Kg in the 1980s to 25 Kg in the 1990s (IBRD, 1994; WARDA, 2003). An estimated 2.1 million tonnes of rice are consumed annually in Nigeria; this has increased since the mid 1980s at an average annual rate of 11 percent of which only 3 percent can be explained by

population growth while the remaining percentage represents a shift in diet towards rice at the expense of the coarse grains (millet and sorghum) and wheat (WARDA, 2003). Erenstein and Lancon (2002) noted this shift and posited that the most important contributory factors are rapid urbanization and the associated changes in family occupational structures. The resultant increases in the opportunity cost of family members' time makes convenience foods such as rice to rise in prominence in the family menu.

Unfortunately, Nigerian rice output is low and declining by 3.4 percent in 1997 (CBN, 1998). Odii and Nwosu (1996) noted that the decline is traceable to inefficient use of farm resources, labour shortages and severe scarcity of resources, poor crop management practices and poor capital base. Moreover, WARDA (2002) opined that inconsistency, shifting between open and protectionist trade policy characterize rice policy in Nigeria. As a result yield potential are not fully achieved on rice farms although high yielding varieties and the associated technologies exist and are already being used by the farmers (Nwaru, 2002). Consequently, Nigeria has depended heavily on imported rice to meet her consumption needs and has become the World's largest importer of rice (WARDA, 2003). That Nigeria has remained a net importer of rice with well over 150.15 billion naira spent annually (FOS, 2000) is indicative of the declining self-sufficiency.

Continued increase in rice production has been proposed through a number of options. Carsky (1992) posited that it would be possible through continued expansion into high potential areas especially the inland valley bottoms in the Midwest and Southeast and the alluvial lowlands along the Niger and Benue Rivers. Iheke (2006) noted that additional gains could be achieved through investment in water control particularly small-scale systems in inland valleys. In deed, rice-growing environments in Nigeria are usually classified into rain fed, upland, rain fed lowland, irrigated lowland, deep water and swamp (Cobley, 1976; WARDA, 1999). Inland valleys are potential agro ecosystems that have substantial impact on African food production especially rice. IITA (1988), Izac, et al, (1991) and Windmeijer and Andriesse (1993) noted that substantial increases in rice production in Sub Sahara Africa would come from inland valleys as they have the potential for increased rice productivity.

Carsky (1992) described inland valleys as small valleys that are located near the coast and do not have long flood plains. They are in the upper reaches of watersheds having no large flood plains typical of large rivers or salinity and sulphur problems typical of coastal valleys (Carsky and Masajo, 1992). According to Andriesse (1986) an inland valley starts at a water source as a stream flow valley, which further downstream becomes a river over flow valley. WARDA (1978) and Carsky (1992) noted that though a substantial amount of research has been conducted on rice, there have been fewer studies on rice production in the inland valleys; placing rice production efforts in the inland valleys at less than 10 percent while between 14 to 22 percent was concentrated on each of the other rice ecosystems; upland and lowland. For instance, in a recent study on rice production, (Idiong, 2006) only categorized rice-growing environments into upland or lowland (swamps) rain fed or irrigated, neglecting inland valleys.

Therefore, the objective of this study is to compare the technical and allocative efficiencies as well as the mean output and the returns to scale of the rice farmers in Abia State of Nigeria according to the upland, lowland and inland valley production systems. Technical efficiency refers to the ability of production units to produce maximum outputs from a given set of inputs. It indicates all the undisputed gains obtainable by simply gingering up the management (Farrel, 1957; Iheke, 2006). Observed differentials in technical efficiency may be due to the differences in managerial ability, employment of different levels of technology as indicated by the quality and type of resources used, differences in environmental conditions such as soil quality, rainfall, temperature, solar radiations and precipitation or non technical and non economic factors such as sicknesses which

may prevent the user of the resources from working hard enough, thus failing to achieve the best level of output (Nwaru, 1993). Allocative efficiency refers to the ability of the resource user to choose the optimum combination of inputs consistent with the relative factor prices (Onyenweaku, 1994). It has to do with the extent to which farmers make efficient decisions by using inputs up to the level at which their marginal contribution to the value of production is equal to the factor costs. The product of technical and allocative efficiencies is production efficiency, which measures the success of the production unit in choosing an optimal set of inputs and the gains that can be obtained by varying the input ratios on certain assumptions about the future price structure.

It is believed that the productivity of the farmers in general and rice farmers in particular could be enhanced through enhancing their technical and allocative efficiency in response to better information and education (Idiong, 2006). With the difficulties encountered by farmers in developing countries for developing and adopting improved technologies due to resource poverty, efficiency has become a very significant factor in increasing productivity (Ali and Chandry, 1990). The drive is for the farmers to allocate their resources to those productive ventures that earn higher returns for each unit of resource spent. There might be re-allocation of available resources if they expect to benefit more from such economic actions. Idiong (2006) observed that a few published empirical works have attempted to compare efficiency between or among rice production systems in Nigeria generally and in Cross River State of Nigeria in particular. This he attempted to do but stopped only at the comparison of upland and lowland rice production systems leaving out the inland valleys. This study sought to fill this gap.

## MATERIALS AND METHODS

The study was carried out in Abia State of Nigeria. The State lies between latitude 5° 25' North and Longitude 7° 30' East. It is divided into Ohafia, Umuahia and Aba Agricultural Zones. The predominant soil of the area is sandy loam while the natural vegetation is the tropical rainforest (Iheke, 2006) and is characterized by two distinct seasons; dry and wet seasons. The dry season lasts from November to March while the wet season lasts from April to October.

The settlement pattern in most part of Abia State is still rural and farming is the predominant occupation of the inhabitants. Most families are involved in one farming activity or the other as a primary or secondary occupation. The region is blessed with favourable warm climate and sufficient moisture ideal for the growing of tree crops, root and tuber crops, cereals, vegetables, nuts and food crops including rice. Livestock are also kept especially on a smallholder basis. The crops are typically grown on smallholder plots. Most crops are grown in mixtures. Rice stands out as a crop essentially grown sole.

Ohafia Agricultural Zone is well noted as the major area of rice production in Abia State of Nigeria. Men and women are involved in the production, processing and marketing of rice in the Zone. The production systems are inland valleys, upland and swamp. The inland valleys are small valleys that do not have long flood plains, located in the upper breaches of watersheds. They usually start at a water source like a stream flow valley, which further down stream becomes a river overflow valley. Swampland arises due to water logging as a result of the topography of the soil and the soil characteristics. The uplands are rain fed or irrigated lands not prone to water logging. They are ideal for the growing of such arable

crops as maize, cassava, yam and upland rice that does not tolerate water logged soils.

A multi-stage sampling technique was used in choosing the sample. Ohafia Agricultural Zone was purposively selected for being the major rice production area in the State. Two Local Government Areas in the Zone, based on performance in rice production, were purposively selected for the study. From each of the chosen LGAs, 3 blocks were randomly selected from which 6 ADP cycles were randomly chosen. Five villages in each cycle were randomly selected. A rapid appraisal of the study area was undertaken and questions posed to village heads, resident agricultural extension agents and key informants helped in preparing the list of rice farmers in each chosen village. This list formed the sampling frame from which a sample of rice farmers was selected using simple random sampling procedure. In all, 142 rice farmers comprising of 46 inland valley, 41 upland and 55 swamp rice farmers were selected.

Preliminary visits were made to the study locations before commencing actual data collection. The visits helped the researchers familiarize themselves with the study locations and establish helpful public relations with village heads, resident agricultural extension agents, key informants and field guides. At this stage, field enumerators were recruited, trained and assigned to the study locations. Also data collection instruments consisting of well-structured questionnaire and interview schedule were pre-tested to standardize them and to give the enumerators adequate orientation. This made for easy understanding by the respondents and easy administration by the field enumerators.

The cost route approach was used in data collection for the entire production period from April to December 2005. By this method, contacts were made with the respondents forth nightly. At each contact,

efforts were made to determine and record relevant pieces of information from the respondents. The research instruments found useful at the end of the fieldwork were used for further analysis. Data collected were those on socio-economic characteristics of the respondents such as age, sex, household size, educational background and farming experience. Others were on farm inputs like fertilizer, labour use, farm size, capital assets, paddy prices, credit and extension services, costs and returns (input and output) arising from rice production in the production systems.

For the technical efficiency, the additive multiplicative dummy variable approach suggested by Gujarati (1970) and Maddala (1988), which has been used widely by researchers (Baggi, 1982; Onyenweaku, 1994; Nwaru, 2003; Iheke, 2006) was used rather than the traditional method of fitting separate models and testing the equality of coefficients between them. Although some studies in agriculture have expressed the production function in many ways such as the linear, semi-log, exponential and cobb-douglas forms, the cobb-douglas function appears to be in greater use than the other functional forms because in most cases, it satisfies statistical, economic and econometric conditions better (Sankhayan, 1998). Moreover, it has been found by economists to be most suitable in analyzing production problems of industries and agriculture. It is hence used in this study.

The implicit functional form of the model is (Onyenweaku, 1994):

$$Y = f(X_1 X_2, X_3, X_4, X_5, D, ei) \quad (1)$$

The log linear cobb-douglas functional form is given by:

$$\ln Y = \ln A_0 + B_0 D + A_1 \ln X_1 + B_1 D \ln X_1 + A_2 \ln X_2 + B_2 D \ln X_2 + A_3 \ln X_3 + B_3 D \ln X_3 + A_4 \ln X_4 + B_4 D \ln X_4 + A_5 \ln X_5 + B_5 D \ln X_5 + ei \quad (2)$$

Where in equations (1) and (2), Y is the output of rice (Kg); In is the natural Logarithm, Ao is the intercept or constant term; Bo is the coefficient of the intercept shift dummy or neutral technical efficiency parameter and D is the dummy variable which takes the value of unity for inland valley and zero for upland; unity for inland valley and zero for swamp and unity for upland and zero for swamp. X1 is size of farmland (ha); X2 is family labour (mandays); X3 is hired labour (mandays); X4 is other inputs (N) (planting materials and other expenses like seeds, fertilizer, agro chemicals, etc); X5 is capital inputs (N) (depreciation charges on farm machinery, implements and tools, interest on loan, land rent); X1D, X2D, X3D, X4D, X5D are the slope shift dummies for farmland, family labour, hired labour, other inputs and capital inputs respectively. Ai ( $i = 1, 2, \dots, 5$ ) is the coefficient of the ith variable and ei is the stochastic error term assumed to satisfy all the assumptions of the classical linear regression model.

If the coefficient of the dummy variable, D (in the additive form) is significant, it means that there is a difference in the technical efficiency of the farmer groups. If it is positive, this implies that the production function for rice farmer groups denoted as unity has larger intercept term denoting a higher level of technical efficiency than the group denoted as zero and vice versa. If  $B_0 = 0$  and all  $B_i (i = 1, 2, \dots, 5) = 0$ , then the two farmer groups are represented by the same production function. If  $B_i = 0$  but  $B_0 \neq 0$ , the two groups of farmers face neutral production function. If at least one  $B_i \neq 0$ , the two groups of farmers are facing factor biased or non-neutral production function (Onyenweaku, 1994).

For the allocative efficiency, the Cobb-Douglas functional form was estimated for each production system. The logarithmic form of the function is given by:

$$\ln Y = b_0 + b_1 \ln X_1 + b_2 \ln X_2 + b_3 \ln X_3 + b_4 \ln X_4 + b_5 \ln X_5 + e_i \quad (3)$$

Where all factors are as previously defined in equations (1 and 2).

The cobb-douglas functional form of equation (3) was estimated for deriving the allocative efficiency, determined by equating the marginal value product (MVP) of the ith input to its price or marginal factor cost (MFC). That is (Onyenweaku, 1994),  $MVP_{xi} = P_{xi}$  (4)

$MVP_{xi} (i = 1, 2, \dots, 5)$  = the marginal value product of the ith input =  $PY_{fi}$ .

$f_i = \delta Q / \delta X_i$  = Marginal physical product (MPP) of the ith input. The marginal physical product (MPP) based on the double log functional form is given by

$$MPP = b_i (\bar{Y} / \bar{X}) \quad (5)$$

Where  $b_i$  is the coefficient of the ith variable,  $\bar{Y}$  is the geometric mean of output and  $\bar{X}$  is the geometric mean of the ith variable;  $P_{xi} (i = 1, 2, \dots, 5)$  is the unit price or marginal factor cost of the ith input and PY is unit price of output. According to Onyenweaku (1994) and Nwaru (2003), for all the resources measured in physical terms, the allocative efficiency index,  $W_{ij}$ , for each farmer type is given as:

$$MVP_{xi} = PY_{fi} = W_{ij} \quad (6)$$

$$P_{xi} \quad P_{xi}$$

Where  $i$ , is a particular resource,  $j$  is the farmer group and all other variables are as previously defined. For any resource that is measured in monetary or value terms, the unit input price becomes irrelevant and equation (5) translates to:

$$MVP_{xi} = PY_{fi} = W_{ij} \quad (7)$$

In this study, the dependent variable, Y, was measured in physical terms while other inputs and capital inputs were measured in value or monetary terms. Accordingly, the marginal value products of the resources measured in value terms are directly equal to their allocative efficiency indices. This is because the marginal value products were already deflated by the unit

factor prices since the value of these factors are the products of the quantity employed and the unit factor prices.

Maximum or absolute allocative efficiency for a particular farmer group is confirmed with respect to a given resource if  $W_{ij} = 1$ . The resource is over-utilized if  $W_{ij} < 1$  and under-utilized if  $W_{ij} > 1$ . The farmer groups would have achieved equal allocative efficiency if  $W_{i1} = W_{i2}$ . To show the extent to which a particular resource should be increased or reduced from the current level of use in order to achieve maximum allocative efficiency, we evaluate the following formula:  $K_{ij} = (1-W_{ij})100$  (8)

Where  $K_{ij}$  is the percentage by which the level of use of a particular resource should be increased or decreased to achieve the objective of maximum allocative efficiency. A negative  $K_{ij}$  implies that an increased employment of the resource is required and vice versa. If  $K_{ij} = 0$ , then absolute allocative efficiency has been achieved.

The analysis of variance was used to test the significance for the mean output of rice from the production systems. It is given by:

$$F_{cal} = \frac{\{\sum n_j (\bar{Y}_j - \bar{Y})^2\} / (K-1)}{\{\sum \sum (Y_{ji} - \bar{Y}_j)^2\} / (N-k)} \quad (9)$$

where,  $\{\sum n_j (\bar{Y}_j - \bar{Y})^2\} / (K-1)$  = estimated variance from "between" the mean,

$\{\sum (Y_{ji} - \bar{Y}_j)^2\} / (N-k)$  = estimated variance from "within" the samples,  $\bar{Y}$  = mean output from the  $j$ th production system

$\bar{Y}$  = mean output from the production systems (pooled sample mean)

$Y_{ij}$  = individual output of the farmers in the  $j$ th production system

$K$  = number of production systems

$n_j$  = number of farmers in the  $j$ th production system

$N = \sum n_j$  = total number of farmers

Decision rule: If  $F_{cal} < F_{tab}$ , accept the null hypothesis i.e we accept that the means are not significantly different, otherwise reject the null hypothesis.

## RESULTS AND DISCUSSION

### Technical efficiency of upland and swamp rice farmers

The estimated production function of the upland and swamp rice farmers is presented in Table 1. The intercept, hired labour, other inputs, capital inputs, intercept shift dummy, slope shift dummies for farmland and capital are significant at 1 percent; farmland is significant at 5 percent while slope dummies for other inputs and hired labour are significant at 10 percent. It has an R<sup>2</sup> value of 0.9525 which implies that 95.25 percent of the variation in output is explained by the independent variables.

That the coefficient of the intercept shift dummy is statistically significant at 1 percent implies that a shift in technology exists between the upland and swamp rice farmers. The positive sign of this coefficient implies that there is a shift in neutral technical efficiency parameter to a higher level for the upland rice farmers. This group of farmers has therefore achieved higher technical efficiency. This conclusion conforms to the findings from Onyenweaku (1994) and Nwaru (2003).

The slope shift dummies for farmland, hired labour, other inputs and capital inputs are statistically significant, implying a difference in the slope shift coefficients of these resources. This means that the upland and swamp farmers are characterized by factor-biased or non neutral production functions. Hence, both groups of farmers are characterized by different production functions. Furthermore, the slope shift dummies for other inputs and capital inputs are negative which implies a higher level of use intensities of these resources by the swamp rice farmers while those for

farmland, family labour and hired labour are positive indicating lower use intensities of the resources by the swamp rice farmers. The implication is that the swamp farmers can improve on their performance by increasing their level of use intensities of other inputs and capital inputs and reducing their level of use intensities of farmland, family labour and hired labour.

### **Technical efficiency inland valley and swamp rice farmers**

The estimated production function for the inland valley and swamp rice farmers are summarized and presented in Table 2. The coefficient of multiple determination ( $R^2$ )

was 0.9146 which implies that 91.46 percent of the variation in rice output, is accounted for by the independent variables. The F-ratio is significant at 1 percent which attests to the overall significance of this estimated function. Farmland, hired labour, other inputs and capital inputs were significant and positive. The implication is that increase in their utilization would lead to increase in rice output. The intercept dummy is statistically insignificant implying that no shift in technology exists between the inland and swamp rice farmers. Both groups of farmers have equal technical efficiency and have the same production function.

Table 1: Estimated production function for the upland and swamp rice farmers

Variable	Parameter	Coefficient	t-ratio
Intercept	A0	-1.719	-4.57***
Farmland	A1	0.057	2.40***
Family labour	A2	0.007	-0.36
Hired labour	A3	0.092	3.47***
Other inputs	A4	0.174	3.49***
Capital inputs	A5	0.832	13.11***
Intercept dummy (D)	B0	7.765	7.60***
(Farmland)D	B1	0.783	6.76***
(Family labour)D	B2	0.019	0.30
(Hired labour)D	B3	-0.078	-1.67*
(Other inputs)D	B4	-0.127	-1.84*
(Capital inputs)D	B5	-0.767	-6.79***
	R2		0.9525
	R-2		0.9463
	F-ratio		153.20***

Source; Survey data, 2005.

\*\*\*, \*\*, \* = Statistically significant at 1, 5 and 10 percent respectively.

Table 2: Estimated production function for inland valley and swamp farmers

Variable	Parameter	Coefficient	t-value
Intercept	A0	-1.719	-3.53***
Farmland	A1	0.057	1.85*
Family labour	A2	-7.20E-3	-0.28
Hired labour	A3	0.092	2.68***
Other inputs	A4	0.174	2.70***
Capital inputs	A5	0.832	10.12***
Intercept dummy (D)	B0	1.310	1.28

(Farmland)D	B1	0.216	2.75***
(Family labour)D	B2	0.047	1.17
(Hired labour)D	B3	-0.045	-0.86
(Other inputs)D	B4	0.497	4.19***
(Capital inputs)D	B5	0.719	-7.37***
	R2		0.9146
	R-2		0.9041
	F-ratio		86.67***

Source: Survey data, 2005

\*\*\*, \*\*, \* Statistically significant at 1, 5 and 10 percent respectively.

### Technical efficiency inland valley and upland rice farmers

The estimated production function for the inland and upland rice farmers is presented in Table 3. The intercept shift dummy is statistically significant at 1 percent implying that a shift in technology exists between the inland valley and upland rice farmers. Moreover, the intercept dummy has a negative coefficient.

Table 3: Estimated production function for upland and inland valley farmers

Variable	Parameter	Coefficient	t-value
Intercept	A0	6.047	5.14***
Farmland	A1	0.840	5.99***
Family labour	A2	0.011	0.16
Hired labour	A3	0.013	0.28
Other inputs	A4	0.048	0.81
Capital inputs	A5	0.065	0.56
Intercept dummy (D)	B0	-6.455	-4.43***
(Farmland) D	B1	-0.567	-3.62***
(Family labour)D	B2	0.029	0.37
(Hired labour)D	B3	0.031	0.49
(Other inputs) D	B4	0.624	5.58***
(Capital inputs) D	B5	0.048	0.38
	R2		0.9129
	R-2		0.9001
	F-ratio		71.45***

Source: Computed from Survey data, 2005

\*\*\*, \*\*, \* statistically significant at 1, 5 and 10 percent respectively.

The slope dummies for farmland and other inputs are statistically significant at 1 percent. This means that the inland and upland farmers are characterized by factor biased or non neutral production functions. There is a lower level of use intensity of farmland by the inland valley farmers and higher use intensities of family labour, hired labour, other inputs and capital inputs by them. The result shows that ample opportunities exist for the farmers to increase their productivity and income through improvements on their technical efficiency. This can be achieved by putting in place policies that will enable them to increase their use of those resources currently at lower levels of use intensities and vice versa.

### Allocative efficiency of the farmers in the production systems

The estimated production functions of the inland valley, upland and swamp farmers were summarized and presented in Table 4. This Table indicates that 84.69 percent, 96.10 percent and 94.71 percent of the variations in rice output in inland valleys, upland swamp farms respectively were explained or accounted for by the independent variables. The F – ratio is significant which attests to the overall significance of the regression result. This implies that the data fit the model and that the independent variables are important explanatory factors of the variations in rice output. All the variables were significant for the upland farm while farmland, other inputs and capital were significant for the inland valley rice farmers. Only hired labour was insignificant for the swamp rice farmers.

Table 4: Estimated production functions for the three group of rice farmers

Variable	Parameter	Inland	Upland	Swamp
Intercept	A0	-0.409 (-0.39)	6.046 (7.53)***	-1.632 (-4.50)***
Farmland	A1	0.273 (3.27)***	0.840 (8.78)***	0.064 (2.79)***
Family labour	A2	0.040 (1.11)	277.641 (2.07)**	-0.011 (-0.53)
Hired labour	A3	0.044 (0.88)	645.651 (3.88)***	0.106 (4.25)***
Other inputs	A4	0.672 (5.83)***	735.412 (2.21)**	0.221 (4.41)***
Capital inputs	A5	0.112 (1.84)*	3385.920 (8.62)***	0.764 (12.98)***
	R2	0.8469	0.9610	0.9471
	R-2	0.8278	0.9554	0.9435
	F-ratio	44.27***	172.38***	261.38***

Source: Survey data, 2005. Figures in parenthesis are the t-ratios

\*\*\*, \*\*, \* = Statistically significant at 1, 5 and 10 percent respectively.

From the coefficients in Table 4, the allocative efficiency indices were derived and presented in Table 5. This Table depicts that none of the three farmer groups achieved absolute allocative efficiency in the use of farm resources. The upland farmers are the least allocatively efficient with respect to all the farm resources. This farmer group under-utilized all the farm resources; that is they used less than the profit maximizing level. The inland valley rice farmers achieved their best allocative efficiency in the use capital inputs while the swamp farmers achieved their best allocative efficiency in the use of hired labour. The inland and swamp rice farmers under-utilized farmland, other inputs and capital. The swamp rice farmers over-utilized hired labour. To achieve maximum allocative efficiency and hence maximum profit, policies and programmes that would enable the inland farmers increase their use of farmland, other inputs and capital inputs by 978.3 percent, 655.0 percent and 188.9 percent respectively should be put in place. Such policies and programmes should help the upland farmers to increase their use of farmland, family labour, hired labour, other inputs and capital inputs by 3097.2 percent, 186950.6 percent, 497751.6 percent, 734588.9 percent and 9257077.5 percent respectively. It

should equally enable the swamp farmers to increase their use of farmland, other inputs and capital inputs by 163.9 percent, 159.2 percent and 2424.8 percent respectively and reduce their use of family and hired labour by 107.5 and 152 percents respectively.

Table 5: Allocative efficiency indices of the farmer groups

Farmer group	Inland valley	Upland	Swamp
a) Marginal physical product (MPP)			
Farmland	336.982	999.132	82.463
Family labour	2.281	9742.22	-0.392
Hired labour	2.435	25929.769	4.419
Other inputs	0.081	78.826	0.027
Capital inputs	0.038	993.218	0.263
b) Price of milled rice (N/kg)	96	96	96
c) Marginal value product (MVP) (N)			
Farmland	32350.272	95916.672	7916.448
Family labour	218.976	935253.12	-37632
Hired labour	233.76	2489257.824	424.224
Other inputs	7.776	7567.296	2.592
Capital inputs	2.976	95348.928	25.248
d) Marginal factor cost (MFC) (N)			
Farmland	3000	3000	3000
Family labour	500	500	500
Hired labour	500	500	500
Other inputs	1.03	1.03	1.03
Capital inputs	1.03	1.03	1.03
e) Allocative efficiency indices (AEI)			
Farmland	10.783	31.972	2.639
Family labour	NS	1870.506	NS
Hired labour	NS	4978.516	0.848
Other inputs	7.55	7346.889	2.517
Capital inputs	2.889	92571.775	24.513
f) Required change in AEI			
Farmland	-9.783	-30.972	-1.639
Family labour	NS	-1869.506	NS
Hired labour	NS	-4977.516	0.152
Other inputs	-0.562	7345.889	-1.517
Capital inputs	-1.889	92570.775	-23.513

Source: Survey data, 2005.

NS = not significant

### Returns to scale of the farmers in the production systems

The elasticity of production for the farmers in the production systems from which their returns to scale were derived are presented in Table 6. It shows that none of the defined farmer groups is operating at constant returns to scale. Farmers in the different production systems are operating at increasing return to scale ( $\sum E_p > 1$ ), suggesting that they are operating in region one of the total product curve which is an irrational region to rest production. The implication is that they can improve on their productivity by increasing their overall employment of farm resources.

Table 6: Elasticity of production of the farmers based on the production systems

Variable	Inland	Upland	Swamp
Farmland	0.273	0.840	0.068
Family labour	0.040	277.641	-0.011
Hired labour	0.044	645.651	0.106
Other inputs	0.671	735.412	0.221
Capital inputs	0.112	3386.920	0.764
$\sum E_p$	1.14	5046.464	1.148

Source: computed from survey data, 2005

### Mean output of rice from the production systems

The test of significance in the mean output of rice from the production systems was realized through analysis of variance (ANOVA) and the result is presented in Table 7. The table revealed the calculated F – value was 2.074 and the tabulated value, 3.00. Therefore, since the calculated F- value was less than the tabulated value ( $F_{cal} < F_{tab}$ ), the null hypothesis is accepted. Hence there is no significant difference in the mean output of rice from the various production systems.

Table 7: ANOVA test of significance in the output of rice by production systems

Source of Variation	Degree of Freedom	Sum of squares	Mean Square	F cal	F tab
Between	2	9187012.42	4593506.21	2.074	3.00
Within	139	307858775.60	2214804.86		
Total	141	317044888			

Source: Survey data, 2005.

### CONCLUSION

Results indicate that the upland rice farmers are technically more efficient than the swamp and inland rice farmers and that there is no difference in technical efficiency between the swamp and inland rice farmers. Furthermore, resources were poorly allocated by these rice farmers in each of the production systems: inland valleys, upland and swamp environments. None of the farmer groups achieved absolute allocative efficiency. There was no significant difference in the mean output of rice from the production systems; upland, inland valley and swamp while each operated in region one on the production surface indicating that overall, resource levels could be increased to achieve higher levels of productivity in each system.

Therefore, economic policies and programmes that could encourage the reallocation and if possible the redistribution of farm production inputs for increased farm productivity and efficiency should be put in place. Such policies should be appropriate enough to grant rice farmers increased access to farmland. They should enable them employ the use of more farm resources since there is increasing return to scale, be targeted more at the upland rice farmers and seek opportunities for exploring the swamp and inland valleys more.

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8/15/2010

# Knowledge Discovery in Al-Hadith Using Text Classification Algorithm

Khitam Jbara

Department of Computer Science, King Abdullah II School for Information Technology, The University Of Jordan  
P.O. Box 710481 Amman 11171 Jordan.

[ktjlc2000@yahoo.com](mailto:ktjlc2000@yahoo.com)

**Abstract:** Machine Learning and Data Mining are applied to language datasets in order to discover patterns for English and other European languages; Arabic language belongs to the Semitic family of languages which differs from European languages in syntax, semantic and morphology. One of the difficulties in Arabic language is that it has a complex morphological structure and orthographic variations. This study is conducted to examine knowledge discovery from AL-Hadith through classification algorithm in order to classify AL-Hadith to one of predefined classes (books), where AL-Hadith is the saying of Prophet Mohammed (peace and blessings of Allah be upon him) and the second religious source for all Muslims, so because of its importance for Muslims all over the word knowledge discovery from AL-Hadith will make AL-Hadith more understandable for both Muslims and nonmuslims. [Journal of American Science 2010;6(11):409-419]. (ISSN: 1545-1003).

**Keywords:** AL-Hadith, classification, stem, feature, class, expansion, training set.

## 1. Introduction

Information Retrieval (IR) is the discipline that deals with retrieval of unstructured data, especially textual documents, in response to a query, which may itself be unstructured like sentence or structured like Boolean expression. The need for effective methods of automated IR has grown in the last years because of tremendous explosion of the amount of unstructured data (Greengrass, 2000).

Text mining is a class of what is called nontraditional (IR) strategies (Kroeze, et al., 2003). The goal of these strategies is to reduce the required effort from users to obtain useful information from large computerized text data sources. Also text classifications is a subfield of data mining which refers generally to the process of deriving high quality of information from a text, which is typically derived through the dividing of patterns and trends through methods such as statistical pattern learning.

However; text classification is one of the most important topics in the field of natural language processing, where the purpose of its Algorithm is to assign each document of text dataset to one or more pre-specified classes. More formally if  $d_i$  is a document of set of documents D and  $\{c_1, c_2, \dots, c_n\}$  is The set of all classes, then text classification assigns one category  $c_j$  to a document  $d_i$  and in multi-subjects classification  $d_i$  can be assigned to more than one class from a set of classes.

Text classification techniques are used in many applications, including e-mail filtering, mail routing, spam filtering, news monitoring, sorting

through digitized paper archives, automated indexing of scientific articles, classification of news stories and searching for interesting information on the web (Khreisat, 2006).

Also, an important research topic appears in this field called Automatic text classification (ATC) because of the inception of the digital documents. Today, ATC is a necessity due to the large amount of text documents that users have to deal with (Duwairi, 2006).

According to the growth of text documents and Arabic document sources on the web, information retrieval becomes an important task to satisfy the needs of different end users; while automatic text (or document) categorization becomes an important attempt to save human effort required in performing manual categorization.

In this paper, a knowledge discovery algorithm for AL-Hadith is proposed in order to classify it to one of predefined classes (books), this algorithm consists of two major phases; the training phase and Classification phase. Experiments will be conducted on a selected set of AL-Hadith from Al-Bukhari book, where thirteen books were chosen as classes in order to run these experiments. The evaluation of the proposed algorithm is carried out by comparing its results to Al-Bukhari classification.

This paper is organized as follows; related work is represented in section 2, while section 3 represents the proposed classification system, and section 4 analyze experiments and results, finally section 5 demonstrates conclusion.

## 2. Related Work

Most of nowadays classifiers were built for English or European languages. For example, Zhang (2004) builds a Naïve Bayes (NB) classifier, which calculates the posterior probability for classes then the estimation is based on the training set that consists of pre-classified documents, in his system testing phase the posterior probability for each class is computed then the document is classified to the class that has the maximum posterior probability.

Isa, et al. (2008) explore the benefits of using enhanced hybrid classification method through the utilization of the NB classifier and Support Vector Machine (SVM). While Lam, et al. (1999) built a neural network classifier addressing the classifier drawbacks and how to improve its performance.

Bellot, et al. (2003) propose an approach that combines a named entity recognition system and an answer retrieval system based on Vector Space model that uses some knowledge bases, while Liu, et al. (2004) focus on solving the problem of using training data set to find representative words for each class, also (Lukui, et al. 2007) explore how to improve the executing efficiency for classification methods.

On the other hand, Yu-ping, et al. (2007) propose a multi-subject text classification algorithm based on fuzzy support vector machines (MFSVM).

In the Arabic language field, AL-Kabi, et al. (2007) present a comparative study that represents the efficiency of different measures to classify Arabic documents. Their experiments show that NB method slightly outperforms the other methods, while AL-Mesleh (2007) proposes a classification system based on Support Vector Machines (SVMs), where his classifier uses CHI square as a feature selection method in the pre-processing step of text classification system procedure.

El-Halees (2006) introduces a system called ArabCat based on maximum entropy model to classify Arabic documents, and Saleem et al. (2004) present an approach that combines shallow parsing and information extraction techniques with conventional information retrieval, while Khreisat (2006) conducts a comprehensive study for the behavior of the N-Gram Frequency Statistics technique for classifying Arabic text document.

Hammo, et al. (2002) design and implement a Question Answering system called QARAB.EL-Kourdi, et al. (2004) build an Arabic document classification system to classify non-vocalized Arabic web documents based on NB algorithm, while AL-Kabi, et al. (2005) represent an automatic classifier to classify the verses of Fatiha and Yaseen Chapters to predefined themes, where the system is based on

linear classification function (score function), and (Hammo, et al. 2008) discuss the enhancement of Arabic passage retrieval for both diacritized and non-diacritized text, they propose a passage retrieval approach to search for diacritic and diacritic-less text through query expansion to match user's query.

## 3. Proposed Classification System

The proposed system consists of four phases; first one is the preprocessing phase. Second phase is the training phase where the learning database is constructed which contains the weights of features representing a class. The input for this phase is a set of pre-classified documents. Third phase is the classification phase in which the resulted training database of previous phase is used with the classification method to classify targeted Hadith, also a query expansion occurs in this phase. Finally, data analyzing and evaluation phase. These phases are shown in figure 1.

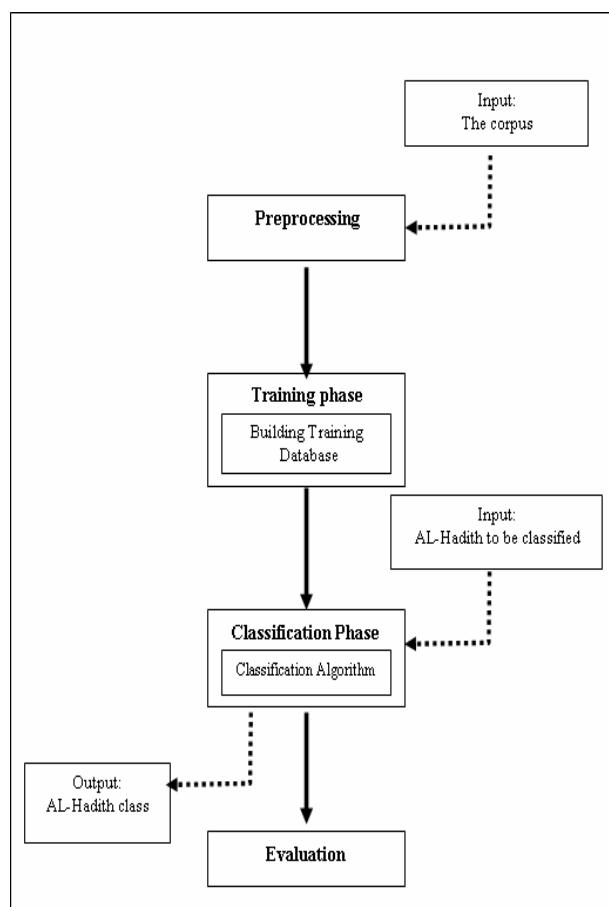


Figure 1. An overview of proposed system phases.

We can define the corpus that contains a set of Ahadith (plural of AL-Hadith) as in definition 1. Figure 2 shows an example of Hadith from the book of food that will be used in the illustration of each step of the proposed system.

## **Definition 1: Corpus Definition**

Suppose corpus  $\mathbf{C} = \{H_1, H_2, H_3, \dots, H_n\}$ . Where  $H_i$  represents the  $i$ th tested Hadith in  $\mathbf{C}$ ,  $n$  is the number of tested Hadith in the  $\mathbf{C}$  and  $i: 1.....n$ .

Suppose  $\mathbf{H}_j = \{w_1, w_2, w_3, \dots, w_m\}$ . Where  $w_d$  represents the  $d$ th word in AL-Hadith  $\mathbf{H}_j$ ,  $m$  is the number of words in  $\mathbf{H}_j$  and  $d: 1 \dots m$ .

حدثى إسحاق بن إبراهيم: أخبرنا روح بن عبادة: حدثنا ابن أبي ذئب، عن سعيد المقيرى، عن أبي هريرة رضى الله عنه:

انه من بقوم بين ابييهم شاه مصلينه، قد عوه، فابي ان يأكل و قال:  
خرج رسول الله صلى الله عليه وسلم من الدنيا ولم يشبع من خبر  
الشاعر.

Figure 2. Example of AL-Hadith from the book of food.

### 3.1 Preprocessing Phase

In this section the preprocessing techniques are introduced, preprocessing will be conducted on each Hadith used in the training and testing sets. This stage is necessary before the classification phase can be applied to discover knowledge from AL-Hadith and it consists of several sub phases:

1. **Removing Sanad:** this process is done manually and aims to remove Sanad which is a part of

AL\_Hadith that refers to the chain of names of persons who have transmitted AL-Hadith.

2. **Tokenization:** which aims to divide AL\_Hadith into tokens (words); AL-Hadith tokenization was easily resolved since each token can be identified as a string of letters between white spaces.
  3. **Removing punctuation and diacritical marks:** removing diacritical and punctuation marks is important since those marks are prevalent in Ahadith and have no effect on determining AL\_Hadith class.
  4. **Removing stop words:** Stop words are words that found in AL-Hadith and have no discriminative meaning (AL-Kabi, et al., 2005). In the proposed system a list of stop words is built manually, it consists of Arabic pronouns, prepositions, names of people (companions of Prophet Mohammed) and places were mentioned in AL-Hadith corpus. After removing stop words from AL\_Hadith, the remaining words (terms) are considered as features.
  5. **Stemming:** In this step the stems of features are extracted, stem extraction implemented is considered as light stem extraction which depends on removing some prefixes or suffixes from the word to relate the word to its stems, we used the stemming algorithm proposed by (Al-Serhan, et al., 2003). The result of stem extraction was filtered to eliminate the incorrect stems (roots less than three characters). The resulted stems will be used in the query expansion process which will be discussed in details in section 3.3.2, Table 1 shows all steps of preprocessing for AL-Hadith that is presented in figure 2.

Table1. Results of preprocessing phase steps for AL-Hadith in figure 2

Step	Result of the step
Removing Sanad	أنه من بقوم بين أيديهم شاة مصلية، فدعوه، فلبي أن يأكل وقال: خرج رسول الله صلى الله عليه وسلم من الدنيا ولم يشبع من خيز الشعير.
Tokenization	{“أنه”, “من”, “يقوم”, “بين”, “أيديهم”, “شاة”, “مصلية”, “،”, “فدعوه”, “،”, “فلبي”, “أن”, “يأكل”, “وقال”, “:”, “خرج”, “رسول”, “الله”, “صلى”, “الله”, “عليه”, “ وسلم”, “من”, “الدنيا”, “ولم”, “يشبع”, “من”, “خيز”, “الشعير”, “.”}.
Removing Punctuation and Diacritical Marks	{ انه ، من ، بقوم ، بين ، ايديهم ، شاة ، مصلية ، فدعوه ، فابي ، ان ، يأكل ، وقال ، خرج ، رسول ، الله ، صلى ، الله ، عليه ، وسلم ، من ، الدنيا ، ولم ، يشبع ، من ، خيز ، الشعير }
Removing Stop Words	{ من ، بقوم ، ايديهم ، شاة ، مصلية ، فدعوه ، فابي ، يأكل ، خرج ، الله ، صلى ، عليه ، وسلم ، الدنيا ، يشبع ، خيز ، الشعير }
Stemming (valid stems)	{ ايدي ، دنيا ، شعير }

### 3.2 Training Phase

Supervised classification exploits the predefined training documents that belong to specific class to extract the features which represent a class. Therefore, every class will have a feature vector representing it, and then these features will be reduced using one of the features selection techniques. Feature vectors will be used later by the classification algorithm in the testing phase.

Supervised classification has its difficulties; one main problem is how to be sure that trained document actually belongs to a specific class. In this study this problem is resolved by conducting it on a set of Ahadith that has been classified by the famous AL-Hadith scientist AL-Bukhari who gave us a good base to evaluate the proposed algorithm.

Training phase consists of two main stages; first one is executed once to produce Inverse Document Frequency (IDF) matrix for the corpus while the second one is executed for each training set.

#### 3.2.1 Corpus IDF Matrix

After conducting the preprocessing phase a list of features for each Hadith in the corpus is

produced that will be used in the classification process. Building the IDF matrix for AL-Hadith corpus is done only one time and it will be used in the classification process every time the IDF value for a feature is needed. The IDF value for a given feature is computed according to equation (1).

$$\text{IDF}_i = \log \left( \frac{N}{DF_i} \right) \quad (1)$$

Where

N: number of Ahadith in the corpus.

DF<sub>i</sub>: Number of Ahadith in the corpus containing feature i.

Fewer documents containing a given feature will produce a larger IDF value and if every document in the collection contains a given feature, feature IDF will be zero, in other words the feature which occurs in every document in a given collection is not likely to be useful for distinguishing relevant from non-relevant documents. Table 2 shows the IDF matrix structure.

Table 2. Corpus IDF matrix.

Feature	Pre-defined Classes(Books)					
	Book1	Book2	Book3	....	Bookc	Feature redundancy
Feature1	Log (N/DF <sub>1</sub> )	log (N/DF <sub>1</sub> )	log (N/DF <sub>1</sub> )	.....	log (N/DF <sub>1</sub> )	([DF <sub>1</sub> ]/N)*100
Feature2	Log (N/DF <sub>2</sub> )	log (N/DF <sub>2</sub> )	log (N/DF <sub>2</sub> )	.....	log (N/DF <sub>2</sub> )	([DF <sub>2</sub> ]/N)*100
Feature3	Log (N/DF <sub>3</sub> )	log (N/DF <sub>3</sub> )	log (N/DF <sub>3</sub> )	.....		
Feature4	Log (N/DF <sub>4</sub> )	log (N/DF <sub>4</sub> )	log (N/DF <sub>4</sub> )	.....		
Feature5	Log (N/DF <sub>5</sub> )	log (N/DF <sub>5</sub> )	log (N/DF <sub>5</sub> )	.....		
.....				.....		
.....				.....		
.....				.....		
.....				.....		
Feature <sub>n</sub>	Log (N/DF <sub>N</sub> )	log (N/DF <sub>N</sub> )	log (N/DF <sub>N</sub> )	.....	log (N/DFc)	([DF <sub>n</sub> ]/N)*100

#### 3.2.2 Weight calculations for training sets features

The proposed system depends on using a set of Ahadith as training documents to extract representative words for each book (class) and compute their weights. The weight of a given feature in a given document is calculated as (TF×IDF) because this weighting schema combines the importance of TF and IDF at the same time, and the features training weights is computed according to equation (2).

$$TW_{bi} = TF_{bi} \times IDF_i \quad (2)$$

Where:

TW<sub>bi</sub>: feature i training weight in training set b.

TF<sub>bi</sub> : feature i frequency in training set b.

IDF<sub>i</sub>: feature i inverse document frequency calculated earlier (IDF matrix).

Features that will be considered to include their weights in training weights must satisfy the feature redundancy threshold 45, that's mean that feature redundancy must be less than 45.

Table 3 shows training weights for features in the training set b in general, while Table 4 shows training weights for features in a training set from the book of food.

Table3.Training weights for features in training set b.

Feature	IDF	TF	Bookb
			TW
Feature1	IDF1	TF <sub>b1</sub>	$TW_{b1} = TF_{b1} * IDF1$
Feature2	IDF2	TF <sub>b2</sub>	$TW_{b2} = TF_{b2} * IDF2$
Feature3	IDF3	TF <sub>b3</sub>	$TW_{b3} = TF_{b3} * IDF3$
Feature4	IDF4	TF <sub>b4</sub>	$TW_{b4} = TF_{b4} * IDF4$
.....			
Feature n			$TW_{bn} = TF_{bn} * IDF_n$

Table 4. Training weights for features in a training set from the book of food.

The book of food (training set No.1)			
Feature	IDF	TF	TW
يأكل	1.80	8	14.39
الدنيا	1.84	1	1.84
شاة	1.97	4	7.90
مر	2.12	1	2.12
ابدفهم	2.22	1	2.22
خنز	2.34	4	9.37
فألى	2.42	1	2.42
الشعير	2.52	2	5.04

### 3.3 Classification Process

The classification process consists of four steps as shown in Figure 3. First step is computing query weights where feature's weight in targeted AL-Hadith is found. Second step is the expansion process where the stems are used to expand the query. Third step is calculating the similarity coefficient for each feature in AL-Hadith to be classified, and the final step is finding the cumulative similarity for AL-Hadith over the predefined classes (books).

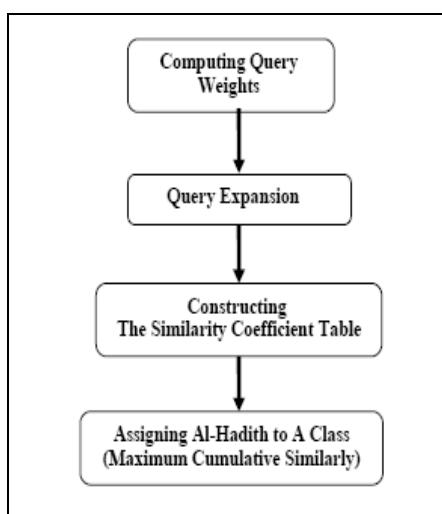


Figure 3.Classification process steps

#### 3.3.1 Computing Query Weights

A feature weight in the query (specific Hadith) is calculated according to equation (3) :

$$Q_h W_i = T F_{hi} \times I D F_i \quad (3)$$

Where:

$Q_h W_i$ : feature i weight in AL-Hadith h (Hadith to be classified).

$T F_{hi}$  : feature i frequency in AL-Hadith h

$I D F_i$  : inverse document frequency calculated by equation (1).

Query weights as shown in Table 5 will be computed for each feature in AL-Hadith to be classified. Feature frequency (TF) depends on AL-Hadith features occurrence while Inverse document frequency (IDF) is a global value referenced from IDF matrix.

Table 5. Query weights table for mined Hadith

#	Feature	IDF	Feature Redundancy	TF	QW
1	الدنيا	1.84	1.44	1	1.84
2	الشعير	2.52	0.30	1	2.52
3	الله	Feature redundancy >45	80.02	2	0
4	ابدفهم		0.61	1	2.22
5	يقوم	2.64	0.23	1	2.64
6	خنز	2.34	0.45	1	2.34
7	خرج	1.53	2.95	1	1.53
8	شاة	1.97	1.06	1	1.97
9	صلى	Feature redundancy >45	68.89	1	0
10	فابى		0.38	1	2.42
11	فدعوه	3.12	0.08	1	3.12
12	مر	2.12	0.76	1	2.12
13	مصلبة	3.12	0.08	1	3.12
14	وسلم	Feature redundancy >45	68.58	1	0
15	يأكل		1.59	1	1.80
16	يسبع	2.64	0.23	1	2.64

#### 3.3.2 Query Expansion

The process of query expansion depends mainly on using the stems of features to expand the searching area. The stems for all features in the training set and AL-Hadith to be classified were produced in the preprocessing phase.

In the expansion process the newly added stems in the expanded training set will have the same weights for its origin feature. In other words, if we have the couple  $\{(W, S), (W, TW)\}$  where S is the stem of word W and TW is the training weight for W

from the training weights Table then the weight for stem S will be the same weight of W.

The same procedure is applied to stems in expanding the query set where stem S will have the same weight of its origin word W from the query weight Table. The extended query weights for AL-Hadith sample are shown in Table 6.

Table 6. Extended query weights table for mined Al-Hadith

#	Feature	IDF	Feature Redundancy	TF	QW
1	الدنيا	1.84	1.44	1	1.84
2	الشاعر	2.52	0.30	1	2.52
3	الله		Feature redundancy >45	80.02	0
4	ابدیهم	2.22		0.61	1
5	بقوم	2.64		0.23	1
6	خنز	2.34		0.45	1
7	خرج	1.53		2.95	1
8	شاة	1.97		1.06	1
9	صلی		Feature redundancy >45	68.89	0
10	فابی	2.42		0.38	1
11	福德عوه	3.12		0.08	1
12	مر	2.12		0.76	1
13	مصلیة	3.12		0.08	1
14	وسلم		Feature redundancy >45	68.58	0
15	باکل	1.80		1.59	1
16	پشیع	2.64		0.23	1
17	دنیا		Feature No. 1		1.84
18	شاعر		Feature No. 2		2.52
19	ابدی		Feature No. 4		2.22

### 3.3.3 Constructing the Similarity Coefficient Table

In the proposed system the cosine similarity coefficient is used, where the similarity between two documents (document (D) & query (Q)) is actually the cosine of the angle (in N-dimensions) between the 2 vectors and can be calculated according to equation (4) (Baarah, 2007):

$$\text{sim}(D, Q) = \sum_{i=1}^n (w_{di} \times w_{qi}) \quad (4)$$

Where i denote the query feature and n is the number of feature in the query Hadith.

Table 7 shows similarity coefficient for features in the mined AL-Hadith in general, while Table 8 shows the similarity coefficient for features in AL-Hadith illustrated in figure 2 against the training set from the book of food shown in section 3.2.2.

Table 7: Similarity coefficient for features for mined Hadith in general.

Feature	Pre-defined Themes			
	Book1	Book2	...	Book13
Feature1	Sim1=Q <sub>b</sub> W <sub>1</sub> *T <sub>1</sub> W <sub>1</sub>			QbW <sub>1</sub> *T <sub>13</sub> W <sub>1</sub>
Feature2	Sim2=Q <sub>b</sub> W <sub>2</sub> *T <sub>1</sub> W <sub>2</sub>			QbW <sub>2</sub> *T <sub>13</sub> W <sub>2</sub>
Feature3	Sim3=Q <sub>b</sub> W <sub>3</sub> *T <sub>1</sub> W <sub>3</sub>			QbW <sub>3</sub> *T <sub>13</sub> W <sub>3</sub>
Feature4	Sim4=Q <sub>b</sub> W <sub>4</sub> *T <sub>1</sub> W <sub>4</sub>			QbW <sub>4</sub> *T <sub>13</sub> W <sub>4</sub>
Feature5	Sim5=Q <sub>b</sub> W <sub>5</sub> *T <sub>1</sub> W <sub>5</sub>			QbW <sub>5</sub> *T <sub>13</sub> W <sub>5</sub>
Feature <sub>n</sub>	Sim n=Q <sub>b</sub> W <sub>n</sub> *T <sub>1</sub> W <sub>n</sub>			
	$\sum_{i=1}^n \text{Sim}$			

### 3.3.4 Assigning AL\_Hadith to a class

After constructing the similarity coefficient table for AL-Hadith to be classified against the predefined classes, the cumulative similarity weights for mined Hadith will be found against each of those classes. The cumulative similarity values indicate common features between AL\_Hadith to be classified and the predefined books.

After finding the cumulative weight for the mined Hadith with correspondence to each predefined book (class), AL-Hadith will be assigned to the book with the maximum cumulative weight, because maximum cumulative weight is an indication of larger common features between the training set and the mined AL-Hadith feature set.

Table 8. Similarity coefficient for features in the example Hadith against training set from the book of food.

#	Feature	Similarity coefficient
1	الدنيا	3.39
2	الشاعر	12.69
3	الله	0.00
4	ابدیهم	4.92
5	بقوم	0.00
6	خنز	21.93
7	خرج	0.00
8	شاة	1.97
9	صلی	0.00
10	فابی	2.42
11	福德عوه	0.00
12	مر	2.12
13	مصلیة	0.00
14	وسلم	0.00
15	باکل	1.80
16	پشیع	0.00
17	دنیا	3.39
18	شاعر	12.69
19	ابدی	4.92
	Cumulative similarity	72.26

#### 4. Experiments and Results

In this section, an overview is given for AL-Hadith corpus content that is used in this study to run the experiments of the proposed classifying algorithm, and details of experiments are also illustrated.

##### 4.1 Content of AL-Hadith Corpus

AL-Hadith corpus that is used in running the experiments consist of thirteen books (classes). Ahadith were taken from Sahih AL-Bukhari which is the most well known Hadith book all over the Islamic world and the most trusted Hadith book for researchers in this field. Twelve of those books were included in AL\_Kabi (2007) study while the Book of the (Virtues of the Prophet and His Companions) is added to the experiment in this study with 143 additional Ahadith.

Table 9 shows statistical information of books included in the experiments along with its name in English and Arabic as it was used by AL-Bukhari in his Sahih. The testing corpus has 1321 Hadith distributed over 13 books (classes).

##### 4.2 Classification Methods Applied to AL-Hadith Corpus

One of the researches in AL-Hadith classification field is done by (AL-Kabi, et al., 2007), in which AL-Kabi did not mention an accurate description of AL-Hadith corpus or the stop words they used in their experiments. Therefore , in this study an implementation for their classification algorithms was done also.

The following subsections represents in details the three methods have been implemented in this study.

**4.2.1.AL-Kabi method :** this method was proposed by AL-Kabi and his Colleagues on AL-Hadith classification(AL-Kabi, et al., 2007). This method is based mainly on using the stems of Ahadith words to calculate the IDF, the weighting of the feature in training phase and the classification phase.

**4.2.2. Word based classification (WBC):** this method uses the words of AL-Hadith after going through the preprocessing phase without stemming stage. The words occurrences after preprocessing are used in the calculation of IDF and in the weighting process. Stems of the words are not used in this method neither in building the training database nor in applying the classification algorithm.

**4.2.3. Stem expansion classification (SEC):** It is the proposed method in this study. In which words and stems are used. Words are used in IDF and features weight calculations for both training and query sets, but stems are used in query expanding process, the expansion process was discussed in details in section 3.3.2.

Table 9. List of books in AL-Hadith corpus

Book (Class)Name	اسم الكتاب	Doc No.	No. of distinct features after stop words removal
The Book of Faith	كتاب الإيمان	38	938
The Book of Knowledge	كتاب العلم	76	1946
The Book of Praying	كتاب الصلاه	115	2137
The Book of Call to Praying	كتاب الأذان	38	574
The Book of the Eclipse Prayer	كتاب الكسوف	24	715
The Book of Almsgiving	كتاب الزكاه	91	2267
The Book of Good Manners	كتاب الأدب	225	5258
The Book of Fasting	كتاب الصوم	107	1905
The Book of medicine	كتاب الطب	92	1895
The Book of Food	كتاب الطعام	91	1894
The Book of Pilgrimage (Hajj)	كتاب الحج	231	4885
The Book of Grievance	كتاب المظالم	40	906
The Book of the Virtues of the Prophet and His Companions	كتاب المناقب	143	3410

##### 4.3 Experiments Specifications

Hadith corpus that is used in this study consists of 1321 Hadith distributed over thirteen books (classes). The system is considered as supervised classification since training sets are used to apply learning algorithm to build the leaning database which will be used for the classification algorithm.

In the experiments author uses (90%) of each Ahadith class as training set while the rest (10%) of each class is used as testing set for the classification system. Of course, for each class the (10%) Ahadith in the testing set are not included in the training set or training phase calculations.

For each class five training - testing sets combination are chosen to run SEC algorithm, which means that for each class five separable experiments will be run, which gives variation for system testing.

It is important to mention that the same training - testing sets combination is used with the other two classification methods (AL-Kabi's,WBC) which is an important aspect to insure fair comparison among different methods against the proposed one.

#### 4.4 Performance Measurements

In order to demonstrate the efficiency of any classification algorithm measurements are needed to compare the proposed system's outcome with others. The most popular measurements in text classification algorithm are recall, precision and F-measure that are used in this study.

Recall and precision based on the concept of relevance. Precision is defined as the ratio of relevant documents retrieved to all documents retrieved while Recall is defined as the ratio of relevant items retrieved to all relevant items in a corpus.

There are obvious trade-off between recall and Precision. If the system retrieves all the documents in a corpus then the system will retrieve all relevant documents in the corpus, in this case the recall will be perfect.

On the other hand, since there are only small proportions of documents in a collection that are truly relevant to the given query, retrieving everything will give a very low precision (Greengrass, 2000).

Because of this trade-off between recall and precision a combination of good precision and good recall is needed. In the best case we would like to retrieve all the relevant documents and to discard non-relevant documents, this combination of recall and precision is found in F-measure (Harmonic mean).Precision, recall and F-measure are calculated according to equation presented by (Al-Mesleh, 2007) as follows

$$\text{Precision (P)} = A / (A + B).$$

$$\text{Recall (R)} = A / (A + C).$$

$$\text{F-measure (Harmonic mean)} = (2 \times P \times R) / (P + R)$$

The meaning of parameters used in recall and precision calculations are shown in Table 10.

Table 10. Recall and Precision Parameters.

System says...	In reality, the document is...	
	Relevant	Irrelevant
document is relevant	A	B
document is irrelevant	C	D

#### 4.5 Comparisons and Results Analysis

In this section we introduce the comparisons between SEC and the other two methods (AL-Kabi's and WBC), in order to show the preference of the proposed system over AL-Kabi's and WBC method.

##### 4.5.1 Stem Expansion vs. Word based classification

The proposed system (SEC) outperform WBC for 11 out of 13 books in precision, while WBS achieve better precision for The Book of Grievance and the book of Knowledge as shown in Figure 4 . This result is predicted because using the stem expansion phase gives a large morphology variation for the words that can resulted in retrieving more documents that belong in reality to other classes.

The proposed algorithm (SEC) overcomes the side effect of expanding the query using stemming, by the weighting strategy adopted, where stems in the expansion phase are giving the same weights of its original word in AL-Hadith.

SEC achieved precision value of 1 for The Book of Call to Praying and enhances the precision of 11 classes by 45% in average.

As shown in Figure 5 SEC achieves better F\_measure values for all classes against WBS method and enhances the F\_measure by 49% in average.

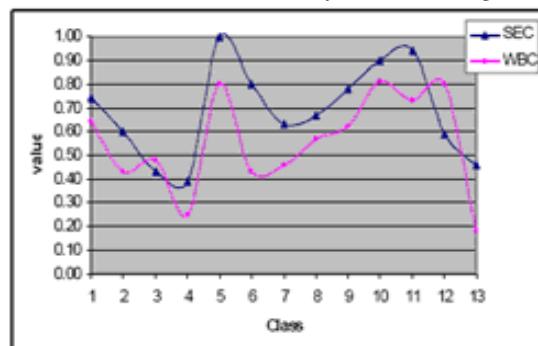


Figure 4. Precision comparison for stem expansion vs. word based classification.

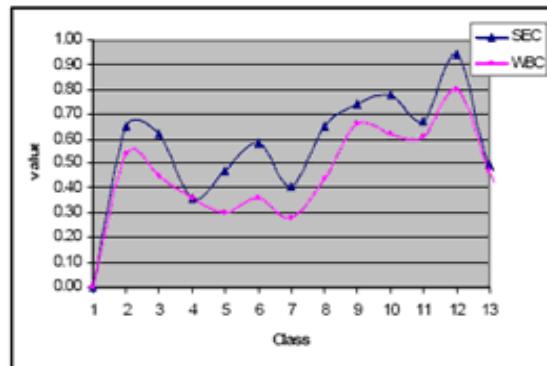


Figure 5. F\_measure comparison for stem expansion vs. word based classification.

#### 4.5.2 Stem Expansion vs. AL-Kabi Classification

After implementing AL-Kabi's method for the same training - testing sets used to examine SEC, comparisons are conducted as shown in Figure 6 and 7.

Figure 6 represents the precision graph of the two methods, where AL-Kabi's method achieved better precision for The Book of Knowledge and The Book of Grievance, while SEC out performed AL-Kabi's method in 11 out of 13 classes.

This behavior can be justified by the fact that those two books have small number of Ahadith and since the experiments are conducted on Ahadith in a closed domain (Sahih AL\_Bukhari), author believes that if those classes have a larger number of Ahadith the superb of SEC will appear.

In addition AL-Kabi used stems of words for term weighting in the preprocessing phase, which means that term frequency used in the training process was the number of the stems occurrence in the training set. In contrary, in SEC the words occurrences were used for the weighting process in preprocessing phase while the stems were used in the expansion process for both training and testing sets.

Since stem expansion is used for both training and testing sets in the proposed system, more non-related documents are presented to be retrieved, which justifying AL-Kabi's method achieves better precision for two classes out of thirteen classes . As Shown in Figure 7, SEC method outperformed AL-Kabi F\_Measure in all the 13 classes.

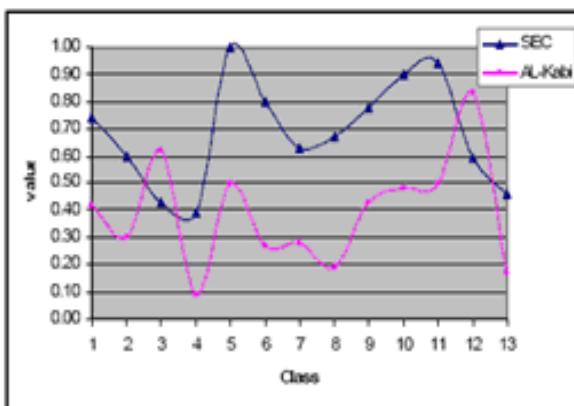


Figure 6. Precision Comparison for Stem expansion vs. AL-Kabi's classification

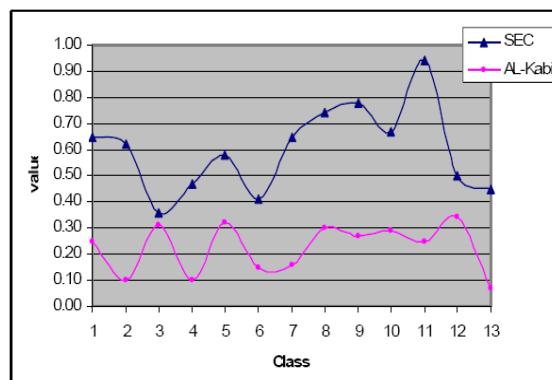


Figure 7. F\_Measure comparison for stem expansion vs. Al-kabi's classification.

#### 5. Conclusions

Arabic language is considered as one of the languages that will never distinguish and few researches were made on Arabic corpus linguistics. However, it is the official language of twenty Middle Eastern and African countries and is the religious language of all Muslims, regardless of their origin.

A classification method called Stem Expansion is proposed in this study, in order to discover knowledge from AL-Hadith by assigning each Hadith to one book (class) of predefined classes. SEC is considered as supervised classification method.

In this study a corpus containing 1321 ahadith from thirteen books from Sahih AL-Bukhari is selected and each Hadith is assigned to one class. Sahih AL-Bukhari is used as the base for deciding the correctness of classification results.

The results of the proposed system (SEC) are compared with the results of two other methods; one proposed by AL-Kabi and the other is word based classification technique (WBC). The comparison shows that SEC was better against WBC and AL-Kabi in recall for all classes while WBC and AL-Kabi achieve better precision for only two out of thirteen classes, and SEC achieves better F\_Measure for all the thirteen classes against the other two methods (WBC and AL-Kabi).

The results show that SEC performed better in classifying AL\_Hadith against existing classifications methods (WBC and AL-Kabi) according to the most reliable measurements (recall, precision, and F\_Measure) in text classification field.

#### Acknowledgements:

Grateful thanks, gratitude and sincerest appreciation to Dr. Azzam T. Sleit and Dr. Bassam H. Hammo for their guidance.

**Corresponding Author:**

Khitam M.Jbara.  
 Department of Computer science.  
 The University Of Jordan.  
 P.O. Box : 710481 Amman 11171 Jordan.  
 Amman,Jordan.  
 E-mail: [ktjlc2000@yahoo.com](mailto:ktjlc2000@yahoo.com)

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8/25/2010

# Organic amendment effect on soil properties and yield of potato (*Solanum tuberosum*) under irrigated condition: a case study from Kombolcha, Eastern Harergie, Ethiopia

Eyasu Mekkonen<sup>1</sup>, Fassil Kebede<sup>2,3,\*</sup> and Nurhussien Taha<sup>2</sup>

<sup>1</sup>Kombolcha Agricultural TVET College, Kombolcha, Eastern Harrargie; <sup>2</sup> Department of Land Resource Management and Environmental Protection, Mekelle University, Ethiopia  
<sup>\*</sup> Corresponding Author: Address: E-mail- [fjimamu@gmail.com](mailto:fjimamu@gmail.com)

**Abstract:** Field experiment was conducted in 2005/06 cropping season in Kombolcha to understand the comparative effect of organic and inorganic sources of soil ameliorant for managing surface soil crust under basin and furrow irrigation practices to boost potato production. A factorial experiment was conducted on plots of 12 m<sup>2</sup> (4 m x 3 m) and arranged in RCBD with three replicates, which combine irrigation methods and soil amendments. The treatments were the control (no amendment), FYM, *chat* residue (decayed leaves of *Chata edulis*) and sediment (sub surface inorganic material locally known as ‘decay dimma’). Results have, therefore, revealed that FYM and *chat* made compost significantly ( $p \leq 0.05$ ) improved moisture content, bulk density, porosity and infiltration rate over the sediment amended plot and the control. However, yield harvested from plots, which were amended with *chat*-made compost was significantly ( $p \leq 0.05$ ) lower than FYM under furrow irrigation practice. [Journal of American Science 2010;6(11):420-425]. (ISSN: 1545-1003).

**Keywords:** Chata edulis, decay dimma, organic amendment, potatoes, soil properties, irrigation

## Introduction

The total area of land under *chat* cultivation in Ethiopia, in the year 1997/98 was estimated at 78,570 hectare (Central Statistics Authority, 1997/98). In Oromiya region, mainly East and West Hararghe zones are the most important centres of *chat* production (East Hararghe zone alone contributes 53.4% of the total production area) in Ethiopia (Dechassa, 2001). It can be grown rain fed and/or irrigated and the crop could be planted both in home garden and in the field. Tremendous quantity of the *chat* residue is left after consumption as solid waste material, which is readily available in various states of decomposition at dumping site near farmlands, around homestead and along the roadside on the way from Aweday town to Harer.

Continuous harvesting of *chat* exhausts and debilitates the mother plant unless fertilizer is used to maintain soil fertility. Manure is applied on *chat* every year based on the availability of manure or compost. Farmers prefer organic fertilizers (manure and compost) as compared to inorganic (chemical) fertilizers. The volume of manure available is limited and too scarce to satisfy the needs of farmers. Thus, farmers in Hararghe prepare their own compost and use this to improve soil fertility. Farmers usually use both organic and inorganic fertilizers to improve the soil fertility status whenever they grow cash crops. In addition, subsurface sediment, which is unintentionally added into irrigated land, is regarded by farmers as soil ameliorant. This ameliorant is

formed from as alluvial deposition at the sub-surface soil layer. It is mainly drawn from hand dug wells in the form of water suspension while pumping is taking place. Based on the farmers' experience the sediment has improved fertility of the soil as well as the structure of the soil (Pers. Comm.).

## Materials and Methods

The study was conducted in 2005/6 in Kombolcha, which is located 542 km east of Addis Ababa with an altitude between 1200 and 2460 masl, Latitude 42° 07' 0" E and Longitude of 9° 25' 60" N.

### 3.1 Bulk density, porosity and moisture content determination

Determination of dry bulk density and porosity was made for the profile pit, which was opened on the irrigated at the depths (0-15, 15-30, 30-45 and 45-60 cm) by using core samplers (diameter = 3.75 cm; height = 5 cm). Similarly, samples were taken in treatment-wise from depth plowing depth (0-30 cm) using the same core samplers to check the treatment effect on bulk density and total porosity. And the soil moisture content was determined using gravimetric method.

### 3.2 Experimental design and layout

The field experiment was conducted in 2005/6 during the normal growing season (Jan – mid April) for potato under irrigation practice and treatments were arranged in RCBD, with three replicate and eight treatments ( $4 \times 2$  factorial experiments). Each form of treatment was applied on plot size of  $12 \text{ m}^2$  [3

$\text{m} \times 4 \text{ m}]$  and the inter block and between plot spacing was 1 m and 0.5 m, respectively (Fig 1). All plots were also designed for methods of basin and furrow irrigation practices. The experimental site was chosen as the land has been irrigated for more than a decade (Pers. comm.). It also represents the major soil type of the study area where vegetables are mainly grown.

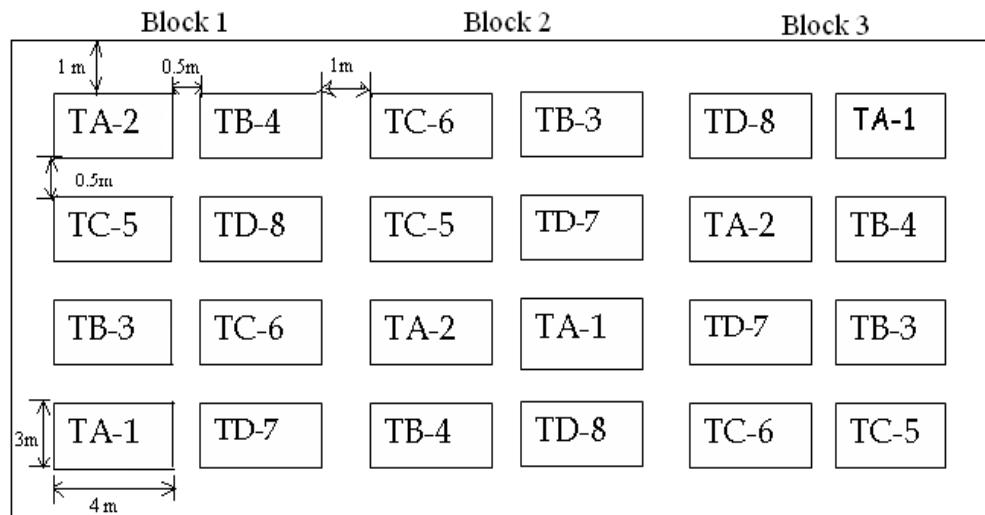


Fig 1. Layout of the field experiment

Under this experiment, two forms of organic compost were used as soil ameliorant (Farm Yard Manure (FYM) and *Chat* residue or decomposed leaves of *Catha edulis F.*) and sediment (inorganic material from subsurface soil, locally called ‘dicay dimma’ to mean ‘qey afer’). The latter was manually collected from hand dug-well, which is located at a distance of 75 m from the experimental site and is different from the source of irrigation water for the experiment. They were incorporated into the surface soil with rate (dry mass) of 1.5 ton/ha, 2.5 ton/ha and 1.3 ton/ha, respectively. The method of soil amendment was partly adopted from the existing farmers’ knowledge and experience. Description of the treatments is summarized in Table 1.

Table 1. Descriptions of treatment combinations

Treatment ID	Treatment combination	
	Surface soil crust management practices	Irrigation practices
TA-1	Control	Furrow
TA-2	Control	Basin
TB-3	FYM [chopped wheat straw and cattle dung]	Furrow
TB-4	FYM [chopped wheat straw and cattle dung]	Basin
TC-5	Residue of <i>chat</i> ( <i>Catha edulis F.</i> )	Furrow
TC-6	Residue of <i>chat</i> ( <i>Catha edulis F.</i> )	Basin
TD-7	Sediment [decay dimma]	Furrow
TD-8	Sediment [decay dimma]	Basin

All plots have also received equal amount of mineral fertilizer: DAP and urea with rate of 100 and 150 kg/ha, respectively; and half of the urea and full dose of DAP was applied at the time of sowing while the remaining amount of urea was top dressed just before the 3<sup>rd</sup> irrigation cycle.

On the field experimental plots, tuber seed of same variety (*Solanum tuberosum L.*) was sown with rate of 2 and 1.67 ton/ha under the basin and furrow irrigation practices, respectively. Each furrow plots had four ridges and sowing was done along the shoulders of two rows of the middle ridges in staggered arrangements while the remained were planted up on the inward side of the two border ridges. In both cases the planting was made at 0.4 m spacing. On the other hand, the basin plots were sub-divided into two equal sections for ease of water management. The main water channel, which is 0.5 m wide, was designed in a row along with the replication where as inlet channels between the experimental plots. The mode of water application and the amount of water was based on the existing irrigation practices by using engine pump as water lifting device from the hand dug well. At each irrigation cycle, roughly flow rate of 2.25 lit/sec that usually preferred by farmers, was allowed to irrigate both the furrow and basin plots for equal time (105 sec per plot). In the case of furrow irrigation practice, the furrow-flow was diked at the end to check the outflow.

## Results and Discussions

### 4.1 Growth response

Both the soil amendment and irrigation practices have a significant effect on the average plant height when measured at the flowering stage. Based on mean comparison, plants grown over plots, which were amended with FYM and *chat* residues, were significantly taller than those from the control plots under basin irrigation practices. The shortest average plant height was recorded from the control plot under furrow irrigation practice (Fig. 2). However, no significant difference was obtained from plots, which were amended with sediment and *chat* residues under the same irrigation practice. Similarly, those which have been raised over FYM amended plots were significantly taller than those from the other treatments under furrow irrigation practices.

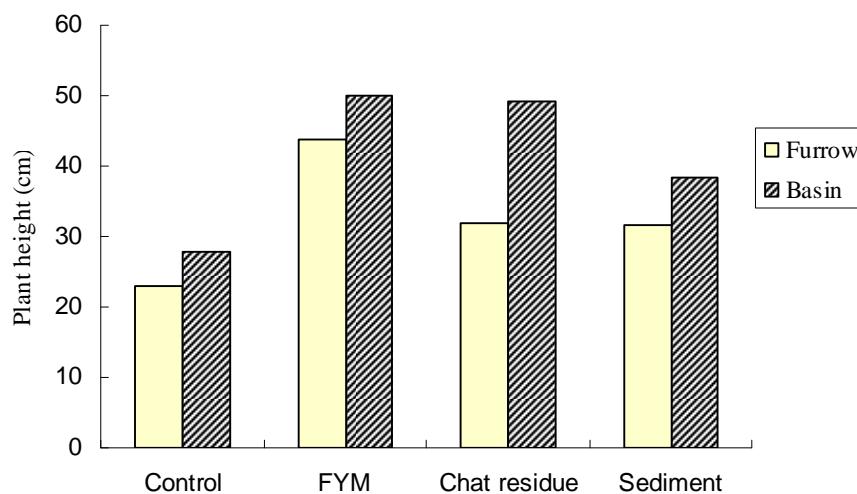


Fig 2. Treatment effect on growth of plant height

In general, those plants which have been grown under basin irrigation practices were more vigorous than plants from furrow irrigation. This may be due to the fact that plants over the furrow ridges relatively bears more roots than shoots in search of soil moisture as more proportion of the applied water is deep percolated (FAO, 1985).

The number of tubers per plant was not affected by the irrigation practices as well as the soil ameliorants. Based on mean comparison, all the treatment combinations had no any significant effect on the number of tubers per plant. Similarly, the mean comparison revealed that soil amendment practice has no significant effect on individual tuber weight with in each irrigation practice while more tuber weight was obtained from FYM amended plots. On the other hand, the irrigation practice had significant effect on individual tuber weight from which more weight was recorded from plots, which were managed under basin irrigation practices. The crops which have been grown under basin irrigation practices produce larger tubers than those from furrow irrigation practices while considering all the management practices.

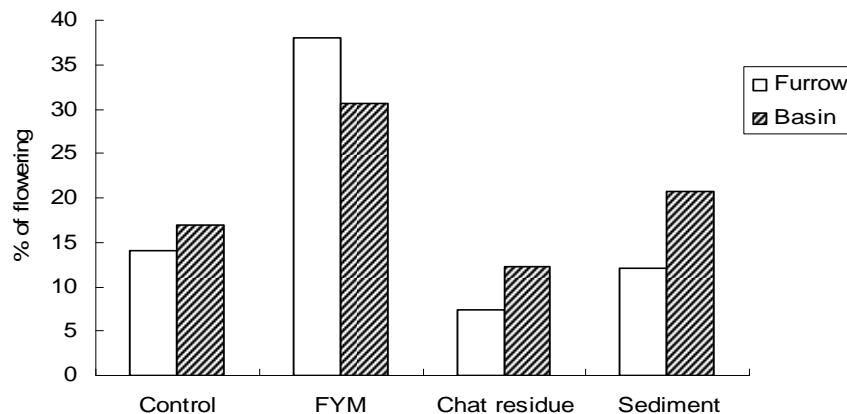


Fig 3. Effect of soil amendment on percent of flowering

Unlike the type of irrigation practice, the crust management practices have a significant effect on the rate of flowering. Based on mean comparison, plants raised over FYM amended plots were significantly effective to bear flower under both irrigation practices (Fig 3). They bear 80 to 60 % more flowering than those which have grown over plots amended with *chat* residue under the furrow and basin irrigation practices, respectively. However, there was no significant difference among the control, sediment and *chat* residue under both irrigation practices. In spite of the fast growth in height of plants which were grown under *chat* amended plots, rate of flowering was relatively late as compared to the FYM. This may be due to the oversized height growth on cost of tuber formation through excess irrigation water application (Khurana et. al. 2003).

#### 4.2 Yield response

Both soil amendments and irrigation practices have significant effect on tuber yield. Based on mean comparison (Table 2), yield obtained from plots amended with FYM was significantly different from the treatment of *chat* residue under basin irrigation while maximum records on yield harvested over the other treatments. The yield from FYM amended plots was significantly higher than the remaining amendments under furrow irrigation practices.

Table 2. Growth and yield response of potato (*Solanum tuberosum*) to different soil amendments under furrow and basin irrigation practices

Treatment combination	Plant height (cm)	Flower (%)	No. of tuber per plant	Tuber wt.* (gram)	Tuber wt. per plant (kg)	Total yield (tones/ha)
Control +Furrow	23.1 <sup>d</sup>	14 <sup>b,c</sup>	12.7 <sup>a</sup>	46.7 <sup>c</sup>	0.59 <sup>d</sup>	7.5 <sup>b</sup>
Control +Basin	27.9 <sup>cd</sup>	17 <sup>b,c</sup>	14.3 <sup>a</sup>	74.1 <sup>abc</sup>	1.07 <sup>ab</sup>	11.8 <sup>ab</sup>
FYM + Furrow	43.8 <sup>ab</sup>	38 <sup>a</sup>	14.7 <sup>a</sup>	81.9 <sup>abc</sup>	1.17 <sup>ab</sup>	13.8 <sup>a</sup>
FYM +Basin	50.1 <sup>a</sup>	30.7 <sup>ab</sup>	12.7 <sup>a</sup>	107.1 <sup>a</sup>	1.38 <sup>a</sup>	14.7 <sup>a</sup>
<i>Chat</i> residue + Furrow	31.8 <sup>cd</sup>	7.3 <sup>c</sup>	10.7 <sup>a</sup>	47.7 <sup>bc</sup>	0.53 <sup>d</sup>	7.6 <sup>b</sup>
<i>Chat</i> residue + Basin	49.3 <sup>ab</sup>	12.3 <sup>c</sup>	13 <sup>a</sup>	74.7 <sup>abc</sup>	0.95 <sup>bc</sup>	10.8 <sup>ab</sup>
Sediment + Furrow	31.5 <sup>cd</sup>	12 <sup>c</sup>	12 <sup>a</sup>	57.2 <sup>bc</sup>	0.65 <sup>cd</sup>	7.6 <sup>b</sup>
Sediment + Basin	38.5 <sup>b,c</sup>	20.7 <sup>abc</sup>	12.3 <sup>a</sup>	91.7 <sup>ab</sup>	1.11 <sup>ab</sup>	12.2 <sup>a</sup>
R <sup>2</sup>	0.75	0.79	0.47	0.72	0.75	0.63
P ≤ 0.05	0.0094	0.025	0.168	0.015	0.019	0.034

Levels not connected by same letter are significantly different at 5 % level

\* Average of individual tuber weight

Based on mean comparison, significantly higher yield was harvested from FYM amended plots under the furrow irrigation practice (Table 2). Moreover, there was no significant difference on yield due to amendment under the basin irrigation practice while the highest yield was harvested from FYM amended plots. Similarly, there was no significant difference on yields from the control, sediment and *chat* residue amended plots under both irrigation practices, while more yield was obtained from the last two soil ameliorants.

Unlike comparison of the maximum yield, mean comparison has shown that relatively low yields were harvested from *chat* amended plot, while it is significantly lower as compared to the FYM amended plot under furrow irrigation practices. This may be due to the effect of excess soil moisture condition together with the sealing effect of the crust, which further deteriorates the hydrologic condition of the soil with in the rooting zone, where tuber is normally formed. This in turn could affect the crop to be more vulnerable with blight and cut worm attack (Khurana et al. 2003). In contrast, maximum yield was gained from plots, which were amended with *chat* made compost have received less suspension load than the other.

Being acquainted with the already existing irrigation practice, farmers are mainly preferring basin irrigation practice to cultivate the crop, which is locally known as '*Ketare*'. Results have also shown that higher yield was harvested from plots, which were managed under basin irrigation practices than furrow irrigation practices in each soil amendment.

In addition, highest yield was harvested from FYM amended plots under both irrigation practices over the others. The yield reduction can be explained by the effect of pronounced moisture stress under the furrow irrigation practice. This may be explained by the lower bulk density of the furrow ridges, which allows the soil water to escape from the ridges. In general, it has been indicated that optimum supply of water is the key factor in production of the potato; both over supply and deficiency of water are harmful as the crop requires readily available water throughout the growing season (Khurana et. al. 2003).

#### **4.3 Effects of compost and sediment on some physical and chemical properties of the amended soil**

As is shown in Table 3, soils treated with *chat* residue and FYM have significant effect on infiltration rate. Based on mean comparison there was no significant difference between soil amendment with *chat* residue and FYM while higher rate of infiltration was recorded from plots treated with *chat* residue. Treatments of FYM and *chat* residue have a significant effect on infiltration rate over the control and sediment treated soil. However, there was no significant difference between the two forms of compost. The results agreed with several studies that soil particle aggregation, water holding capacity, drainage, nutrient retention, and plant root growth were all increased when organic compost was incorporated (Keith, 1997; Wayne et. al., 1999). Soil amended with *chat* residue has significantly lower bulk density than the remaining treatment under basin irrigation practices, and similarly soils amended with *chat* residues and FYM have shown lower bulk density than the control and sediment under furrow irrigation practices. Records of initial moisture content (v/v) within the depth of 0-30 cm were found as 38.1 %, 38.4 %, 35 % and 38.2 % that measured under the control, FYM, *chat* residue and sediment amended plots, respectively. The total time required to attain the basic infiltration rate for each measurement was ranged from 140 up to 200 minutes.

Table 3. Effect of soil amendment on soil physical properties under furrow and basin irrigation

Treatment combination	Bulk density (g/cm <sup>3</sup> )	Porosity (%)	% moisture content (v/v)		In. rate (cm/hr)	Basic In. rate (mm/hr)
			0-15 cm	15-30 cm		
Control +Furrow	1.27bc	51.9bc	13.1c	22.4b	-	-
Control +Basin	1.42a	46.5d	25.8b	24.5bc	10.8b	2.2a
FYM + Furrow	1.07d	59.5a	21.7bc	25.7bc	-	-
FYM +Basin	1.3ab	50.7cd	28.1ab	32.1ab	16.2a	2.8a
Chat residue + Furrow	1.05d	60.3a	26.8ab	29.4b	-	-
Chat residue + Basin	1.15cd	56.6ab	30.5a	35.6a	14.5a	3.2a
Sediment + Furrow	1.31ab	50.7cd	14.6c	25.5bc	-	-
Sediment + Basin	1.33ab	49.8cd	17.6c	26.4bc	9.7b	2.4a
R2	0.82	0.81	0.75	0.81	0.97	0.93
P< 0.05	0.0123	0.0123	0.039	0.0032	0.0002	0.058

Levels not connected by same letter are significantly different at 0.05 levels

### Conclusions

This experiment has tried to demonstrate that *chat* residue can benefit potato production by virtue of its soil moisture conservation and improvement in soil organic matters thereby reduce the risk of soil encrustation as frequent abstraction of suspension laden irrigation water from the hand dug well is minimized. It also indicated that production of potato under basin irrigation practice is better than the furrow irrigation practice, in terms of tuber yields. Although, the yield obtained from *chat* amended plot has shown to be less, addition of *chat* residue on crusted surface soil has shown improvement up on similar soil physical properties, which play a key role to suppress soil encrustation. The yield obtained from would rather imply the need to conduct further research by integrating the surface crust management options with improved irrigation water management. Although, the yield obtained from *chat* amended plot has shown to be less, addition of *chat* residue on crusted surface soil has shown improvement up on similar soil physical properties, which play a key role to suppress soil encrustation.

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9/9/2010

## Nutritive Aspects of *Oxalis corniculata* L. Used by Tribals of Central India During Scarcity of Food.

Ashok k. Jain<sup>1</sup>, Preeti Tiwari Barua<sup>2</sup> and Mudasir Bashir<sup>3</sup>

<sup>1</sup>Professor, School of Studies in Botany, Jiwaji University Gwalior -474011, Madhya Pradesh, India  
E- mail: [asokjain2003@yahoo.co.in](mailto:asokjain2003@yahoo.co.in);

<sup>2</sup>Assistant Professor, Department of Biotechnology, IPS Academy Indore-452012, Madhya Pradesh, India  
E- mail: [preetibarua26@gmail.com](mailto:preetibarua26@gmail.com);

<sup>3</sup>Research Scholar, Plant Tissue Culture Laboratory, School of Studies in Botany, Jiwaji University Gwalior-474011, Madhya Pradesh, India  
E-mail: [mudasirbot@gmail.com](mailto:mudasirbot@gmail.com)

**Abstract:** Reports on ethnobotanical surveys reveal that a good number of plant species are being used by various tribal communities as emergency food. The present work deals with some parameters regarding nutritive value of leaves of *Oxalis corniculata*. L. used as alternative vegetable during emergency by some tribes of central India. The leaves have been found to be rich in moisture ( $82.42 \pm 0.5\%$ ), total carbohydrate ( $24.67 \pm 0.4\%$ ), crude protein ( $22.28 \pm 0.5\%$ ), crude lipid ( $23.7 \pm 0.5\%$ ), sodium ( $1.12 \pm 0.02\%$ ), potassium ( $2.17 \pm 0.31\%$ ), calcium ( $2.5 \pm 0.08\%$ ), nitrogen ( $3.56 \pm 0.70\%$ ) and magnesium ( $0.25 \pm 0.03\%$ ). [Journal of American Science 2010;6(11):435-437]. (ISSN: 1545-1003).

**Key words:** Nutritive status; *Oxalis corniculata*; Tribes; Scarcity of food.

### 1. Introduction

Analysis of the work done all over the world on survey among aboriginal societies, and scrutiny of ethnobotanical literature had brought about the record of several hundred wild edible plants, which not only satisfy hunger of the people but have been proved nutritious too. It has been noticed that several tribal communities, who still live in undisturbed forest areas possess the traditional food habit. This probably emphasizes on sound nutritional status of wild edible plants, consumed by tribals as regular food or supplementary food. Seeds of certain indigenous species rich in protein are more or less equal to that of almonds (Oommachan and Masih, 1998). It was observed that during adverse conditions when food is not available due to drought, flood or other calamities, these tribes go for consuming other herbal edibles. The nutritional composition of a large number of Plants used in emergency by various tribes in different parts of the world has been evaluated. Kundaji and Rao (1954), Barrau (1959), Mai *et al.*, (1960), Rajaram and Janardanan (1991), Vadivel and Janardanan (2000), Lockett *et al.*, (2000), Ogle *et al.*, (2001), Rehman *et al* (2006). .

Madhya Pradesh is a tribal rich

state where a large number of tribal communities are living in various forest pockets. Reports on ethnobotanical surveys reveal that tribal communities use a good number of plant species as emergency food. Patole and Jain (2002) enumerated nearly 45 plant species consumed by tribals and other rural people residing in Pachmarhi Biosphere Reserve of Madhya Pradesh. It includes the species of *Bauhinia*, *Cassia*, *Phoenix*, *Ficus*, *Polygonum*, *Rhus*, *Amaranthus*, *Oxalis* etc. Nutritional value of several such species has now been estimated and their consumption seems to be beneficial to tribals. The present work deals with analysis of nutritive value of leaves of *Oxalis corniculata*.L, consumed during emergency by some tribal communities of central India.

*Oxalis corniculata* L. commonly known as Amboti, Indian sorrel, (Family Oxalidaceae) is a herb with creeping stem, rooting at nodes. Leaves long petioled, Leaflets with white hairs on margins and lower portion of the middle vein, petioles pubescent 2-4 cm long. Its leaves serve as the major means of food for the tribals during emergency since long back, and proven to save the life of tribals during severe famine and extreme scarcity. The leaves of the plant are eaten both raw as salad and cooked. Leaves

are also used for making sandwiches; chutney pickles and is considered as cooking refrigerant by the Sahariya tribe of central India. Its leaves are used to cure fever, piles, Scurvy and dysentery (Kirtikar and Basu, 1975).

## 2. Material and Methods

The plant material of *Oxalis corniculata* was collected from various tribal inhabited forest localities of central India and identified with the help of floras, authentic literature and herbarium of School of Studies in Botany, Jiwaji University Gwalior (M.P). The parts of plants, used by tribals for edible purposes were analyzed for the estimation of various components. Moisture content, crude protein, total carbohydrates, crude lipids were estimated following the standard methods (AOAC 1990). Total nitrogen was estimated by modified Kjelhdal method (Isvaran and Marwaha 1980).

### Estimation of Potassium, Sodium and Calcium (Flame photometer method)

One gm of powdered plant material was taken in a conical flask and digested in tri acid mixture i.e. Sulphuric acid ( $H_2SO_4$ ), Nitric acid ( $HNO_3$ ) and Per Chloric acid ( $HClO_4$ ) in the ratio of 9:3:1. The white colourless digested material was filtered and was made up to 100 ml by adding distilled water, 10 ml of aliquot was taken and diluted to 25 ml with distilled water and analyzed on digital Flame Photometer by using filters of potassium, sodium and calcium separately.

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## 3. Results

Nutritional composition of leaves of *Oxalis corniculata* (Table -1) shows that it contains high moisture content ( $82.42 \pm 0.5\%$ ), which is in conformity with earlier reported range of 81.4 - 90.3% in some Nigerian green leafy vegetables (Ifon and Bassir 1980).

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Nutritional component	Concentration %
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Crude proteins	$22.28 \pm 0.5$
Crude Lipids	$23.75 \pm 0.5$
Sodium	$1.12 \pm 0.02$
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The data comprise the mean value  $\pm$  SD of three replicates

The crude protein content in leaves of *Oxalis corniculata* ( $28\% \pm 0.5$ ) was found more than *Ipomoea aquatica* leaves i.e. 17.84% (Vishwakarma and Dubey 2009). The crude lipid content ( $23.75 \pm 0.5\%$ ) was observed more in comparison to the previously reported values 13.4-17.6% in non-conventional leafy vegetables in Maharashtra (Kulkarni et al., 2003). The carbohydrate Content was found to be ( $24.67 \pm 0.4\%$ ), which is less in comparison to *Ipomoea batatas* leaves 82.85% and *Corchorus tridens* 75.00% (Asibey et al., 1999). The leaves of *Oxalis corniculata* exhibit rich in mineral contents like Sodium ( $1.12 \pm 0.02\%$ ), Potassium ( $2.17 \pm 0.31\%$ ), Calcium ( $2.5 \pm 0.08\%$ ), Nitrogen ( $3.56 \pm 0.70\%$ ) and Magnesium ( $0.25 \pm 0.03\%$ ), these mineral components are vital in regulating various metabolic pathways in human body. Thus observations indicate that consuming *oxalis corniculata* during scarcity of food probes to be neutracentric. The species grow in wild thus can be collected easily. Tribals also store its dried parts so that it can be consumed at any time. Mineral composition of leaves also indicates that all required mineral

## 4. Discussions

The present study indicates that the leaves of *Oxalis corniculata* are rich from nutritional point of view and can be used as supplementary food source to deal with the problems of limited food production in the tribal areas during emergency. As such there is a treasure of knowledge with the various tribal communities of world regarding the multifarious use of wild plants. There are several other wild plant

species used by the tribal which are edible and easily accessible during adverse conditions like drought, famine etc. Some such species of Central India are *Rhus parviflora*, *Achyranthes aspera*, *Amaranthus viridis* and *Boerhaavia diffusa*. When such wild species are accepted by the people as edibles like other cultivated ones they can play an important role in solving the various food problems world over.

#### **Acknowledgements:**

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#### **Corresponding Author:**

Prof. Ashok Kumar Jain  
School of studies in Botany  
Jiwaji University Gwalior-474011,  
Madhya Pradesh, India.  
E-mail: [asokjain2003@yahoo.co.in](mailto:asokjain2003@yahoo.co.in);

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E- mail: [asokjain2003@yahoo.co.in](mailto:asokjain2003@yahoo.co.in);

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E-mail: [asokjain2003@yahoo.co.in](mailto:asokjain2003@yahoo.co.in);

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## Effects of sports participation on psychological stress in female students in region 3 of Kermanshah

Ali Feyzkhademi<sup>1</sup>, Saadat Hajipoor<sup>1</sup>, Shahram Azimi<sup>2</sup>, Mehrdad Jalalian<sup>3, 4, 5</sup>

<sup>1</sup>Faculty Member of Izeh Branch, Islamic Azad University, Izeh, Iran

<sup>2</sup>Lecturer, Sama Branch (Kermanshah), Islamic Azad University, Kermanshah, Iran

<sup>3</sup>Research Center of Iranian Blood Transfusion Organization, Khorasan-e Razavi Blood Center, Mashhad, Iran

<sup>4</sup>Department of Community Health, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor D.E., Malaysia

<sup>5</sup>Editorial Office, Electronic Physician Journal, Mashhad, Iran

[khademisport@gmail.com](mailto:khademisport@gmail.com)

**Abstract:** The aim of this study was to investigate the effects of sports participation on the psychological stress levels of female students 15-18 years old. Psychological stress is defined as a collection of nonspecific reactions against organisms in reflections and exposure to any factor that should be faced. Stress control includes several factors, and, in particular, sports participation is thought to be effective. This quasi-experimental research was performed using pre-test plan-test, after-test, and control groups. Research subjects for the control group were 30 people chosen randomly. The subjects of stress were tested by a 40-item stress questionnaire and then tested in step independent variable "Sports participation" included volleyball education and skills training for three months and three weekly sessions of 75-90 minutes. This was carried out to investigate its effect on the dependent variable "stress." We did not observe any statistically meaningful difference between the mean scores of stress-control group and experiment group scores at pre-test in  $p<0.05$ ; however, statistically meaningful differences were observed between the mean scores of stress control group and experiment group scores on the post test stage ( $p<0.05$ ) and between the mean scores of stress in the control group pre-test and post-test in ( $p<0.05$ ). In addition, A statistically meaningful difference was observed statistically meaningful differences were observed between the mean scores of stress in the experiment group pre-test and post-test ( $p<0.05$ ). [Journal of American Science 2010;x(x):xx-xx]. (ISSN: 1545-1003). [Journal of American Science 2010;6(11):438-441]. (ISSN: 1545-1003).

**Keywords:** Sports Participation; Psychological stress; Students

### 1. Introduction

Although advances in science, technology, and industrialization have brought valuable improvements to society, they have also presented numerous complications. One complication is decreased mobility and physical activity. Sedentary lifestyles can lead to physical and mental health issues and impairments. Mental energy cannot be separated from physical energy. Fatigue, disappointment, lack of exercise, and the inability to make decisions are related. The opposite theorem is also true: with suitable condition, progress is possible and leads to excellence. Skills steadily lead to confidence and physical fitness is a big step toward having a psychological fitness.

Participation in sports often has therapeutic effects on children and adolescents suffering from emotional or developmental disabilities. Children, teenagers, and even adults gain the opportunity through sports to strengthen their physical, psychological, and social faculties. On this basis, the study seeks to evaluate whether participation in a relatively long-term physical activity changes the stress on student athletes. The researchers chose

volleyball as the sport, since it is a preferred activity for students in Kermanshah.

Low mobility, especially in girls, can cause many mental disorders and vice versa; mental illness can cause physical symptoms. Therefore, sports participation can be therapeutic for children and youngsters. For this reason, many groups spend leisure hours on such activities after tedious days of work. Even in subjects who suffer from depression, sports can reduce psychological discomfort through exercise. Low mobility and immobility have been forcibly imposed on human beings parallel to the development of technology. Thus their physical and mental health is subject to this sensitive position. With exercise periods, they can revive their physical conditions and reestablish mental balance (Tondnevis, 1992).

The aim of this study is to evaluate the effects of exercise participation on stress levels in female athletes. Specific objectives include:

- Evaluating pre-participation stress levels in subjects before volleyball training and practice.

- Measuring post-participation stress levels in subjects after volleyball training and practice.
- Determining whether participation in sports (volleyball) reduces stress in subjects.

To achieve the objectives, the following research hypothesis was proposed:

“Sports participation (volleyball) has a meaningful effect on the reduction of subjects stress.”

## 2. Material and Methods

This study examines the effect of sport participation on the mental stress levels of students. Therefore, it was conducted as a quasi-experimental study and consisted of two groups - control and experiment - with pre-participation and post-participation measurements. Thirty subjects in the control group were selected randomly from 120 Esteghlal High School students. They were ages 15-18 years with a mean age of 16.53. Thirty subjects were also selected at random from the same population to be in the experiment group. The experiment group (mean age 16.26) participated in girls volleyball classes at Kermanshah City Azadi Stadium. Measurement instruments in this study included two questionnaires:

- Personal information questionnaire
- Personal stress questionnaire

Information was captured via the questionnaire from June-September 2003. Researchers collected the pre-test questionnaire after the first meeting with the control and experiment groups. However, before distributing the questionnaires, they did interviews to ensure none of the students had participated in regular exercises before. The experiment group participated in volleyball skills training within 32 sessions, each lasting 70-90 minutes. At the last session, post-test questionnaires were distributed to the two groups and were collected after completion by the examinees

## 3. Results

This study was done as a quasi-experimental research using control and experiment groups with pre-participation and post-participation questionnaires. Information from questionnaires regarding stress levels was analyzed and quantitatively converted using descriptive statistics (mean and standard deviation) and inferential statistics (student t-test).

Table 1. Pre-participation in the control and experiment groups

Evidence Groups	XΣ	N	$\bar{X}$	S	T
Control	293	30	9/77	3/52	
Experiment	278	30	9/27	4/27	0/495

As shown in Table 1, since the t value (0.495) is less than table critical value (1.671) at 5% alpha level, it will not reject the zero assumption; the study hypothesis based on differences between the pre-participation mean scores of control and experiment groups is not confirmed. The differences observed between the control and experiment groups' mean scores of stress during the pre-participation phase are not statistically significant.

Table 2. Post-participation measurements of control and experiment groups

Evidence	XΣ	N	$\bar{X}$	S	T
Control	281	30	9/37	3/39	
Experiment	217	30	7/23	3/67	2/338

As shown in Table 2, since the t value (2.338) was more than the table critical value (1.671) at 5% alpha level, it rejects the zero assumption; the study hypothesis based on differences between the post-participation mean scores of control and experiment groups is confirmed. The differences observed between the control and experiment groups' mean scores of stress during post-participation testing are statistically meaningful.

As shown in Table 3, since the t value (0.747) is less than the table critical value (1.671) at 5% alpha level, it will not reject the zero assumption; the study hypothesis based on differences between the pre-participation and post-participation mean scores of control and experiment groups is not confirmed. The differences observed between the control and experiment groups' pre-participation and post-participation mean scores are not statistically meaningful.

Table 3. Results of the control and experiment groups at pre-participation and post-participation

Evidence	XΣ	N	$\bar{X}$	S	T
Pre test	293	30	9/77	3/52	
Post test	281	30	9/37	3/39	0/747

As shown in Table 4, since the t value (4.169) was more than the table critical value (1.671) at 5% alpha level, it will reject the zero assumption; the study hypothesis based on differences between the pre-participation and post-participation mean scores of experiment groups is confirmed. The

differences observed between the pre-participation and post-participation mean scores of experiment groups are statistically meaningful

Table 4 - Experiment group pre-participation and post-participation test results

Evidence	$X\Sigma$	N	$\bar{X}$	S	T
Pre test	278	30	9/27	4/27	
Post test	217	30	7/23	3/67	4/169

Finally, the mean scores of stress in the control group's pre-participation and post-participation phases are 9.77 and 9.37 respectively, and for experiment group are 9.27 and 7.23 respectively

#### 4. Discussions

Mc Mahan (1990) conducted many studies of the benefits of exercise and concluded that intense aerobic exercise can improve the development of self-esteem and reduce depression (quoting from Habibian, 2000). Skeleton et al. (1991) studied the role of tae kwon do belt ranks on the level of aggression in children 9-11 years old. The results showed that as students progressed through rising levels of combat, aggression levels reduced. The reason for this was found during the taekwondo exercises. Brook and Heim (1996) performed a preliminary study of 16 children with asthma. They found those who participated in sports had more positive self perceptions and were less anxious and dealt better with their illness than individuals who did not participate in sports (quoting Shamlu, 1999). Nora et al. (1995) found increases of 13-14 percent in aerobic power, decreased joint pain, and improved levels of anxiety and depression after 12 weeks of aerobic training. In another study (1997) they reported 10-20 percent cardio-respiratory improvements along with effective reductions of depression, anxiety, fatigue, and stress after subjects participated in an aerobic fitness program.

Cooks and Colin (1998) reported a linear relationship between confidence and motor skills performance; with higher motor skills, confidence increases (quoting from Namazizade and Naghavi, 2002). Zibery (1993) has compared the mood status of a selected group of elite athletes to non-athletes. The results showed meaningful differences in anger, hostility, power, activity, and fatigue between the two groups but there was no meaningful difference between the groups in terms of tension, anxiety, and confusion. Saheb Alzamani (1995) divided students aged 13-15 into two groups and trained the control group in karate instruction for three months. After

practicing karate for three months, students showed reduced levels of aggression.

Mozayeni (1374) studied female athletes and non-athletes and found that depression was higher in non-athletes than athletes. Exercise is a very effective antidepressant. Qoli Allah Poor (1997) compared the aggression of athletes competing in basketball, karate and boxing with non-athletes and found meaningful differences between athletes and non athletes. Habibian (2000) in a study entitled "Comparison of stress between athlete and non-athlete female students" concluded that female athletes have less stress than non-athletes. Ali Faiz Khademi (2005) in "Loneliness and source of control (internal-external) in male athletes of 18-12 years for group and solo sports in city of Kermanshah" reported that the mean of loneliness feelings is higher in solo-sport athletes than in group-sport athletes. Group-sports athletes have more external sources of control than solo-sports athletes. Also there was a meaningful difference between individual athletes and team athletes in reported feelings of loneliness and sources of control (internal-external).

This study examined the effects of sports participation on stress in female students. Analysis of the results and mean scores of the stress levels of subjects by t-test shows that female students' sports participation (specifically in volleyball) has a meaningful effect in reducing incidence of stress and can be 95% confirmed. Participation in sports is closely related to mental health, especially the prevention of disorders, and is a valuable tool to improve physical health. It seems the cause of stress reduction in the experiment group of students is regular physical activity that led to adjusted physical and psychological characteristics. Exercise causes excitement and joy and is a special vitality that can be a factor in reducing psychological disorders.

#### Acknowledgements:

Authors are grateful to the Islamic Azad University for its support to carry out this work.

#### Corresponding Author:

Ali Feyzkhademi.  
Izeh Branch, Islamic Azad University ,Izeh, Iran.  
E-mail: [khademisport@gmail.com](mailto:khademisport@gmail.com)

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9/15/2010

# Plotting An Improved Orbital Elements Through Speckle Interferometry For Two Binary Systems, 00122+5337=Bu 1026ab And WDS 04136+0743 =A1938.

**<sup>1</sup>S. PATTNAIK, S. K. KAMILA<sup>2</sup>, G. S. ROY<sup>3</sup>, B. B. Acharya<sup>4</sup>**

<sup>1</sup>Pathani Samanta Planetarium, Bhubaneswar, Orissa (INDIA),

<sup>2</sup>Department of Physics, ITER, Bhubaneswar, Orissa (INDIA),

<sup>3</sup>Department of Physics, Govt. (Auto) college, Bhawanipatana, Orissa (INDIA)

<sup>4</sup>Christ college, Cuttack, Orissa (INDIA)

[subhendu\\_patnaik@yahoo.com](mailto:subhendu_patnaik@yahoo.com)

**ABSTRACT:** The effect of atmospheric turbulence on the diffraction-limited imaging of celestial bodies is one of the major problems in observational astronomy. The speckle interferometric technique was introduced in the 1970s to solve this problem. The technique is used to decode the diffraction-limited spatial Fourier spectrum and image features of the celestial objects, using a series of short-exposure (< 20 ms) images. Since most common binary orbit periods vary from 10 to 30 years, a large number of these binary systems, studied using the speckle data, completed one or two revolutions. In this study, an algorithm developed by the Indian Institute of Astrophysics (IIA) and an algorithm developed by Hartkopf's group at Georgia State University were used to plot the orbits of two binary systems, 00122+5337=Bu 1026AB and WDS 04136+0743 = A1938. The orbital parameters of these binary systems have been calculated using speckle data and other interferometric data. The former algorithm is based on standard least square technique with iterative improvement of the orbital parameters. Unlike the latter algorithm, the former algorithm does not require any previous knowledge of the period and the eccentricity of the binary systems. The results of this comparative study have shown that both algorithms generate almost the same orbital parameters. However, the algorithm developed by the IIA requires fewer steps to calculate the orbital parameters of these binary systems. [Journal of American Science 2010;6(11):442-448]. (ISSN: 1545-1003).

**Key words:** close binaries: visual stars: Speckle interferometric technique

## 1. Introduction

One of the major problems in observational astronomy is atmospheric seeing, blurring and twinkling of celestial objects. The light from these objects is significantly affected by the micro-thermal fluctuations in the atmosphere, which is highly turbulent and optically inhomogeneous. The resolution of the images of these objects is reduced by a factor of 20 due to the effects of atmospheric seeing (Saha 2000). To reduce the effects of atmospheric seeing, Labeyrie (1970) introduced a new observational technique, speckle interferometry, which is based on taking short exposure (less than 20 ms) images, specklegrams. Speckle interferometry freezes the atmosphere, but produces an instantaneously distorted image. The specklegrams produced are then processed using Fourier-domain methods, which allow regaining the true diffraction-limited image of the object. In this study, orbits of two binary systems, 00122+5337=Bu 1026AB and WDS 04136+0743 = A1938, have been plotted using a computer program, developed by the Indian Institute of Astrophysics (IIA). The orbital

parameters of these binary stars have been compared to the orbital parameters generated by another computer program developed by Hartkopf et al. (1989, 1996) at Georgia State University.

The most well-known advantage of speckle interferometry is probably its ability to resolve binary stars at or near the diffraction limit of telescopes. This has resulted in a powerful synergy between short-period visual and long-period spectroscopic binaries, leading to stellar masses and more effective characterization of the empirical mass-luminosity relation for stars. In addition to the ability to reach the diffraction limit of a telescope, interferometry has provided the possibility of significant improvement of precision of measurement, yielding typical errors of 0.5° in position angle and 0.5% in separation.

The time base of routine speckle interferometric observations of binary stars is now in excess of 30 years. As a result of this long series of observations, which are of exceptional accuracy and precision, are now being further refined, often resulting in significant changes in the orbital elements.

Improvements to the orbital elements based on these data, coupled with the parallaxes (primarily from the *Hipparcos* program), has yielded masses for these binaries with smaller errors. Orbital elements, ephemerides, predicted radial velocity curves, and model-dependent masses have been calculated and presented for two binary systems. Also elements and ephemerides for an additional two systems are presented. These two orbits, while better than previously published determinations, are still be regarded as provisional.

The effect of atmospheric turbulence on the diffraction-limited imaging of celestial bodies is one of the major problems in observational astronomy. The speckle interferometric technique was introduced in the 1970s to solve this problem. The technique is being used to decode the diffraction-limited spatial Fourier spectrum and image features of the celestial objects, using a series of short-exposure (< 20 ms) images. Since most common binary orbit periods vary from 10 to 30 years, a large number of these binary systems, studied using the speckle data, have already completed one or two revolutions. Now let us give a brief account of collection of data.

## 1 Speckle Formation

When observing a point source and a continuum of wave components pass through a telescope's aperture, the superposition of these components leads to a pattern of constructive and destructive interference. In telescopes, the incoming light is approximately a plane wave since the source of the light is so far away. The intensity pattern of these constructive and destructive interference rings is known as the Airy diffraction pattern (Figure 1).

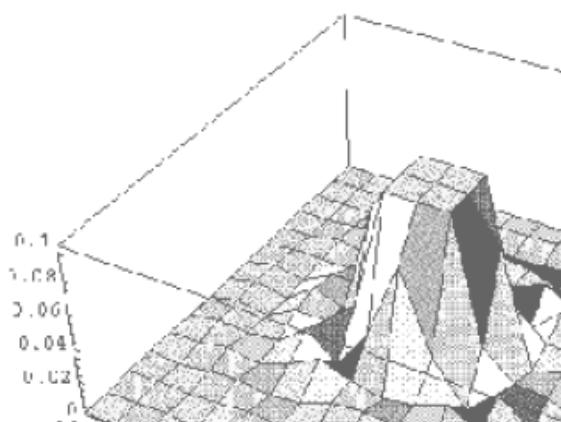


Figure 1. The airy diffraction pattern.

All telescopes have an inherent limitation to their angular resolution due to the diffraction of light at the telescope's aperture. The resolution of a telescope is characterized by the width of the Point Spread Function (PSF), which is the order of,

$$1.22 \lambda / r_o \quad (1.1)$$

Where  $\lambda$  is the wavelength of light and  $r_o$  is the average size of the turbulence cell, which is the order of 10 cm.

A wave plane propagating through the atmosphere of Earth is distorted by the Bmicro structures of refractive index inhomogeneity, called eddies. This plane wave reaches the pupil of the telescope with random patches of uniform phases. Each patch phase of the plane wave with a diameter  $r_o$ , known as *Fried's parameter* or *atmospheric coherence diameter*, is independent of the rest of the patch phases of the plane wave. Thus, the aperture of the telescope is subdivided into a set of sub-apertures. The resultant interference patterns produced by all patch phases of the plane wave consists the speckle image of the object. The measurement of the atmospheric coherence diameter is crucial to estimate the seeing at any astronomical site. This diameter can be calculated using speckle interferometric technique and the following equation,

$$\sigma = 0.342 (r_o/\lambda)^2 \quad (1.2)$$

Where  $T\sigma$  is wave plane coherence area calculated by ratioing the area of telescope aperture to the estimated number of speckles. Generally, the effective resolution of a telescope is affected by two factors, the PSF of the atmosphere and the telescope's aperture. If the telescope's diameter,  $D$ , is smaller than the atmospheric coherence diameter,  $r_o$ , the resolution will be the true diffraction limited resolution,  $1.22 \lambda/D$ . On the other hand, if  $D$  is larger than  $r_o$ , the resolution of the telescope will be affected by the atmospheric turbulence, which suppresses the telescope diameters. The seeing disc in a large telescope is equal to  $1.22\lambda/r_o$ .

## 2. Data Collection

Most of the data used in these calculations are tabulated in the Washington Double Star Catalog (WDS; [Worley & Douglass](#))<sup>[1]</sup> Database and the Third Catalog of Interferometric Measures of Binary Stars ([Hartkopf, McAlister, & Mason](#))<sup>[2]</sup>. The US Naval Observatory (USNO) speckle camera, initially described by [Douglass, Hindsley, & Worley \(1997\)](#)<sup>[3]</sup>, has been upgraded with a new intensified CCD and reduction software package as described by Germain et al [4][5]. All data taken with the 0.7 m

telescope at the USNO produced real-time directed vector autocorrelations (DVA; see Bagnulo et al.)[6] and were reduced in the manner described in Douglass et al. Speckle data from CHARA taken with the 4.0, 3.8, and 2.5 m telescopes have been taken with the CHARA speckle camera (McAlister et al.)[7]; these data were postprocessed using the DVA algorithm. USNO speckle data from the McDonald 2.1 m telescope were taken with the USNO speckle camera and also post processed with the DVA algorithm.

### 3. Speckle Interferometry Applications To Binary Stars

Binary stars are systems of two close stars gravitationally bound together and moving around each other. Generally, the two stars of the system have unequal brightness. The brighter star is more massive called the primary, while the fainter is less massive called the secondary or the companion. Binary systems can be classified into four types based on the techniques used for their discoveries- visual, spectroscopic, eclipsing, and astrometric. The relative positions of the visual binary stars can be plotted from long-term observations to determine their orbits. Due to their gravitational boundness, the relative positions of these binaries change over the years. [10][11]Baize; Saha et al.) [12][13]. This technique allows accurate astrometric study of close visual binary stars. It has revolutionized the field of binary star astronomy. The Center for High Angular Resolution Astronomy (CHARA) of the Georgia State University has been a major contributor of the interferometric measurements of thousands of binary stars since it begun by McAlister (1976). Some binary stars data used for this study are from CHARA. The interferometric measurements of binary stars allow deducing the apparent orbit of these binaries, using Kepler's third law,

$$4\pi^2 a^3 = G (m_1 + m_2) T^2 \quad (1.3)$$

where  $a$  is the semi-axis of the orbit,  $G$  is the gravitational constant,  $T$  is the period, and  $m_1$  and  $m_2$  are the masses of the stars. These stars's masses, as well as their orbital parallax, can also be identified using other spectroscopic elements.

The position of the companion of a binary system vis-à-vis the primary is determined by two coordinates, the angular separation  $\theta$ , and the position angle,  $\rho$  (fig-2). Due to the mutual gravitational boundness, both stars move around the mass of the system, barycenter. The motion of the secondary star with respect to the primary describes the true elliptic orbit. Using Kepler's laws, the orbital elements of a binary system can be identified. These elements are

crucial in determining the masses and the parallax of the individual stars.

The projection of the true orbit on the plane of the sky, the tangent plane to celestial sphere, is referred to as the apparent orbit. The apparent orbit can be determined using the semi-axis, eccentricity, position angle of the major axis, and the two coordinates of the center of the ellipse with respect to the primary star. (fig-3) We know that the general equation of the ellipse of the orbit can be expressed by,

$$Ax^2 + 2 Hxy + By^2 + 2Gx + 2Fy + 1 = 0 \quad (1.4)$$

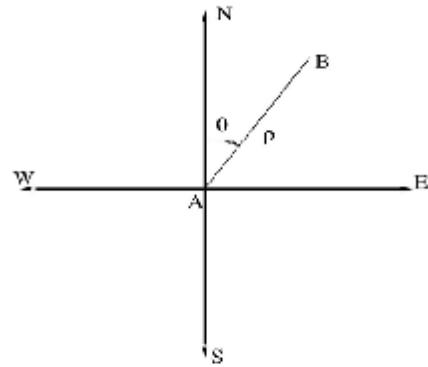


Figure 2. Part of the celestial sphere where A is the primary and B is the companion. AN defines the direction of the north celestial pole.

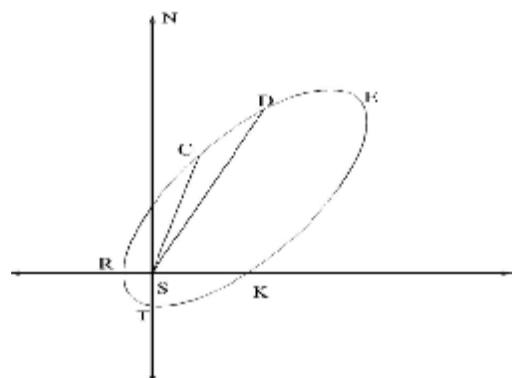


Figure 3. Apparent orbit of a binary star. where S represents the primary star.

The above equation 1.4, has five independent constants,  $A$ ,  $H$ ,  $B$ ,  $G$ , and  $F$ . If the companion is at  $C$ , the angular separation,  $\rho$ , and the position angle,  $\theta$ , can be determined using the observation measurements. Finding these parameters allows identifying the rectangular coordinates  $x$  and  $y$  of  $C$  according to,

$$x = \rho \cos \theta \text{ and } y = \rho \sin \theta \quad (1.5)$$

Theoretically, five observations spread over the orbit are sufficient to determine the five constants,  $A$ ,  $H$ ,  $B$ ,  $G$ , and  $F$ . However, a large number of observations spread over many years are required to determine accurate orbit.

### 3 Algorithms For Plotting The Orbits of Binary Stars

Various algorithms have been used to determine the elements of the orbit of a binary system. Hartkopf et al. [10][11] utilized a method based on 3-D grid search technique and visual measurements along with the interferometric data to calculate and plot the orbits of binary system. If the period,  $T$ , eccentricity,  $e$ , and the time of the periastron passing,  $\tau$ , are given, the four Thiele-Innes elements,  $A$ ,  $F$ ,  $B$ , and  $G$ , semimajor axis,  $a$ , orbital inclination,  $i$ , the longitude of ascending node,  $\Omega$ , the argument of periastron passage,  $\omega$ , can be determined by the least square method. Given  $(T, e, \tau)$  and a set of observations  $(t, x_i, y_i)$ , the eccentric anomaly  $E$  is found using the following equation,

$$M = E - e \sin E \quad (1.6)$$

Where  $M = 2\pi/T(t - \tau)$  is the mean anomaly of the companion at a time  $t$ .

The normalized rectangular coordinates  $X_i$ ,  $Y_i$ , are determined by the following equations,

$$\begin{aligned} X_i &= \cos(E) - e \\ Y_i &= \sqrt{1 - e^2} \sin E \end{aligned} \quad (1.7)$$

The four Thiele-Innes elements  $A$ ,  $F$ ,  $B$ , and  $G$  are found by a least squares solution of the equations,

$$\begin{aligned} X &= AX_i + FY_i \\ Y &= BX_i + GY_i \end{aligned} \quad (1.8)$$

The orbital elements are then deduced from these Thiele-Innes elements.

However, Hartkopf's[10] method requires a previous knowledge of the period of the binary system. (Saha et al. )[14] used another algorithm based on least square method to obtain the plots and orbital calculations. The normal equations are solved using

cracovian matrix elimination technique. This algorithm produces results similar to the results produced by the Kowalsky's algorithm, the inversion method, but it involves a fewer numbers of steps. This algorithm is based on minimizing the sum of squares of residual with respect to each constant and obtaining five equations.

The algorithm used by Saha et al. [14] is the first algorithm to use cracovian matrix elimination technique in an orbital program. This program was written by Dr. A. V. Raveendran from the Indian Institute of Astrophysics. The method has a system of giving different weightage to data obtained from different sources. This algorithm eliminates high residues data.

### 4. Method Of Orbital Calculation

Orbital elements are determined using a "three-dimensional adaptive grid search" technique, as described in [Hartkopf et.al.\[10\]](#). The routine for the determination of formal errors has been changed to one adapted from the analysis programs of [Tokovinin \[15\]](#) and yields more reliable errors leading to relatively accurate result. Many of the systems presented here have extensive histories of visual observation that, although of lower accuracy than the interferometric data, provide crucial information in the determination of the orbital period. Given a set of elements  $P$ ,  $T$ , and  $e$ , the four Thiele-Innes elements ( $A$ ,  $F$ ,  $B$ , and  $G$ ) hence the four remaining geometric elements ( $a$ ,  $i$ ,  $\Omega$ , and  $\omega$ ) can be determined by the method of least squares.

Relative weights are assigned to each astrometric observation based on the telescope aperture and observing technique. Visual observations made with larger telescopes are found to be more accurate than those made using smaller instruments. A weight of 1.0 is assigned to a visual observation made with a "large" (aperture 45 cm) telescope, while a weight of 0.5 is given to visual observations made with a "small" (aperture < 45 cm) telescope. Relative weights of interferometric versus visual measures were initially determined by [Hartkopf et al. \[10\]](#) based on rms residuals for eight very well-observed binaries. These relative weights have been born out in subsequent orbital analyses as well. A Center for High Angular Resolution Astronomy (CHARA) interferometric measure made using a 4 m class telescope is therefore given an initial weight of 20, while CHARA or USNO measures made with 2 m class telescopes and *Hipparcos* measures are given one-half this weight. Other interferometric measures are given weights of 5 (a more multidimensional weighting scheme is currently under consideration).

Weights for measures averaged from several nights' data are scaled by  $\sqrt{n}$  and weights for measures noted as being of poor quality are reduced by 50%.

## 5. Result and Discussion

### Plotting Orbits of Binary Systems

In this study, two binary systems orbits have been plotted, 00122+5337=Bu 1026AB and WDS04136+0743 = A1938. The data were obtained from the Fourth Catalog of Interferometric Measurements of Binary Stars, which began in 1982 as an internal database at the Georgia State University Center for High Angular Resolution Astronomy (CHARA). These orbits were compared to the ones obtained by Hartkopf's team (Hartkopf et al. 1989, 1996).

**00122+5337=Bu 1026AB (HD761).** Speckle data for this system date back to 1975, so they cover about  $\frac{1}{2}$  revolution and define the period well. For this system, 36 interferometric measurements, from 1975 to 2000, have been used for the orbit calculations. Most of these observations are speckle measurements. The data shows almost uniform variation of  $\rho$  and  $\theta$ . This system is found to have a period of about 60 years. The system's approaching periastron is around 1986. Figure 4 illustrates the apparent orbit of HD761 and Figure 5 represents the radial velocity curves of the same system.

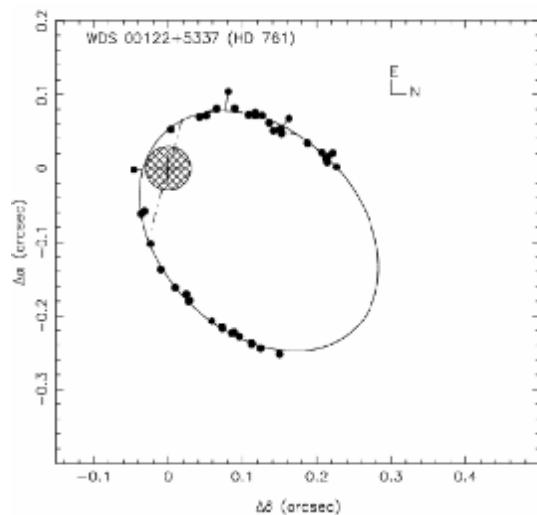


Figure 4. The apparent orbit of HD761 generated by IIA's program

In Figure 4, the x and y scales are in arsecs. The dot-dash line denotes the line of nodes. The shaded circle centered in the orbit represents the Rayleigh limit of the telescope. Figure 6 illustrates the

plot of the mean anomaly (radian) vs. the epoch. The orbital parameters of HD761 generated by IIA's program and Hartkopf's program are given in table 1.

Both programs generate slightly different orbital parameters for HD761. The slight difference between these results can be explained by the fact that Hartkopf's team used more visual and speckle measurements in their orbits calculations.

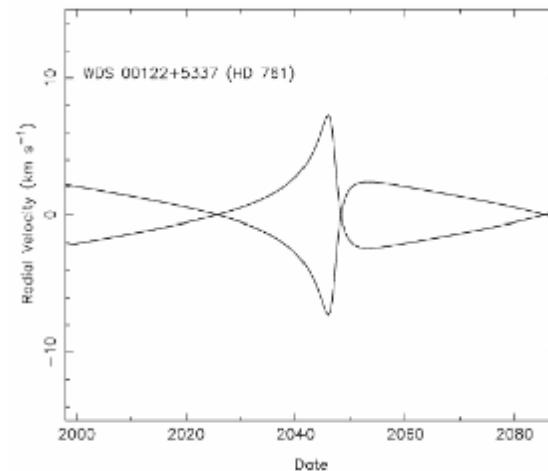


Figure 5. Radial velocity curves of HD781 generated by IIA's Program

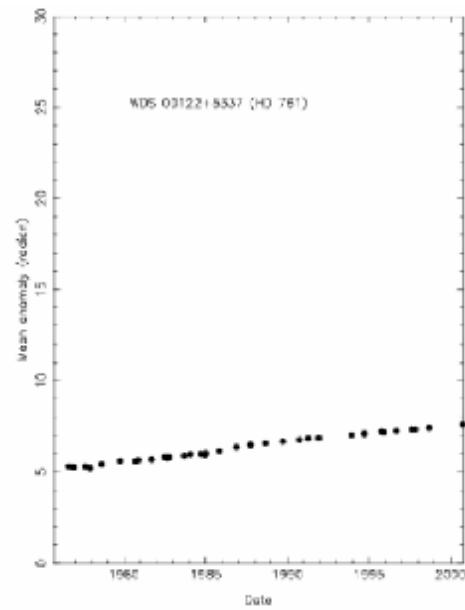


Figure 6. The mean anomaly in radian vs. the epoch.

Table 1. Orbital parameters of HD761.

	P (year)	a (arcsec)	i ( $^{\circ}$ )	$\Omega(^{\circ})$	T	e	$\omega(^{\circ})$
Hartkopf's Algorithm	66.84	0.2514	42.77	254.9	1986.542	0.8282	255.2
IIA's Algorithm	59.78	0.2266	34.57	47.50	1986.9626	0.7491	103.49

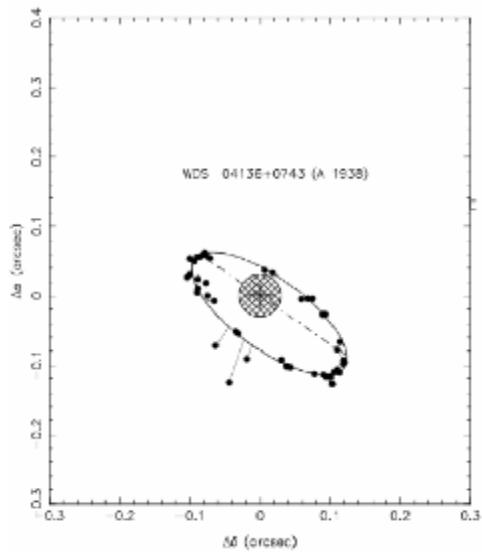


Figure 7. The Apparent orbit of HD26690 generated by IIA's program

*WDS 04136+0743 = A1938 (HD26690).* The speckle data for this system date back to 1975, so they cover about 3/2 revolution and define the period very well. For this system, 36 interferometric measurements, from 1975 to 1995, have been used for the calculation of orbits.

Most of these observations are speckle measurements. The data shows almost uniform variation of  $\rho$  and  $\theta$ . This system was found to have a period of about 7.18 years. The system's approaching periastron3 is around 1990. Figure 7 illustrates the apparent orbit of HD26690 and Figure 8 represents the radial velocity curves of the same system.

The orbital parameters of HD 26690 generated by IIA's program and Hartkopf's program are given in table 2.

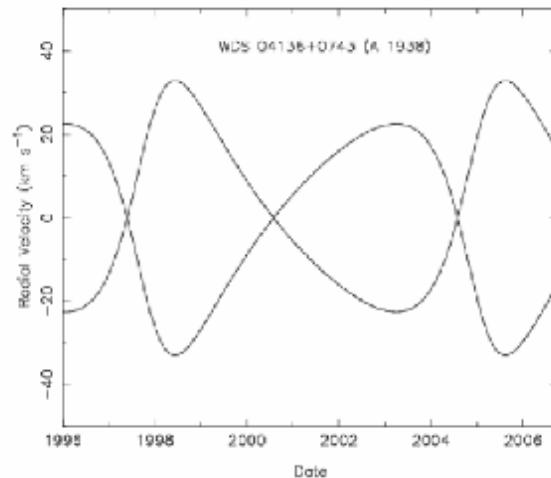


Figure 8. Radial velocity curves of HD 26690 generated by IIA's program.

## 6. Conclusion and Future Work

In this study, an algorithm developed by the IIA and another algorithm developed by hartkopf's group have been used to plot the orbits of four binary systems, 00122+5337=Bu 1026AB AND WDS 04136+0743 = A1938. The orbital parameters of these binary systems have been calculated using speckle data and other interferometric data. Both programs generate slightly different orbital parameters for the four binary systems. This slight difference can be explained by the fact that Hartkopf's team used more visual and speckle measurements in their orbits calculations. It is needless to mention that more speckle data are crucial to calculate the orbital parameters of binary systems with more accuracy.

Table 2. Orbital parameters of HD 26690. Both programs generated almost similar results

	P (year)	a (arcsec)	i ( $^{\circ}$ )	$\Omega(^{\circ})$	T	E	$\omega(^{\circ})$
Hartkopf's Algorithm	7.1788	0.1355	67.16	144.88	1990.625	0.3395	303.40
IIA's Algorithm	7.1807	0.1366	67.56	145.12	1990.6856	0.3242	305.15

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9/17/2010

# Hepatoprotective and Therapeutic Activity of *Origanum syriacum* Aqueous Extract in Paracetmol Induced cell Damage in Albino Mice

Abeer Y. Ibrahim<sup>1</sup>, Nermeen M. Shaffie<sup>2</sup> and Hemaia M. Motawa<sup>3</sup>

<sup>1</sup>Medicinal and Aromatic Plants Dept., Pharmaceutical and Drug Industries Division, National Research Centre.

<sup>2</sup>Pathology Department, Medical researches Division, National Research Centre, Egypt.

<sup>3</sup>,Pharmacognosy Department, Pharmaceutical and Drug Industries Division, National Research Centre, 12622, Cairo, Egypt.

[abeeryousry@yahoo.com](mailto:abeeryousry@yahoo.com)

**Abstract:** Ethnomedically genus *Origanum* L. is one of the most commonly used herb in many countries as a stimulant, analgesic, antitussive, expectorant, sedative, anti-inflammatory and antihelminthic agent. The hepatoprotective and therapeutic effects of *Origanum syriacum* aqueous methanolic extract on paracetamol induced liver cell damage in mice with respect to antioxidant status was investigated. Mice were treated with extract and sylimarin in recommended dose after or before paracetamol administration (400mg/ kg/ day). Lipid peroxides concentration was considerably decreased due to the elevation of reduced glutathione concentration(GSH) and enhancing of glutathione reductase(GR), glutathione transferase(GST), glutathione peroxidase (GPx)and superoxide dismutase(SOD) activities as compared to paracetamol or sylimarin treated mice. Liver function parameters are still in the normal levels in extract treated mice as compared to control. Using extract as a treating agent after subjecting mice to paracetamol gave better results, the liver tissue showing a nearly normal liver tissue except for a little cellular infiltrate around main blood vessels while sylimarin showing a noticeable dilatation of blood vessels that are surrounded by fibrosis and cellular infiltration. Liver tissue from mouse received *Origanum* extract and then paracetamol showing mild dilatation of blood sinusoids and cellular infiltration around main blood vessels while sylimarin treated mice showed marked dilatation of blood sinusoids, vacuolar degeneration in many of the hepatocytes and focal necrotic areas among the hepatocytes. In conclusion, *Origanum syriacum* extract has potent therapeutic activity than hepatoprotective activity and it is more effective than sylimarin in two cases. The plant extract was screened for its phytochemical constitutions.

[Abeer Y. Ibrahim<sup>1</sup>, Nermeen M. Shaffie<sup>2</sup> and Hemaia M. Motawa. Hepatoprotective and Therapeutic Activity of *Origanum syriacum* Aqueous Extract in Paracetmol Induced cell Damage in Albino Mice. Journal of American Science 2010;6(11):449-458]. (ISSN: 1545-1003).

**Key words:** Hepatoprotective, Antioxidant, Oregano, Paracetamol, Therapeutic

## 1. Introduction

As the etiology and pathogenesis for many diseases are unclear, there has been increasing interest in the potential therapeutic and protective agents. Antioxidant compounds are usually employed in the food industry to prevent undesirable changes due to oxidation reactions. In recent years, there is a wide interest in finding natural compounds that could replace synthetic antioxidants. Herbs and spices are employed as food ingredient to flavour sausages, meats and salads; some studies have reported that they contain a wide variety of compounds that have shown to have beneficial health effects. It has recently become clear that one of the values of many spices is that they contain natural antioxidants, which provide protection against harmful free radicals. Oregano is an herbaceous plant native to the Mediterranean regions (Baytop, 1999) used as a medicinal plant with healthy properties like its powerful anti-bacterial and anti-fungal properties ( Elgayyar et al., 2001& Sokovic et al., 2002), antihelminthic agent ( Khanna et al., 2007) and anti-inflammatory ( Robledo

et al., 2005). Cervato et al.(2000) have found some antiradical activity in aqueous and methanolic extracts of *Oregano* leaves also Bendini et al. (2002) reported that ethanolic extracts under selected conditions showed antioxidant activity. *Origanum* species are traditionally used as sedative, diuretic, degasifier, sweater and antiseptic also in the treatment of gastrointestinal diseases and constipation (Baytop, 1999). Drug-induced liver injury is a potential complication of virtually every prescribed medication, because the liver occupies a central role in the metabolic disposition of all drugs and foreign substances. Most of the hepatotoxic chemicals damage liver cells mainly by lipid peroxidation and other oxidative damages and this applies also to paracetamol which is a widely used analgesic/antipyretic agent regarded as generally safe when used at therapeutic levels (Danque et al., 1993) while representing the drug of choice in children. However, paracetamol hepatotoxicity is the leading cause of drug-induced liver failure in the western countries and an acute or cumulative overdose can

cause severe liver injury with the potential to progress to liver failure (Lee, 2004). The main toxicity mechanism advocated for include the Cyp2E1 metabolic activation of the reactive metabolite, N-acetyl-p-benzoquinone imine which depletes cellular glutathione and then covalently binds to critical cellular proteins and macromolecules (Cohen and Khairallah, 1997, Paglia and Valentine, 1967) followed by proteins alkylation, namely mitochondrial proteins on its turn, triggers (Park et al., 2005), then formation of reactive oxygen species into the mitochondria (Knight et al., 2001). These events are primarily based on the dysfunction of the cellular  $\text{Ca}^{2+}$  homeostasis, with enhancement of the cytosolic  $\text{Ca}^{2+}$  concentration, noxious translocation of Bax and Bid to the mitochondria and peroxynitrite formation too. Superoxide anions insofar generated can dismutate to form molecular oxygen and hydrogen peroxide, which then require electrons from GSH molecules to be reduced to water by glutathione peroxidase enzyme and brings about a significant increase of mitochondrial glutathione disulfide (GSSG) levels (Griffith, 1980, Habig et al., 1974). The present study aims to investigate hepatoprotective and therapeutic effects of *Origanum syriacum* aqueous methanolic extract on paracetamol induced liver cell damage in mice with respect to antioxidant status in liver tissue.

## 2. Material & Methods:

### Plants extract preparation

The aerial parts of *Origanum syriacum* were defatted using petroleum ether then the defatted powder was extracted with 20% aqueous methanol. The crude methanolic extract was concentrated using rotary evaporator under reduced pressure then the concentrated extract was used in phytochemical screening and biological studies.

### Animals

Male albino mice weighing  $30\pm 35\text{g}$  were housed in polypropylene cages, each cage was contained ten mice in case of  $\text{LD}_{50}$  assessment while it was contained eight mice in case of hepatoprotective study. Animals were fed on standard diet, temperature through the housing was controlled at  $24^\circ\text{C}$ , relative humidity  $65\pm 5\%$  and light/dark cycles (12/12hrs). This study was approved by Medical Research Ethics Committee, National Research Center, Egypt, under registration no. 10 033

### Experimental design

Male albino mice (25-30g) were obtained from animal house of national Research Centre. Animals were kept for one day under the condition of experiment then they were intraperitoneally injected with 0.5ml of different solution used in experiment using infantile syringe. Animals were divided into three main groups includes negative control group, normal group treated with extract or sylimarin and treated group. Each subgroup and control group was contained eight mice. The

control group was injected with saline solution (0.5ml/day/ 5 days). The second main group is healthy normal group which was divided into three subgroups, the first one was injected with extract of medicinal plant prepared in saline solution (0.5ml of 1/10 extract  $\text{LD}_{50}/\text{day}/5\text{days}$ ), the second was injected with silymarin in a recommended dose (25mg/kg) for 5days prepared in 0.5ml saline solution while the third group was injected with paracetamol as a super saturated solution in 0.9 % saline at the dose of 500mg/ kg body weight. The third main group was treated group which contain four subgroups, the first one was treated with medicinal plant extract( 0.5ml of 1/10 extract  $\text{LD}_{50}/\text{day}/5\text{days}$ ) then was injected with paracetamol at a dose as mentioned above, blood samples, livers were collected after 48hrs of paracetamol injection. The second subgroup was treated with paracetamol then was injected with plant extract (0.5ml of 1/10 extract  $\text{LD}_{50}/\text{day}/5\text{days}$ ) after 48hrs of paracetamol injection blood samples and livers were collected after 24hr of last injection. The third subgroup was treated with silymarin (0.5ml of 25mg/ kg/ day/ 5days) then was injected with paracetamol in a dose of 500mg/ kg b.wt. while blood samples and livers were collected after 48hrs of paracetamol injection. The fourth subgroup was treated with paracetamol then was injected with silymarin (0.5ml contain dose of 25mg/ kg/ day/ 5days) after 48hrs of paracetamol injection, blood samples and livers were collected after 24hr of last injection.

### Acute toxicity assay

The acute toxicity test for *Origanum* extract was carried out to evaluate any possible toxicity. Swiss albino mice ( $n = 10$ ) of either ten mice were subjected to a 24 hour fast with water before initiating the test then was administered with different doses of the extract by increasing or decreasing the dose according to the response of animal (Bruce, 1985). The dosing patron was 500, 1000, 1500, 2000, 2500 and 3000mg/ kg by intraperitoneally injection while the control group received only the normal saline. All groups were observed for any gross effect or mortality during 48h.

### Biochemical assessment

At the end of the experimental period, animals were fasted for 12 h and blood samples were obtained from the experimental and control mice by puncturing retro-orbital plexus. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in serum were measured with kits (Reitman and Frankel, 1957). After collection of blood samples mice's livers were, collected then immediately excised, rinsed in ice cold normal saline. Liver homogenate(5%) was prepared in bidistilled water using potter-Elvehjem homogenizer with Teflon pestle. Protein concentration was measured as described, by Sedlack and Lindsay (1968). GPx activity was assayed spectrophotometrically at 340nm (Paglia and Valentine, 1967) and the amount of the

enzyme converting 1 $\mu$ mol GSH per min per mg protein was taken as 1 activity unit. GR activity was measured spectrophotometrically at 340nm (Goldberg and Spooner, 1983) and the amount of the enzyme reducing 1 $\mu$ mol GSSG per min per mg protein was regarded 1 activity unit as elsewhere described. GST activity was measured spectrophotometrically at 340nm (Habig et al., 1974) and the amount of the enzyme that conjugate 1, chloro-2, 4-dinitrobenzene with reduced glutathione per min per mg protein was regarded 1 activity unit. SOD was measured at 560nm (Fridovich , 1974) as the reduction suppression rate of nitrotetrazolium blue and for 1 unit of activity, the amount of protein was taken which provided 50% inhibition of nitrotetrazolium blue reduction under standard conditions. GSH concentration was measured spectrophotometry at 405nm (Griffith, 1980). and the unit of concentration was mg/g tissue using Ellman's reagent (5,5'-dithiobis 2-nitrobenzoic acid; DTNB), which was reduced by thiol groups to form 1 mol 2-nitro 5-mercaptopbenzoic acid/mol thiol and with maximal absorption at 412 nm. Malondyaldehyde (MDA) determination in liver was assayed by spectrophotometric method at 534nm (Ohkawa et al., 1979). and the unit of concentration was  $\mu$ mol/ g tissue.

#### Statistical analysis

Data were analyzed by one-way ANOVA test for comparisons among means at  $p \leq 0.05$ .

#### Histopathological assessment:

Specimens of liver and kidney from all animals were dissected immediately after death. All the specimens were fixed in 10% neutral-buffered formal saline for 72 hours at least, washed in distilled water and then dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin wax. Serial sections of 6  $\mu$ m thick were cut and stained with Haematoxylin and eosin (Drury and Wallington, 1980) for histopathological investigation.

Histochemical investigation by using Periodic acid Schiff's reagent (Mac-Manus and Cason, 1950) was performed to evaluate the mucopolysaccharide content in these tissues.

Images were captured and processed using Adobe Photoshop version 8.0.

### 3. Results

#### 1) Effect of OSE on antioxidant parameters:

Administration of paracetamol significantly decreased glutathione concentration also considerably reduces GR, GST, GPx and SOD activities while it elevated protein concentration and lipid peroxides concentration in liver tissue as compared to control group.

Data presented in table (1) shows the elevation of glutathione production in mice treated with extract and sylimarin as compared to paracetamol induced mice or healthy group administered saline, the healthy group administered OSE or sylimarin has the same trend of

results. GR was significantly enhanced by administration of OSE and sylimarin but it was magnified by OSE administration more than sylimarin administration, sylimarin administration has approximately the same activity when administered as hepatoprotective or therapeutic agent but OSE is superior to sylimarin in two cases.

Paracetamol administration significantly decreased GST by 68% as compared to healthy group. Administration of OSE or sylimarin significantly enhanced GST and there was no significant difference between GST activities when OSE was administrated without liver injury or used as therapeutic drug after paracetamol administration, it means that GST was magnified to be in a healthy level. Also there was no significant difference in GST when sylimarin was administered as hepatoprotective or therapeutic agent. The best recorded activity was in case of OSE administration as therapeutic drug.

Data presented in table (1) shows that, paracetamol decreased GPx by 72% while OSE and sylimarin induced GPx in healthy animal treated with OSE or sylimarin also GPx was induced to be nearly ve<sup>-</sup> control group in paracetamol induced liver injury mice when treated with OSE. Sylimarin as protective agent gave results nearly the same to OSE as therapeutic agent, while it gave the least GPx activity when administered as therapeutic agent.

Injection of paracetamol highly decreased SOD by about 82% while OSE and sylimarin administration significantly enhanced SOD to be more than healthy group in healthy mice treated with OSE and sylimarin also SOD was elevated in liver injured mice when they were treated with OSE as hepatoprotective or therapeutic agent. Sylimarin also induced SOD and the hepatoprotective effect was more sufficient than therapeutic effect but OSE is superior to sylimarin in enhancing SOD activity either protective or therapeutic agent.

The obtained results indicates that injection of paracetamol significantly induced LPC by 90.5% while treating mice with extract or sylimarin didn't enhance liquid peroxide production so LPC is still nearly to control level in healthy treated mice also treating mice with extract or sylimarin protect liver from increasing of lipid peroxide as a response of liver injury with paracetamol in both methods of treatments, as hepatoprotective or therapeutic. *Origanum syriacum* extract significantly decrease LPC as compared to paracetamol group and there is no significant difference between ve- control group (injected with saline) and group treating with extract as hepatoprotective agent, these results are true with sylimarin. Treating mice with extract as therapeutic agent showed the best result in decreasing LPC as compared to paracetamol group.

The obvious mentioned results shows that the extract significantly increase all determined antioxidant enzyme activities and glutathione concentration so it Table (1): Antioxidant activity of *Origanum syriacum* extract in paracetamol induced - liver injury in mice

significantly reduces lipid peroxide concentration in both cases, hepatoprotective or therapeutic agent, and it is superior than sylimarin and the best result was

Parameter Groups	Glutathione concentration mg/g tissue Mean± SD	Protein concentration mg/ g tissue	Glutathione reductase activity μmol/mg protein/min	Glutathione transferase activity μmol/mg protein/min	Glutathione peroxidase activity μmol/mg protein/min	Superoxide dismutase activity U/mg protein
Control (ve <sup>-</sup> )	2.84±0.063 <sup>a</sup>	182.19±1.41 <sup>a</sup>	3.5±0.14 <sup>a</sup>	2.66±0.09 <sup>a</sup>	1.43±0.067 <sup>a</sup>	12.14±0.72 <sup>a</sup>
Paracetamol group (ve+control)	0.55±0.05	275.32±1.44	1.18±0.13	0.84±0.04	0.39±0.02	2.15± 0.11
<i>Origanum</i> extract (ve+control)	4.09±0.001 <sup>a</sup>	185.26±2.46 <sup>a</sup>	12.21±0.2 <sup>a</sup>	4.34±0.16 <sup>a</sup>	2.66±0.08 <sup>a</sup>	40.04±1.18 <sup>a</sup>
Sylimarine (ve <sup>+</sup> )	3.73±0.002 <sup>a</sup>	182.19±2.02 <sup>b</sup>	8.22±0.19 <sup>a</sup>	4.96±0.09 <sup>a</sup>	3.08±0.09 <sup>a</sup>	29.17±0.66 <sup>a</sup>
Extract as hepatoprotective agent	3.19±0.001 <sup>a</sup>	177.4±1.08 <sup>a</sup>	9.93±0.21 <sup>a</sup>	3.26±0.18 <sup>a</sup>	1.09±0.14 <sup>a</sup>	64.27±0.76 <sup>a</sup>
Sylimarin as hepatoprotective agent	2.92±0.06 <sup>c</sup>	175.33±0.99 <sup>a</sup>	8.67±0.22 <sup>a</sup>	4.63±0.07 <sup>a</sup>	1.33±0.05 <sup>ac</sup>	39.37±0.82 <sup>a</sup>
Extract as therapeutic agent	3.65±0.08 <sup>a</sup>	191.33±1.64 <sup>a</sup>	11.53±0.5 <sup>a</sup>	6.58±0.26 <sup>a</sup>	1.62±0.07 <sup>a</sup>	50.58±0.46 <sup>a</sup>
Sylimarin as therapeutic agent	2.48±0.09 <sup>a</sup>	158.43±1.68 <sup>a</sup>	8.49±0.22 <sup>a</sup>	3.94±0.05 <sup>a</sup>	0.92±0.06 <sup>a</sup>	46.78±0.89 <sup>a</sup>

Data are presented as the means±S.D  
compared to control

a P<0.001, compared to paracetamol group b P<0.05,

c n.s., compared to control

recorded in case of extract as therapeutic agent.

Injection of paracetamol in an over dose significantly increased protein production in liver tissue while it was decreased when these animals were treated with extract or sylimarin as hepatoprotective agents and sylimarin as therapeutic agent although it was significantly increased when extract used as therapeutic agent. There was no significant difference between ve<sup>-</sup> control group and healthy groups treated with OSE and sylimarin also between OSE and sylimarin as hepatoprotective agents, this means that both of OSE and sylimarin have the same effect on protein production in liver tissue.

## 2) Effect of OSE on Liver function

Glutamic-oxaloacetic transaminase was significantly enhanced by paracetamol as mentioned in table (2)

while it was still at the control level when extract was administered as therapeutic agent also GOT was around the recorded values of healthy group when it was administered as hepatoprotective agent. Administration of sylimarin increased GOT when it was administered as hepatoprotective or as therapeutic drug, as compared to healthy group administered saline. The obtained results indicate that *Origanum* extract reduced the danger effect of paracetamol on liver by 72.9% as hepatoprotective and by 74.8% as therapeutic agent. *Origanum* extract showed the same effect on glutamic- pyruvate transaminase so there was no significant difference between healthy group treated with saline or healthy group treated with extract, treating liver injured

animals with extract prevent elevation of GPT by paracetamol administration. It is clear from the recorded activities that treating animals with

sylimarin was less sufficient than *Origanum* extract when used as hepatoprotective or therapeutic agent.

Table (2): Liver function of *Origanum syriacum* extract in paracetamol induced - liver injury in mice

Groups Parameter	Control (ve-) Mean±SD	Paracetamol treated group	<i>Origanum</i> extract	Sylimarin	Extract as hepato- protective agent	Sylimarin as hepato- protective agent	Extract as therapeutic agent	Sylimarin as therapeutic agent
Glutamic- oxalacetic transaminase	50.83 ±0.79 <sup>a</sup>	206.85 ±0.79	50.98 ±0.96 <sup>c</sup>	53.96 ±0.75 <sup>a</sup>	55.85 ±1.07 <sup>a</sup>	60.05 ±0.83 <sup>a</sup>	52.4 ±0.6 <sup>a</sup>	65.62 ±1.27 <sup>a</sup>
Glutamic- pyruvic transaminase	44.33 ±0.63 <sup>a</sup>	179.97 ±2.00	43.62 ±1.19 <sup>c</sup>	45.45 ±0.56 <sup>c</sup>	46.27 ±0.89 <sup>b</sup>	47.36 ±0.68 <sup>a</sup>	45.36 ±0.75 <sup>c</sup>	49.31 ±0.63 <sup>a</sup>

Data are presented as the means±S.D

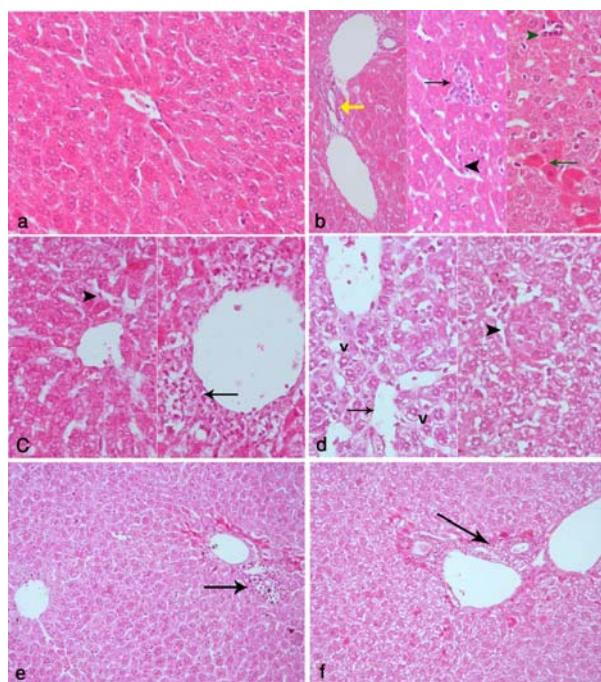
b P<0.05, compared to control

a P<0.001, compared to paracetamol group

c n.s. , compared to control

### Histopathological Results:

Using paracetamol had a marked damaging effect on many organs in the body. In liver tissue its effect appears in the form of marked dilatation of the main blood vessels with fibrosis around and multiple foci of cellular infiltration, proliferation of kuppfer cells in blood sinusoids and acidification of some hepatocyte cytoplasm (Fig. 1,b).



**Figure 1:** (a) is a section of liver tissue from a control rat showing the normal structure of it, where the hepatocytes are arranged in the form of plates radiating from the central vein. (b) is a section of liver tissue from a rat received paracetamol showing marked dilatation of blood vessels with fibrosis around (yellow arrow), focal cellular infiltration (black arrow), proliferation of kuppfer cells in blood sinusoids (black arrow head) and acidification of some hepatocyte cytoplasm (green arrow). Signs of apoptosis were observed in the form of DNA fragmentation (yellow arrow head) and karyolysis (white arrows). (c) is a section of liver tissue from a rat received origanum extract and then paracetamol showing mild dilatation of blood sinusoids (arrow head) and cellular infiltration around main blood vessels (arrow). (d) is a section of liver tissue from a rat received sylimarin and then paracetamol showing marked dilatation of blood sinusoids (arrow), vacuolar degeneration in many of the hepatocytes (v) and focal necrotic areas among the hepatocytes (arrow head). (e) is a section of liver tissue from a rat received paracetamol and then origanum extract showing a nearly normal liver tissue except for a little cellular infiltrate around main blood vessels (arrow). (f) is a section of liver tissue from a rat received paracetamol and then sylimarin showing a noticeable dilatation of blood vessels that are surrounded by fibrosis and cellular infiltration (arrow).

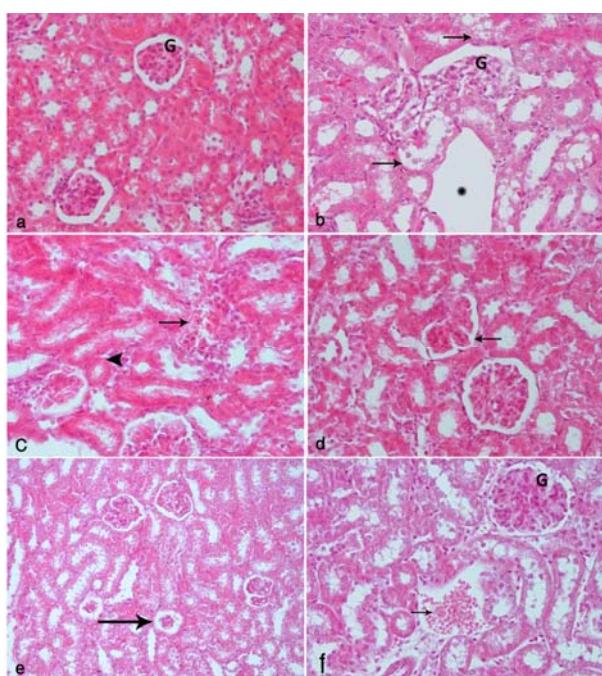
(Hx. & E. X 50, 100)

In our work, examination of renal tissue from mice exposed to paracetamol revealed the presence of deformity of glomeruli and vacuolar degeneration in

tubular lining epithelium in a good number of tubules (Fig. 2,b).

Using origanum extract as a protecting agent before subjecting mice to paracetamol gave moderate results as mild cellular infiltration specially around blood vessels in liver tissue (Fig. 1,C) and some deformed glomeruli in renal tissue (Fig. 2,C) were still noticed.

Using this extract as a treating agent after subjecting mice to paracetamol gave better results. Liver tissue showed quite normal liver tissue except for only very mild cellular infiltrate around blood vessels (Fig. 1,e) and renal tissue showed normal appearance except for a few glomeruli that appeared atrophied (Fig. 2,e).



**Figure 2:** (a) is a section of renal tissue of a control rat showing the normal structure of this tissue composed of glomeruli (G) and different types of tubules. (b) is a section of renal tissue of a rat received paracetamol showing deformation of glomeruli (G), vacuolar degeneration of epithelial lining of the tubules and large gaps denoting edema. (c) is a section of renal tissue of a rat received origanum extract and then paracetamol showing that most of the tubules appear normal (arrow head), while glomeruli show some deformity and/or hemorrhage (arrow). (d) is a section of renal tissue of a rat received sylimarin and then paracetamol showing lobulation of some glomeruli (arrow). (e) is a section of renal tissue of a rat received paracetamol and then origanum extract showing a nearly normal renal tissue except for atrophy of a few glomeruli. (f) is a section of renal tissue of a rat received paracetamol and then sylimarin showing

hemorrhage (arrow) in interstitial tissue. Some of the epithelial lining of tubules show vacuolar degeneration.

(Hx. & E X 50, 100)

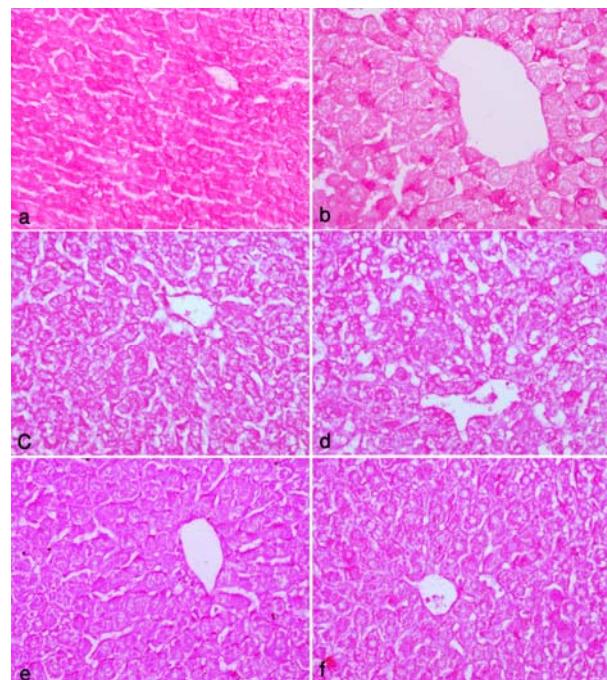
On the other hand, using sylimarin as a protective agent gave less effective results than *Origanum* as in liver tissue marked dilatation of blood sinusoids, vacuolar degeneration in many of the hepatocytes, focal necrotic areas among the cells (Fig. 1,d) and lobulation of some glomeruli in renal tissue (Fig. 2,d) were still present.

Using sylimarin as a therapeutic agent gave better results than those obtained from using it as a protective agent, although still less than those obtained from *Origanum* extract as dilatation of blood vessels with fibrosis and cellular infiltration in liver tissue (Fig. 1,f) hemorrhage and vacuolar degeneration of the epithelial lining of some tubules in renal tissue (Fig. 2,f) was observed.

Results obtained from *Origanum syriacum* extract as a protecting and a treating agent were much better than those obtained from sylimarin used as a protecting and a treating agent respectively.

#### Histochemical results:

The histochemical results of the present work confirmed those of the histopathological investigations as Periodic acid Schiff reagent showed that injection of mice with paracetamol caused marked depletion of mucopolysaccharide content of cells both in liver tissue (Fig. 3, b) and in renal tissue (Fig. 4, b).

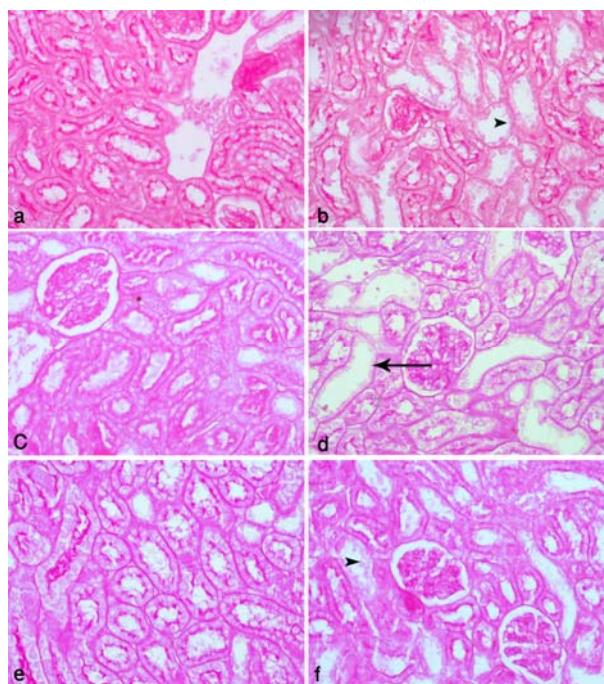


**Fig. 3:** (a) is a section of liver tissue from a control rat showing the normal component of mucopolysaccharides in hepatocytes. (b) is a section of

liver tissue from a rat received paracetamol showing marked depletion of mucopolysaccharides in most of the hepatocytes. (c) is a section of liver tissue from a rat received origanum extract and then paracetamol showing a mild decrease in mucopolysaccharide content in hepatocytes (d) is a section of liver tissue from a rat received sylimarin and then paracetamol showing a moderate decrease in mucopolysaccharide content in hepatocytes if compared with control group. (e) is a section of liver tissue from a rat received paracetamol and then origanum extract showing restoration of the normal mucopolysaccharide content in liver tissue. (f) is a section of liver tissue from a rat received paracetamol and then sylimarin showing a more or less same result as the previous group.

#### (Periodic acid Schiff X 100)

Using origanum extract before subjecting animals to paracetamol led to moderate amelioration in the mucopolysaccharide content in hepatocytes (Fig. 3, C) and renal cells (Fig. 4, C), while using it after injection of paracetamol led to restoration of the normal content of mucopolysaccharides in both liver tissue (Fig. 3, e) and renal tissue (Fig. 4,e).



**Fig. 4:** (a) is a section of renal tissue of a control rat showing the normal content of mucopolysaccharides in renal tissue, being localized at the basement membranes of renal tubules and Bowmen's capsules and also in the brush borders of proximal convoluted tubules. (b) is a section of renal tissue of a rat received paracetamol showing loss of positive reaction of the stain at the site of brush borders of many tubules (arrow head). (c) is a section of renal tissue of a rat received

origanum extract and then paracetamol showing a positive reaction of the stain at the normal places but with less density of the stain if compared with the control group. (d) is a section of renal tissue of a rat received sylimarin and then paracetamol showing a weak positive reaction of the stain and complete loss of it at the site of brush border of some tubules (arrow). (e) is a section of renal tissue of a rat received paracetamol and then origanum extract showing restoration of the normal content of mucopolysaccharide in renal tissue. (f) is a section of renal tissue of a rat received paracetamol and then sylimarin showing a loss of positive reaction of the stain at the site of brush borders of some tubules (arrow head).

#### (Periodic acid Schiff X 100)

Using sylimarin as a protective agent led to slight amelioration of mucopolysaccharide content in hepatocytes (Fig. 3, d) and in cells of renal tissue (Fig. 4, d). Using the same drug as a therapeutic agent gave better results, although they are less than those obtained from origanum extract in both liver tissue (Fig. 3, f) and in renal tissue (Fig. 4, f).

#### 4. Discussion

Drug-induced liver injury (DILI) is a major health problem that challenges not only health care professionals but also the pharmaceutical industry and drug regulatory agencies. According to the United States Acute Liver Failure Study Group (Rumack, 2004), DILI accounts for more than 50% of acute liver failure, including hepatotoxicity caused by overdose of acetaminophen (39%) and idiosyncratic liver injury triggered by other drugs (13%). Because of the significant patient morbidity and mortality associated with DILI, the U.S. Food and Drug Administration (FDA) has removed several drugs from the market (Elgayar et al., 2001). Paracetamol is widely used as an analgesic and antipyretic agent. However, accidental or intentional intake of high doses often causes acute hepatocellular necrosis with high morbidity and mortality (Park et al., 2005& Qiu et al., 2001). It was reported that many mechanisms are involved in paracetamol hepatotoxicity, Jaeschke et al. (2003) showing that the toxicity is mediated by CYP450 metabolism of paracetamol to N-acetyl-p-benzoquinone imine which covalently binds to critical proteins leading to inactivation of these proteins, especially after GSH depletion. CYP2E1 is usually assumed to be the most active CYP450 in catalyzing the metabolism of paracetamol to hepatotoxic NAPQI (Xia Chen et al., 2009).

The histopathological results of the present work go in coincidence with these reported findings as multiple foci of cellular infiltration, proliferation of Kupffer cells in blood sinusoids and acidification of some hepatocyte cytoplasm can be explained by the fact that the toxicity of paracetamol is mediated by generation of rather toxic

metabolite, N-acetyl- p-benzoquinone imine, whose detoxification may lead to a dramatic depletion of hepatic GSH (Yokozawa and Dong, 2001).

Deformity of glomeruli and vacuolar degeneration in tubular lining epithelium in a good number of tubules observed in sections of renal tissue from animals subjected to paracetamol are in coincidence with Ortiz *et al.* (2000) who stated that an acute paracetamol overdose can lead to potentially lethal liver and kidney failure in humans and experimental animals and in severe cases to death. Paracetamol is a phenacetin metabolite. Phenacetin was considered one of the most nephrotoxic analgesics. Tubular cell loss is a characteristic feature of both acute renal failure and chronic renal disease and is observed when cell death predominates over mitosis. Apoptosis is an active form of cell death that offers the opportunity for therapeutic intervention.

There is increasing evidence to suggest that the endoplasmic reticulum stress apoptotic pathway is important in the kidney, specifically in tubular epithelial cells. It has been found that the expression of GADD153, a marker of endoplasmic reticulum stress, is increased in tubular epithelial cells during paracetamol-induced apoptosis. (Corinal *et al.*, 2004).

The Importance of antioxidant constituents of plant materials in maintaining health and in protecting against many diseases and cancer is raising interest among scientists, food manufacturers and consumers, as the trend of the future is moving towards functional food with specific health effects (Kahkonen *et al.*, 1999). In our present study, we evaluate *Origanum syriacum*, aqueous methanolic extract, hepatoprotective and therapeutic activities as compared to sylimarin which recommended as hepatoprotective drug. The antioxidant activity of extract in liver tissue was used as indicator for amelioration of liver to be healthier as compared to ve<sup>r</sup> control group and paracetamol induced liver injury group. It increased GST, GR, SOD, GSH and decreased lipid peroxides.

Glutathione-S-transferases are multifunctional enzymes, which play a key role in cellular detoxification. The enzyme protect cells against toxicant by conjugating them to glutathione, thereby neutralizing their electrophilic sites, and rendering the products more water soluble, the glutathione conjugates are metabolized further to therapeutic acid and then excreted. The enzyme is comprised of both cytosolic and microsomal enzyme.

Cellular glutathione peroxidase is a member of peroxidases enzyme whose function is to detoxify peroxides in cell because peroxides can decompose to form highly reactive radicals, the GP<sub>X</sub> play a critical role in protecting cell from free radical damage. It catalyzes the reduction of H<sub>2</sub>O<sub>2</sub> to organic peroxides

using glutathione as a source of reducing equivalents (Paglia and Valentine, 1967).

Superoxide dismutase is metalloenzyme that catalyzes oxygen and hydrogen peroxide and thus form a crucial part of the cellular antioxidant defense mechanism that prevent body from disease linked to oxidative stress. Lipid peroxide concentration was determined as indicator for oxidative status of liver tissue.

Using *Origanum* extract as a protecting agent gave moderate results in both liver and renal tissue as mild cellular infiltration specially around blood vessels in liver tissue and some deformed glomeruli in renal tissue were still noticed, while using it as a therapeutic agent gave better results because liver tissue showed quite normal appearance except for only very mild cellular infiltrate around blood vessels and renal tissue appeared normal except for a few atrophied glomeruli.

*Origanum* effect on liver status may due to the high content of polyphenol in *Origanum* extract. It has a high content of total phenols and flavonoids also *Oregano* extracts (aqueous and methanol extracts) have very high polyphenol content while anthocyanins and catechins represent a smaller amount (Cervato *et al.*, 2000). Rosmarinic acid is a dominant component detected in Oregano aqueous tea also the Oregano aqueous extract contains eriocitrin, apigenin-7-O-glucoside, luteolin-7-O-glucoside, Caffeic acid, quercetin, luteolin and apigenin so the aqueous tea infusions of *Oregano* represented a good source of the compounds with significant antioxidant activity (Kulisic *et al.*, 2006)

In conclusion, the results indicate that the sufficient activity of *Origanum* extract in hepatic protection against administration of paracetamol in an over dose as liver injury drug. All results shows the role of *Origanum* extract in liver amelioration to be in a healthy status and it is more effective than sylimarin as hepatoprotective or therapeutic drug but it is superior as therapeutic than hepatoprotective also it is sufficient to decrease the oxidative stress on liver as mentioned in magnification of glutathione-antioxidant system and detoxification in liver with glutathione path way.

#### Correspondence to:

Abeer Y. Ibrahim

Medicinal and Aromatic Plants Dept., Pharmaceutical and Drug Industries Division, National Research Centre, 12622, Cairo, Egypt.

[abeeryousry@yahoo.com](mailto:abeeryousry@yahoo.com)

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**07/30/2010**

## Water quality status of Golden Key Lake in Clement Town, Dehradun, Uttarakhand

Avnish Chauhan\*, Mayank Pawar\* and Showkat Ahmad Lone

\* Department of Applied Sciences, College of Engineering, Teerthanker Mahaveer University, Moradabad-244001  
Department of Environmental Science, Uttarakhand College of Science and Technology, Dehradun-248001

**Abstract:** An attempt has been made to understand to provide information on the physico-chemical characteristics of Golden Key Lake which is being used for aquaculture, were studied between Nov 2008 to Feb 2009. All the parameters has been correlated with each other and each parameters has shown correlation matrix with different parameters at selected sites.

[Avnish Chauhan, Mayank Pawar and Showkat Ahmad Lone. Water quality status of Golden Key Lake in Clement Town, Dehradun, Uttarakhand. Journal of American Science 2010;6(11):459-464]. (ISSN: 1545-1003).

**Keywords:** TDS, TSS, pH, DO, COD, Ca, Mg, K, Golden Key Lake.

### Introduction:

Physico-chemical features of water and sediment play important role in structure and functioning of the lake ecosystem Okram, *et al.*, 2003). Water is one of the most abundant compounds found in nature covering approximately three-fourths of surface of the earth (Beebi *et al.*, 2004). Water is the elixir of life, a precious gift of nature of mankind and millions of other species living on the earth. It is fast becoming a scarce commodity in most part of the world (Usharani *et al.*, 2010). Water is an essential requirement of human and industrial development and also it is one of the most delicate parts of the environment (Das and Acharya, 2003). Attention on water contamination and its management has become a need of the hour because of for reaching impact on human health (Sinha and Srivastava, 1995). Continuous assessment of physical, chemical and biological parameters of water is an essential part of current water quality control programmes. These efforts lead to accumulation of considerable information which cannot usually produce direct judgmental determination of water quality (Sharifi, 1990). In the present study the physico-chemical status of Golden Key Lake, Clemet Town, Dehradun, Uttarakhand State of India was investigated.

### Material and Methods:

**Study Area:** Dehradun is capital of Uttarakhand, famous for its beauty, basmati rice, litchi and also a centre of various research institutes as well. It is bounded in the north by the higher range of lesser Himalaya and in the south by the younger Shivalik ranges. The river Yamuna and Ganga from the

valley's western and eastern boundaries in the NW and SE direction, respectively. Geographically the Doon valley lies between latitude 29° 55'N and 38° 30'N, longitude 77° 35'E and 78° 20'E covering an area of about 3088 sq. km, with a population of 12,82,143 (as per 2001 census). (Chauhan, 2008).

**Study Sites:** The study carried out between November 2008 to February 2009 to asses the physico-chemical characteristics of Golden Key Lake in Clement Town of Dehradun. The lake divided in to two compartment I<sup>st</sup> & II<sup>nd</sup>, the I<sup>st</sup> compartment has an area about 2000 m<sup>3</sup> while the II<sup>nd</sup> compartment has an area about 4500 m<sup>3</sup>. The lake is used for aquaculture purpose and various species like *catla*, *catla*, rohu, silver carp etc. are cultured here and various types of trees and plant grow on its bank. Four sites were selected for evaluation the physico-chemical properties of Golden Key Lake, site 1 lies in east direction of lake whereas site 2 is located in the centre of the lake while site 3 is located in the south-west direction of the lake and site 4 is located in north direction of lake.

### Result and discussion:

Physico-chemical parameters were studied during November 2008 to February 2010 for the samples collected from four selected sites of Golden Key Lake. Physico-chemical parameters for the selected sites are presented in Table-1-4, while correlation matrix values among these parameters are given in Table 5-8.

pH value observed between 6.5 to 7.10, 6.9 to 7.08, 7.01 to 7.43 and 7.54 to 8.0 at site 1, 2, 3 and 4, respectively. TDS value was reported between as 120.0 to 146.0, 308.0 to 400.00, 200.1 to 217.0 and 363.0 to 400.0 mg/L at site 1, 2, 3 and 4, respectively. DO was reported as between 20 to 2.9, 5.8 to 6.7, 5.9 to 6.9 and 5.9 to 6.1 mg/L at site 1, 2, 3 and 4, respectively, whereas the value of COD was reported as ranged between 200 to 290.0, 100.0 to 104.0, 112.0 to 115.0 and 109.0 to 110.8 mg/L at site 1, 2, 3 and 4, respectively. While in case of chloride was ranged between 54.6 to 65.1, 17.9 to 26.1, 9.9 to 19.1 and 5.9 to 12.7 mg/L at site 1, 2, 3 and 4,

respectively. Negi et al., have studied 13 physico-chemical parameters viz. water temperature, DO, Free CO<sub>2</sub>, alkalinity, TDS, total hardness, chloride, phosphates and nitrates of Hinval freshwater stream and Ganga River water during 2005-2007. Nagdali and Gupta also reported some important physico-chemical parameters and nutrients of Metropole of famous Nainital Lake of Uttarakhand, India. Okram et al., (2003) also studied the physico-chemical parameters of Waithou Lake in Manipur state of India on monthly basis. Spence (1967) reported that pH of the oligotrophic lakes ranges from 4.8 to 8.0 whereas in the eutrophic lake he range is 7.7 to 9.6.

Table: 1, showing monthly variations in physico-chemical characteristics of Golden Key Lake at site 1.

Parameters	Months			
	Nov. 2008	Dec. 2008	Jan. 2009	Feb. 2009
Temperature (°C)	14.20	16.30	10.20	20.10
Transparency (cm)	10.00	9.30	9.00	9.00
TDS (mg/L)	120.00	130.00	141.00	146.00
TSS (mg/L)	400.00	600.00	673.00	674.00
pH (mg/L)	7.01	7.10	6.50	6.70
Total Hardness (mg/L)	280.00	300.00	331.00	341.00
Total Alkalinity (mg/L)	560.00	590.00	594.00	600.00
Chloride (mg/L)	54.60	60.10	63.20	65.10
Calcium (mg/L)	179.00	184.00	160.00	164.00
Magnesium (mg/L)	24.54	28.18	34.20	35.70
Sodium (mg/L)	37.00	40.00	43.00	47.00
Potassium (mg/L)	10.00	12.00	14.00	14.00
Dissolved Oxygen (mg/L)	2.80	2.90	2.00	2.10
Chemical Oxygen Demand (mg/L)	200.00	250.00	263.00	290.00
Nitrate-Nitrogen (mg/L)	1.80	1.03	2.10	2.70
Phosphate-Phosphorus (mg/L)	1.01	1.03	1.00	1.20

Table: 2, Monthly variations in physico-chemical characteristics of Golden Key Lake at site 2.

<b>Parameters</b>	<b>Months</b>			
	Nov. 2008	Dec. 2008	Jan. 2009	Feb. 2009
Temperature (°C)	16.00	16.90	17.60	18.40
Transparency (cm)	65.00	61.00	58.10	57.90
TDS	400.00	390.00	309.00	308.00
TSS	300.00	342.00	300.00	310.00
pH	7.08	6.90	7.00	7.00
Total Hardness	214.00	234.00	241.00	253.00
Total Alkalinity	260.00	281.00	287.00	293.00
Chloride	17.90	21.90	26.10	25.20
Calcium	142.00	156.00	157.00	160.00
Magnesium	17.49	18.90	20.40	22.5
Sodium	17.00	19.00	23.00	25.00
Potassium	1.00	5.00	5.00	5.00
Dissolved Oxygen	6.70	6.10	5.8	5.90
Chemical Oxygen Demand	102.00	104.00	100.00	101.00
Nitrate-Nitrogen	0.06	0.07	0.09	0.08
Phosphate- Phosphorus (mg/L)	0.006	0.007	0.007	0.006

Table: 3, Monthly variations in physico-chemical characteristics of Golden Key Lake at site 3.

<b>Parameters</b>	<b>Months</b>			
	Nov. 2008	Dec. 2008	Jan. 2009	Feb. 2009
Temperature (°C)	15.00	17.00	18.00	18.70
Transparency (cm)	74.00	73.00	65.00	64.80
TDS	200.10	210.00	211.00	217.00
TSS	100.00	107.00	113.00	117.00
pH	7.43	7.01	7.02	7.03
Total Hardness	216.00	222.00	231.00	233.00
Total Alkalinity	260.00	287.00	289.00	290.00

Chloride	9.90	13.70	17.30	19.10
Calcium	143.00	121.00	127.00	132.00
Magnesium	25.02	24.50	25.20	24.50
Sodium	17.10	17.10	18.10	20.10
Potassium	1.00	3.00	4.00	4.00
Dissolved Oxygen	6.90	6.30	6.10	5.90
Chemical Oxygen Demand	115.00	114.00	112.00	112.00
Nitrate-Nitrogen	0.04	0.06	0.06	0.08
Phosphate- Phosphorus (mg/L)	0.002	0.002	0.005	0.007

Table: 4, Monthly variations in physico-chemical characteristics of Golden Key Lake at site 4.

Parameters	Months			
	Nov. 2008	Dec. 2008	Jan. 2009	Feb. 2009
Temperature (°C)	16.80	17.90	19.00	19.20
Transparency (cm)	85.00	83.00	76.20	75.40
TDS	400.00	380.00	363.00	367.00
TSS	10.00	14.00	21.00	23.00
pH	7.54	8.00	7.90	7.60
Total Hardness	216.00	240.00	215.00	219.00
Total Alkalinity	240.00	249.00	253.00	259.00
Chloride	5.90	11.80	12.10	12.70
Calcium	102.00	143.00	121.00	126.00
Magnesium	27.70	23.50	22.80	22.50
Sodium	15.00	16.00	16.00	20.00
Potassium	2.00	6.00	9.00	7.00
Dissolved Oxygen	6.10	5.90	6.00	6.00
Chemical Oxygen Demand	109.00	109.00	109.00	110.80
Nitrate-Nitrogen	0.02	0.05	0.07	0.09
Phosphate- Phosphorus (mg/L)	0.005	0.004	0.005	0.005

Table-5, Correlation matrix of physic-chemical parameters at site-1.

	Temp	Trans	TDS	TSS	pH	Hard	Alka	Chloride	Ca	Mg	Na	K	DO	COD
NO <sub>3</sub> -N <sub>2</sub>														
Trans	-0.115													
TDS	0.232	-0.945												
TSS	0.123		-0.999	0.935										
pH	0.328	0.673	-0.772	-0.649										
Hard	0.189	-0.929	0.997	0.917	-0.814									
Alk	0.267	-0.984	0.921	0.987	-0.543	0.894								
Chloride	0.255	-0.977	0.988	0.97	-0.695	0.976	0.970							
Ca	0.188	0.668	-0.809	-0.643	0.987	-0.849	-0.554	-0.723						
Mg	0.161	-0.930	0.996	0.918	-0.828	1.000	0.890	0.973	-0.860					
Na	0.421	-0.873	0.976	0.863	-0.681	0.970	0.876	0.956	-0.751	0.962				
K	0.076	-0.978	0.982	0.971	-0.800	0.979	0.938	0.983	-0.806	0.982	0.917			
DO	0.118	0.736	-0.866	-0.713	0.977	-0.900	-0.635	-0.791	0.994	-0.909	-0.813	-0.860		
COD	0.387	-0.955	0.967	0.954	-0.589	0.945	0.974	0.989	-0.631	0.939	0.959	0.944	-0.709	
NO <sub>3</sub> -N <sub>2</sub>	0.240	-0.377	0.656	0.350	-0.731	0.694	0.321	0.539	-0.828	0.691	0.724	0.548	-0.821	0.504
PO <sub>4</sub> -P	0.859	-0.443	0.634	0.438	-0.198	0.615	0.529	0.609	-0.340	0.593	0.787	0.481	-0.403	0.687
														0.668

Whereas, Temp = Temperature, Trans = Transparency, TDS = Total Dissolved Solids, TSS = Total Suspended Solids, Hard = Hardness, Alka = Alkalinity, Ca = Calcium, Mg = Magnesium, Na = Sodium, K = Potassium, DO = Dissolved Oxygen, COD = Chemical Oxygen Demand, NO<sub>3</sub>-N<sub>2</sub> = Nitrate-Nitrogen, PO<sub>4</sub>-P = Phosphate-Phosphorus.

Table-6, Correlation matrix of physic-chemical parameters at site-2.

	Temp	Transp	TDS	TSS	pH	Hardn	Alka	Chloride	Ca	Mg	Na	P	DO	COD	NO <sub>3</sub> -N <sub>2</sub>
Transpar	-0.948														
TDS	-0.905	0.908													
TSS	-0.031	-0.025	0.391												
pH	-0.290	0.423	0.003	-0.895											
Hardness	0.988	-0.960	-0.853	0.115	-0.434										
Alkalini	0.956	-0.980	-0.831	0.185	-0.534	0.987									
Chloride	0.909	-0.991	-0.925	-0.056	-0.368	0.915	0.945								
Calcium	0.905	-0.950	-0.741	0.328	-0.657	0.958	0.988	0.909							
Magnesi	0.993	-0.905	-0.899	-0.094	-0.199	0.966	0.915	0.861	0.852						
Sodium	0.983	-0.941	-0.966	-0.202	-0.149	0.950	0.915	0.923	0.843	0.981					
Potassiu	0.800	-0.904	-0.642	0.436	-0.769	0.877	0.940	0.874	0.977	0.725	0.730				
DO	-0.877	0.982	0.834	-0.137	0.544	-0.913	-0.963	-0.980	-0.957	-0.813	-0.861	-0.951			
COD	-0.492	0.494	0.801	0.853	-0.543	-0.376	-0.336	-0.568	-0.201	-0.517	-0.641	-0.098	0.399		
NO <sub>3</sub> -N <sub>2</sub>	0.797	-0.926	-0.915	-0.208	-0.245	0.790	0.836	0.969	0.789	0.742	0.849	0.775	-0.929	-0.680	
PO <sub>4</sub> -P	0.028	-0.331	-0.052	0.464	-0.705	0.141	0.302	0.380	0.396	-0.093	0.000	0.577	-0.501	0.169	0.447

Whereas, Temp = Temperature, Trans = Transparency, TDS = Total Dissolved Solids, TSS = Total Suspended Solids, Hard = Hardness, Alka = Alkalinity, Ca = Calcium, Mg = Magnesium, Na = Sodium, K = Potassium, DO = Dissolved Oxygen, COD = Chemical Oxygen Demand, NO<sub>3</sub>-N<sub>2</sub> = Nitrate-Nitrogen, PO<sub>4</sub>-P = Phosphate-Phosphorus.

Table-7, Correlation matrix of physico-chemical parameters at site-3.

	Temp	Trans	TDS	TSS	pH	Hard	Alkal	Chlor	Calcium	Magnesi	Sodium	Potassium	DO	COD
NO <sub>3</sub> -N <sub>2</sub>														
Transpar	-0.885													
TDS	0.977	-0.788												
TSS	0.990	-0.928	0.961											
pH	-0.883	0.615	-0.880	-0.810										
Hardness	0.972	-0.969	0.912	0.989	-0.775									
Alkalini	0.935	-0.703	0.926	0.877	-0.992	0.846								
Chloride	0.990	-0.938	0.952	0.999	-0.812	0.994	0.879							
Calcium	-0.581	0.230	-0.594	-0.462	0.894	-0.421	-0.831	-0.468						
Magnesi	-0.319	-0.083	-0.512	-0.273	0.398	-0.127	-0.382	-0.237	0.372					
Sodium	0.791	-0.823	0.804	0.859	-0.437	0.831	0.541	0.845	0.000	-0.341				
Potassiu	0.981	-0.861	0.936	0.954	-0.931	0.950	0.967	0.960	-0.683	-0.223	0.667			
DO	-0.997	0.849	-0.985	-0.979	0.910	-0.953	-0.955	-0.977	0.629	0.369	-0.764	-0.982		
COD	-0.949	0.981	-0.867	-0.967	0.755	-0.994	-0.826	-0.976	-0.482	0.022	-0.786	-0.943	0.926	
NO <sub>3</sub> -N <sub>2</sub>	0.939	-0.754	0.985	0.937	-0.796	0.874	0.851	0.922	0.874	-0.590	0.866	0.866	-0.945	-0.816
PO <sub>4</sub> -P	0.854	-0.945	0.812	0.918	-0.511	0.926	0.615	0.915	0.945	-0.129	0.962	0.770	-0.819	-0.907
	0.833													

Whereas, Temp = Temperature, Trans = Transparency, TDS = Total Dissolved Solids, TSS = Total Suspended Solids, Hard = Hardness, Alka = Alkalinity, Ca = Calcium, Mg = Magnesium, Na = Sodium, K = Potassium, DO = Dissolved Oxygen, COD = Chemical Oxygen Demand, NO<sub>3</sub>-N<sub>2</sub> = Nitrate-Nitrogen, PO<sub>4</sub>-P = Phosphate-Phosphorus.

Table-8, Correlation matrix of physico-chemical parameters at site-4.

	Temp	Trans	TDS	TSS	pH	Hardness	Alkalinity	Chloride	Calcium	Mg	Na	K	DO	COD	NO <sub>3</sub> <sup>-</sup>
N <sub>2</sub>															
Trans	-0.970														
TDS	-0.981	0.928													
TSS	0.988	-0.992	-0.945												
pH	0.252	-0.054	-0.419	0.103											
Hardness	-0.144	0.379	0.073	-0.271	0.647										
Alkalinity	0.882	-0.740	-0.918	0.802	0.618	0.325									
Chloride	0.902	-0.769	-0.924	0.831	0.560	0.294	0.997								
Calcium	0.442	-0.210	-0.504	0.316	0.762	0.823	0.804	0.785							
Magnesium	-0.932	0.816	0.952	-0.869	-0.529	-0.217	-0.992	-0.997	-0.733						
Sodium	0.722	-0.721	-0.582	0.770	-0.281	-0.096	0.558	0.617	0.294	-0.626					
Potassium	0.919	-0.847	-0.978	0.860	0.575	0.000	0.915	0.907	0.544	-0.931	0.409				
DO	-0.405	0.170	0.490	-0.270	-0.837	-0.831	-0.788	-0.759	-0.992	0.709	-0.184	-0.555			
COD	0.586	-0.623	-0.420	0.661	-0.475	-0.198	0.369	0.436	0.119	-0.448	0.977	0.226	0.000		
NO <sub>3</sub> -N <sub>2</sub>	0.979	-0.944	-0.928	0.977	0.149	-0.090	0.864	0.895	0.470	-0.917	0.843	0.834	-0.410	0.726	
PO <sub>4</sub> -P	0.195	-0.429	-0.100	0.330	-0.713	-0.990	-0.287	-0.247	-0.790	0.172	0.225	0.000	0.816	0.333	0.167

Whereas, Temp = Temperature, Trans = Transparency, TDS = Total Dissolved Solids, TSS = Total Suspended Solids, Hard = Hardness, Alka = Alkalinity, Ca = Calcium, Mg = Magnesium, Na = Sodium, K = Potassium, DO = Dissolved Oxygen, COD = Chemical Oxygen Demand, NO<sub>3</sub>-N<sub>2</sub> = Nitrate-Nitrogen, PO<sub>4</sub>-P = Phosphate-Phosphorus.

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# Influence of freeze-shocked mesophilic lactic starter bacteria and adjunct lactobacilli on the rate of ripening Gouda cheese and flavor development

El-Sayed El-Tanboly, Mahmoud El-Hofī, Y. B. Youssef,\*Wahed El-Desoki, and \*\*Reda A. Jalil

Dairy Science Department, National Research Center, Dokki, Cairo, Egypt.

\*Dairy Science Department, Al-Azhar Univ., Agriculture Faculty, Assuet Branch, \*\*Chamber of Food Industries, 1195 Cornish El-Nil, Beaulac, Cairo, Egypt.

[tanboly1951@yahoo.com](mailto:tanboly1951@yahoo.com)

**Abstract:** The objective of the present study was to determine the effects of *Lactobacillus acidophilus* on the sensory attributes, ripening time, and composition of Gouda cheese and to investigate the survival of *L. acidophilus* during ripening. Five types of Gouda cheeses, control cheese (Tc), made with mesophilic lactic starter bacteria, Ta1, Ta2, Tb1 and Tb2 cheeses made using modified mesophilic lactic starter bacteria by freeze-shocked at -10°C/-20°C for 24, 96 hrs and probiotic Lactobacillus, as adjunct culture. Cheese samples were assessed for microbiological and compositional properties, proteolysis, and sensory evaluation at different ripening stages. The composition and the pH value were almost identical between control and experimental vats within a single trial cheese. Characterization of proteolysis of gel electrophoresis of cheese samples at various stages of ripening showed that the extent of casein degradation varied between samples in all cheeses,  $\alpha_{S1}$ -Casein was more extensively degraded than  $\beta$ -casein. However, levels of soluble nitrogen (SN/TN) increased with ripening period for all cheeses, only moderate enhancement of proteolysis as in amino acid -N in all trials. The formation of non protein nitrogen (NPN/TN) was slightly increased compared to control at the end of ripening. Organoleptic evaluation showed that probiotic cheese had higher sensory evaluation than control cheese, without probiotic strain. The population of Lactobacillus survived to numbers  $> 10^7$  cfu/g, which is necessary for positive effects on health. These results showed that the contribution of modified mesophilic lactic starter bacteria by freeze-shocked and probiotic strain as adjunct culture can be successfully used in production of Gouda cheese.

[El-Sayed El-Tanboly, Mahmoud El-Hofī, Y. B. Youssef, Wahed El-Desoki, and Reda A. Jalil. Influence of freeze-shocked mesophilic lactic starter bacteria and adjunct lactobacilli on the rate of ripening Gouda cheese and flavor development. Journal of American Science 2010;6(11):465-471]. (ISSN: 1545-1003).

Keywords: Physically freeze-shock mesophilic starter, probiotic bacteria, proteolysis Gouda cheese.

## 1. Introduction

Cheese is an excellent dietary source of high-quality protein, vitamins and minerals such as absorbable dietary calcium. Gouda Dutch type semi hard cheese is a traditional, creamery, hard cheese. It is round with very smooth, yellow, waxed rind. The flavor is sweet and fruity. As time passes, the taste intensifies and becomes more complex. Mature Gouda (18 months plus) is coated in black wax which provides a stark contrast to the deep yellow interior. Gouda is considered to be one of the world's great cheeses. A great deal of research in Cheddar cheese technology is devoted towards the addition of adjunct cultures which may accelerate ripening times or to improve flavor (Wilkinson, 1993). The use of attenuated starters was first proposed by Petterson and Sjöström (1975) to accelerate the ripening of Svecia, a Swedish semi-hard cheese; these authors attenuated

cells by heat treatment. Besides heat treatment, other methods to achieve attenuation have been studied including freezing and thawing, freeze or spray drying, lysozyme treatment, use of solvents, and natural and induced genetic modification. Freezing cells at sub-optimal conditions reduces the viability of lactic acid bacteria. Stressed cells do not contribute significantly to acid production during cheese making, but may retain protease and peptidase activity (El-Tanboly 1991). Frozen cells may lyses to a greater extent than non-frozen cells, and thus release intercellular enzymes (Barteis *et al.*, 1987). Dairy foods, including in particular, fermented milks and yogurt are among the best accepted food carriers for probiotic cultures. The aim of this study was to develop new probiotic foods, particularly, the production of high quality Gouda cheese containing high levels of probiotic bacteria. The dairy products

with probiotic bacteria recognition as functional foods that provide health benefits beyond basic nutrition and the emerging clinical evidence to their potential in preventing some diseases have notably enlarged their consumption and stimulated innovation and new product development (Boylston *et al.*, 2004; Ong *et al.*, 2007). Although yogurt and fermented milks have received the most attention as carriers of probiotic bacteria, some cheese varieties such as Gouda, white and Cheddar cheeses (Gomes *et al.*, 1995; Kasmoglu *et al.*, 2004; Ong *et al.*, 2007). Cheeses have a number of advantages over fermented milks as a delivery system for viable probiotic microorganisms, because they generally have higher pH and buffering capacity, more solid consistency, and relatively higher fat content (Ong *et al.*, 2007; Joutsjoki, 2009). These features give protection to probiotic bacteria during storage and passage through the gastrointestinal tract. To exert positive health effects, the microorganisms need to be viable, active, and sufficiently abundant, in concentrations of at least  $10^6$  cfu/g throughout the shelf life (Vinderola *et al.*, 2000; Narvhus, 2009). The aims of this study was to influence of physically modified mesophilic lactic starter bacteria by freeze-shocked and probiotic strain of Lactobacillus, as adjunct on the rate of ripening Gouda cheese and flavor development.

## 2. Materials and Methods

### Mesophilic lactic starter bacteria and adjunct lactobacilli conditions

The mixed strains of mesophilic lactic starter bacteria 022 and adjunct lactobacilli used for experiments were obtained from the Production Laboratory of Dairy Biopreparation in Olsztyn, Poland. Bacteria were inoculated at 2% (v/v) into sterile 10%(w/v) reconstituted non-fat milk (RNFM). It was subcultured at least twice for 18 hrs at 23°C before treatment. Overnight adjunct lactobacilli (37°C for 16 h) were obtained from (MRS) broth. Cells were harvested by centrifugation at 8,000 x g for 20 min at 4°C. The resultant pellet was washed twice with saline solution (0.9% NaCl in distilled water) and resuspended in 10% sterile skim.

### Mesophilic lactic starter bacteria modification

Biomass cells of mixed mesophilic lactic starter bacteria 022 were physically modified by freeze-shocked at -10, -20°C for 24 and 96 hrs. thawed the following experimental moring at 40°C and added just prior to renneting.

### Gouda cheese manufacturing

Cheeses were manufactured according to the standard procedure Fox *et al.*, (2004) from three trials, T<sub>c</sub> (control) of milk with modified mesophilic lactic starter bacteria, T<sub>a1</sub> (-10°C/24 hr), T<sub>a2</sub> (-20°C/24 hr), T<sub>b1</sub> (-10°C/96 hr) and T<sub>b2</sub> (-20°C/96 hr) made using

modified mesophilic lactic starter bacteria and probiotic Lactobacillus, as adjunct culture.

### Microbiological analysis

Samples cheeses were tested for counts of mesophilic lactic starter bacteria, *L. acidophilus* and coliform bacteria using standard methods (Vanderzant & Splittoesser, 1992). Plate count agar was used for enumeration of mesophilic lactic starter bacteria. Plates were incubated aerobically at 30°C for 48 h. *L. acidophilus* was counted on acidified (pH 5.4) MRS agar and incubated anaerobically at 37°C for 3 days. For the count of coliform bacteria, violet red bile agar was used and incubated aerobically at 37°C for 48 h.

### Chemical analysis of Gouda cheese

pH was measured by pH-meter 646 with glass electrodes, Ingold, Knick, Germany. Titratable acidity (°SH) was done with Soxhlet Hankel method as described by (IDF,1993). Moisture content and cheese fat content was determined according to (IDF, 1986). Secondary proteolysis was measured by nitrogen fraction in cheese. Total nitrogen content (TN) was determined according to method of Kjeidahl, soluble nitrogen at pH 4.6 (SN), Non protein nitrogen (NPN), Peptide-N and Amino acid-N (AAN) was estimated according to as described by (IDF, 1993).

### Organoleptic assessment of Gouda cheese

The cheese were evaluated organoleptically by a team of experienced cheese graders. The cheese samples were characterized by appearance of body, texture and flavor during ripening period. Cheese samples were analyzed chemically, when fresh and after 3 and 6 weeks.

## 3. Results and Discussion

### Gross chemical composition of Gouda cheese

The composition of Semi hard cheese was almost identical for control and experimental vats within modified mesophilic lactic starter bacteria and probiotic Lactobacillus, as adjunct culture. The composition was similar between trials with a moisture content ranging 40-41 % , fat 28-30 %, salt in moisture 6.9-8.7 %, protein 24.6-29.4 % and PH 5.4-5.8 at 6 weeks of ripening. However, the production schedules were not altered because of the added modified mesophilic starter and probiotic Lactobacillus, as adjunct culture as illustrated in Table (1). Similar results were described by Degheidi *et al.*, (2007).

### Microbiological analysis

Initial numbers of *L. acidophilus* inoculated into the milk were  $10^5$ - $10^6$  cfu ml<sup>-1</sup>, but they grew rapidly during the one week of ripening and reached to  $10^7$ - $10^8$  cfu g<sup>-1</sup> in trials cheeses, respectively. Rapid growth of *L. acidophilus* might be due to the fermentation of lactose by modified mesophilic starter bacteria. It is well known that lactobacilli grow best

under acidic conditions (Mäkeläinen, *et al.*, 2009). The viable cell numbers of *L. acidophilus* began to decrease after two weeks of ripening, because of the decrease in moisture level, increase in salt content, and the low ripening temperature. Although *L. acidophilus* decreased until the end of the ripening period, it did not decrease below  $10^7$  and  $10^6$  cfu g<sup>-1</sup> in trials cheeses, respectively. As indicated earlier, it is necessary to maintain the viability of *L. acidophilus* at  $\geq 10^7$  cfu g<sup>-1</sup> of cheese, to call the cheese probiotic (Jatila *et al.*, 2009). There were no differences between the trials cheeses for the number of modified mesophilic starter bacteria count during the ripening period. Also, survival and growth of modified mesophilic starter bacteria was similar to that of the *L. acidophilus* at different stages of ripening for trials and Tc cheeses. Similar results were described by Degheidi *et al.*, (2007). Modified mesophilic starter bacteria showed a decline after the one week of ripening. This reduction might be due to the low growth ability of modified mesophilic starter bacteria under acidic conditions (Mundt, 1986). Coliform bacteria were not detected in any of the samples in the present study.

#### **Proteolysis of Semi hard cheese during ripening**

##### **(A) primary proteolysis**

Characterization of proteolysis of gel electrophoresis of cheese samples at various stages of ripening are shown in Fig.1. Polyacryamide gel electrophoresis (PAGE), as well as stacking gel electrophoresis (SGE), showed cheese made with modified mesophilic starter and probiotic *Lactobacillus*, as adjunct culture did not show any distinct proteolysis of  $\alpha_{S1}$ -casein but slight proteolysis was evident as a very faint band in trials Tb1 and Tb2 after just salting of ripening. There was no evidence of proteolysis of  $\beta$ -casein in 0-day old cheese for any trials. After 3-weeks of ripening, Ta1, Ta2, Tb1, Tb2 and Tc had a distinct proteolysis of  $\alpha_{S1-1}$  peptide.

A  $\beta$ -1 peptide appeared as very faint band in all trials.  $\gamma$ -casein were present in all cheeses after 6-weeks ripening. Major differences were observed in amount intact  $\alpha_{S1}$ - and  $\beta$ -casein. Ta1 and Ta2 showed extensive degradation of  $\alpha_{S1-1}$  peptide and increased intensities of  $\gamma_2$ - Casein and  $\beta$ -1 peptide bands. Trials and had smaller amount of  $\alpha_{S1}$ - and  $\beta$ -Casein present as shown in Fig. (1). These results are in agreement with those of Jensen and Ardö (2009). The forgoing results of Polyacryamide gel electrophoresis (PAGE) of cheese samples treated with modified mesophilic bacteria and probiotic culture at different stages of ripening indicate that the proteolysis of both  $\alpha_{S1}$ -casein and  $\beta$ -casein increased during ripening,  $\beta$ -casein was more resistant to hydrolysis than  $\alpha_{S1}$ -

casein which rapidly degraded during ripening, there are also increasing amount of some low-mobility peptides were detected in the  $\gamma$ -casein regions of all cheese samples.

##### **(B) Secondary proteolysis**

Addition of modified mesophilic starter bacteria and probiotic *Lactobacillus*, as adjunct culture, increase soluble-N levels over those in the control in several trials. The rate of proteolysis in Ta2 and Tb1 was higher in the first stage of ripening than when ripening had progressed. On contrary, the rate of proteolysis in Tc , Tb1 and Tb2 was lower in the first stage of ripening (Fig. 2). The data indicated that the Non protein-N values generally increased slightly for Tb1 and Tb2 compared to the control (Tc) at the end of ripening period (Fig. 2). On the both previous cheeses trials contained approximately 6.85 to 8.04% of the Total-N contents at the end of ripening time. Furthermore, it was observed also that the levels of Non protein-N were 32.21 to 38.67% of soluble-N. The accumulation of Peptide-N was increased slightly in modified mesophilic starter and probiotic *Lactobacillus*, as adjunct culture trials than control. Tb2 was greatest being 0.446 and 7.29% (relative to Total-N and soluble-N at the beginning of ripening of ripening time, increased to 2.495 and 14.075% after 6 weeks ripening, respectively.

It was found to increase rapidly in the course of ripening mainly due to the breakdown of protein and peptide. Enhancement of proteolysis was observed only in Tb1 and Tb2 approximately 4.664 and 26.315% increased to 6.601 and 33.920% at the end of ripening period (Fig. 2). Only slight enhancement of proteolysis in T1 and Ta2.

A comparison between the results and those by other investigators would reveal similar influences , Gagnaire *et al.*, (2009) who reported that a heat treated culture of *Lb. helveticus* could be used to increase proteolysis and enhancement of cheese flavour without introducing bitter taste in Swedish hard cheese . This might be due to the results of cell lyses and release of intracellular proteinase of modified starter into surrounding cheese matrix, high level and specificities (Gagnaire *et al.*, 2009).

In view of the foregoing available evidence, it could be concluded that a combination of rennet, regular and modified mesophilic starter bacteria and probiotic *Lactobacillus* was successful in accelerating maturation of Gouda cheese. They were mainly responsible for accelerating casein breakdown and contribute to hydrolysis of medium sized peptides to amino acids nitrogen . it is also clear that maturation time for semi hard cheese can be halved by using modified starter and can improve flavour intensity and reduce bitterness (El-Tanboly *et al.*, 2010).

Table (1) The changes in chemical composition during ripening of Gouda cheese made from modified mesophilic lactic bacteria and probiotic culture during ripening

*Trials	Ripening period (weeks)	Composition (%)				**FDM (%)	***S/M (%)
		fat	protein	Moisture	salt		
TC	0	27.5	22.83	42.70	2.10	47.99	4.92
	3	27.5	24.09	39.04	2.39	45.11	6.12
	6	31.5	25.56	37.36	3.79	50.29	10.14
Ta1	0	26.5	19.89	44.53	1.95	47.77	4.38
	3	28.5	22.96	44.15	2.95	51.03	6.68
	6	30.8	24.58	40.11	3.24	51.43	8.08
Ta2	0	27.0	20.24	45.95	2.25	49.59	4.90
	3	28.3	23.91	44.31	2.42	50.82	5.46
	6	28.3	25.07	41.25	3.59	48.17	8.70
Tb1	0	26.0	24.31	41.46	2.36	44.41	5.69
	3	27.5	28.42	40.05	2.95	45.87	7.29
	6	28.0	29.41	39.86	3.39	46.56	8.50
Tb2	0	27.5	19.84	41.37	2.16	46.90	2.22
	3	27.5	27.44	40.80	2.48	46.45	6.08
	6	30.0	27.93	40.04	2.66	50.01	6.92

Ta1: -10°C/24 hr, Ta2: -20°C/24 hr, Tb1: -10°C/96 hr and Tb2: -20°C/96 hr \*\*FDM (%): Fat dry matter

\*\*\*S/M (%): Salt in moisture

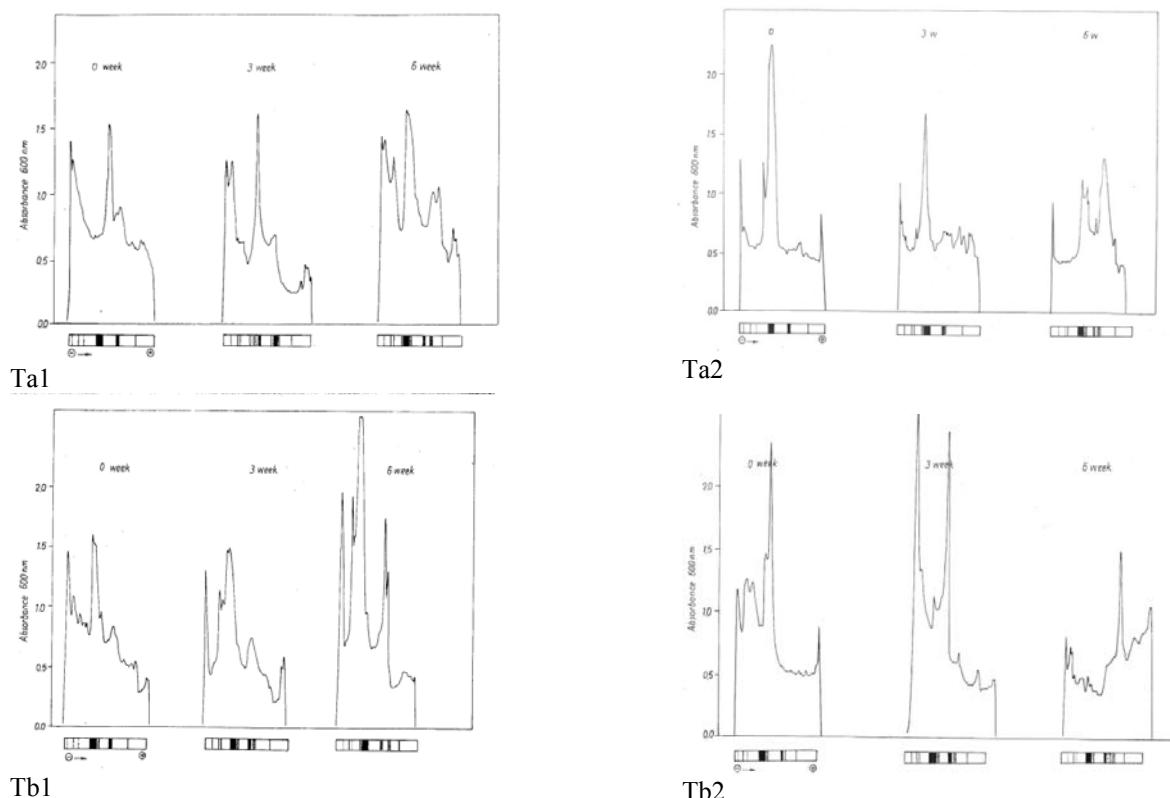


Fig. 1. Densitometric scans of PAGE during semi hard cheese ripening made with modified mesophilic bacteria and probiotic culture (Tc, Ta1, Ta2, Tb1 and Tb2).

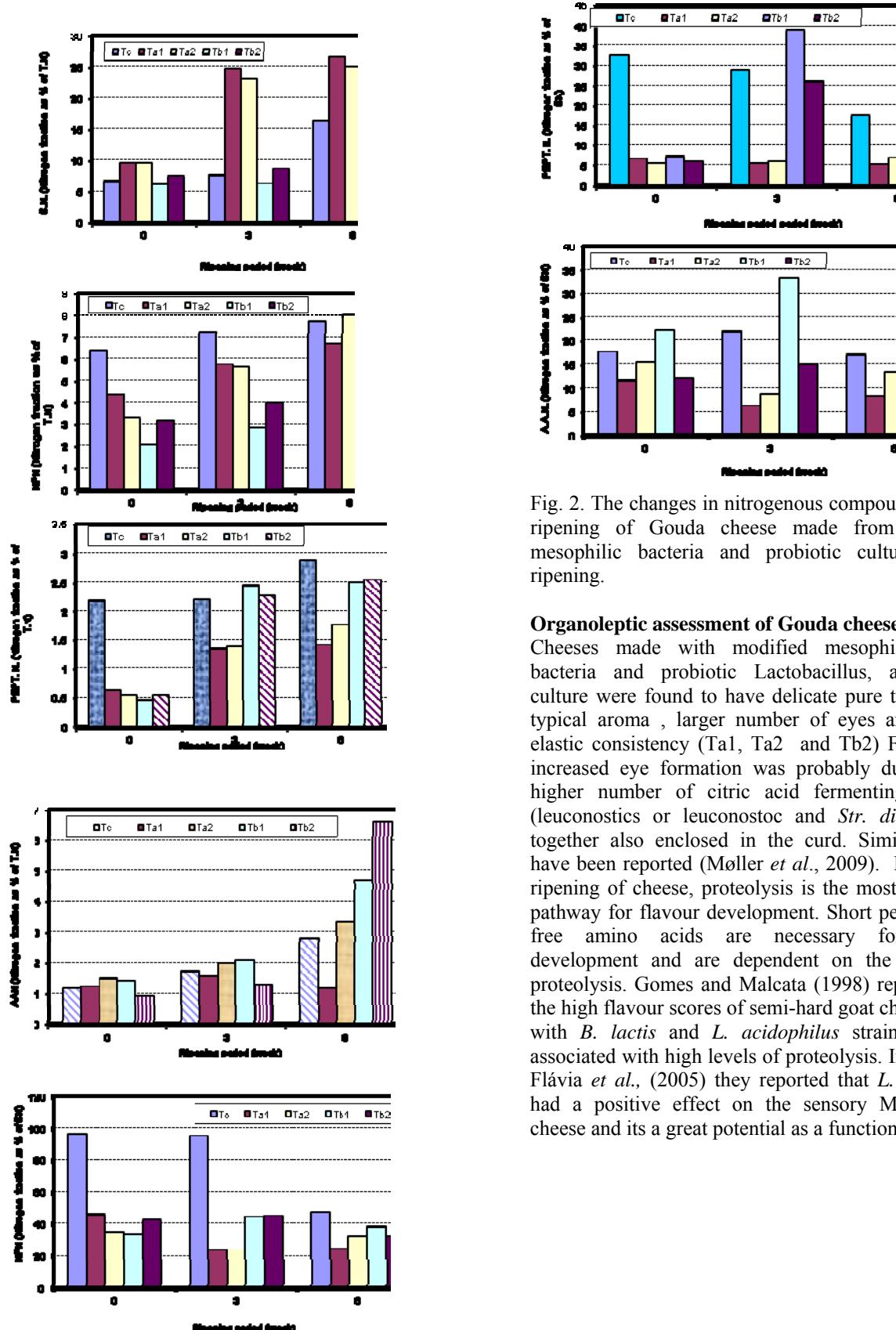


Fig. 2. The changes in nitrogenous compounds during ripening of Gouda cheese made from modified mesophilic bacteria and probiotic culture during ripening.

#### Organoleptic assessment of Gouda cheese

Cheeses made with modified mesophilic starter bacteria and probiotic *Lactobacillus*, as adjunct culture were found to have delicate pure taste, clean typical aroma, larger number of eyes and normal elastic consistency (Ta1, Ta2 and Tb2) Fig. 3. The increased eye formation was probably due to the higher number of citric acid fermenting bacteria (leuconostics or leuconostoc and *Str. diacetilylactis* together also enclosed in the curd. Similar results have been reported (Møller *et al.*, 2009). During the ripening of cheese, proteolysis is the most important pathway for flavour development. Short peptides and free amino acids are necessary for flavour development and are dependent on the extent of proteolysis. Gomes and Malcata (1998) reported that the high flavour scores of semi-hard goat cheese made with *B. lactis* and *L. acidophilus* strain Ki were associated with high levels of proteolysis. In addition, Flávia *et al.*, (2005) they reported that *L. paracasei* had a positive effect on the sensory Minas fresh cheese and its a great potential as a functional food.

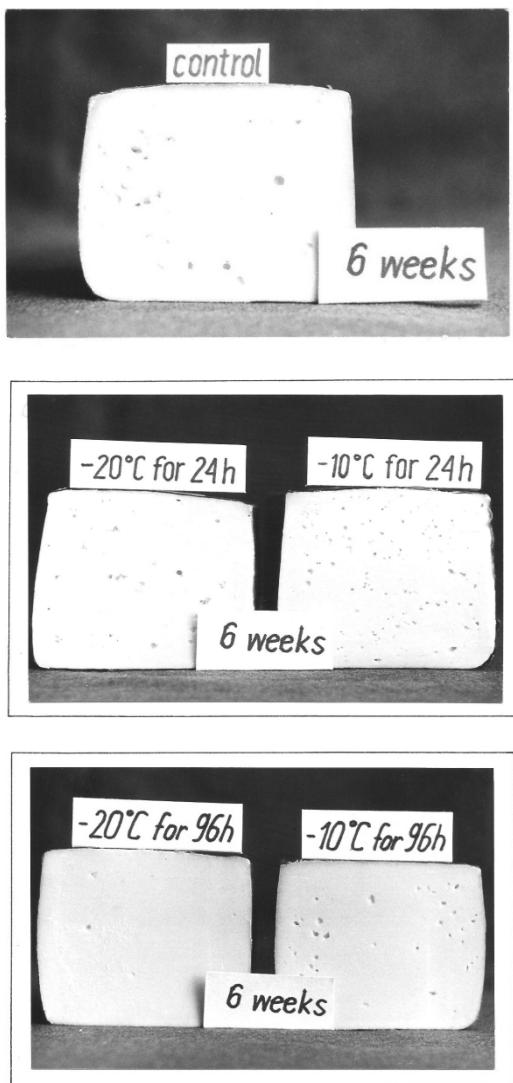


Fig. 3. Texture of 6 weeks old Gouda cheese made with modified mesophilic bacteria and probiotic culture.

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9/22/2010

# Gap Analysis for Protected Areas of Andhra Pradesh, India for conserving biodiversity

C. Sudhakar Reddy

Forestry and Ecology Division, National Remote Sensing Centre, Indian Space Research Organisation, Balanagar,  
Hyderabad -500 625, India  
[drsudhakarreddy@gmail.com](mailto:drsudhakarreddy@gmail.com)

**Abstract:** A gap analysis was carried out to assess the Protected Area (PA) network system in Andhra Pradesh, India. The decisive factors of vegetation type distribution, elevation and endemism was used to determine the representativeness of PA system. In Andhra Pradesh, vegetation cover occupies 23.03% of geographical area and distributed in Coastal Plains, Deccan Plateau and Eastern Ghats. There are 27 PAs for conservation in Andhra Pradesh. The total area protected for biodiversity is about 12,555 km<sup>2</sup> or 4.56% of geographical area of Andhra Pradesh. Of the three physiographic regions, Eastern Ghats represents very high area under PAs which was estimated as 7811.38 km<sup>2</sup> followed by Deccan plateau of 3526.89 km<sup>2</sup>. Three main forest types (semi evergreen forests, thorn forests and dry evergreen forests) missing in the existing PA network were identified. Moist deciduous forests of Eastern Ghats of northern Andhra Pradesh were under-represented in PAs. The land area in an elevation range of 900m-1527m was not included in PA network. Of the 103 species of endemics, 64 species were not included in PA system. Many PAs are experiencing threat from invasive species, forest fires, grazing pressure etc. There is a need to consider for possible ways for effective conservation and to extend the present PA network system in India.

[Journal of American Science 2010;6(11):472-484]. (ISSN: 1545-1003).

**Keywords:** gap area; vegetation; protected area; semi evergreen forests; Andhra Pradesh; India

## 1. Introduction

International treaties call for the conservation of biodiversity in all its manifestations including ecosystem level diversity. Conservation biology is concerned with developing the scientific support for conservation policy and management decisions. Conservation efforts have focused on maintaining biodiversity through establishment of networks of protected areas. Protected areas (PAs) are the priority centers of biodiversity and wildlife conservation. The criteria of biodiversity inventory, vegetation type distribution, topographic variability, climatic gradient and biotic pressure are basic stuff in conservation planning. These surrogates can be effective at representing other aspects of biodiversity, such as ecological uniqueness, species distributions, species diversity and contiguous intact natural habitats (Kirkpatrick and Brown, 1994, Wessels *et al.* 1999). Conservation planning exercises also call for data on the distribution of PAs. But many PA systems across the world were chosen based on socio-economic and aesthetic criteria (Oldfield *et al.* 2004). Gap analysis is a comprehensive approach for assessing conservation needs. The gap analysis has been undertaken by few researchers (Hunter and Yonzon, 1993; Fearnside and Ferraz, 1995; Powell *et al.* 2000; Scott *et al.* 2001; Bon and Gaston, 2005)

India is one of the mega-diversity nations in the world. India has 590 PAs (ca. 500 wildlife sanctuaries and 90 national parks. PAs of India cover 156,700 km<sup>2</sup>, roughly 4.95% of the total geographical area (<http://en.wikipedia.org.2010>). There is a pressing requirement to identify gap areas of high biological richness to declare new Protected Areas in India. Spatially explicit inventories of vegetation types and land cover permit comparisons between particular vegetation distributions and distributions of land cover, land-cover change, expected changes and issues of protected areas. With the current trend of increasing rate of deforestation and loss of habitats in densely populated developing countries, there is a urgent need to generate database which is available for planning, decision making and further objective oriented requirements. Satellite remote sensing along with Geographic Information System (GIS) provides a cost and time effective solution to collect, process and integrate database in an effective manner. In the present study, an attempt was made to identify 'gap areas' in PA network of Andhra Pradesh, India which covers a major part of Deccan Plateau, Eastern Ghats and East Coast regions by integrating spatial and non spatial databases.

## 2. Material and Methods

The study was conducted in Andhra Pradesh, is the largest State of southern India. The State of Andhra Pradesh (The land of Telugu people) is situated in the middle of eastern half of the Indian Peninsula lying between  $12^{\circ} 41' - 19^{\circ} 54' N$  latitudes and  $76^{\circ} 46' - 84^{\circ} 45' E$  longitudes. It is bounded by the Bay of Bengal in the east, Tamil Nadu in the south, Karnataka in the west, and Maharashtra, Chhattisgarh and Orissa in the north. Administratively, Andhra Pradesh has 23 districts which were grouped into three zones: (1) *Circars or Coastal Andhra* with nine districts, i.e. East Godavari, Guntur, Krishna, Nellore, Prakasam, Srikakulam, Vizianagaram, Visakhapatnam and West Godavari (2) *Rayalaseema* with four ceded districts, i.e. Anantapur, Chittoor, Cuddapah and Kurnool (3) *Telangana* (Deccan or erstwhile Nizam's Dominions of Hyderabad State) with 10 districts, i.e. Adilabad, Hyderabad, Karimnagar, Khammam, Mahabubnagar, Medak, Nalgonda, Nizamabad, Rangareddy and Warangal.

Geographically, the State is categorized into three regions, namely: (1) the *Coastal Plains* (along the east coast, a low-lying area from Srikakulam to Nellore) mainly of agricultural land and mangroves (2) the *Eastern Ghats*, forming a chain of discontinuous range of hills along the coast with good vegetation, and (3) the *Deccan Plateau* consisting of agricultural lands, scrub and deciduous forests, which cover part of Kurnool (excl. Nallamalais), Anantapur districts (excl. Nigidi hills) and the major part of Telangana. The total forest cover of the State is  $44,419 \text{ km}^2$ , which occupies 16% of the total geographical area of  $275,068 \text{ km}^2$  (FSI, 2003). The highest peak in Andhra Pradesh is Sambarikonda (1527 m), found in RV nagar range of Visakhapatnam district.

Preparation of vegetation type and land use map was accomplished through visual interpretation of multi-season IRS P6 LISS III images as part of project on "biodiversity characterization at landscape level" (Reddy et al. 2008). The protected area information was accessed from the Protected Areas Database (Anonymous, 2007). In the present study vegetation type information, elevation data (<http://www.landcover.org/data/srtm/>) and endemism was used to determine 'gap areas' for Protected Areas network (Reddy et al. 2006).

### 3. Results

In Andhra Pradesh, vegetation cover occupies 23.03% of total geographical area. While forest cover of the State is estimated as  $44,334 \text{ km}^2$ . The area under forest cover is proportionately 16.12% of total geographical area (Table 1). The forest types found in Andhra Pradesh are Semi

Evergreen, Moist Deciduous, Dry Deciduous, Dry Evergreen, Thorn, Teak mixed, Bamboo mixed, mangrove, riverine forest, woodland, savannah and forest plantation (Table 2). Most abundant forest type was Dry Deciduous forest (Fig. 1) which comprises 73.36% of the total forest area, followed by Moist Deciduous forest of 10.98%. Scrub/shrub land occupies significant area, which is about 6.54% of total geographical area of State. There are about 18,443 wetlands, accounts for  $11,572.50 \text{ km}^2$  (4.21%) of total area of State.

Table 1. Areal extent of Vegetation and Land Use of Andhra Pradesh, India

Sl.no.	Class	Area (km <sup>2</sup> )	% of Area
1	Forest	44334	16.12
2	Scrub	17982.9	6.54
3	Grassland	1032.7	0.38
	Subtotal	63349.5	23.03
4	Wetland	11572.5	4.21
	Other Land		
5	Use	200146	72.76
	Subtotal	211718.5	76.97
	Grand total	275068	100

Table 2. Areal extent of forest types of Andhra Pradesh, India

Class	Area (km <sup>2</sup> )	% of Area
Semi Evergreen forest	1585.4	3.58
Moist Deciduous forest	4865.85	10.98
Dry Deciduous forest	32524.03	73.36
Thorn forest	66.42	0.15
Dry Evergreen forest	221.55	0.5
Teak mixed forest	461.14	1.04
Bamboo mixed forest	654.02	1.48
Mangrove	329	0.74
Riverine forest	1209.09	2.73
Forest Plantation	1156.99	2.61
Woodland	397.59	0.9
Savannah	862.92	1.95
Grand total	44334	100

#### 3.1 Total coverage of PAs

There are 27 PAs declared for conservation in Andhra Pradesh, comprising 22 wildlife sanctuaries and 5 national parks (Table 3; Fig.2). The areas of the individual PAs range from 1.42 to  $3,568 \text{ km}^2$ . Taking into account the wildlife sanctuaries and

national parks, the total area protected for biodiversity is about 12,555 km<sup>2</sup> or 4.56% of geographical area of Andhra Pradesh. Of the 63,349.5 km<sup>2</sup> of natural vegetation in State, 11,773 km<sup>2</sup> (18.6%) of area was under PA network. Of the three physiographic regions, Eastern Ghats represents very high area under PAs which was estimated as 7811.38 km<sup>2</sup> (62.2% of total PA) followed by Deccan plateau of 3526.89 km<sup>2</sup> (28%). Altogether an area of 1287.83 km<sup>2</sup> (10.3%) was demarcated for PA system in coastal plains. Nagarjunasagar Srisailam Tiger Reserve is largest tiger reserve of the country, occupies 28% of the total PA of Andhra Pradesh. Of the five PAs of coastal plains, Nelapattu wildlife sanctuary is smallest with an area of 4.58 km<sup>2</sup> and Kolleru is largest with an area of 308.55 km<sup>2</sup>. Of the eight wildlife sanctuaries in Deccan Plateau (Telangana), Pakhal (860.2 km<sup>2</sup>) and Eturnagaram (803 km<sup>2</sup>) are occupying 47.42% area. The PA area under grassland and wetlands are accounted as 6.14 km<sup>2</sup> and 782.1 km<sup>2</sup> respectively.

Table 3. Protected Areas of Andhra Pradesh and representation of Vegetation types

Protected Area	Area (km <sup>2</sup> )	% of PA	Habitat/Veg. type	Elevation (m)				
<b>Coastal Plains</b>								
Coringa	235	1.87	Mangroves, Wetland	<100	Kawal	893	7.11	Dry deciduous forest, Scrub 100-600
Krishna	200	1.59	Mangroves, Wetland	<100	Pranahita	136.02	1.08	Dry deciduous forest, Scrub 100-200
Pulic平 lake	469	3.74	Wetland	<100	Sivaram	29.81	0.24	Dry deciduous forest, Scrub 100-200
Nelapattu	4.58	0.04	Wetland	<100	Kinnerasani	635.4	5.06	Dry deciduous forest, Scrub 100-400
Kolleru	308.55	2.46	Wetland	<100	Eturnagaram	803	6.4	Dry deciduous forest, Scrub 100-200
Sub total	1217.1	9.69			Pakhal	860.2	6.85	Dry deciduous forest, Scrub 200-400
<b>Eastern Ghats</b>								
Nagarjunasagar Srisailam Tiger Reserve	3568	28.42	Dry deciduous forest, Scrub	100-800	Manjira	20	0.16	Dry deciduous forest, Scrub 400-600
Gundla Brahmewaram	1194	9.51	Dry Deciduous forest, Moist deciduous forest, Scrub	300-900	Pocharam	129.8	1.03	Dry deciduous forest, Scrub 400-600
Rollapadu	6.14	0.05	Grassland	200-300				
Rajiv Gandhi National Park	2.3	0.02	Dry deciduous forest, Scrub	400-500				

KBR National Park	1.425	0.01	Dry deciduous forest, Scrub	500-600
Mrugavani National Park	3.6	0.03	Dry deciduous forest, Scrub	500-600
MHV National Park	14.59	0.12	Dry deciduous forest, Scrub	500-600
Sub total	3526.9	28.09		
Grand total	12555	100		

### 3.2 Coverage by vegetation type distribution

The semi evergreen forests of Andhra Pradesh were not covered under PA network. Semi evergreen forests were seen only in the northern parts of Andhra Pradesh above an elevation of 900m to 1500m and found in Chintapalle, Gudem, Lankapakala, Sambarikonda, Upper Sileru, Sapparla hills of Visakhapatnam, Dummakonda, Maredumilli, Peddakonda hills of East Godavari. Total area covered by this forest is 1,585.4 km<sup>2</sup> (3.58% of total forest area). The mean annual rainfall in semi evergreen forests is high as compared with other forest types, which is in the range of 1300mm-1700mm.

Moist Deciduous forests are found extensively in Eastern Ghats of northern Andhra Pradesh region, parts of Khammam, Warangal, Adilabad, Gundlabrahmeswaram of Nallamalais and Talakona RF in Chittoor district. The area coverage is about 4,865.85 km<sup>2</sup> (10.98% of total forest area). These forests were exists between elevation of 600m to 900m and mean annual rainfall of 1000mm-1300mm. Moist deciduous forests of East Godavari, Visakhapatnam, Srikantham, Vizianagaram and West Godavari districts and Mothugudem of Khammam district were unique and not present in PA network. Sal mixed deciduous forests of parts of Srikantham (Seethampet and Donubayi) and Vizianagaram districts also significant and can be prioritized for protection.

The dry deciduous forests are conspicuous throughout Andhra Pradesh with supremacy in Telangana and Rayalseema districts. It occurs at an altitude of around 200m-600m. It is spreading over an area of 32,524 km<sup>2</sup> (73.36% of total forest area). Dominance of species differs across different regions. Red Sanders (*Pterocarpus santalinus*) is gregarious species and forms Red Sanders mixed forest in Seshachalam hills of Cuddapah and Chittoor districts. Teak and Bamboo often occurs gregariously and characterize similar associations of dry deciduous

forests. Even though, 20 PAs were represented in dry deciduous forests, still forests like Nigidi hills of Anantapur, Mahadevpur-Kaleshwaram of Karimnagar, Narsapur of Medak, Manchippa of Nizamabad and Chathakonda, Venkatapuram of Khammam need to be considered in view of their community distinctness, geographical situation and topographic variability.

Thorn forests are prevails mostly in Anantapur, Chittoor, Cuddapah, Kurnool and Nalgonda districts. This is characteristic of the dry areas with low rainfall and high temperatures. There is no any PA for thorn forests. The forests of Erramalais of Kurnool, parts of Palakonda hills of Cuddapah and Nagalapuram hills of Chittoor can be prioritized for conservation. Dry Evergreen forest is a unique forest shows dominance of deciduous elements and small leathery-leaved evergreen trees with short trunks and the dense shrubby undergrowth. Dry evergreen forests of Vinukonda range of Guntur district and Kondapalle of Krishna district need to be considered for PA network.

Mangroves are unique systems of east coast, distributed mainly in East Godavari, West Godavari, Krishna, Guntur and parts of Prakasham districts. Total area covered by mangroves is 329 km<sup>2</sup>. The major area of Mangrove was under protection in Coringa wildlife sanctuary and Krishna wildlife sanctuary. Grasslands are mostly found in Rollapadu sanctuary and environs of Kurnool district and in hill tops of Eastern Ghats of northern Andhra Pradesh. Grasslands of Dummakonda and Peddakonda of East Godavari and Gudem, Sapparla, Sileru and Sambarikonda of Visakhapatnam are potential areas for PA network.

### 3.3 Coverage by Elevation

Most of Andhra Pradesh (91.73%) is covered by land with an elevation of <600 m (asl) but only 3.76% of this area corresponds to PA status (Table 4), (Fig. 3). The land between elevation of 300-600m represents 2% of area coverage under PAs. The proportion of PA is very less (0.80%) in an elevation range of 600-900m. However, about 75% of the land between elevation of 900m and 1527m in Eastern Ghats of northern Andhra Pradesh has vegetation cover, but there is no single PA. The geographical area of elevation above 900m occupies 3598.72 km<sup>2</sup> (1.31% of total TGA) need to be considered for declaration of 'Biosphere Reserve'. Coastal plains were received consideration of forest managers and important wetlands of international/national importance were included in PA network.

Table 4. Proportion of PAs with reference to elevation range and geographical area of Andhra Pradesh

Elevation Range	Geographic area (km <sup>2</sup> )	% of geographic area	% of PA network
<100	63156.85	22.96	0.44
100-300	91065.96	33.11	1.32
300-600	98110.11	35.67	2
600-900	19136.18	6.96	0.8
>900	3598.72	1.31	0
Total	275068	100	4.56

### 3.4 Coverage by Endemism

Endemism is a special criterion in conservation of any area. The word '*endemic*' is ascribed to any taxon, which has a restricted distribution. Because of their narrow distributional zonation, endemic species became important target for global conservation efforts. Of the 27 Protected Areas in Andhra Pradesh, Sri Venkateshwara wildlife sanctuary/national park, Nagarjunasagar-Srisailam tiger reserve, Gundlabrahmeswaram wildlife sanctuary, Sri Lankamalleshwara wildlife sanctuary, Peninsula Narasimha wildlife sanctuary and Pakhal wildlife sanctuary represents endemic species (Table 5). Sri Venkateshwara wildlife sanctuary represents 24 endemic species, which are edaphically and climatically adapted to occur in small ecological refugium. So, there is an urgent need to elevate the status of Sri Venkateshwara wildlife sanctuary and national park as 'Seshachalam Biosphere Reserve'. Of the 103 species of endemics, 64 (62%) species were not included in PA network. Of the 45 red listed (threatened) species, 24 (53%) were not covered in PA network. The upper hills of Visakhapatnam district represents 27 endemic species and seven endangered species, needs immediate concern.

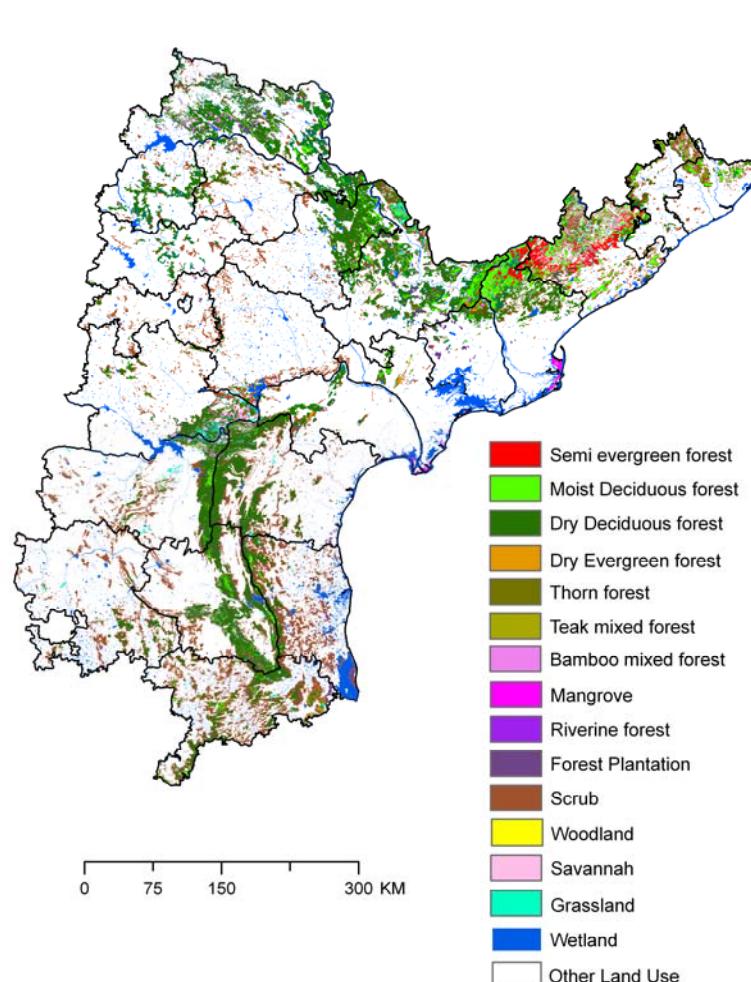
Table 5. Distribution of Endemic species of Andhra Pradesh

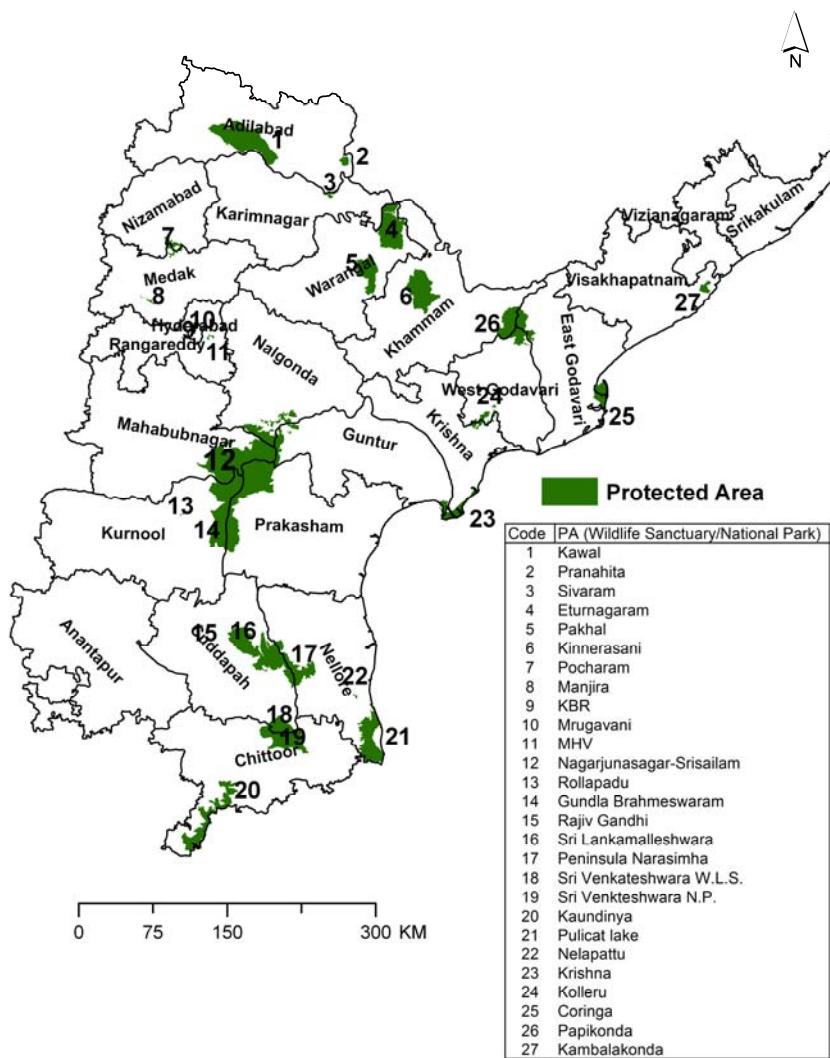
TAXON	Protected Area	Red Data Book category	Veg.type			
<i>Acacia campbellii</i> Arn.	N.A.	Vu	DD			
<i>Albizia thompsonii</i> Brandis	Gundlabrahmeswaram	Vu	DD			
<i>Alphonsea madraspatana</i> Bedd.	N.A.	-	MD			
<i>Alysicarpus mahabubnagarensis</i> Raghavarao et al.	N.A.	-	DD			
				<i>Alysicarpus monilifer</i> (L.) DC. var. <i>cuddapahensis</i> Almeida & Almeida	N.A.	- DD
				<i>Andrographis beddomei</i> Clarke	Nagarjunasagara-Srisailam and Sri Lankamalleshwara	- DD
				<i>Andrographis nallamalayana</i> Ellis	Nagarjunasagara-Srisailam and Gundlabrahmeswaram	- DD
				<i>Aphyllorchis montana</i> (Thw.) Reichb.f.	Sri Venkateshwara	Vu DD
				<i>Argyreia arakuensis</i> Balakr.	N.A.	- SEG
				<i>Argyreia srinivasanii</i> Subba Rao et Kumari	N.A.	- SEG
				<i>Arthraxon depressus</i> Stapf ex Fischer	N.A.	Vu DD
				<i>Arthraxon lanceolatus</i> (Roxb.) Hochst. var. <i>echinatus</i> (Nees) Hack.	N.A.	- DD
				<i>Arundinella setosa</i> Trin. var. <i>lanifera</i> Fischer	N.A.	Vu DD
				<i>Atylosia cajanifolia</i> Haines	N.A.	Vu MD
				<i>Barleria morrisiana</i> Bor ex Fischer	N.A.	- DD
				<i>Boswellia ovalifoliolata</i> Balakr. & Henry*	Sri Venkateshwara	En DD
				<i>Brachystelma glabrum</i> Hook.f.	N.A.	- DD
				<i>Brachystelma volubile</i> Hook.f.	N.A.	- DD
				<i>Bridelia cinerascens</i> Gehrm.	Sri Venkateshwara	- DD
				<i>Bulbophyllum kaitense</i> (Wight) Reichb.f.	Sri Venkateshwara	Vu DD
				<i>Bupleurum andricum</i> Nayar & Banerji	N.A.	- SEG
				<i>Caralluma indica</i> N.E.Br.	N.A.	- DD
				<i>Caralluma lasiantha</i> N.E. Br.	N.A.	- Thorn
				<i>Ceropegia spiralis</i> Wight.	Nagarjunasagara-Srisailam and Sri Venkateshwara	Vu DD
				<i>Chamaesyce linearifolia</i> Soják var. <i>nallamalayana</i> (Ellis) V.S. Raju & P.N. Rao	Nagarjunasagara-Srisailam	- DD

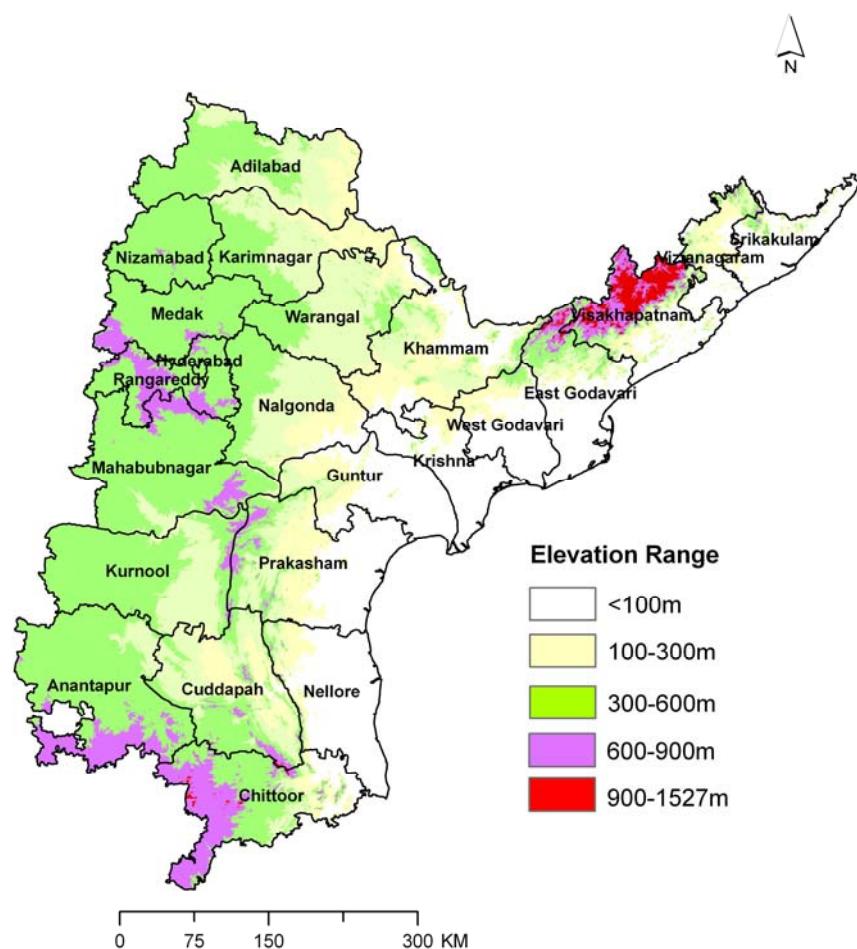
<i>Chamaesyce senguptiae</i> (Balakr. & Subr.) V.S. Raju & P.N. Rao	Nagarjunasagara-Srisailam and Gundlabrahme swaram	-	DD	<i>Eriolaena lushingtonii</i> Dunn	Nagarjunasagara-Srisailam	Vu	DD
<i>Chrysopogon velutinus</i> (Hook.f.) Bor	N.A.	-	DD	<i>Glochidion tomentosum</i> Dalz.	N.A.	Vu	SEG
<i>Cleome chelidonii</i> L.f. var. <i>pallai</i> C.S. Reddy & V.S. Raju	Pakhal	-	DD	<i>Habenaria ramayana</i> Ramachandrachary & Wood	Nagarjunasagara-Srisailam	-	DD
<i>Cleome viscosa</i> L. var. <i>nagarjunakondensis</i> Sund.-Ragh.	Sri Venkateshwar a	-	DD	<i>Halophila ovalis</i> (R.Br.) Hook.f. ssp. <i>ramamurthiana</i> Ravi & Ganesan	N.A.	-	Aquatic vegetation
<i>Commelinia hirsuta</i> Clarke	N.A.	Vu	DD	<i>Heterostemma deccanense</i> (Talb.) Swarup & Mangaly	N.A.	En	MD
<i>Cordia domestica</i> Roth	N.A.	-	DD	<i>Hildegardia populifolia</i> (Roxb.) Schott. & Endl.	Sri Venkateshwar a	En	DD
<i>Corymborkis veratrifolia</i> (Reinw.) Bl.	N.A.	Vu	DD	<i>Hybanthus vatsavayii</i> C.S. Reddy	Pakhal	-	DD
<i>Crotalaria clarkei</i> Gamble	N.A.	-	SEG	<i>Indigofera barbieri</i> Gamble	Sri Venkateshwar a	Vu	DD
<i>Crotalaria filipes</i> Benth.	N.A.	En	DD	<i>Iseilema venkateshwarii</i> Satyavathi	N.A.	-	DD
<i>Crotalaria longipes</i> Wight & Arn.	N.A.	En	DD	<i>Isonandra villosa</i> Wight	N.A.	Vu	MD
<i>Crotalaria madurensis</i> Wight var. <i>kurnoolica</i> Ellis & Swamin.	Nagarjunasagara-Srisailam	-	DD	<i>Kalanchoe cherulkondensis</i> Subbarao & Kumari	N.A.	-	MD
<i>Crotalaria paniculata</i> Willd. var. <i>nagarjunakondensis</i> Thoth.	Nagarjunasagara-Srisailam	-	DD	<i>Lasianthus truncatus</i> Bedd.	N.A.	-	MD
<i>Crotalaria rigida</i> Heyne ex Roth	N.A.	Vu	DD	<i>Lasiococca comberi</i> Haines ( <i>Homonoia comberi</i> (Haines) Merr.)	N.A.	-	MD
<i>Croton scabiosus</i> Bedd.	N.A.	-	DD	<i>Leucas diffusa</i> Benth.	N.A.	-	MD
<i>Cyathocline maniliana</i> C.Raju & R. Raju	N.A.	-	DD	<i>Leucas indica</i> (L.) R. Br. var. <i>nagalapuramiana</i> (Chandr. & Sriniv.) Moulali & Pullaiah	N.A.	-	DD
<i>Cycas beddomei</i> Dyer	Sri Venkateshwar a	Vu	DD	<i>Leucas mollissima</i> Wall. var. <i>mukherjiana</i> Subbarao & Kumari	N.A.	-	SEG
<i>Cycas sphaerica</i> Roxb.	N.A.	-	MD	<i>Leucas mollissima</i> Wall. var. <i>sebastiana</i> Subbarao & Kumari	N.A.	-	SEG
<i>Decalepis hamiltonii</i> Wight. & Arn.*	Nagarjunasagara-Srisailam and Sri Venkateshwar a	En	DD	<i>Leucas mollissima</i> Wall. var. <i>silvestriana</i> Subbarao & Kumari	N.A.	-	SEG
<i>Decaschistia cuddapahensis</i> Paul & Nayar	Sri Venkateshwar a	-	DD	<i>Leucas mollissima</i> Wall. var. <i>mukherjiana</i> Subbarao & Kumari	N.A.	-	SEG
<i>Decaschistia rufa</i> Craib	Sri Venkateshwar a	En	DD	<i>Leucas mollissima</i> Wall. var. <i>silvestriana</i> Subbarao & Kumari	N.A.	-	SEG
<i>Dendrobium ovatum</i> (Willd.) Kranz	Sri Venkateshwar a	Vu	DD	<i>Leucas mollissima</i> Wall. var. <i>silvestriana</i> Subbarao & Kumari	N.A.	-	SEG
<i>Dicliptera beddomei</i> Clarke	Nagarjunasagara-Srisailam	-	DD	<i>Leucas mollissima</i> Wall. var. <i>mukherjiana</i> Subbarao & Kumari	N.A.	En	SEG
<i>Dimorphocalyx kurnoolensis</i> R. Raju & Pullaiah	N.A.	-	DD	<i>Leucas mukherjiana</i> Subbarao &			

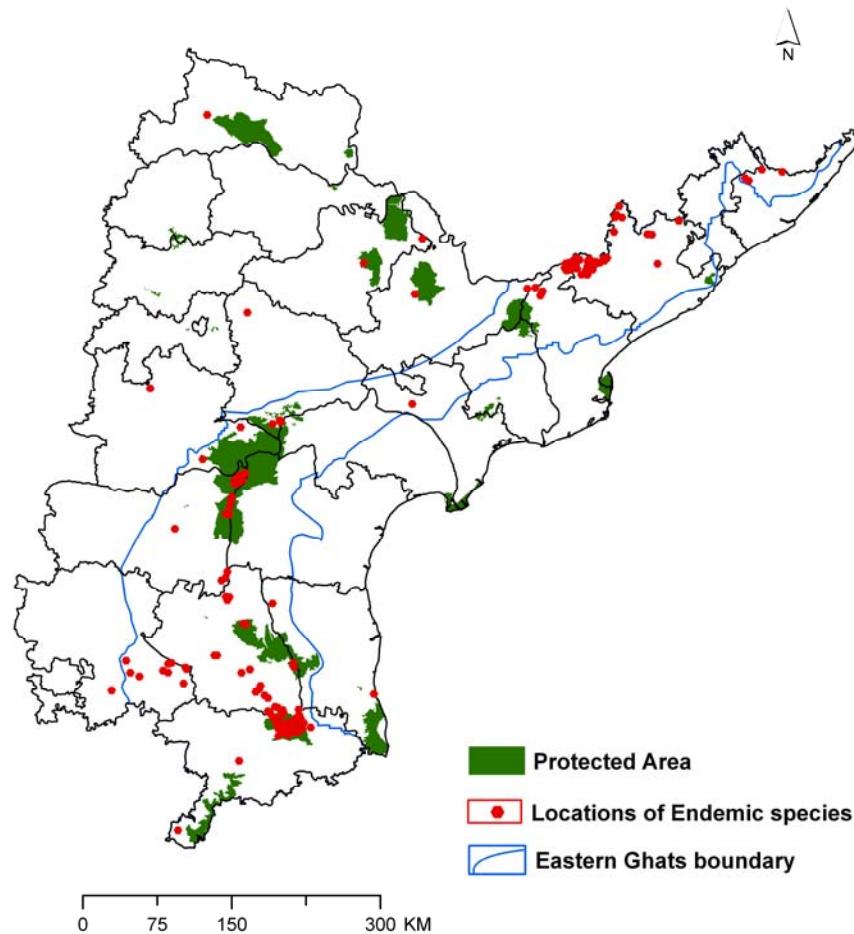
Kumari					Narasimh.				
<i>Lipocarpha reddyi</i>	N.A.	-	DD		<i>Polycarpaea corymbosa</i> var. <i>yadagiriense</i> C.S.	N.A.	-	Thorn	
Hooper					Reddy, Y.N.R.				
<i>Maytenus bailadilliana</i>	N.A.	-	MD		Varma & V.S.				
(Narayan & Mooney) D.C.S.					Raju				
Raju & Babu					<i>Pterocarpus santalinus</i> L.f.*	Sri Venkateshwara and Peninsula	En	DD	
<i>Memecylon jadhavii</i> K.N.	N.A.	-	MD		<i>Rhynchosia beddomei</i> Baker	Sri Venkateshwara	Vu	DD	
Reddy, C.S. Reddy & V.S. Raju					<i>Rostellularia vahlii</i> (Roth) Nees var. <i>rupicola</i> Ellis	Nagarjunasagara-Srisailam	-	DD	
<i>Memecylon madgolense</i> Gamble	N.A.	-	MD		<i>Shorea tumbuggaia</i> Roxb.*	Sri Venkateshwara	En	DD	
<i>Mimosa barberi</i> Gamble	N.A.	-	MD		<i>Syzygium alternifolium</i> (Wight) Walp.*	Sri Venkateshwara	En	DD	
<i>Murdannia juncoidea</i> (Wight)	N.A.	Vu	DD		<i>Terminalia pallida</i> Brandis*	Sri Venkateshwara	En	DD	
Rolla Rao & Kammathy					<i>Toxocarpus roxburghii</i> Wight & Arn.	N.A.	En	SEG	
<i>Nilgirianthus circarensis</i> (Gamble) Bremek.	N.A.	-	SEG		<i>Trichosanthes anaimalaiensis</i> Bedd.	N.A.	CR	DD	
<i>Nothopogea hyneana</i> (Hook.f.) Gamble	N.A.	Vu	SEG		<i>Tripasia reticulata</i> Smith var. <i>parviflora</i>	N.A.	-	DD	
<i>Oianthus disciflorus</i> Hook.f.	Nagarjunasagara-Srisailam	-	DD		Santapau				
<i>Ophiorrhiza chandrasekharanii</i>	N.A.	-	SEG		<i>Tripogon jacquemontii</i> Stapf	N.A.	Vu	DD	
Subbarao & Kumari					<i>Tripogon wightii</i> Hook.f.	Sri Venkateshwara	Vu	DD	
<i>Oropetium roxburghianum</i> (Steud.) S.M.Phillips	N.A.	Vu	DD		<i>Urginea nagarjuna</i> Hemadri & Swahari*	N.A.	Vu	DD	
<i>Panicum fischeri</i> Bor	Sri Venkateshwara	Vu	DD		<i>Vanilla wightiana</i> Lindl.	Sri Venkateshwara	Vu	DD	
<i>Paraphyparrhenia bellariensis</i> (Hack.) Clayton	N.A.	Vu	DD		<i>Wendlandia angustifolia</i> Wight & Arn.	N.A.	Ex	DD	
<i>Pavetta madrassica</i> Bremek.	N.A.	-	DD		<i>Wendlandia gamblei</i> Cowan	N.A.	-	SEG	
<i>Pentanema indicum</i> (L.) Ling var. <i>sivarajanianum</i> C. Raju & R. Raju	Nagarjunasagara-Srisailam	-	DD						
<i>Phlebophyllum jeyaporensis</i> (Bedd.) Bremek.	N.A.	En	SEG						
<i>Phyllanthus indofischeri</i> Bennet*	Sri Venkateshwara and Gundlabrahme swaram	Vu	DD						
<i>Phyllanthus narayanaswamii</i> Gamble	N.A.	En	SEG						
<i>Pimpinella tirupatiensis</i> Balakr. & Subram.*	Sri Venkateshwara	En	DD						
<i>Polycarpaea corymbosa</i> var. <i>longipetala</i> Srinivas. &	Sri Venkateshwara	-	DD						

\*Species marked with asterisk are prioritised for conservation, C.A.M.P., 2001. Andhra Pradesh; Jadhav *et al.* 2001. Ex: Extinct; CR: Critically Endangered; En: Endangered; Vu: Vulnerable; N.A.: No Protected Area Available.

**Figure 1. Vegetation type and Wetland map of Andhra Pradesh**

**Figure 2. Map of Andhra Pradesh showing districts and Protected Area network**

**Figure 3. Elevation range map overlaid on districts of Andhra Pradesh**

**Figure 4. Endemic species locations and Protected Areas in Andhra Pradesh**

#### 4. Discussion

Most PA systems around the world have a tendency to over-represent highland areas (Fearnside and Ferraz, 1995; Powell et al. 2000; Scott et al. 2001) and the study results show a different pattern for Andhra Pradesh. Study found that there is no PA in the elevation above 900m, which is representing semi evergreen forests, moist deciduous forests and savannah. The area above 900m elevation harbours 29 endemic species.

Total PA coverage was low when compared with the commonly used target of 10% (Miller, 1984) with only 4.56% of geographical area of the Andhra Pradesh having PA status. But total proportion of PAs with reference to natural vegetation infer 18% of the area was included under conservation. The analysis of vegetation type distribution reveals that dry deciduous

forests were represented in 20 PAs, where as moist deciduous forests were noticed in two PAs only (Gundlabrahmeswaram and Papikonda). The percentage of PA above 600m, need to be expanded in view of varied vegetation types, species diversity and terrain complexity. Three main vegetation types of State (semi evergreen forests, thorn forests and dry evergreen forests) missing in the existing PA network were identified. Moist deciduous forests of Eastern Ghats of northern Andhra Pradesh were under-represented in PA system (Fig. 4). Gap analysis also determined the number of endemic and threatened plant species currently not protected. Considering vegetation type distribution, elevation and endemism, Upper Godavari and Upper Visakha hills (Eastern Ghats of northern Andhra Pradesh) can be prioritised to the status of 'biosphere reserve' along

with Seshachalam hills (Eastern Ghats of southern Andhra Pradesh). Many PAs have low levels of protection with multifarious threat from invasive species, habitat degradation, forest fires, grazing, illegal extraction of wood, shifting cultivation etc. Deciduous forests are vulnerable ecosystems because of very high economic potential. According to the Directorate of Revenue Intelligence, Govt. of India, the smuggling of Red Sanders wood from Sri Venkateswara Wildlife Sanctuary to South East Asia has emerged as a significant area of concern. During 2003-04, the Directorate of Revenue Intelligence had seized 151 MTs of Red Sanders wood valued at Rs.6 crores (approx.) in the illegal market in India. During 2004-05, 347 MTs of Red Sanders valued at Rs.13.88 crores (approx.) was seized. During 2005-06, the smuggling of Red Sanders continued and 449 MT of Red Sanders valued at Rs. 17.98 crores was seized (<http://www.dri.nic.in>). Pranahita wildlife sanctuary of Adilabad district, had witnessed loss of forest cover of 248 ha area during 1993-2004 (Giriraj et al. 2007). In PAs of Andhra Pradesh, aggressive colonization by invasive plant species (*Hyptis suaveolens*, *Lantana camara*, *Chromolaena odorata*, *Prosopis juliflora*, *Ageratum conyzoides*, *Cassia tora*, *Cassia uniflora*, *Parthenium hysterophorus*) are posing survival threat to native biodiversity. Aquatic habitats also suffering by invasion of *Eichhornia crassipes*, *Alternanthera philoxeroides*, *Ipomoea carnea*, *Typha angustata*, *Pistia stratiotes* etc (Reddy, 2008).

Analysis of endemism also provides a strong platform for the necessity of new protected areas to safeguard unique biodiversity of Andhra Pradesh. To save the biodiversity from human interference, we need to resolve for strong conservation efforts. Consequently, there is need to consider ways to create a network of continuums of Protected Areas in India.

The advent of the remote sensing has made it possible to prepare precise vegetation type maps. The study demonstrated the usefulness of information of vegetation types, elevation and endemism for the conservation prioritisation. The study suggests for declaration of new PAs in semi evergreen forests, moist deciduous forests, dry evergreen forests and under-represented vegetation types. Further, it is recommended that the habitats of endemic species can be preserved by appropriate conservation planning.

#### **ACKNOWLEDGEMENT**

I am grateful to Dr. V. Jayaraman, Director, NRSC, Dr. P.S. Roy, Deputy Director, NRSC and Head, Forestry & Ecology Division, NRSC, Hyderabad for suggestions and encouragement. Thanks are due to Sri P.S. Rao, Additional Chief Conservator of Forests, Andhra Pradesh Forest Department for inspiration and support and to Global

Land Cover Facility Programme, University of Maryland for SRTM data. The present work is carried out as part of national project on "Biodiversity characterization at landscape level using satellite remote sensing and GIS" with financial support of Department of Space and Department of Biotechnology, Government of India.

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29/9/2010

# Knowledge Discovery in Al-Hadith Using Text Classification Algorithm

Khitam Jbara

Department of Computer Science, King Abdullah II School for Information Technology, The University Of Jordan,  
P.O. Box 710481 Amman 11171 Jordan.

[ktjlc2000@yahoo.com](mailto:ktjlc2000@yahoo.com)

**Abstract:** Machine Learning and Data Mining are applied to language datasets in order to discover patterns for English and other European languages, Arabic language belongs to the Semitic family of languages, which differs from European languages in syntax, semantic and morphology. One of the difficulties in Arabic language is that it has a complex morphological structure and orthographic variations. This study is conducted to examine knowledge discovery from AL-Hadith through classification algorithm in order to classify AL-Hadith to one of predefined classes (books), where AL-Hadith is the saying of Prophet Mohammed (Peace and blessings of Allah be upon him (PBUH)) and the second religious source for all Muslims, and because of its importance for Muslims all over the world knowledge discovery from AL-Hadith will make AL-Hadith more understandable for both Muslims and nonmuslims.

[Khitam Jbara. Knowledge Discovery in Al-Hadith Using Text Classification Algorithm. Journal of American Science 2010;6(11):485-494]. (ISSN: 1545-1003).

**Keywords:** AL-Hadith, classification, stem, feature, class, expansion, training set.

## 1. Introduction

Information Retrieval (IR) is the discipline that deals with retrieval of unstructured data, especially textual documents, in response to a query, which may itself be unstructured like sentence or structured like Boolean expression. The need for effective methods of automated IR has grown in the last years because of tremendous explosion of the amount of unstructured data (Greengrass, 2000).

Text mining is a class of what is called nontraditional (IR) strategies (Kroeze, et al., 2003). The goal of these strategies is to reduce the required effort from users to obtain useful information from large computerized text data sources. Also text classifications (TC) is a subfield of data mining which refers generally to the process of deriving high quality of information from a text, which is typically derived through the dividing of patterns and trends through methods such as statistical pattern learning.

However; text classification is one of the most important topics in the field of natural language processing (NLP), where the purpose of its Algorithm is to assign each document of text dataset to one or more pre-specified classes. More formally if  $d_i$  is a document of set of documents  $D$  and  $\{c_1, c_2, \dots, c_n\}$  is the set of all classes, then text classification assigns one category  $c_j$  to a document  $d_i$  and in multi-subjects classification  $d_i$  can be assigned to more than one class from a set of classes.

Text classification techniques are used in many applications, including e-mail filtering, mail routing, spam filtering, news monitoring, sorting through digitized paper archives, automated indexing of scientific articles, classification of news stories and searching for interesting information on the web (Khreisat, 2006).

Also, an important research topic appears in this field called Automatic text classification (ATC) because of the inception of the digital documents. Today, ATC is a necessity due to the large amount of text documents that users have to deal with (Duwairi, 2006).

According to the growth of text documents and Arabic document sources on the web, information retrieval becomes an important task to satisfy the needs of different end users; while automatic text (or document) categorization becomes an important attempt to save human effort required in performing manual categorization.

In this paper, a knowledge discovery algorithm for AL-Hadith is proposed in order to classify it to one of predefined classes (books), this algorithm consists of two major phases; the training phase and Classification phase. Experiments will be conducted on a selected set of AL-Hadith from Al-Bukhari book, where thirteen books were chosen as classes in order to run these experiments. The evaluation of the proposed algorithm is carried out by comparing its results to Al-Bukhari classification.

This paper is organized as follows; related work is represented in section 2, while section 3 represents the proposed classification system, and section 4 analyze experiments and results, finally section 5 demonstrates conclusion.

## 2. Related Work

Most of nowadays classifiers were built for English or European languages. For example, Zhang (2004) builds a Naïve Bayes (NB) classifier, which calculates the posterior probability for classes then the estimation is based on the training set that consists of pre-classified documents, in his system testing phase the posterior probability for each class is computed then the document is classified to the class that has the maximum posterior probability.

Isa, et al. (2008) explore the benefits of using enhanced hybrid classification method through the utilization of the NB classifier and Support Vector Machine (SVM). While Lam, et al. (1999) built a neural network classifier addressing the classifier drawbacks and how to improve its performance.

Bellot, et al. (2003) propose an approach that combines a named entity recognition system and an answer retrieval system based on Vector Space model and uses some knowledge bases, while Liu, et al. (2004) focus on solving the problem of using training data set to find representative words for each class, also (Lukui, et al. 2007) explore how to improve the executing efficiency for classification methods.

On the other hand, Yu-ping, et al. (2007) propose a multi-subject text classification algorithm based on fuzzy support vector machines (MFSVM).

In the Arabic language field, AL-Kabi, et al. (2007) present a comparative study that represents the efficiency of different measures to classify Arabic documents. Their experiments show that NB method slightly outperforms the other methods, while AL-Mesleh (2007) proposes a classification system based on Support Vector Machines (SVMs), where his classifier uses CHI square as a feature selection method in the pre-processing step of text classification system procedure.

El-Halees (2006) introduces a system called ArabCat based on maximum entropy model to classify Arabic documents, and Saleem et al. (2004) present an approach that combines shallow parsing and information extraction techniques with conventional information retrieval, while Khreisat (2006) conducts a comprehensive study for the behavior of the N- Gram Frequency Statistics technique for classifying Arabic text document.

Hammo, et al. (2002) design and implement a Question Answering (QA) system called

QARAB.EL-Kourdi, et al. (2004) build an Arabic document classification system to classify non-vocalized Arabic web documents based on Naïve Bayes algorithm, while AL-Kabi, et al. (2005) represent an automatic classifier to classify the verses of Fatiha and Yaseen Chapters to predefined themes, where the system is based on linear classification function (score function), and (Hammo, et al. 2008) discuss the enhancement of Arabic passage retrieval for both diacritized and non-diacritized text, they propose a passage retrieval approach to search for diacritic and diacritic-less text through query expansion to match user's query.

## 3. Proposed Classification System

The proposed system consists of four phases; first one is the preprocessing phase. Second phase is the training phase where the learning database is constructed which contains the weights of features representing a class. The input for this phase is a set of pre-classified documents. Third phase is the classification phase in which the resulted training database of previous phase is used with the classification method to classify targeted Hadith, also a query expansion occurs in this phase and the output of it will be the class (book) of targeted AL-Hadith. Finally, data analyzing and evaluation phase. These phases are shown in figure 1. We can define the corpus that contains a set of Ahadith (plural of AL-Hadith) as in definition 1.

### Definition 1: Corpus Definition

Suppose corpus  $C = \{H_1, H_2, H_3, \dots, H_n\}$ . Where  $H_i$  represents the  $i$ th tested Hadith in  $C$ ,  $n$  is the number of tested Hadith in the  $C$  and  $i: 1 \dots n$ .

Suppose  $H_j = \{w_1, w_2, w_3, \dots, w_m\}$ . Where  $w_d$  represents the  $d$ th word in AL-Hadith  $H_j$ ,  $m$  is the number of words in  $H_j$  and  $d: 1 \dots m$ .

Figure 2 shows an example of Hadith from the book of food that will be used in the illustration of each step of the proposed system.

### 3.1 Preprocessing phase

In this section the preprocessing techniques are introduced, preprocessing will be conducted on each Hadith used in the training and testing sets. This stage is necessary before the classification phase can be applied to discover knowledge from AL-Hadith and it consists of several sub phases:

1. **Removing Sanad:** this process is done manually and aims to remove Sanad which is a part of AL\_Hadith that refers to the chain of names of persons who have transmitted AL-Hadith.
2. **Tokenization:** which aims to divide AL\_Hadith into tokens (words); AL-Hadith tokenization was

- easily resolved since each token can be identified as a string of letters between white spaces.
3. **Removing punctuation and diacritical marks:** removing diacritical and punctuation marks is important since those marks are prevalent in Ahadith and have no effect on determining AL\_Hadith class.
  4. **Removing stop words:** Stop words are words that found in AL-Hadith and have no discriminative meaning (AL-Kabi, et al., 2005). In the proposed system a list of stop words is built manually and it consists of Arabic pronouns, prepositions, names of people (companions of Prophet Mohammed) and places were mentioned in AL-Hadith corpus. Then after removing stop words from AL\_Hadith, the remaining words (terms) are considered as features.
  5. **Stemming:** In this step the stems of features are extracted, stem extraction implemented is considered as light stem extraction which depends on removing some prefixes or suffixes from the word to relate the word to its stems, we used the stemming algorithm proposed by (Al-Serhan, et al., 2003). The result of stem extraction was filtered to eliminate the incorrect (roots less than three characters). The resulted stems will be used in the query expansion process which will be discussed in details in section 3.3.2, Table 1 shows all steps of preprocessing for AL-Hadith that is presented in figure 2.

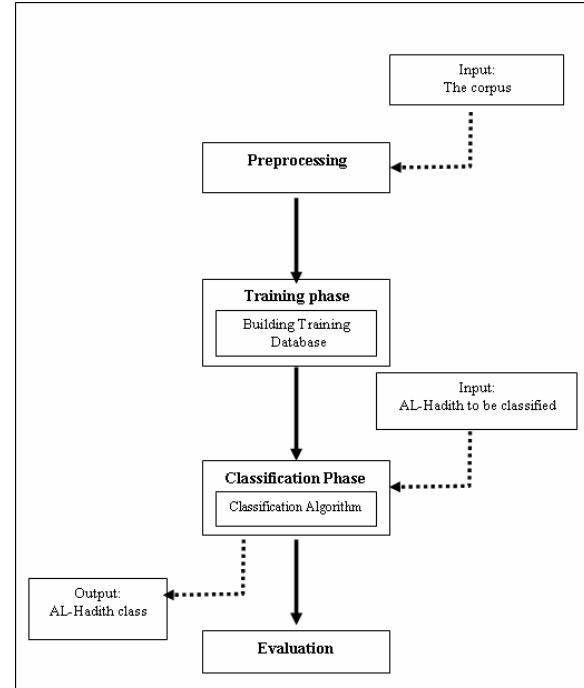


Figure 1. An overview of proposed system phases.

حدثى اسحق بن ابراهيم: أخبرنا روح بن عبادة: حدثنا ابن أبي ذئب، عن سعيد المقيرى، عن أبي هريرة رضي الله عنه:  
 أنه من بقوم بين أيديهم شاة مصلية، فدعوه، فأبى أن يأكل وقال: خرج رسول الله صلى الله عليه وسلم من الدنيا ولم يشبع من خبز الشعير.

Figure 2. Example of AL-Hadith from the book of food.

Table1. Results of preprocessing phase steps for AL-Hadith in figure 2

Step	Result of the step
Removing Sanad	أنه من بقوم بين أيديهم شاة مصلية، فدعوه، فأبى أن يأكل وقال: خرج رسول الله صلى الله عليه وسلم من الدنيا ولم يشبع من خبز الشعير.
Tokenization	{“أنه”, “مر”, “يقوم”, “بين”, “أيديهم”, “شاة”, “مصلية”, “فدعوه”, “أ”, “فأبى”, “أن”, “يأكل”, “وقال”, “خرج”, “رسول”, “الله”, “صلى”, “الله”, “عليه”, “ وسلم”, “من”, “الدنيا”, “ولم”, “يشبع”, “من”, “خبز”, “الشعير”, ”! ”}
Removing Punctuation and Diacritical Marks	{ انه , مر , بقوم , بين , ايديهم , شاة , مصلية , فدعوه , فابى , ان , يأكل , وقال , خرج , رسول , الله , صلى , الله , عليه , وسلم , من , الدنيا , ولم , يشبع , من , خبز , الشعير }
Removing Stop Words	{ مر ، بقوم ، ايديهم ، شاة ، مصلية ، فدعوه ، فابى ، يأكل ، خرج ، الله ، صلى ، عليه ، وسلم ، الدنيا ، يشبع ، خبز ، الشعير }
Stemming (valid stems)	{ ايدي ، دنيا ، شعير }

### 3.2 Training Phase

Supervised classification exploits the predefined training documents that belong to specific class to extract the features that representing a class.

Therefore, every class will have a feature vector representing it, and then these features will be reduced using one of the features selection

techniques. Feature vectors will be used later by the classification algorithm in the testing phase.

Supervised classification has its difficulties; one main problem is how to be sure that trained document actually belongs to a specific class. In this study this problem is resolved by conducting it on a set of AL-Hadith that has been classified by the famous AL-Hadith scientist AL-Bukhari who gave us a good base to evaluate the proposed algorithm.

Training phase consists of two main stages; first one is executed once to produce Inverse Document Frequency (IDF) matrix for the corpus while the second one is executed for each training set.

### 3.2.1 Corpus IDF Matrix

After conducting the preprocessing phase a list of features for each Hadith in the corpus is produced and will be used in the classification process. Building the IDF matrix for AL-Hadith

corpus is done only one time and it will be used in the classification process every time the IDF value for a feature is needed. The IDF value for a given feature is computed according to equation (1).

$$\text{IDF}_i = \log \left( \frac{N}{DF_i} \right) \quad (1)$$

Where

N: number of Ahadith in the corpus.

DF<sub>i</sub>: Number of Ahadith in the corpus containing feature i.

Fewer documents containing a given feature will produce a larger IDF value and if every document in the collection contains a given feature, feature IDF will be zero, in other words the feature which occurs in every document in a given collection is not likely to be useful for distinguishing relevant from non-relevant documents. Table 2 shows the IDF matrix structure.

Table 2. Corpus IDF matrix.

Feature	Pre-defined Classes(Books)					
	Book1	Book2	Book3	....	Bookc	Feature redundancy
Feature1	Log (N/DF <sub>1</sub> )	log (N/DF <sub>1</sub> )	log (N/DF <sub>1</sub> )	.....	log (N/DF <sub>1</sub> )	([DF <sub>1</sub> ]/N)*100
Feature2	Log (N/DF <sub>2</sub> )	log (N/DF <sub>2</sub> )	log (N/DF <sub>2</sub> )	.....	log (N/DF <sub>2</sub> )	([DF <sub>2</sub> ]/N)*100
Feature3	Log (N/DF <sub>3</sub> )	log (N/DF <sub>3</sub> )	log (N/DF <sub>3</sub> )	.....		
Feature4	Log (N/DF <sub>4</sub> )	log (N/DF <sub>4</sub> )	log (N/DF <sub>4</sub> )	.....		
Feature5	Log (N/DF <sub>5</sub> )	log (N/DF <sub>5</sub> )	log (N/DF <sub>5</sub> )	.....		
.....				.....		
.....				.....		
.....				.....		
.....				.....		
.....				.....		
.....				.....		
Feature n	Log (N/DF <sub>N</sub> )	log (N/DF <sub>N</sub> )	log (N/DF <sub>N</sub> )	.....	log (N/DFc)	([DF <sub>n</sub> ]/N)*100

### 3.2.2 Weight calculations for training sets features

The proposed system depends on using a set of Ahadith as training documents to extract representative words for each book (class) to compute their weights. The weight of a given feature in a given document is calculated as (TF×IDF) because this weighting schema combines the importance of TF and IDF at the same time, and the features training weights is computed according to equation (2).

$$TW_{bi} = TF_{bi} \times IDF_i \quad (2)$$

Where:

TW<sub>bi</sub>: feature i training weight in training set b.

TF<sub>bi</sub> : feature i frequency in training set b.

IDF<sub>i</sub>: feature i inverse document frequency calculated earlier (IDF matrix).

Features that will be considered to calculate their training weights must satisfy the feature redundancy threshold 45, that's mean that feature redundancy must be less than 45.

Table 3 shows training weights for features in the training set b in general, while Table 4 shows training weights for features in a training set from the book of food.

Table3.Training weights for features in training set b.

Table 4. Training weights for features in a training set from the book of food.

The book of food (training set No.1)			
Feature	IDF	TF	TW
يأكل	1.80	8	14.39
الدنيا	1.84	1	1.84
شاة	1.97	4	7.90
مر	2.12	1	2.12
ابدیهم	2.22	1	2.22
خنزير	2.34	4	9.37
فأبى	2.42	1	2.42
الشعير	2.52	2	5.04

### 3.3 Classification Process

The classification process consists of four steps as shown in Figure 3. First step is computing query weights where feature's weight in targeted AL-Hadith is found. Second step is the expansion process where the stems are used to expand the query. Third step is calculating the similarity coefficient for each feature in AL-Hadith to be classified, and the final step is finding the cumulative similarity for AL-Hadith over the predefined classes (books).

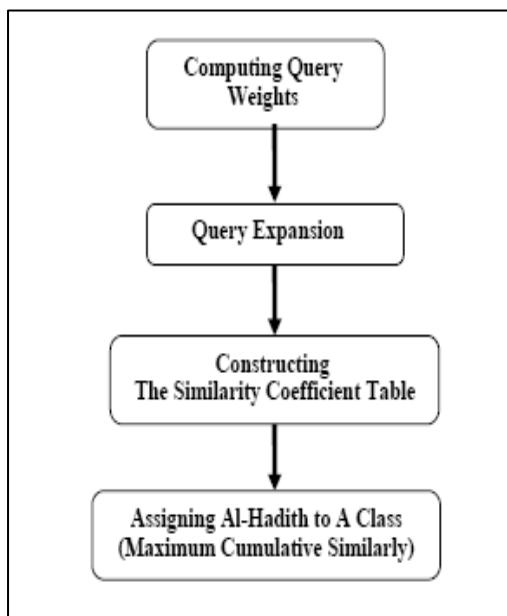


Figure 3. Classification process steps

#### 3.3.1 Computing Query Weights

A feature weight in the query (specific Hadith) is calculated according to equation (3) :

$$Q_h W_i = TF_{hi} \times IDF_i \quad (3)$$

Where:

$Q_h W_i$ : feature i weight in AL-Hadith h (Hadith to be classified).

$TF_{hi}$  : feature i frequency in AL-Hadith h

Bookb			
Feature	IDF	TF	TW
Feature1	IDF1	TFb1	$TWb1 = TFb1 * IDF1$
Feature2	IDF2	TFb2	$TWb2 = TFb2 * IDF2$
Feature3	IDF3	TFb3	$TWb3 = TFb3 * IDF3$
Feature4	IDF4	TFb4	$TWb4 = TFb4 * IDF4$
.....			
Feature n			$TWbn = TFbn * IDFn$

$IDF_i$ : inverse document frequency calculated by equation (1).

Query weights as shown in Table 5 will be computed for each feature in AL-Hadith to be classified. Feature frequency (TF) depends on AL-Hadith features occurrence while Inverse document frequency (IDF) is a global value referenced from IDF matrix.

Table 5. Query Weights Table for Mined Hadith

No.	Feature	IDF	Feature Redundancy	TF	QW
1	الدنيا	1.84	1.44	1	1.84
2	الشعير	2.52	0.30	1	2.52
3	الله	Feature redundancy >45	80.02	2	0
4	ابدیهم	2.22	0.61	1	2.22
5	بقوم	2.64	0.23	1	2.64
6	خنزير	2.34	0.45	1	2.34
7	خرج	1.53	2.95	1	1.53
8	شاة	1.97	1.06	1	1.97
9	صلى	Feature redundancy >45	68.89	1	0
10	فأبى	2.42	0.38	1	2.42
11	قدعوه	3.12	0.08	1	3.12
12	مر	2.12	0.76	1	2.12
13	مصلية	3.12	0.08	1	3.12
14	مسلم	Feature redundancy >45	68.58	1	0
15	يأكل	1.80	1.59	1	1.80
16	يشبع	2.64	0.23	1	2.64

#### 3.3.2 Query Expansion

The process of query expansion depends mainly on using the stems of features to expand the searching area. The stems for all features in the training set and AL-Hadith to be classified were produced in the preprocessing phase.

In the expansion process the newly added stems in the expanded training set will have the same weights for its origin feature. In other words, if we have the couple  $\{(W, S), (W, TW)\}$  where S is the stem of word W and TW is the training weight for W from the training weights Table then the weight for stem S will be the same weight of W.

The same procedure is applied to stems in expanding the query set where stem S will have the same weight of its origin word W from the query

weight Table. The extended query weights for AL-Hadith sample are shown in Table 6.

Feature	Pre-defined Themes			
	Book1	Book2	...	Book13
Feature1	Sim1=Q <sub>b</sub> W <sub>1</sub> *T <sub>1</sub> W <sub>1</sub>			Q <sub>b</sub> W <sub>1</sub> *T <sub>13</sub> W <sub>1</sub>
Feature2	Sim2=Q <sub>b</sub> W <sub>2</sub> *T <sub>1</sub> W <sub>2</sub>			Q <sub>b</sub> W <sub>2</sub> *T <sub>13</sub> W <sub>2</sub>
Feature3	Sim3=Q <sub>b</sub> W <sub>3</sub> *T <sub>1</sub> W <sub>3</sub>			Q <sub>b</sub> W <sub>3</sub> *T <sub>13</sub> W <sub>3</sub>
Feature4	Sim4=Q <sub>b</sub> W <sub>4</sub> *T <sub>1</sub> W <sub>4</sub>			Q <sub>b</sub> W <sub>4</sub> *T <sub>13</sub> W <sub>4</sub>
Feature5	Sim5=Q <sub>b</sub> W <sub>5</sub> *T <sub>1</sub> W <sub>5</sub>			Q <sub>b</sub> W <sub>5</sub> *T <sub>13</sub> W <sub>5</sub>
Feature n	Sim n=Q <sub>b</sub> W <sub>n</sub> *T <sub>1</sub> W <sub>n</sub>			
	$\sum_{i=1}^n \text{Sim}$			

### 3.3.3 Constructing the Similarity Coefficient Table

In the proposed system the cosine similarity coefficient is used, where the similarity between two documents (document (D) & query (Q)) is actually the cosine of the angle (in N-dimensions) between the 2 vectors and can be calculated according to equation (4) (Baarah, 2007):

$$\text{sim}(D, Q) = \frac{\sum_{i=1}^n (w_{di} \times w_{qi})}{\sqrt{\sum_{i=1}^n w_{di}^2} \sqrt{\sum_{i=1}^n w_{qi}^2}} \quad (4)$$

Where  $w_{di}$  denote the query feature and n is the number of feature in the query Hadith.

Table 7 shows similarity coefficient for features in the mined AL-Hadith in general, while Table 8 shows the similarity coefficient for features in AL-Hadith illustrated in figure 2 against the training set from the book of food shown in section 3.2.2.

Table 6. Extended query weights table for mined Al-Hadith

No	Feature	IDF	Feature Redundancy	TF	QW
1	الدنيا	1.84	1.44	1	1.84
2	الشعر	2.52	0.30	1	2.52
3	الله		Feature redundancy >45	80.02	2
4	ايديهم	2.22	0.61	1	2.22
5	بقوم	2.64	0.23	1	2.64
6	خبز	2.34	0.45	1	2.34
7	خرج	1.53	2.95	1	1.53
8	شاة	1.97	1.06	1	1.97
9	صلى		Feature redundancy >45	68.89	1
10	فابي	2.42	0.38	1	2.42
11	قدعوه	3.12	0.08	1	3.12
12	مر	2.12	0.76	1	2.12
13	مصلية	3.12	0.08	1	3.12
14	وسلم		Feature redundancy >45	68.58	1
15	ياكل	1.80	1.59	1	1.80
16	يشبع	2.64	0.23	1	2.64

17	الدنيا	Feature No. 1	1.84
18	الشعر	Feature No. 2	2.52
19	ايدي	Feature No. 4	2.22

Table 7. similarity coefficient for features for mined Hadith in general.

### 3.3.4 Assigning AL\_Hadith to a class

After constructing the similarity coefficient table for AL-Hadith to be classified against the predefined classes, the cumulative similarity weights for mined Hadith will be found against each of those classes. The cumulative similarity values indicate common features between AL\_Hadith to be classified and the predefined books.

After finding the cumulative weight for the mined Hadith with correspondence to each predefined book (class), AL-Hadith will be assigned to the book with the maximum cumulative weight, because maximum cumulative weight is an indication of larger common features between the training set and the mined AL-Hadith features set.

Table 8. Similarity coefficient for features in the example Hadith against training set from the book of food.

No.	Feature	Similarity coefficient
1	الدنيا	3.39
2	الشعر	12.69
3	الله	0.00
4	ايديهم	4.92
5	بقوم	0.00
6	خبز	21.93
7	خرج	0.00
8	شاة	1.97
9	صلى	0.00
10	فابي	2.42
11	قدعوه	0.00
12	مر	2.12
13	مصلية	0.00
14	وسلم	0.00
15	ياكل	1.80
16	يشبع	0.00
17	الدنيا	3.39
18	الشعر	12.69
19	ايدي	4.92
	cumulative similarity	72.26

## 4. Experiments and Results

In this section, an overview is given for AL-Hadith corpus content that is used in this study to run the experiments of the proposed classifying algorithm, and details of experiments are also illustrated.

#### 4.1 Content of AL-Hadith Corpus

AL-Hadith corpus that is used in running the experiments consist of thirteen books (classes). Ahadith were taken from Sahih AL-Bukhari which is the most well known Hadith book all over the Islamic world and the most trusted Hadith book for researchers in this field. Twelve of those books were included in AL\_Kabi (2007) study and the Book of the (Virtues of the Prophet and His Companions) is added to the experiment in this study with 143 additional Ahadith.

Table 9 shows statistical information of books included in the experiments along with its name in English and Arabic as it was used by AL-Bukhari in his Sahih. The testing corpus has 1321 Hadith distributed over 13 books (classes).

Table 9. List of books in AL-Hadith corpus

Book (Class)Name	اسم الكتاب	Doc No.	No. of distinct features after stop words removal
The Book of Faith	كتاب الإيمان	38	938
The Book of Knowledge	كتاب العلم	76	1946
The Book of Praying	كتاب الصلاه	115	2137
The Book of Call to Praying	كتاب الأذان	38	574
The Book of the Eclipse Prayer	كتاب الكسوف	24	715
The Book of Almsgiving	كتاب الزكاه	91	2267
The Book of Good Manners	كتاب الأدب	225	5258
The Book of Fasting	كتاب الصوم	107	1905
The Book of medicine	كتاب الطب	92	1895
The Book of Food	كتاب الطعام	91	1894
The Book of Pilgrimage (Hajj)	كتاب الحج	231	4885
The Book of Grievance	كتاب المظالم	40	906
The Book of the Virtues of the Prophet and His Companions	كتاب المتفق	143	3410

#### 4.2 Classification Methods Applied to AL-Hadith Corpus

One of the researches in AL-Hadith classification field is done by (AL-Kabi, et al., 2007), in which AL-Kabi did not mention an accurate description of AL-Hadith corpus or the stop words list used in their experiments. Therefore, in this study an implementation for their classification algorithm on the corpus used is done.

The following subsections represents in details the three methods have been implemented in this study.

**4.2.1.AL-Kabi method :** this method was proposed by AL-Kabi and his Colleagues on AL-Hadith classification(AL-Kabi, et al., 2007). This method is based mainly on using the stems of Ahadith words to calculate the IDF, the weighting of the feature in training phase and the classification process.

**4.2.2. Word based classification (WBC):** this method uses the words of AL-Hadith after going through the preprocessing phase without stemming stage. The words occurrences after preprocessing are used in the calculation of IDF and in the weighting process. Stems of the words are not used in this method neither in building the training database nor in applying the classification algorithm.

**4.2.3. Stem expansion classification (SEC):** It is the proposed method in this study. In which words and stems are used. Words are used in IDF and features weighting calculations for both training and query sets, but stems are used in query expanding process, the expansion process was discussed in details in section 3.3.2.

#### 4.3 Experiments Specifications

Hadith corpus that is used in this study consists of 1321 Hadith distributed over thirteen books (classes). The system is considered as supervised classification since training sets are used to apply learning algorithm to build the leaning database which will be used for the classification algorithm.

In the experiments author uses (90%) of each Ahadith class as training set while the rest (10%) of each class is used as testing set for the classification system. Of course, for each class the (10%) Ahadith in the testing set are not included in the training set of Ahadith and the training phase calculation.

For each class five testing – training sets combination are chosen to run SEC algorithm, which means that for each class five separable experiments will be run, which gives variation for system testing.

It is important to mention that the same training - testing sets combination is used with the other two classification methods (AL-Kabi's,WBC) which is an important aspect to insure fair comparison among different methods against the proposed one.

#### 4.4 Performance Measurements

In order to demonstrate the efficiency of any classification algorithm, measurements are needed to compare the proposed system's outcome with others. The most popular measurements in text classification algorithm are recall, precision and F-measure that are used in this study.

Recall and precision based on the concept of relevance. Precision is defined as the ratio of relevant documents retrieved to all documents retrieved while Recall is defined as the ratio of relevant items retrieved to all relevant items in a corpus.

There are obvious trade-off between recall and Precision. If the system retrieves all the documents in a corpus then the system will retrieve all relevant documents in the corpus, in this case the recall will be perfect.

On the other hand, since there are only small proportions of documents in a collection that are truly relevant to the given query, retrieving everything will give a very low precision (Greengrass, 2000).

Because of this trade-off between recall and precision a combination of good precision and good recall is needed. In the best case we would like to retrieve all the relevant documents and to discard non-relevant documents, this combination of recall and precision is found in F-measure (Harmonic mean). Precision, recall and F-measure are calculated according to equation presented by (Al-Mesleh, 2007) as follows

$$\text{Precision (P)} = A / (A + B)$$

$$\text{Recall (R)} = A / (A + C)$$

$$\text{F-measure (Harmonic mean)} = (2 \times P \times R) / (P + R)$$

Where the meaning of parameters used in recall and precision calculations are shown in Table 10.

Table 10. Recall and Precision Parameters.

System says...	In reality, the document is...	
	Relevant	Irrelevant
document is relevant	A	B
document is irrelevant	C	D

#### 4.5 Comparisons and Results Analysis

In this section we introduce the comparisons between SEC and the other two methods (AL-Kabi's and WBC), in order to show the preference of the proposed system over AL-Kabi's and WBC method.

##### 4.5.1 Stem Expansion vs. Word based classification

The proposed system (SEC) outperform WBC for 11 out of 13 books in precision, while WBS achieve better precision for The Book of Grievance and the book of Knowledge as shown in Figure 4 . This result is predicted because using the stem expansion phase gives a large morphology variation for the words that can resulted in retrieving more documents that belong in reality to other classes.

The proposed algorithm (SEC) overcomes the side effect of expanding the query using

stemming, by the weighting strategy adopted in the system, where stems in the expansion phase are giving the same weights of its original word in AL-Hadith.

SEC achieved precision value of 1 for The Book of Call to Praying and enhances the precision of 11 classes by 45% in average.

As shown in Figure 5 SEC achieves better F\_measure values for all classes against WBS method and enhances the F\_measure by 49% in average.

##### 4.5.2 Stem expansion vs. AL-Kabi classification

After implementing AL-Kabi's method for the same training -testing sets used to examine SEC, comparisons are conducted as shown in Figure 6 and 7.

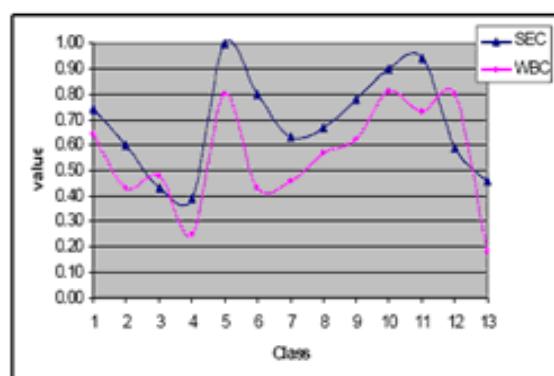


Figure 4. Precision comparison for stem expansion vs. word based classification.

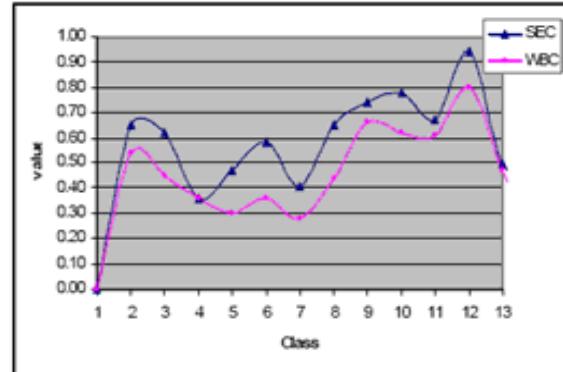


Figure 5. F\_measure comparison for stem expansion vs. word based classification.

Figure 6 represents the precision graph of the two methods, where AL-Kabi's method achieved better precision for The Book of Knowledge and The Book of Grievance, while SEC out performed AL-Kabi's method in 11 out of 13 classes.

This behavior can be justified by the fact that those two books have small number of Ahadith and since the experiments are conducted on Ahadith in a closed domain (Sahih AL\_Bukhari), author

believes that if those classes have larger number of Ahadith the superb of SEC will appear.

In addition AL-Kabi used the stem of words for term weighting in the preprocessing phase, which means that term frequency used in the training process will be the number of the stems occurrence in the training set. In contrary, in SEC the words occurrences is used for the weighting process in preprocessing phase while the stems are used in the expansion process for both training and testing sets.

Since stem expansion is used for both the training and testing sets in the proposed system, more

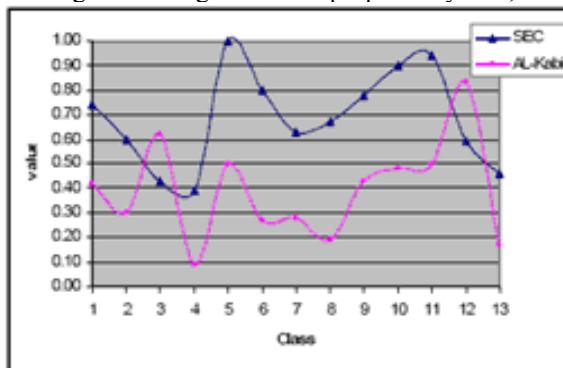


Figure 6. Precision Comparison for Stem expansion vs. AL-Kabi's classification

non-related documents are presented to be retrieved, which justifying AL-Kabi's method achieves better precision for two classes out of thirteen classes . As Shown in Figure 7, SEC method outperformed AL-Kabi F\_Measure in all the 13 classes.

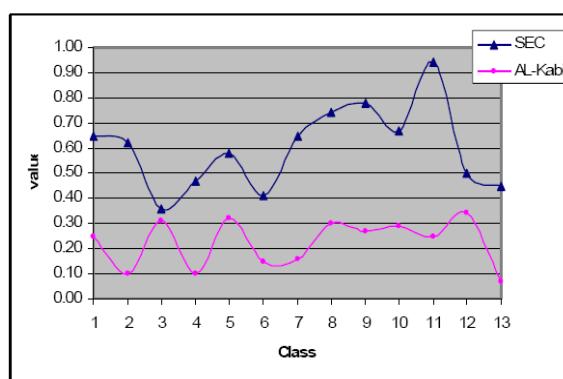


Figure 7. F\_Measure comparison for stem expansion vs. Al-kabi's classification.

## 5. Conclusions

Arabic language is considered as one of the languages that will never distinguish and few researches were made on Arabic corpus linguistics. However, it is the official language of twenty Middle Eastern and African countries and is the religious language of all Muslims, regardless of their origin.

A classification method called Stem Expansion is proposed in this study, in order to discover knowledge from AL-Hadith by assigning each Hadith to one book (class) of predefined classes. SEC is considered as supervised classification method.

In this study a corpus containing 1321 Hadith from thirteen books from Sahih AL-Bukhari is selected and each Hadith is assigned to one class. Sahih AL-Bukhari is used as the base for deciding the correctness of classification results.

The results of the proposed system (SEC) are compared with the results of two methods; one proposed by AL-Kabi and the other is word based classification technique (WBC). The comparison shows that SEC was better against WBC and AL-Kabi in recall for all classes while WBC and AL-Kabi achieve better precision for only two out of thirteen classes, and SEC achieves better F\_Measure for all the thirteen classes against the other two methods (WBC and AL-Kabi).

The results show that SEC performed better in classifying AL\_Hadith against existing classifications methods (WBC and AL-Kabi) according to the most reliable measurements (recall, precision, and F\_Measure) in text classification field.

## Acknowledgements:

Grateful thanks, gratitude and sincerest appreciation to Dr. Azzam T. Sleit and Dr. Bassam H. Hammo for their guidance.

## Corresponding Author:

Khitam M.Jbara.  
Department of Computer science.  
The University Of Jordan.  
P.O. Box : 710481 Amman 11171 Jordan.  
Amman,Jordan.  
E-mail: [ktjlc2000@yahoo.com](mailto:ktjlc2000@yahoo.com)

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24/8/2010

## Efficacy of some Biocontrol Agents on Reproduction and Development of *Meloidogyne incognita* Infecting Tomato

Moussa Lobna\* and Hanaa Zawam\*\*

\*Soils, Water & Environment Research Institute, Agricultural Research Centre (ARC), Giza, Egypt,

\*\* Plant Pathology Research Institute, Agricultural Research Centre (ARC), Giza, Egypt

[mlobnamy@yahoo.com](mailto:mlobnamy@yahoo.com), [hn\\_zawam@yahoo.com](mailto:hn_zawam@yahoo.com)

**Abstract:** Three rhizobacteria and two yeasts isolates were used as biocontrol agents against *Meloidogyne incognita* in laboratory and greenhouse. The used biocontrol agents were identified as *Bacillus amyloliquefaciens*, *Brevibacterium otitidis*, *Sanguibacter inulinus*, *Candida incommunis* and *Wicherhamiella domercqiae*. They inhibited the egg-masses hatching *in vitro* and exhibited strong nematicidal activity by killing the second stage juveniles of *Meloidogyne incognita* to various degrees in greenhouse. The most effective treatment was the complete culture of the four biocontrol agents (propagules and filtrate) suppressed galls and egg-masses formation by 100% *Br. otitidis* reduced galls and egg-masses by 43.7 and 52.19 %, respectively compared with the untreated control. The microorganisms used in greenhouse test reduced nematode populations in the rhizosphere and promoted the growth of tomato plants over the control treatment.

[Moussa Lobna and Hanaa Zawam. Efficacy of some Biocontrol Agents on Reproduction and Development of *Meloidogyne incognita* Infecting Tomato. Journal of American Science 2010;6(11):495-509]. (ISSN: 1545-1003).

**Key words:** Biocontrol; *Meloidogyne incognita*; tomato; Rhizobacteria; yeast

### 1. Introduction:

The root-knot nematodes cause serious damage to important crops world-wide resulting in significant loss of revenue. Resistant cultivars, crop rotation, soil fumigation and chemical nematicides have been used traditionally, for management of the root-knot nematodes. Unfortunately, longevity and slow degradation rate of chemical nematicides created potential environmental and human health concerns, which have forced researchers to find other safe and efficient methods for nematode control. Several soil microbes which produce an array of biologically active compounds can serve as potential biological control agents. Plant growth-promoting rhizobacteria (PGPR) have been identified as an important biological control agent (Johnsson et al., 1998).

A group of important natural enemies of nematode pests, nematophagous bacteria exhibit diverse modes of actions including parasitizing, competing for nutrient uptake, inducing systemic resistance of plants, promoting plant health, producing toxins, antibiotics or enzymes. They act synergistically on nematodes through the direct suppression of nematodes, promoting plant growth, and facilitating the rhizosphere colonization and activity of microbial antagonists (Tian et al., 2007).

Chitinases and glucanases lyse microbial cells and these enzymes have been implicated in the reduction of deleterious and pathogenic rhizosphere microorganisms, creating an environment more

favorable for root growth (Leong, 1986).

Chitin, a glucosamine polysaccharide, is a structural component of fungal cell wall, shells of insects, various crustaceans and nematode eggs. In egg shells of tylenchoid nematodes, chitin is located between the outer vitelline layer and the inner lipid layer and may occur in association with proteins (Bird and Bird, 1991). The breakdown of this polymer by chitinase can cause premature hatching which results in fewer viable juveniles (Mercer et al., 1992).

Bacteria and fungi are also capable of producing lytic enzymes such as chitinases,  $\beta$ (1,3) glucanases, cellulases, lipases and proteases. Some of these enzymes are involved in the breakdown of fungal cell wall by degrading its constituents, such as glucans and chitin, resulting in the destruction of pathogen structures or propagules. Biocontrol bacteria producing protease (Dunne et al., 1998) and chitinase (Rossi et al., 2000) were capable to suppress several plant diseases. The degradation products released can be used by the biocontrol agent to proliferate. Several bacterial proteases have been shown to be involved in the infection processes against nematodes (Tian et al., 2006).

A variety of nematophagous bacterial groups were isolated from soil, host-plant tissues, beside nematodes, their eggs and cysts (Meyer, 2003). They affected nematodes by variety of modes including parasitism, production of toxins, antibiotics or enzymes, hindering the nematode plant-host

recognition, competing for nutrients, inducing systemic resistance of plants and promoting their health. These bacteria had a wide range of suppressive activities on different nematode species, including free-living and predatory nematodes as well as animal and plant parasitic nematodes (Siddiqui and Mahmood, 1999). The objectives of the present study were to impact bioagents that suppress root-knot nematodes and to evaluate the potent antagonistic strain in controlling meloidogyne infesting tomato planted under greenhouse conditions.

## 2. Materials and Methods

### 2.1. Samples

Tomato seedlings (Castel rock) were provided from Horticulture Research Institute (Agric. Res. Center -ARC, Giza, Egypt). The experimental soil was collected from the ARC farm, Giza, Egypt. The soil texture was sandy clay characterized by an EC (2.32 dSm-1), pH (7.9) and available N, P & k of 57.35, 6.76 and 110 mg kg soil-1, respectively. The soil analyses were conducted by the methods described by Page et al. (1982). Five potential bio-control agents of which three bacterial strains of *Bacillus amyloliquefaciens*, *Brevibacterium otitidis* and *Sanguibacter inulinus* and two yeast strains of *Wickerhamiella domercqiae* and *Candida incommunis* were previously isolated by Moussa et al. (2006) and Moussa (2007). These microorganisms were tested *in vitro* and *in vivo* for their effect on controlling *Meloidogyne incognita* which causes the root-knot nematodes.

### 2.2. Biocontrol microorganism propagation on laboratory scale

Both bacteria and yeast strains were cultured individually in 250 ml Erlenmeyer flasks containing 100 ml king's B broth medium as described before by King et al. (1954). Incubation was in a shaker incubator at 28 °C and 150rpm min-1 for 24 hours. The obtained culture suspension contained 107cfu/ml

### 2.3. Nematode larvae extraction

The extraction of juveniles from the soil was accomplished using Jenkins's method (Jenkins, 1964). They were counted under the stereoscopic microscope and data were expressed as juveniles per ml of soil.

### 2.4. Nematode eggs extraction

Nematode Eggs were recovered from excised roots by agitation in 0.5% sodium hypochlorite solution (Jenkins, 1964). The total number of eggs was counted under a stereoscopic microscope and expressed as number of eggs per

gram root.

### 2.5. Nematode stock culture

Nematode population of *M. incognita* was maintained on tomato plants cv. super marmand in a green house at 25 -27 °C. Plants were infested at 2-3 leaves stage by adding egg-masses to roots then covered with soil. After 60 days nematode egg-masses collected from roots by a needle, put in Petri dishes and put it in incubator for hatching at 25°C. The hatched juveniles were collected daily for seven days to laboratory experiment and green house testes.

### 2.6. Screening of antagonisms against egg-masses and juveniles nematodes *in vitro*

Five ml from the complete culture, culture filtrate and culture suspension of strain cells of each biocontrol agent were added to five egg-masses of *M. Incognita* (hand picked) in Petri-dishes (5cm). One Petri dish containing same egg-masses number received 5 ml distilled water to serve as control. Each treatment was applied in three replicates. The nematode percentage inhibition was recorded after 3 days.

The same procedure was applied to test the effect of each biocontrol agent suspension on controlling nematode juveniles. One ml of nematode suspension containing 500 individual juveniles was placed in 8 ml glass vial and completed to 5 ml with each bioagent. Each treatment was held in three replicates. The nematode percent mortality was recorded after 48 hours under a stereoscopic microscope. Morphological changes in eggs and juveniles were observed on an inverted microscope found in Cell Manipulation Lab.

Nematode specimens were examined microscopically through phase contrast system using Olympus IX-70 inverted research microscope equipped with 100W Philips halogen lamp for maximum illumination. The objective phase contrast lens used was of 40X power (LCPlanFI40XPh) while the magnification selector knob was 1.5X power. Observation was carried out using frosted filter, color temperature conversion filter (LBD) and the green interface filter (IF 550) Magnification index: 40X (phase contrast lens) x 5X (built in lens) x 1.5X (magnification selector knob) x 2.5X (camera magnification) = 750X.

### 2.7. Effect of biocontrol agents against *M. incognita* using plastic cups

After 7 days of cultivation the tomato seedlings in steam sterilized sand, 500 freshly hatched juveniles of *M. incognita* poured around the roots of tomato seedlings and also, the different treatments of five biocontrol agents were added. Two

controls were maintained, one with nematode suspension in water and another in the media used for bioagents growth. All treatments replicated three times. After 60 days the plants were uprooted and the roots were washed free from the adhering sand particles. Number of galls, number of egg-masses, number of free nematodes in soil and also the plant weight were determined.

#### 2.8. Effect of biocontrol agents against *M. incognita* under greenhouse conditions

A pot experiment was conducted to explore the effectiveness of the five biocontrol agents to reduce the population density of root-knot nematode juveniles and eggs. Three week old tomato seedlings (*Lycopersicon esculentum*) were transplanted in pots (25cm) which were previously filled with 4kg sandy clay soil. Pots were divided into thirteen groups each comprises six replicates.

The treatments included the bioagents *Bacillus amyloliquefaciens*, *Brevibacterium otitidis*, *Sanguibacter inulinus* and two yeast strains of *Wickerhamiella domercqiae* and *Candida incommunis*. They were individually incorporated into the soil at the rates of 4 ml and 8 ml per pot (10<sup>7</sup> cells/ml). This practice was repeated two times every 15 days. Pots were then, watered weekly twice. Fertilization was practiced after cultivation as Super phosphate (15% P<sub>2</sub>O<sub>5</sub>) at a rate 460 kg/Hectare. Nitrogen Fertilizer was added 35 kg/Hectare (Ammonium sulphate 20.5% N) and potassium Sulphate (48% K<sub>2</sub>O) at a rate 115 kg/Hectare recommended by the Ministry of Agriculture. Prior to biocontrol agent addition, the transplanted tomato seedlings were infested with *M. incognita* by using 500 freshly hatched juveniles that were poured around the roots of tomato seedlings (7 days after tomato transplanting).

After 60 and 120 days from tomato transplantation, the developed plants in each pot were uprooted. The roots were then washed to get rid of the adhering sand particles and to determine numbers of galls, egg-masses and free nematodes in soil.

The tomato rhizosphere soil was collected to determine microbial activity by using the fluorescein diacetate hydrolysis (FDA) method as described by Schnurer and Rosswall (1982), total fungl count on potato dextrose agar (Difco, 1985), total diazotrophs bacteria (Hegazi et al. 1998), chitinase activity in soil (Rodriguez-Kabana et al., 1983), protease activity in soil (Wright and Reddy, 2001) and also fruits weight.

Throughout the pots experiment, the following treatments were statistically arranged in a completely randomized design:  
Control (without root-knot nematodes and bioagents).  
Control + *M. incognita*.

Soil + *M. incognita* + media used for growing bioagents.  
Soil + *M. incognita* + *Bacillus amyloliquefaciens* (4 ml/ pot).  
Soil + *M. incognita* + *Bacillus amyloliquefaciens* (8 ml/ pot).  
Soil + *M. incognita* + *Brevibacterium otitidis* (4 ml/ pot).  
Soil + *M. incognita* + *Brevibacterium otitidis* (8 ml/ pot).  
Soil + *M. incognita* + *Sanguibacter inulinus* (4 ml/ pot).  
Soil + *M. incognita* + *Sanguibacter inulinus* (8 ml/ pot).  
Soil + *M. incognita* + *Wickerhamiella domercqiae* (4 ml/ pot)  
Soil + *M. incognita* + *Wickerhamiella domercqiae* (8 ml/ pot).  
Soil + *M. incognita* + *Candida incommunis* (4 ml/ pot).  
Soil + *M. incognita* + *Candida incommunis* (8 ml/ pot).

#### 2.9. Statistical analysis

The data were analyzed by ANOVA using SPSS version 12 statistical software (SPSS Inc. chicago, Illinois). Differences between treatments were determined by Duncan's Multiple Range Test (DMRT) at 5% significance level. Data Collected were subjected to the statistical analysis according to the standard methods recommended by Gomez and Gomez (1984) using the computer program (Costat). The differences between the mean values of various treatments were compared by Duncan's multiple range test (Duncan, 1955).

### 3. Results:

#### 3.1 Effect of biocontrol agent treatments on *M. incognita* egg-masses *in vitro*

*Meloidogyne incognita* inhibition as affected by five biocontrol agents were studied and presented inTable1. Obviously, the inhibition of nematode egg hatching was affected by the treatment type. In general, the maximum inhibition percentage was mostly achieved by using the yeasts complete culture, as it reached 100% with both *Candida incommunis* and *Wickerhamiella domercqiae*, while by using the suspension of the cells it reached 97.79and 89.91%, respectively.

The treatment with the culture filtrates only of either yeasts suppressed hatching by 79.81 and 96.09%, respectively. On the other hand, the lowest inhibition percentage (72.97%) was achieved by *B. amyloliquefaciens* suspension of microbial cells.

The morphological change of *M. incognita* eggs and juveniles was examined using an inverted microscopy during 7 days of incubation with each microorganism. Deformation of juveniles and eggs that occurred as shown in Figure1. (A) to (E) and (F) to (J), respectively and their untreated controls are represented in Figure2. (A) and (B), respectively. Some eggs appeared to be destroyed, but no inhibition was observed with the water control. Observation through the inverted microscope demonstrated that the microorganisms widely attached to the eggs and juveniles of *M. incognita*.

### 3.2 Effect of biocontrol agents on development of *Meloidogyne incognita* infecting tomato roots *in vivo*

The following experiment was conducted to evaluate the five biocontrol agent treatments previously tested in suppressing root-knot nematode infection and nematode population densities under greenhouse conditions as a pre-field test.

The data presented in Table 2. revealed the highly significant response to the effect of variable treatments to tomato including *B. amyloliquefaciens*, *Brevibacterium otitidis*, *Sanguibacter inulinus*, *Candida incommunis* and *Wickerhamiella domercqiae* which suppressed galling compared to untreated control.

The number of juveniles extracted from roots, number of galls and also number of egg-masses were 100% reduced by the complete culture of all treatments except for *Brevibacterium otitidis* treatment which showed lower suppressive effect, whereas, the tomato plant weights were increasingly improved.

**Table 1. Effect of different biocontrol agent treatments on hatching of *M. incognita* egg-masses *in vitro*.**

Treatment	Bacterial sp.	% inhibition	R
Microbial cells + filtrate	<i>B. amyloliquefaciens</i>	87.95	de
	<i>Brevibacterium otitidis</i>	93.16	c
	<i>Sanguibacter inulinus</i>	93.82	c
	<i>Candida incommunis</i>	100	a
	<i>Wickerhamiella domercqiae</i>	100	ab
	<i>B. amyloliquefaciens</i>	74.37	h
	<i>Brevibacterium otitidis</i>	81.76	f
	<i>Sanguibacter inulinus</i>	89.012	d
	<i>Candida incommunis</i>	79.81	g
	<i>Wickerhamiella domercqiae</i>	96.09	b
Filtrate	<i>B. amyloliquefaciens</i>	72.97	i
	<i>Brevibacterium otitidis</i>	97.07	ab
	<i>Sanguibacter inulinus</i>	86.32	e
	<i>Candida incommunis</i>	97.79	ab
Microbial cell	<i>Wickerhamiella domercqiae</i>	89.91	d
	Media	0.32	d
Control		0	J
LSD			199



Figure 1. Destroyed juvenile (A) and egg (F) of *M. incognita* as a result of using *Bacillus amyloliquefaciens*



Figure 1. Destroyed juvenile (B) and egg (G) of *M. incognita* as a result of using *Brevibacterium otitidis*

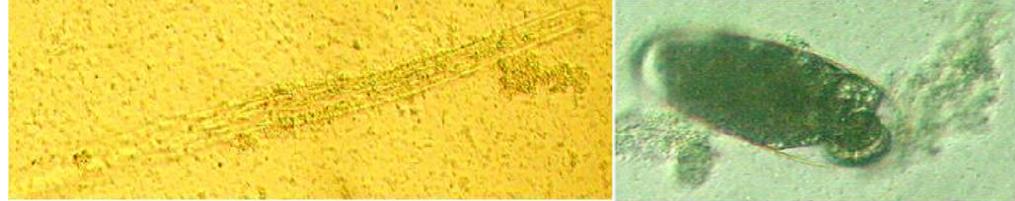


Figure 1. Destroyed juvenile (C) and egg (H) of *M. incognita* as a result of using *Sanguibacter inulinus*

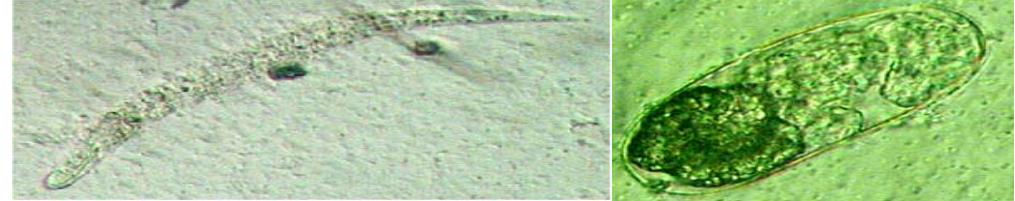


Figure 1. Destroyed juvenile (D) and egg (I) of *M. incognita* as a result of using *Wickerhamiella domercqiae*



**Figure 1. Destroyed juvenile (E) and egg (J) of *M. incognita* as a result of using *Candida incommunis***



**Figure 2. Untreated juvenile (A) and egg (B) of *Meloidogyne incognita***

**Table 2. A Pre-field test for studying the effect of biocontrol agent on development of *M. incognita* infecting tomato roots.**

Treatment	Bacterialsp.	N / 250 cm <sup>3</sup> soil	R	Galls no.	R	egg mass no.	R	Plant wt.	R
Microbial cells + filtrate	S1	0	f	0	e	0	d	6.7	h
	S2	155.7	def	118	c	126.3	c	9.9	g
	S3	0	f	0	e	0	d	7.6	h
	S4	0	f	0	e	0	d	12.3	cfg
	S5	0	f	0	e	0	d	14.1	def
Filtrate	S1	321.7	cd	115.3	c	145.7	c	10.9	fg
	S2	288.3	cde	87.3	d	101	c	9.2	g
	S3	129	ef	17.7	e	25	d	15.4	cd
	S4	371.3	c	116.3	c	152.66	c	12.3	ef
	S5	360	c	167.7	b	225	b	13.7	cde
Microbial cell	S1	66.7	f	18.3	e	31	d	15.5	c
	S2	66.7	f	21.7	e	33	d	12.8	ef
	S3	65.3	f	9	e	14	d	25	a
	S4	593.3	b	84	d	132.3	c	25.4	a
	S5	33.3	f	6.7	e	11.3	d	22.5	b
Control ( nematodes)		2723	a	351	a	438.67	a	6.27	h
LSD 5%		171.573		21.846		53.205		2.183	

S1 (*B. amyloliquefaciens*), S2 (*Brevibacterium otitidis*), S3 (*Sanguibacter inulinus*), S4 (*Wickerhamiella domercqiae*), S5 (*Candida incommunis*).

4. Effect of biocontrol agent treatment on the numbers of nemtode, gall and egg masses after 60 days of cultivation

Among the microbial antagonists used as alternative nematicides, the biological control agents showed limitation in nematode abundance and increased tomato plant weight and height.

Data in Table 3.revealed that all treatments suppressed galling up to 100% compared to the untreated control expect those treatments with *Brevibacterium otitidis*, 8 ml/pot, and *Candida*

*incommunis* at 4.8 ml/pot and media which reduced gall formation with 87.27%, 63.6%, 81.82% and 29.6%, respectively.

The same trend was observed when testing the parameter of egg-masses number and number of juveniles extracted from soil. In case of development in plant parameters, Figure3. shows the highest shoot length being achieved when treated with *Brevibacterium otitidis* 8ml / pot treatment, while the lowest one was with the media treatment. The highest shoot weight was achieved as revealed in Figure 4.

also with *Brevibacterium otitidis*, 8ml / pot treatment, while the lowest was with the *Wickerhamiella domercqiae* at 4 ml /pot treatment.

#### 4.1 Effect of biocontrol agent treatment on the numbers of nemtode, gall and egg masses at harvest time

The inoculation with nematode alone resulted in extensive galling on roots of tomato at harvest. Treatments with the experimental microorganisms reduced the gall formation at harvest as shown in Table 4. Decline in gall formation was from 436 to 16 due to *Sanguibacter inulinus* at 8ml/pot in comparison with untreated control, followed by *B. amyloliquifaciens* at (8ml/pot) and *Candida incommunis* at (8ml/pot). Egg masses production was successfully inhibited due to the application of all microorganisms. The *Sanguibacter inulinus* at

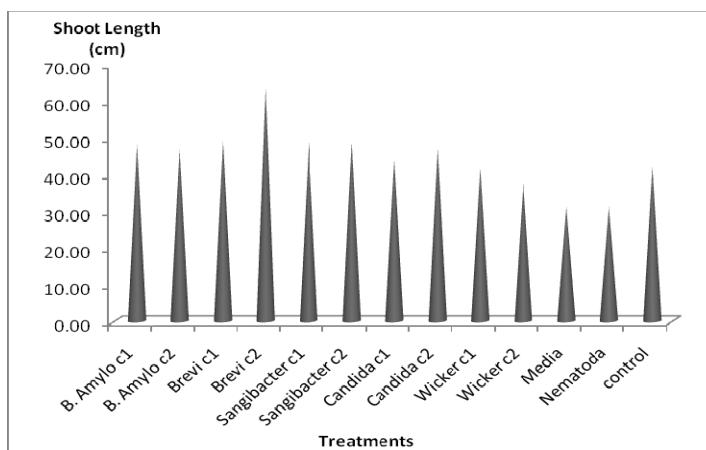
8ml/pot gave the maximum decline in the egg masses, followed by *B. amyloliquifaciens* (8ml/pot) and *Candida incommunis* at 4.8ml/pot and the lowest was with media treatment.

The soil root-knot nematode populations were significantly affected by treatments with microorganisms, as the percent decrease in them was greater than that in galls or egg-masses. The highest decrease in nematode juveniles (n/250 cm<sup>3</sup> soil) occurred with *Brevibacterium otitidis* (8ml/pot) and *Wickerhamiella domercqiae*(8ml/pot) compared to the control infected with nematode. Nevertheless, the nematode development stages inside the root system decreased descending from 7% with *Wickerhamiella domercqiae* at (8ml/pot) to 15% with either *Candida incommunis* (4ml/pot) or *B. amyloliquifaciens* (4ml/pot) compared to with control.

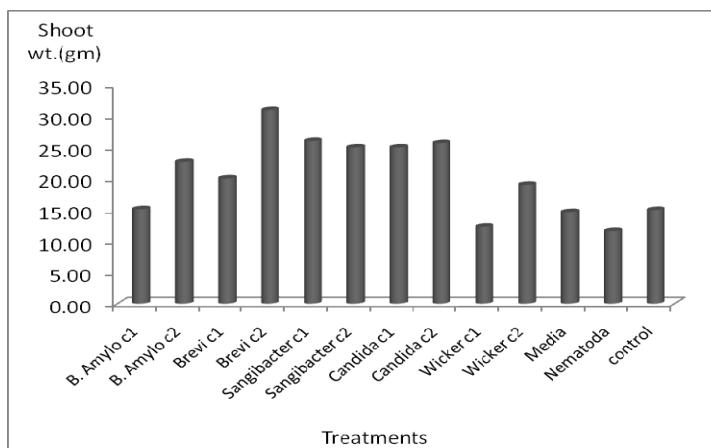
**Table 3. Effect of biocontrol agent treatments on number of nemtodes, galls and egg masses after 60 days of cultivation.**

Treatments	Nematode no. (n/250 cm <sup>3</sup> )	R	Gall no.	R	Egg mass no	R
<i>B. amyloliquefaciens</i> C1	0.00		0.00	E	0	
<i>B. amyloliquefaciens</i> C2	0.00		0.00	E	0	
<i>Brevibacterium otitidis</i> C1	0.00		0.00		0	
<i>Brevibacterium otitidis</i> C2	0.00		3.50		0	
<i>Sanguibacter inulinus</i> C1	0.00		0.00		0	
<i>Sanguibacter inulinus</i> C2	0.00		0.00		0	
<i>Candida incommunis</i> C1	0.00		10.00		0	
<i>Candida incommunis</i> C2	20.00	c	5.00	C	1	c
<i>Wickerhamiella domercqiae</i> C1	0.00		0.00		0	
<i>Wickerhamiella domercqiae</i> C2	0.00		0.00		0	
Media	50.00	b	19.00	C	3	b
Nematodes	70.00	a	27.50	A	5	a
LSD	2.1		1.63		0.47	

C1=4ml/pot., C2=8ml/pot., P1=60days after cultivation, P2=90days after cultivation, P3=120 days after cultivation.



**Figure 3. Effect of 12 different treatments on tomato shoot length achieved after 60 days.**



**Figure4. Effect of 12 different treatments on tomato shoot weight achieved after 60 days.**

**Table 4. Effect of biocontrol agent treatments on the numbers of nematode, galls and egg masses after harvest.**

Treatments	Nematode no. (n/250 cm <sup>3</sup> )	R	Gall no.	R	Egg mass no.	R	NDS	R
<i>B. amyloliquefaciens</i> C1	981	f	39	f	25	e	33	c
<i>B. amyloliquefaciens</i> C2	901	h	32	g	18	g	22	f
<i>Brevibacterium otitidis</i> C1	1200	d	60	c	31	C	19	g
<i>Brevibacterium otitidis</i> C2	881	i	51	d	22	F	18	h
<i>Sanguibacter inulinus</i> C1	1001	e	44	e	30	D	32	c
<i>Sanguibacter inulinus</i> C2	981	g	16	h	12	H	28	d
<i>Candida incommunis</i> C1	980	g	38	f	24	E	33	c
<i>Candida incommunis</i> C2	901	h	38	f	18	g	24	e
<i>Wickerhamiella domercqiae</i> C1	1301	c	51	d	32	C	23	ef
<i>Wickerhamiella domercqiae</i> C2	880	i	49	d	21	F	16	i
Media	2301	b	181	b	136	b	132	b
Nematodes	4101	a	436	a	305	a	218	a
LSD 5%	0.28		2.8		0.78		1.13	

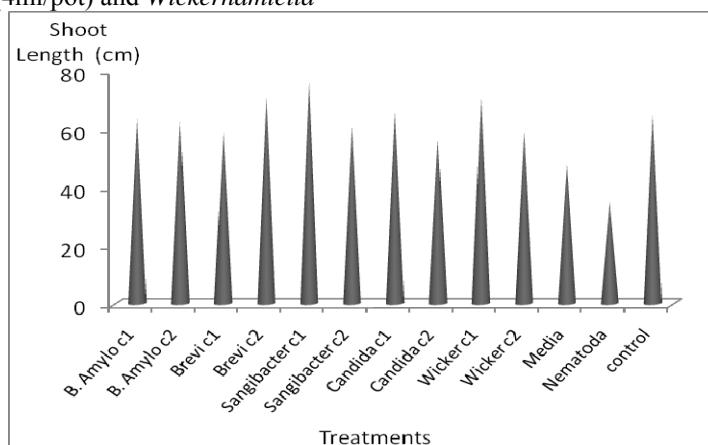
C1=4ml/pot., C2=8ml/pot., P1=60days after cultivation, P2=90days after cultivation, P3=120 days after cultivation, NDS: Nematode development stage.

At harvest, both plant shoot length and weight increased with all the used microorganism treatments compared to nematode infested plant as shown in Figure 5. The highest shoot length (76 cm) being achieved when treated with *Sanguibacter inulinus* at 4ml / pot treatment, followed by (70.67cm) achieved with either *Brevibacterium otitidis* 8ml / pot treatment and *Wickerhamiella domercqiae* 4ml / pot treatment compared to the untreated control (64.67cm), while the lowest one was with the media treatment (47.67cm).

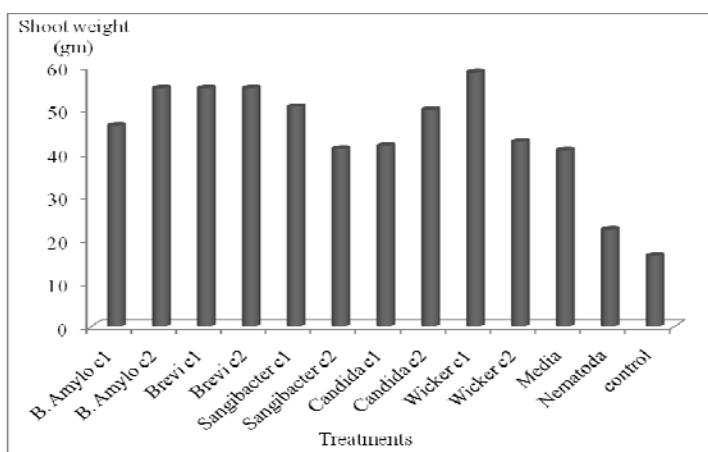
The highest shoot weights were with *Wickerhamiella domercqiae* using first concentration (58.67gm) and *B. amyloliquifaciens* (8ml/pot) recorded (52.33gm) , while *Sanguibacter inulinus* (4ml/pot), *B. amyloliquifaciens* (4ml/pot), *Brevibacterium otitidis* (4ml/pot) and *Wickerhamiella*

*domercqiae* (8ml/pot) recorded 50.67, 46.33 and 42.67gm, respectively, as shown in Figure 6. The lowest shoot weights were 22.33gm obtained from plants infected with nematodes or 16.33 gm obtained from non- infected plants.

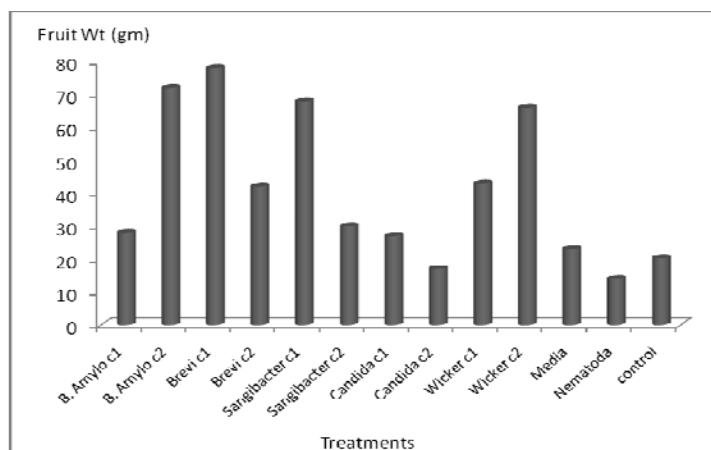
Fruit weights varied significantly due to the used treatments, as the highest fruit weights were obtained with *Brevibacterium otitidis* (4ml/pot), *B. amyloliquifaciens* (8ml/pot), *Sanguibacter inulinus* (4ml/pot) and *Wickerhamiella domercqiae* (8ml/pot) to be 78, 72, 68 and 66 gm respectively, while the lowest fruit weights were obtained from plants infected with nematode (14 gm) and for non-treatment with *Candida incommunis* (8ml/pot) (17 gm) as shown in Figure 7.



**Figure 5. Effect of biocontrol agent on shoot length of tomato at harvest time.**



**Figure 6. Effect of biocontrol agent on shoot weight of tomato at harvest time.**

**Figure 7. Effect of biocontrol agent on fruit weight of tomato.**

#### 4.2 Microbial enzymes detected in rhizosphere during plantation periods

The total microbial activity was assessed by measuring fluorescein diacetate (FDA) hydrolysis releasing fluorescein under the action of microbial enzymes such as proteases, lipases and esterases (Green et al, 2006). The effect of the biocontrol agents activities including bacteria and yeasts added to tomato rhizosphere on fluorescein diacetate compared with that of the controls 1 and 2, beside that of soil amended with media were investigated

through the growth stages of tomato. Data in Table 5. indicate the frequency in the amount of fluorescein resulting according to treatment type. The significant differences indicated that not only the type of treatment affected the activity calculated but both the concentration of the biocontrol added and the intervals of sampling. The *Sanguibacter inulinus* achieved the best activity measured after 90 days using 8ml/pot. All soil rhizosphere treated with those biocontrol agents gave more activity than those untreated (control 1, 2 and that with media only).

**Table 5. Effect of different treatments on microbial activity in soil measured as fluorescein diacetate hydrolysis.**

Treatment name	Activity	Rank
<i>Sanguibacter inulinus</i> c2p2	14.6	A
<i>B.amyloliquefaciens</i> c2p1	13.3	B
<i>Brevibacterium otitidis</i> c2p1	13	C
<i>Brevibacterium otitidis</i> c1p1	12.9	C
<i>Sanguibacter inulinus</i> c2p1	12.9	C
<i>Candida incommunis</i> c1p1	12.7	D
<i>Candida incommunis</i> c2p1	12.7	D
<i>Brevibacterium otitidis</i> c1p2	12.6	De
<i>Wickerhamiella domercqiae</i> c2 p1	12.6	De
<i>Brevibacterium otitidis</i> c2p2	12.5	E
<i>Wickerhamiella domercqiae</i> c1 p1	12.3	F
<i>Brevibacterium otitidis</i> c1p3	12.2	Fg
<i>Candida incommunis</i> c2p3	12.1	Gh
<i>Sanguibacter inulinus</i> c1p1	12.07	Ghi
<i>Wickerhamiella domercqiae</i> c2 p2	12	Hi
<i>B.amyloliquefaciens</i> c2p3	12	Hi
<i>B.amyloliquefaciens</i> c2p2	11.9	Ij
<i>Candida incommunis</i> c2p2	11.9	Ij
<i>B.amyloliquefaciens</i> c1p1	11.8	Jk
<i>B.amyloliquefaciens</i> c1p3	11.8	Jk
<i>B.amyloliquefaciens</i> c1p2	11.7	Kl
<i>Wickerhamiella domercqiae</i> c1 p2	11.6	L
<i>Sanguibacter inulinus</i> c1p2	11.4	M
<i>Brevibacterium otitidis</i> c2p3	11	N
<i>Candida incommunis</i> c1p2	10.9	No
<i>Wickerhamiella domercqiae</i> c2 p3	10.8	Op

<i>Sanguibacter inulinus</i> c2p3	10.7	P
<i>Sanguibacter inulinus</i> c1p3	10.5	Q
<i>Candida incommunis</i> c1p3	10.3	R
<i>Wickerhamiella domercqiae</i> c1 p3	10	S
Medium p1	9.9	S
Control 1 (c1p2)	9.7	T
Medium p2	9.7	T
Control 1 (c1p1)	9.5	U
Medium p3	9.3	V
Control 1 (c1p3)	9.2	Vw
Control 2 (c1p2)	9.1	W
Control 2 (c1p1)	8.1	X
Control 2 (c1p3)	6.1	Y

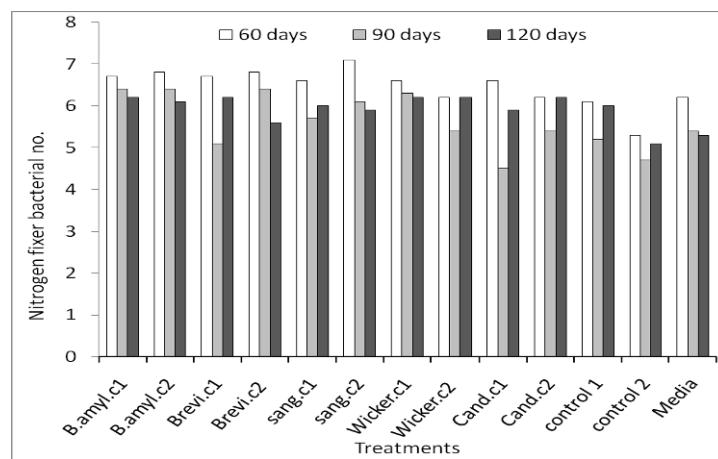
LSD 5% = 0.16

C1=4ml/pot., C2=8ml/pot., P1=60days after cultivation, P2=90days after cultivation, P3=120 days after cultivation.

#### 4.3 Effect of bioagents on diazotrophic bacteria in soil rhizosphere

The diazotrophic bacteria capable of utilizing atmospheric N<sub>2</sub> as their soil nitrogen source (N-fixer) are commonly associated with plants to achieve phyto -nitrogen balance, are affected by soil rhizosphere microflora including the bioagents under study. This experiment was performed to evaluate the effect of bioagents on N-fixing population. As shown in Figure 8, the highest bacterial number among the nitrogen fixing population tested was recorded with *Sanguibacter inulinus* (8ml/pot after 45 days). Along all samples tested, the whole nitrogen fixers' numbers were the maximum after 45 days of cultivation. Obviously, five of the treatments tested (*Bacillus amyloliquifaciens* C1 and C2,

*Brevibacterium otitidis* C2, *Sanguibacter inulinus* C2 and *Wickerhamiella domarcqiae* C1) decreased the diazotrophic bacterial numbers throughout the whole period (120 days). On the other hand, the other five treatments tested (*Brevibacterium otitidis* C1, *Sanguibacter inulinus* C1, *Wickerhamiella domarcqiae* C2, *Candida incommunis* C1 and C2) decreased the diazotrophic bacterial numbers after 90 days but their numbers increased after 120 days. After 45 days the nitrogen fixers' numbers in the soil samples treated with the bioagents exceeded that in the control 1, 2 and that treated with medium. Concerning those untreated, the nematodal presence in the control 2 affected negatively the nitrogen fixers' numbers than those in control 1 with no nematodal invasion.



**Figure 8. Effect of bioagent on Nitrogen fixer bacterial number.**

#### 4.4 Chitinolytic and proteolytic activity criteria in tomato rhizosphere during biocontrol treatments

Data presented in Table 6. And7. shows the influence of different biocontrol agents on excretion

of microbial chitinase and protease, respectively in soil infested with root knot-nematode *M. incognita*. Apparently, the most efficient bioagent in producing chitinase was *Candida incommunis* added at

concentrations of 4ml and 8ml/pot in the second period, followed by the bioagents *Brevibacterium otitidis* and *Wickerhamiella domercqiae* at concentration (4ml, 8ml/pot) in third period. Other less effective bioagents varied in accordance to their type, concentration applied and incubation period in soil. Egg masses production was successfully inhibited due to the application of all microorganisms. The *Sanguibacter inulinus* at 8ml/pot gave the maximum decline in the egg masses, followed by *B. amyloliquifaciens* (8ml/pot) and *Candida incommunis* at (4.8ml/pot) and the lowest with media treatment. The bioagent *B. amyloliquefaciens* showed maximum proteolytic activity at a concentration of 4ml/pot in the third period. Lower protease activity was detected when applying *Brevibacterium otitidis* (4ml/pot, first

period) followed by *Candida incommunis* 8 and 4 ml/pot in the third period). Other bioagent actions decreased dramatically.

#### 4.5 Total number of fungi in rhizosphere soil

Total numbers of fungi were affected by the biocontrol agents added. Their numbers were negatively pronounced with all treatments when compared to control 1 and 2 at first period, while all treatments in the second and third periods decreased fungal numbers as shown in Figure 9. Addition of these two species to the soil seeded with kidney bean and infested with the pathogen increased the percentage of control plants. This was carried out through the process of antibiosis (secretion of antifungal compounds).

**Table 6.Chitinase activity in the presence biocontrol agent**

Treatment name	Activity	Rank
<i>Candida incommunis</i> c1p2	0.6	a
<i>Candida incommunis</i> c2p2	0.42	b
<i>Brevibacterium otitidis</i> c2p3	0.18	c
<i>Brevibacterium otitidis</i> c1p3	0.17	cd
<i>Wickerhamiella domercqiae</i> c1 p3	0.17	cd
<i>Wickerhamiella domercqiae</i> c2 p3	0.17	cd
<i>B. amyloliquefaciens</i> c1p2	0.15	de
<i>Sanguibacter inulinus</i> c2p2	0.14	ef
<i>Brevibacterium otitidis</i> c2p2	0.13	efg
<i>Sanguibacter inulinus</i> c1p2	0.13	efg
<i>Sanguibacter inulinus</i> c1p3	0.13	efg
<i>Brevibacterium otitidis</i> c1p2	0.12	fgh
<i>B. amyloliquefaciens</i> c2p2	0.12	fgh
<i>B. amyloliquefaciens</i> c1p3	0.12	fgh
<i>B. amyloliquefaciens</i> c2p3	0.12	fgh
<i>Candida incommunis</i> c2p3	0.12	fgh
<i>Wickerhamiella domercqiae</i> c1 p2	0.11	gh
<i>Candida incommunis</i> c1p3	0.11	gh
<i>Sanguibacter inulinus</i> c2p3	0.10	h
Medium p1	0.07	i
Medium p2	0.07	i
Medium p3	0.06	I
Control 1 p3	0.037	J
<i>Wickerhamiella domercqiae</i> c2 p2	0.037	J
<i>Wickerhamiella domercqiae</i> c2 p1	0.014	K
<i>Candida incommunis</i> c2p1	0.013	K
<i>Brevibacterium otitidis</i> c2p1	0.013	K
Control 2 p3	0.013	K
<i>B. amyloliquefaciens</i> c1p1	0.012	K
<i>Sanguibacter inulinus</i> c2p1	0.012	K
<i>B. amyloliquefaciens</i> c2p1	0.012	k
<i>Candida incommunis</i> c1p1	0.012	K
<i>Sanguibacter inulinus</i> c1p1	0.012	K
<i>Brevibacterium otitidis</i> c1p1	0.012	K

Control 1 p2	0.011	K
<i>Wickerhamiella domercqiae</i> c1 p1	0.011	K
Control 2 p2	0.011	K
Control 1 p1	0.010	K
Control 2 p1	0.01	K
LSD 5% =	0.02	

C1=4ml/pot., C2=8ml/pot., P1=60days after cultivation, P2=90days after cultivation, P3=120 days after cultivation

**Table 7. Protease activity in the presence biocontrol agent**

Treatment name	Activity	Rank
<i>B. amyloliquefaciens</i> c1p3	54.2	A
<i>Brevibacterium otitidis</i> c1p1	53	B
<i>Candida incommunis</i> c2p3	50	C
<i>Candida incommunis</i> c1p3	48	D
<i>Sanguibacter inulinus</i> c1p3	19.7	E
<i>Wickerhamiella domercqiae</i> c1 p2	19.7	E
<i>Wickerhamiella domercqiae</i> c2 p2	10.3	F
<i>Brevibacterium otitidis</i> c1p3	8.7	G
<i>B. amyloliquefaciens</i> c2p3	7.6	H
<i>Brevibacterium otitidis</i> c1p3	6.2	I
<i>Wickerhamiella domercqiae</i> c1 p3	6.1	I
<i>B. amyloliquefaciens</i> c2p2	5.8	Ij
<i>Sanguibacter inulinus</i> c1p1	5.3	J
<i>Sanguibacter inulinus</i> c2p3	4.60	K
<i>Candida incommunis</i> c2p1	4.21	Kl
<i>Wickerhamiella domercqiae</i> c2p3	3.81	Lm
<i>Brevibacterium otitidis</i> c1p2	3.4	M
<i>Candida incommunis</i> c1p1	2.4	N
<i>Sanguibacter inulinus</i> c1p2	2.3	N
<i>Candida incommunis</i> c2p2	2	No
<i>Wickerhamiella domercqiae</i> c1 p1	1.86	Nop
<i>Candida incommunis</i> c1p2	1.6	Opq
<i>Sanguibacter inulinus</i> c2p1	1.42	Opqr
<i>B. amyloliquefaciens</i> c1p1	1.3	Pqrs
<i>B. amyloliquefaciens</i> c2p1	1.2	Qrs
<i>Brevibacterium otitidis</i> c2p1	1.13	Qrs
<i>B. amyloliquefaciens</i> c1p2	1.03	Qrst
<i>Sanguibacter inulinus</i> c2p2	1	Rstu
Control 1p2	1	Rstuv
Control 1p1	0.9	Stuv
Medium p1	0.8	Tuv
Control 1p3	0.7	Tuv
Control 2p1	0.5	Tuv
Control 2p2	0.45	Tuv
<i>Brevibacterium otitidis</i> c2p2	0.4	Tuv
Medium p2	0.4	Tuv
Control 2p3	0.35	Uv
Medium p23	0.25	V
<i>Wickerhamiella domercqiae</i> c2p1	0.18	V
LSD at 5% =	0.535	

C1=4ml/pot., C2=8ml/pot., P1=60days after cultivation, P2=90days after cultivation, P3=120 days after cultivation

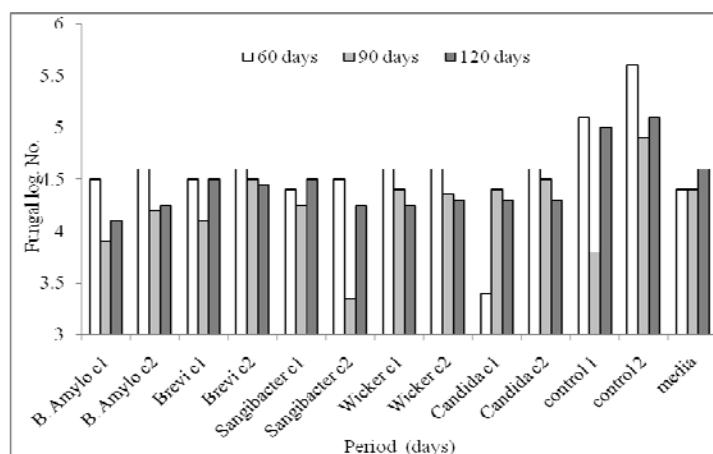


Figure 9. Effect of bioagents on fungal number log.

#### 4. Discussion:

*Meloidogyne incognita* inhibition was affected by the treatment type. The maximum inhibition percentage 100% was mostly achieved by using the complete culture of both *Candida incommunis* and *Wickerhamiella domercqiae*. Similar results were obtained by Shawky et al. (2006) who reported that all the bioagent candidates *Bacillus subtilis*, *Saccharomyces uvarum* and *Saccharomyces ludwigii* proved harmful to *M. javanica* juveniles, egg masses and numbers of galls but the effect magnitude differed from one candidate to another. Jung et al. (2002) showed that *Paenibacillus illinoiensis* had caused 2.5% reduction in egg hatching on the first day and by seventh day there were no hatching eggs found from the 78 eggs/ml used. Mohamed et al. (2008) indicated that the application with the yeast isolates *Pichiagluijillier mondii*, *Pachytrichospora transvaalensis* and *Candida albicans* treatments significantly reduced the number of juveniles *in vitro* after both 24h and 48h. The lethal action of toxic compounds produced by microorganisms on egg *in vitro* noted by Meadows et al. (1989) deserves further exploration.

The morphological change of *M. incognita* eggs and juveniles agreed with the study of Westcott and Kluepfel (1993). They reported that chitinases produced by PGBR was more potent in attacking the eggs rather than the cuticle of *M. incognita*. This might have resulted from the direct damage to the eggs caused by the bacterial or yeast chitinase activity. Also, Mercer et al. (1992) reported that the bacterial lytic enzymes interferes with the egg hatching of *M. hapla* that might had lysed the egg shell including various lipolytic, proteolytic and chitinolytic enzymes, causing an aberrant change in

egg shape and egg rupture.

*In vivo*, the results of evaluating the five biocontrol agent treatments may be referred to the fact that most rhizobacteria act against plant-parasitic nematodes by means of metabolic by-products, enzymes and toxins. The effects of these toxins include the suppression of nematode reproduction, egg hatching and juvenile survival, as well as the direct killing impact on nematode itself (Siddiqui and Mahmood, 1999).

Cronin et al. (1997) reported that purified commercial chitinase inhibited the egg hatching of potato cyst nematode *Globodera rostochiensis* *in vitro* up to 70%. On the other hand, Yong et al. (2004) reported that genus *Sanguibacter* produced chitinase efficiently, hindering the pathogenesis of locusts. Both protease and collagenase had adverse effects on larval motility *in vitro*, when larvae were treated with the enzymes prior to inoculation, protease-treated larvae caused a significant decrease of 40 % in galling (Galper et al. 1990). Rossi et al. (2000) reported that several bacterial proteases have been shown to be involved in the infection processes against nematode. *B. amyloliquefaciens* has been developed to control plant parasitic nematodes on tomato, bell pepper and strawberry (Meyer, 2003). Huang et al. (2005) reported that eggshells of root-knot nematode might be lysed by bacteria that produce various lipolytic, proteolytic and chitinolytic enzymes. These reports indicated that chitinase produced by soil bacteria was associated with the inhibition of egg hatching of root knot nematode. The present results showed the most efficient bioagent in producing chitinase was *Candida incommunis* added at concentrations of 4ml and 8ml/pot in the second period and the bioagent *B. amyloliquefaciens* showed

maximum proteolytic activity at a concentration of 4ml/pot in the third period this agrees with above reports.

Both plant shoot length and weight increased with all the used microorganism treatments compared to nematode infested plant. At harvest, present results showed that *Brevibacterium otitidis* affected the plant showing the highest shoot length and fruit weight, while *Wickerhamiella domerciae* achieved to the plant the highest shoot weight at specific concentrations. Shawky et al. (2006) found that adding any of the antagonistic bacteria had increased the endophytic strains like *Sanguibacter sp.* increased biomass production of plants in the shoot part and good biomass yield. Also, *B. amyloliquefaciens* strain has been shown to induce growth promotion in tomato seedling and reduce severity of diseases caused by several pathogens and elicitation of induced systemic resistance, additionally reduced gall incidence by root-knot nematodes in tomato plants and resulted in increased yield (Kokalis-Burelle and Dickson, 2003). YU et al. (2002) found that *B. amyloliquefaciens* strains produced Iturin A2 molecules that have been used as biocontrol agents to suppress fungal plant pathogens. Also Hiradate et al. (2002) found that those molecules included seven  $\alpha$ - amino acids and one  $\beta$ -amino fatty acid and Iturin A which were produced as a mixture of up to eight isomers.

*Candida incommunis* was reported for its ability to produce IAA and phosphate solubilization that aid in microbial nutrition besides its ability to produce siderophores that acts as antifungal agents that aid in bacterial enrichment (Hassanin et al. 2007). These actions might affect positively the diazotrophs during the third period (120 days) in the present study.

Total numbers of fungi were affected by the biocontrol agents added. These results agree with that of Chen et al. (2006). They reported that *B. amyloliquefaciens* produced lipopeptides, surfactins, bacillomycin D and fengycins as secondary metabolites mainly of known antifungal activity. Similar results were reported by Turner and Messenger (1986), who estimated the ability of *Brevibacterium* to produce phenazine compounds acting as antibiotics. *Candida incommunis* is characterized by its ability to produce siderophores as antifungal agents and inhibitory effects on the growth of the fungal pathogen *Fusarium oxysporum*, according to Hassanin et al. (2007).

Addition of these two species to the soil seeded with kidney bean and infested with the pathogen increased the percentage of control plants. This was carried out possibly through the process of antibiosis (secretion of antifungal compounds).

## 5. Conclusion:

Through this study, clear evidence was presented that the most effective biocontrol treatment was the complete culture (propagules and filterate) of the four biocontrol agents mentioned *Bacillus amyloliquefaciens*, *Brevibacterium otitidis*, *Sanguibacter inulinus*, *Candida incommunis* and *Wickerhamiella domerciae*. These biocontrol agents suppressed galls and egg-masses formation of *Meloidogyne incognita* by 100% and promoted the growth of tomato plants over the control treatment. Therefore, these biocontrol agents can substitute the use of the nematicides used in tomato fields.

## Corresponding author

Moussa Lobna ,  
Soils, Water & Environment Research Institute,  
Agricultural Research Centre (ARC), 9 El Gamaa st.,  
P. O. Box 12112, Giza, Egypt.  
[mlobnay@yahoo.com](mailto:mlobnay@yahoo.com) ,

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9/21/2010

## Phytochemical and Elemental Analysis of *Acalypha wilkesiana* Leaf

\*Madziga, H. A.<sup>1</sup>, Sanni S.<sup>2</sup> and Sandabe U. K.<sup>1</sup>

<sup>1</sup>Department of Veterinary Physiology, Pharmacology and Biochemistry, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria. <sup>2</sup>Department of Veterinary Pharmacology, University of Abuja, Nigeria.

[hannamadziga@yahoo.com](mailto:hannamadziga@yahoo.com)

**ABSTRACT:** Phytochemical and Elemental determination of *Acalypha wilkesiana* was conducted. The result of the Phytochemical analysis of the aqueous leaf extract of *A. wilkesiana* revealed a high presence of carbohydrates, Tannins and Flavonoid, a moderate presence of Phlobatannins, Saponins. Alkaloids and Cardiac glycosides and minute quantity of Terpenes and Steroids. Anthraquinone derivatives was not present. The Elemental analysis showed presence of chloride, sodium, potassium, calcium, iron, magnesium, zinc copper and manganese in moderate quantity while cadmium and lead were not detected. It is therefore concluded that the aqueous leaf extract of *A. wilkesiana* contains Pharmacologically useful active principles elements. Thus the aqueous leaf extract of the plant could play vital roles in health and disease.

[Madziga, H. A.<sup>1</sup>, Sanni S.<sup>2</sup> and Sandabe U. K. Phytochemical and Elemental Analysis of *Acalypha wilkesiana* Leaf. Journal of American Science 2010;6(11):510-514]. (ISSN: 1545-1003).

**Key words:** *Acalypha wilkesiana*, aqueous leaf extract , Phytochemical analysis, Elemental analysis

### INTRODUCTION

*Acalypha wilkesiana* Muell Arg (copper leaf) is a plant from the family Euphorbiaceae. The genus *Acalypha* comprises about 570 species (Riley, 1963), a large proportion of which are weeds while the others are ornamental plants. The plants are found all-over the world especially in the tropics of Africa, America and Asia. The weeds are wild and can be found growing everywhere, while the ornamental species must have been introduced into West Africa from other parts of the world and are cultivated as foliage plants in gardens and greenhouses (Abiodun, 2005). It's a fast growing evergreen shrubs which provides a splash of colour in the landscape with bronze red to muted red, the leaves appear as heart shaped with combination of colour like green, purple, yellow, orange, pink or white depending on cultivation.

Some of the species are well known in traditional medicine and a few have actually appeared in the homeopathic pharmacopoeia of United States (1941) and India (1971). *A. wilkesiana* was reported to be used in the treatment of hypertension, especially in Managing the abnormal sodium and potassium metabolism that accompany hypertension (Ikewuchi et al, 2005).

However few studies have mentioned the phytochemical constituents and elemental studies of *A. wilkesiana*. Akinde (1986) reported the presence of sesquiterpene, monoterpenes, triterpenoids and polyphenols. Adesina et al (2000) reported the presence of gallic acid, corilagin, geranin, quercentin, 3-O-rutinoside and Kaempferol in the leaves of *A. wilkesiana*.

In another study, Oladunmoye (2006) reported the presence of saponins, tannins, anthraquinan and glycoside in the leaves of *A. wilkesiana*. *A. wilkesiana* has antibacterial and antifungal properties (Akinde, 1986, Alade and Irobi, 1993; Adesina et al, 2000, Ogundaina 2005, Oladunmoye 2006).

The leaves of *A. wilkesiana* are popularly used in the north eastern Africa in the treatment of skin infections. This study is therefore designed to investigate the phytochemical and elemental constituent of *A. wilkesiana* obtained from Maiduguri, Nigeria.

### MATERIALS AND METHODS

#### Plant collection and identification

Fresh leaves of *A.wilkesiana* were collected in the month of June from University of Maiduguri staff quarters. It was identified and authenticated by a botanist from the Department of Forestry, University of Agriculture Makurdi, Benue State. The Plant was then air dried under room temperature for two weeks after which it was pulverized using wooden mortar and pestle.

#### Aqueous Extract Preparation

The powdered sample (200g) was mixed with 1 litre of distilled water in a round bottom flask. A reflux condenser was attached to the flask and inserted with a heating mantle. The mixture was reflux for one hour and filtered with Watman filter paper No. 1. The reflux was done twice again using new distilled water at each stage. The filtrate was then evaporated to dryness using oven at 50°C to a dark viscous substance. The yield was 22.85% w/w.

The resultant extract was concentrated and stored in a specimen bottle at room temperature until used.

### Determination of the Phytochemical constituent

The extract was evaluated for the presence of Carbohydrate, tannins, flavonoids, phlobatannins, cardiac glycosides, saponins, alkaloids, terpenes, steroids and anthraquinone using simple qualitative and quantitative methods of Trease and Evans (1989) and Sofowora (1993).

### Determination of the elemental constituent of *A. wilkesiana* leaf.

Air dried sample (15grams) of *A. wilkesiana* was put in a labeled crucible and heated in a hotspot furnace at 550°C for 3 hours. The sample was removed and cooled in a dessicator. Half (0.5) gram of the ashed sample was digested in a 250mls beaker with 20cm<sup>3</sup> of aqua-regia (mixture of HCl and HNO<sub>3</sub> in a ratio 3:1) and 10 cm<sup>3</sup> of 30% H<sub>2</sub>O<sub>2</sub> was added. The beaker was then covered with watch glass and heated on a hot plate at 90°C for about 1 hour so that the volume is reduced to 2cm<sup>3</sup> in Fume cupboard until a clear digest was obtained. The content was then filtered after cooling and deionized water added and made up to 100mls in volumetric flask. The

elemental analysis was done using Sp-9-single beam atomic absorption spectrophotometer (Philip/pye Unicam Ltd, England). The elemental concentrations were determined by a standard calibration curve method (Sunderman, 1973; Kolthoff and Elving 1976).

### RESULT

The result of the Phytochemical analysis obtained from the aqueous leave extract of *A. wilkesiana* indicated that carborhydrates, tannins and flavonoids were highly present in the extract. Phlobatanins, cardiac glycosides, saponins and alkaloids were present in medium quantity while terpenes and steroids occurred in minute quantity. Anthroquinone were not present in the extract. (Table 1).

The result of the elemental analysis of the leave of *A. wilkesiana* showed a very high concentration of chloride, sodium and potassium ions. Calcium, iron, magnesium and zinc were in medium concentration while copper and manganese were in minute concentration. Lead and cadmium were not detected. (Table 2).

**Table 1:** Phytochemical constituents of *A. wilkesiana* aqueous leaf extract

	Chemical Components	Extract
1.	<b>Test for Carbohydrates</b>	
i)	Molisch's test	+
ii)	Barfoed's test-for monosaccharide	-
iii)	Fetiling's test for free reducing sugar	+++
iv)	Standard test for combined reducing sugar	++
v)	Test for Ketones	+
vi)	Test for Pentoses	-
2.	<b>Test for Tannins</b>	
i)	Ferric Chloride test	+
ii)	Formaldehyde Test	-
iii)	Chlorogenic acid test	-
3.	<b>Test for Flavonoid</b>	
i)	Lead acetate test	+++
ii)	Sodium Hydroxide	-
iii)	Ferric Chloride	+++
iv)	Pew test	+++
4.	<b>Test for Saponins</b>	
i)	Froth test	++
5.	<b>Test for Phlobatannins</b>	
i)	Hydrochloride acid test	-
ii)	Lime Water Test	++
6.	<b>Test for Cardiac glycosides</b>	
7.	<b>Test for Alkaloid</b>	
i)	With meger's reagent	+
ii)	With Dragendorff's reagent	++
8.	<b>Test for Terpenes and Steroids</b>	

i)	Lieberman – Burchard test-	+
ii)	Salkowski's test	-
9.	<b>Test for Anthraquinone derivatives</b>	
i)	Brontrager's test – to show the presence of free anthraquinone	-
ii)	To show the presence of free and or combined anthraquinone	-
iii)	To show the presence of anthraquinone derivatives In a reduced form which are not easily hydrolysed	-

**Table 2: Elemental Concentration of *A. wilkesiana* Leaf**

Elements	Concentration Mg/L	WHO Standard Concentration Mg/L
1. Chloride (Cl)	3550	-
2. Sodium (Na)	2530	4 – 5
3. Potassium (K)	390	0.1 - 1.0
4. Calcium (Ca)	30.8855	360 - 800
5. Iron (Fe)	9.6728	0.5 – 50
6. Magnesium (Mg)	5.4068	-
7. Zinc (Zn)	1.9787	15 - 50
8. Copper (Cu)	0.4720	1 - 3
9. Manganese (Mn)	0.0825	10 - 20
10. Cadmium (Cd)	0.00	10 - 35
11. Lead (Pb)	0.00	1 - 2

## DISCUSSION

The Phytochemical test result indicated the presence of carbohydrate in the extract; carbohydrate is reported to have numerous roles in living things, such as the storage and transport of energy (starch, Glycogen) and structural components (cellulose in plants, chitin in animals). Additionally carbohydrates and their derivatives play major roles in the working process of the immune system, fertilization, pathogenesis, blood clotting and development (Maton *et al.*, 1993).

The presence of tannins in the aqueous extract of *A. wilkesiana* leaf implies that the extract can be pharmacologically useful as astringents. Tyler (1988) reported that the astringent activity of tannins

is by precipitating proteins, thereby protecting the underlying tissue leading to improvement of wound healing.

Tannins inhibit microbial proliferation by denaturation of enzymes involved in microbial metabolism (Awosika, 1991). Tannins also have shown potential antiviral (stephone *et al.*, 2004 and Lin *al* *et* .,2004), antibacterial (Akiyama *et al* .,2001and Funatogawa *et al.*, 2004) antiparasitic and anticancer effects (Bhagavathi *et al.*,1999; Ling-lihy *et al.*,2000 and Susumu *et al.*, 2005,) Tannins including gallo and ellagic acid (epigallitannins) are inhibitors of HIV replication. Flavonoids have been referred to as nature's biological response modifiers because of its ability to modify the body's reaction to

allergies, viruses, and carcinogens. They show anti-allergic, anti-inflammatory microbial and anti-cancer activity (Yamamoto and Gaynor , 2002 ). However some research indicated that only small amount of flavonoids are necessary to see its medical benefits. Taking large dietary supplements provides no extra benefit and may pose some risks (David stauth, 2007). Cardiac glycosides have been used in the treatment of congestive heart failure, constipation, edema and microbial infections (Robinson, 1967 and Franstisk, 1991). It may be possible that the aqueous extract of *A. wilkesiana* could be useful in the treatment of this ailment since it contains cardiac glycoside.

In dogs and cats, cardiac glycosides are indicated for their negative chronotropic effect in supraventricular arrhythmias such as atrial fibrillation. They slow the rate of impulse conduction through the A.V. node and allow the ventricular rate to fall below the atrial and so restore more efficient pumping (Aliu, 2007). Saponins have expectorant properties which have been used in the treatment of upper respiratory tract infection (Trease and Evans, 1989). They also have antibacterial activities (Birk and Petri, 1980) thus have been used in the treatment of microbial infections.

Alkaloids are pharmacologically useful. They are the local anaesthetic, CNS stimulant (Cocaine, nicotine, coffiene, etc), analgesic e.g. Morphine and antimalarials e.g. guanine (<http://en.wikipedia.org/wiki/Alkaloid>). Steroids (anabolic steroids) have been observed to promote nitrogen retention in Osteoporosis and in animals with wasting illness (Aliu, 2007).

The result of the elemental analysis of the aqueous extract of *A.wilkesiana* indicated the presence of macro and micro nutrients. Macro nutrients such as sodium, potassium and calcium regulate the fluid balance of the body and thereby influence cardiac output (Sanni , 2007). Restriction of sodium intake or an increase in Potassium intake exert remarkable anti-hypertensive effect (Schroever , 1976). Calcium ions plays an important physiological and biochemical processes such as neuromuscular excitability blood coagulation, secretary processes etc (Sanni, 2007).

Proper extracellular fluid and periosteal concentration of calcium and phosphate ions are required for bone mineralization (Robert *et al.*, 2000).

Elements such as iron, zinc and manganese are essential because they are important in several enzyme reactions as co-factors (Robert *et al.*, 2000). Potassium has an oxidizing effect, it act as astringent and can destroy organic poisons especially alkaloids (Aliu, 2005).

## CONCLUSION

This study shows that *A. wilkesiana* contains pharmacologically active principles and elements which are used extensively in chemotherapy and which are useful in health and disease in humans and animals. Therefore the aqueous extract of this plant could be of immense medicinal value.

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9/29/2010

# Nutritional studies on some different sources of iodine on productive performance, ruminal fermentation and blood constituents of Buffalo. 1 – Effect of two different iodine levels on productive and reproductive performance of buffalo cows.

**Kh. I. I. Zeedan<sup>1</sup>, O. M. El-Malky<sup>2</sup>, Kh. M. M. Mousa<sup>1</sup>, A. A. El.Giziry<sup>1</sup> and K. E.I. Etman<sup>1</sup>**

1- Department of Animal Nutrition Research.

2- Department of Buffalo Research.

Animal Production Research Institute, Agricultural Research Centre, Dokki, Giza, Egypt.

[khzeedan@yahoo.com](mailto:khzeedan@yahoo.com)

**Abstract:** This study was conducted to evaluate the effect of feeding buffalo cows on ration supplemented with two levels from iodine (I) during late pregnancy (three months before parturition) and postpartum period (six months after parturition) on nutrients digestibility, some blood constituent, birth weight of their offspring, Concentrations of immunoglobulin in colostrums, milk (yield and composition) and reproductive parameters. Eighteen buffalo cows (2-4 lactations) in late pregnancy period were selected to carry out the experimental work. The animals were divided into three similar groups (6 female buffaloes in each). Concentrate feed mixture (CFM), berseem hay (BH) and rice straw (RS) were given to animals as a control ration ( $I_0$ ) without supplementation, while the other groups  $I_1$  and  $I_2$  received the control ration with iodine at levels of 0.3 and 0.5 mg I per kg DM intake /h/d, respectively. Results indicated that supplementation ration of buffalo cows with different levels of I had improved the digestibility of all nutrients, TDN, DCP at pre and post partum, feed efficiency, increased milk yield, 7% fat correct milk yield and its composition. Birth and weaning weight of calves in treated groups were higher than that control group. Immunoglobulin concentration in colostrums indicated higher values with animals feed supplemented rations than those fed the control. Moreover, addition of I improved RBC, WBC, Hb, PCV, plasma total protein, globulin, glucose, T<sub>3</sub> and T<sub>4</sub>. Supplemented rations of buffalo cows with 0.5 mg I/h/d tend to significantly ( $P < 0.05$ ) higher in actual milk yield, 7% FCM yield, fat %, protein %, lactose %, SNF % and TS %, while supplemented with 0.3 mg I/h/d appeared to the same higher trend with no significantly differences. Moreover, better feed efficiency was observed with animals fed supplemented rations. The periods required for fetal membrane expulsion was significantly reduced in  $I_2$  group when compared to  $I_1$  or control groups. Moreover, only control group showed a case of abortion and still birth, while treated dams delivered 100% healthy calves. Buffaloes of group  $I_2$  had the least ( $P < 0.05$ ) calving interval due to the shorter intervals for uterine involution, onset of the 1<sup>st</sup> postpartum heat and days open. Iodine supplementation showed significant differences among groups in studied parameters such as NSPC and CI. Mean period elapsed from calving until placenta drop significantly decreased  $I_2$  than the control group. Generally, it concluded that I supplementation for ration of buffalo cows improved immunity, nutrients digestibility, calves birth weight and increased milk (yield and composition) and showed better feed efficiency as well as higher some traits of reproductive performance.

[Kh. I. I. Zeedan, O. M. El-Malky, Kh. M. M. Mousa, A. A. El.Giziry and K. E.I. Etman. Nutritional studies on some different sources of iodine on productive performance, ruminal fermentation and blood constituents of Buffalo. 1 – Effect of two different iodine levels on productive and reproductive performance of buffalo cows. Journal of American Science 2010;6(11):515-530]. (ISSN: 1545-1003).

**Keywords:** buffalo cows, iodine, performance, reproductive parameters, blood components, milk yield, digestibility, immunity.

## 1. Introduction

Buffalos are the most favored milk animal in Egypt, as most Egyptians prefer buffalo milk to cow milk because of its white color, sweet taste and high fat percentage.

Iodine is a key component in the formation of thyroid hormones that contributes to brain development during fetal life and metabolism

thereafter. On the other hand, thyroid hormones are major regulators of development, metabolism and homeostasis. Studies addressed that thyroid hormones influence both aspects of development; growth. A lack of iodine indirectly influences growth rate, milk production and feed consumption. Other deficiency symptoms include poor conception rates, abortions, longer gestation periods and the birth of

dead, weak or hairless calves. Moreover, pregnant animals are more susceptible to iodine deficiency than non-pregnant animals, which in turn increase the incidence of prepartum and postpartum reproductive disorders. Also Lactating dairy cattle require more iodine than beef cattle because approximately 10% of the iodine intake is normally excreted in milk, and this percentage may increase as milk production increases.

NRC (1994, 1998, 2001) reported that the iodine requirements for dairy cows 0.5 mg/kg dry matter (DM). Sanchez (1995) reported that iodine requirement is 0.6 mg /Kg of DM for lactating cows and 0.25 for calves, and growing heifers. Although cattle can tolerate iodine far in excess of their requirements, a level of 50 mg I/ Kg of DM has been suggested as the maximum tolerable level. Who added that low levels of iodine in the diet affect reproduction of cows, causing irregular oestrous cycles, low conception rates as well as desorbed fetuses, abortions, stillbirths or calves may be born blind, weak or with goiter. Male fertility may be also affected, decreasing libido and semen quality. Severe iodine deficiency may reduce milk yield or growth rate. Georgievskii et al. (1990) found that the principal function of iodine is determined by its presence in the thyroid hormones. These hormones are known to regulate basic metabolism, consumption of carbohydrates, proteins and fats, and heat formation processes, and to affect growth, development and the reproductive function. The effect of hormones on metabolism involves synthesis of respiratory and other enzymes, which affect intracellular processes of oxidation, oxidative phosphorylation and protein synthesis. Gaffarov and Saliev (1979) indicated that supplement of iodine for cow rations increased the digestibility of all nutrients. Gasanova (1985) reported that increasing the dietary level of iodine from basal values 0.11-0.16 mg /Kg DM to 0.59 - 0.65 mg / Kg DM improved feed utilization for milk production. Hetzel(1990) reported that iodine deficiency disorders is the term now used, instead of goiter (enlarged thyroid), to denote all the effects of I<sub>2</sub> deficiency on growth and development.

Hoption (2006) reported that thyroidal hormones play a major role in the growth and development of brain and central nervous systems, control of several metabolic processes including carbohydrate, fat, protein, vitamin and mineral metabolism. Iodine deficiency affects reproductive capacity, brain development and progeny as well as growth. Smith and Chase (2004) reported that early research confirmed that nutrition played an important role in reproduction, but in most cases severe

nutritional deficiencies were required to cause reproductive problems. They also said that reproduction is influenced through iodine's action on the thyroid gland. Inadequate thyroid function reduces conception rate and ovarian activity. Lobb and Dorrington (1992) and Monge and Monniaux (1995) reported that the animals suffer from iodine deficiency then ovary become atrophy and decrease the milk production and there will be decrease calcium absorption capacity i.e. low calcium and high phosphorus in blood. Iodine acts on the thyroid gland. Metabolic activity of body also decreases slow growth due to Iodine deficiency. If we supply iodine, increase the function of thyroid gland, maintain the balance of Ca and P level, and increase metabolic activity of body, body weight and animal come in heat early. So hormone will be less effective in Iodine deficient cow. The mechanism by which massage brings back cows ovary to function is not clearly understood, but is probably the result of activation of intrinsic ovarian factors.

Therefore, the present work aimed to study the effect of iodine dietary supplementation on birth weight of their offspring, milk (yield and composition), nutrients digestibility, immunoglobulin status, blood constituents and reproductive performance of Egyptian buffalo's cows.

## 2. Material and Methods

### The experimental procedures:

This study was carried out at EL-Gemmaiza Experimental Station belonging to Animal Production Research Institute, Agricultural Research Centre, Giza, Egypt. Eighteen Egyptian lactating buffalo cows (2-4 lactations) were used in this study. Animals were chosen in late pregnancy period at approximately 90 days prepartum and divided randomly into three similar experimental groups, (6 animals in each group) to evaluate the effect of using iodine in feeding buffalo cows on nutrients digestibility, some blood parameters, birth weight of their offspring, immunoglobulin status, milk yield, milk composition and reproductive parameter. The control ration consisted of concentrate feed mixture (CFM), berseem hay (BH) and Rice straw (RS)which was given to buffaloes, (I<sub>0</sub>) without additives, while the other groups I<sub>1</sub> and I<sub>2</sub> fed the control ration supplemented with two levels of Iodine : 1<sup>st</sup> is 0.5 mg sodium iodide per kg DM intake /h/d (0.3 mg I per kg DM intake /h/d) and 2<sup>nd</sup> is 0.8 mg sodium iodide per kg DM intake /h/d /h/d (0.5 mg I per kg DM intake /h/d)respectively. I were well mixed with some of the ground concentrate feed mixture before feeding. Animals were individually fed according to Kearn (1982). The animals were left

for 4 weeks (as a preliminary period) on the same diet before receiving any samples. The experimental treatments lasted nine months (three months before the expected calving date and continued up to six months of lactation period) to investigate the effect of I supplementation on productive and reproductive performance of buffaloe cows.

#### **Management and feeding:-**

All animals were housed in semi open pens until time of delivery then they were transferred to the maternity unit. Water was offered freely in water troughs except at the milking time. After delivery all buffalo cows were allowed to nurse their calves for only one week postpartum (period of colostrums intake) thereafter, the dams were transferred to the milking unit and milked twice daily at 7a.m and 5 p.m. and they were subjected to the regular managerial practices of the breeding stock. Dams were weighted before and after calving as well as their new born calves were also weight and recorded immediately after calving until weaning (90days). Milk yield was recorded. Composite and representative samples of milk (morning and evening samples) were mixed by ratio of 1% weight of milk and analyzed biweekly for fat, protein, lactose, solids non fat, total solids and ash using Milkoscan apparatus. Energy of milk was calculated by using the formula of Overman and Sanmann (1926) where energy of milk:

$$(Kcal) = 115.3 (2.51 + \text{fat \%}).$$

Actual milk yield was converted into 7% fat corrected milk (FCM) using the formula given by Raafat and Saleh, (1962) as follows:

$$7\% \text{ FCM} = 0.265 \text{ milk yield} + 10.5 \text{ fat yield.}$$

Two digestibility trials were carried out at 60 and 180 days of the feeding trials using buffalo cows from all groups to determine the nutrients digestibility and nutritive value of the experimental rations. Acid Insoluble Ash (AlA) technique according to (Van Keulen and Young, 1977) was used as a marker for the determination of the nutrients digestibility. Digestibility of DM as well as all nutrients was determined with the following equations:

$$\% \text{ Nutrient digestibility} = 100 - (\% \text{ DM digestibility} \times \% \text{ Nutrient in feces}) / \% \text{ Nutrient in feed}.$$

At the 60 and 180 days of the trial, fecal samples were collected from the rectum twice daily every 10 hr at 7:00 and 17:00 hr starting at the 3<sup>rd</sup> day of the collection period. Feed and fecal samples were dried, ground and kept for later analysis. Chemical composition of the different ingredients and feces samples were analyzed according to A. O. A. C. (1990).

#### **Colostrums analysis:-**

Colostrums samples were collected within one hour of parturition (first milking) from each dam at the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> day postpartum for immunoglobulin studies. Determination of immunoglobulin's Ig A, Ig M and Ig G in colostrums was applied by Bovine radial immunodiffusion (RID) kit according to the procedure outlined by the manufacturer (The Binding Site Ltd, Birmingham, UK). The principle of the technique was derived from the work of Mancini *et al.* (1965) and Fahey and McKelvey (1965).

#### **Blood sampling:-**

Blood samples were collected biweekly via the jugular vein from each buffalo cows during late pregnancy (LP) and postpartum (PP) period. Count of red blood cell (RBC) and white blood cell (WBC) was determined using haemocytometer, packed cell volume (PCV%) was estimated using micro-hematocrit tube and micro hematocrit centrifuge at 10000 rpm for 5 min, while concentration of hemoglobin (Hb) was carried out using (Super+Ior®, Sahli's method) according to Sahli, (1905). Blood plasma was carefully separated after centrifugation at 4000 r.p.m. for 20 minutes, and then stored at -20 C° until analysis for the different blood parameters. Plasma was used for determination of Glucose (Trinder, 1969), total protein (Armstrong and Carr 1964), blood urea nitrogen (Fawcett and Scott, 1960), albumin (A) (Drury, 1974), cholesterol (Kostner *et al.*, 1979) and triglycerides (Schalm *et al.*, 1975). Direct radioimmunoassay technique was performed for determination of progesterone, (P4) estradiol 17β, triiodothyronine (T<sub>3</sub>) and thyroxin (T<sub>4</sub>) hormones in representative plasma samples. Kits of "Diagnostic Products Corporation, (DCP) Los Angles, USA " with ready antibody coated tubes were used according to the procedure outlined by the manufacturer.

#### **Measurement of reproductive traits:-**

During postpartum period, the reproductive tract was rectally palpated once every two days till 21 postpartum and once every three days after that in order to assess the uterine involution according to El-Fadaly (1978). All experimental buffalo cows were observed twice daily for estrous activity and buffalo cows in heat were inseminated 12 h after estrus detection. Animals were examined for pregnancy by rectal palpation after 50 days of insemination.

Immediately after parturition, the interval elapsed for complete fetal membranes drop (hour) and uterine involution period (day) were recorded. Also, the interval from calving to first detected estrus (day), service period length (day), number of services

per conception (NSPC), days open, pregnancy rate (%) and calving interval (CI/day) were recorded.

#### **6- Statistical analysis:**

Data were analyzed using GLM procedures of the SAS (SAS, 1996). Means were separated by using Duncan's multiple range test (Duncan, 1955).

### **3. Results and Discussions**

#### **Chemical composition of feedstuffs:-**

The chemical composition of CFM, BH and RS (Table 1) are within the normal ranges reported in Egypt by several workers (El-Hosseiny, *et al.*, 2008 and Zeedan, *et al.*, 2008 and 2010).

#### **Nutrients digestibility and nutritive values:-**

Results obtained from Table (2) indicated that digestibility of DM, OM, CF and EE was not influenced by I supplementation at 60 days. While digestibilities of CP and NEF were significantly higher ( $P<0.05$ ) for  $I_2$  than  $I_0$ . Also, nutritive value as TDN was significantly ( $P<0.05$ ) for  $I_2$  than  $I_1$  and  $I_0$ . At 180 days the digestibility of all nutrients and nutritive value (TDN and DCP) tended to significantly ( $P<0.05$ ) increase for the  $I_2$  compared to the  $I_0$ . These results were in agreement with those obtained by Lubin *et al.* (1979), Gasanova (1985) and Lenina (1986). Pattanaik *et al.* (2001) found that digestibility of DM, OM, CP and EE was not influenced by I supplementation and were comparable to the control values during the first metabolism trial (90 days). During the second trial, conducted at 165 days post-feeding, the digestibility of nutrients tended to increase for the I supplemented group  $I_{50}$  compared to the control, but the differences were non-significant. El-Hosseiny, *et al.* (2008) found that supplement of iodine for young camel, the digestibility of nutrients and nutritive value (TDN and DCP) tended to increase, but the differences were non-significant. Also Gaffarov and Saliev (1979) indicated that supplement of iodine for cow rations increased the digestibility of all nutrients.

The improving of digestibility of most nutrients may be due to its effect on rumen bacteria especially rumen proteolytic bacteria and increasing the number of rumen cellulolytic bacteria (Hungate, 1968 and Martinz and Church, 1969). Lawrence and Fowler (1997) reported that thyroid hormones influence the function of most organs and stimulate the basic metabolic rate through regulation of the metabolism of carbohydrates, proteins and lipids. Improvement of TDN and DCP might be due to the higher values of digestibility values of all nutrients by supplementation with different levels of I

supplementation. Hoption (2006) reported that thyroidal hormones play a major role in the control of several metabolic processes including carbohydrate, fat, protein, vitamin and mineral metabolism. On the other hand, Sawal *et al.* (1996) found that digestibility of all organic nutrients decreased with female Gaddi sheep receiving iodine.

#### **Birth weight of born calves and dams:-**

Data of body weight (dams and calves) and daily gain of calves are summarized in Table (3). The dam body weight before parturition; late pregnancy (LP) was insignificant in all experimental groups. The same trend was observed with fetal fluid, but dam body weights after parturition, post-partum (PP) were significant ( $P<0.05$ ). Also, fetal fluid was not significant in all treatments. Percentages of calving loss (CBW + fetal fluid) / DBW before parturition were 9.30%, 9.66% and 10.48 % for groups  $I_0$ ,  $I_1$  and  $I_2$ , respectively. Differences between groups in Calf birth weight (CBW) and CBW / Dam post-partum were significant ( $P<0.05$ ) (Table 3). Group  $I_2$  achieved greater values of CBW / Dam post-partum, calf birth weight, weaning weight, total gain of calf and daily gain of calf than other groups. Improvement percentage was 8.62 and 12.07% for daily gain of calves. The newly born calves from treated dams I showed higher CBW, weaning weight, total gain, daily gain and better vitality in comparison with the control group. Generally the calves from treated dams showed higher CBW, CBW / Dam post-partum, weaning weight, total gain and daily gain than the control group. The supplementation has improved the thyroid function at calving and weaning in the buffaloes, increased the digestion protein, TDN (60 and 180 days), concentrations of immunoglobulin in colostrums, milk protein and milk lactose led to increase the calf birth weight and calf weaning weight. Gilles *et al.* (2009) found that the selenium and iodine administration in prepartum cows may enhance the calf immune defences by improving the maternal mineral status. Sultana *et al.* (2006) found that the highest live weight gain was found in Lugol's iodine treated group (7.09%) followed by iodide salt treated group (5.28%) and common salt treated group (4.94%). They added that the lowest live weight gain was determined in control group (No iodine treatment). They added that the reason why might be due to anabolic effect of iodine on weight gain. Hoption (2006) reported that thyroidal hormones play a major role in the growth and development of brain and central nervous systems, control of several metabolic processes including carbohydrate, fat, protein, vitamin and mineral metabolism. Iodine deficiency affects reproductive capacity, brain

development and progeny as well as growth Lobb and Dorrington (1992) and Monget and Monniaux (1995) reported that the animals suffer from Iodine deficiency then decrease the milk production and there will be decrease calcium absorption capacity i.e. low calcium and high phosphorus in blood. Iodine acts on the thyroid gland. Metabolic activity of body also decreases slow growth due to Iodine deficiency. If we supply iodine, increase the function of thyroid gland, maintain the balance of ca and p level, and increase metabolic activity of body, body weight. Also, Mc Dowell, (2003) and Underwood and Suttle, (2001) found that both hormones have multiple functions in the energy metabolism of cells, in the growth, as a transmitter of nervous stimuli, and as an important factor in brain development. Also, Gasanov (1991) reported that Supplemented (injections of tetravitan and supplements of trivitan, potassium iodide, sodium selenite, calcium triphosphate, proserine, cobalt chloride or zinc sulphate) buffaloes had increased calf birth weight, decreased calving problems, increased milk yield and colostral density, and blood immunoglobulin concentrations. He added that buffaloes were deficient in selenium, cobalt and iodine which affected immune.

#### **Concentrations of immunoglobulin in colostrums:-**

As shown in table (4) concentrations of all Immunoglobulin (Ig) fractions in colostrum of buffaloes showed a descending trend with sequence sampling days after parturition. IgA, IgM and IgG were detected increasing for  $I_1$  and  $I_2$  compared to  $I_0$ . IgG were significantly ( $P < 0.05$ ) in group  $I_2$  in comparison with other groups. The control group  $I_0$  had minimal concentration of IgA, IgM and IgG than other treated groups. The supplementation has improved the thyroid function at calving in the pregnant cows, increased the digestion both protein and TDN at 60 days led to increase immunoglobulin. Northeim *et al.* (1985) reported that the immune status of the newborn calves is dependent upon the passage of immunoglobulins from dams to the calves through the ingestion of colostrum and its subsequent absorption from small intestine. Nocek *et al.* (1984) demonstrated that IgG has been related to pre-weaning growth. Consumption of colostrum is essential to provide animals with the antibodies and other proteins that calves need to stay healthy. The amount of colostrum and immunoglobulin, or IgG consumed determines amount of passive immunity and resistance to disease. When calves consume insufficient amounts of IgG from colostrum within the first 24 hours of life, calves are much more susceptible to developing disease and possibly dying.

A major reason that pre-weaning mortality is higher than optimum (defined as less than 5% of calves born alive) is due to inadequate IgG intake (Quigley, 2005).

Estimates of Ig in the current study are greater than those obtained by Salama *et al.* (1997) who observed that Ig contents in colostrum of Egyptian buffaloes at 24, 48 and 72 hr after calving were 26.1, 20.0 and 16.4 mg/ml in premiparous and 26.0, 14.3 and 10.9 in pluriparous buffaloes. Gasanov (1991) reported that Supplemented (injections of tetravitan and supplements of trivitan, potassium iodide, sodium selenite, calcium triphosphate, proserine, cobalt chloride or zinc sulphate) buffaloes had decreased calving problems, increased colostral density, and blood immunoglobulin concentrations. Who added that buffaloes were deficient in selenium, cobalt and iodine which affected immune.

Generally Subsequently, the amount of IgG in dam's colostrum depended mainly upon prepartum administration of immunopotentiators, and in calves depended mainly upon consumption of colostrum directly after parturition. Also, iodine supplementation may be improved the thyroid function led to increase concentrations of immunoglobulin in colostrums.

#### **Milk yield and composition:-**

Data presented in Table (5) showed that the actual milk, milk composition percentage and yields, 7% fat corrected milk (7% FCM) yield and milk energy (Kcal / Kg milk) of lactating buffalo cows. The actual milk yield (AMY) and 7% fat corrected milk (7% FCM) yield of dairy buffalo cows were significantly ( $P < 0.05$ ) higher of  $I_2$  than that of  $I_0$ . Similar results were obtained by Monastyrev (1980), Gasanova (1986), Kobeisy and Shetaewi (1992), Kobeisy *et al.* (1992) and Allam *et al.* (2003) they reported that addition of I to the ration of lactating cows and buffaloes increased milk production and 4 or 7% fat correct milk yield. Improvement and increasing in milk yield was observed with animal gropes fed supplemented ration with I, being 14.94 % and 28.67% with groups  $I_1$  and  $I_2$ , respectively. The same increasing trend was shown with 7% FCM yield. The respective values were 16.00% and 45.5. Also Golubev and Sedov(1984), Drebickas *et al.*(1993) and Androsova (1994) showed that addition of potassium iodide to ration of lactating cows increase milk production by 10 – 18%.

The increase in milk yield with I supplementation may be due to higher nutrients digestibility at 180 days (Table 2) and increase in  $T_3$  and  $T_4$ . Hoption (2006) reported that thyroidal hormones play a major role in the control of several

metabolic processes including carbohydrate, fat, protein, vitamin and mineral metabolism. Georgievskii *et al.* (1990) reported that the principal function of iodine is determined by its presence in the thyroid hormones. These hormones are known to regulate basic metabolism, consumption of carbohydrates, proteins and fats the effect of hormones on metabolism involves synthesis of respiratory and other enzymes, which affect intracellular processes of oxidation, oxidative phosphorylation and protein synthesis. Also, Lawrence and Fowler (1997) reported that thyroid hormones influence the function of most organs and stimulate the basic metabolic rate through regulation of the metabolism of carbohydrates, proteins and lipids. Gasanova (1985) reported that increasing the dietary level of iodine from basal values 0.11-0.16 mg /Kg DM to 0.59 - 0.65 mg / Kg DM improved feed utilization for milk production.

Percentages and yield milk constituent (fat, protein, lactose, SNF, TS and milk energy) were significantly ( $P < 0.05$ ) higher in groups  $I_2$  than those of  $I_0$  in (Table 5). Allam *et al.* (2003) reported that supplementing ration with iodine significantly ( $P < 0.05$ ) increase percentages fat, protein and energy contents. Angelow *et al.* (1993) reported that the differences between control and iodine (0.2 mg / kg as KI) for daily yield of milk fat and protein were significant. Todorova *et al.* (1995) found that iodine deficiency decreased milk fat, protein yield of ewes. Ehlers *et al.* (1994) observed that there was trend towards higher milk protein yield which was attributed to higher thyroxin for dairy cows given diet supplemented with iodine. Kobeisy and Shetaewi (1992) found that milk fat% and milk TS% increased by iodine supplemented group in Egyptian buffaloes at mid lactation. Kobeisy *et al.* (1992) found that iodine supplementation in ration buffaloes increase percentages milk constituent (fat, protein, lactose and TS) compared the control group. On the other hand, Norouzian *et al.* (2009) pointed that no significant differences observed in all treatment by different level of iodine, for milk yield and milk composition. Also Nudda *et al.* (2009) found that milk yield and composition were not influenced by KI supplementation, but the first level of KI supplementation increased the content of milk fat significantly compared with that of the unsupplemented group.

#### **Feed intake and feed efficiency:-**

Data in Table (6) indicated that lower dry matter intake was obtained for  $I_1$  and  $I_2$  than that of  $I_0$ , with no significant differences among groups. Data presented in Table (6) showed that the DMI decreased with animal groups fed  $I_1$  and  $I_2$ .

Decreasing in DMI might be due decreasing intake of rice straw with there groups because of I supplementation make a favorable effect for animals to get the best ingredient from ration and also might be to higher digestibility and nutrients value for experimental rations. Norouzian *et al.* (2009) found that decrease of dry matter intake related to feeding high levels of iodine but no significant difference was observed between treatments for DMI. These results are in agreement with El-Hosseiny, *et al.* (2008), Allam *et al.* (2003) and Meyer *et al.* (2008) they found that I supplementation had no significant effect on feed intake. Total intake from both TDN and Total DCP showed no significant difference with I supplementation. Results obtained herein are in agreement with that of El-Hosseiny, *et al.* (2008) and Allam *et al.* (2003).

Feed efficiency was expressed as amount of either milk yield or 7% milk yield produced from one kg DM intake. Results obtained in Table (6) showed that the feed efficiency was significantly ( $p < 0.05$ ) higher with animal groups fed  $I_2$  than those fed  $I_0$ , but the differences between groups fed  $I_0$  and  $I_1$  were not significant. Also, differences between groups fed  $I_1$  and  $I_2$  were not significant. Generally, iodine supplementations tend to higher feed efficiency. Improvement in feed efficiency for groups consumed I supplementation might be attributed to lower DM intake and higher milk yield.

The same previous trend was observed with feed efficiency when expressed as 7% FCM/kg DCP, being 8.89, 9.98 and 12.13 for gropes fed rations  $I_0$ ,  $I_1$  and  $I_2$ , respectively. These results are in harmony with Allam *et al.* (2003) and Lenina (1986) they reported that supplements of I for cattle improve feed efficiency compared to control groups.

#### **Blood parameters:-**

Data in Table (7) illustrated that count of (RBC and WBC), Hb and PCV were significantly ( $P < 0.05$ ) increased on LP and PP in treatment. This effect could be due to increased thyroid hormone and functions of hormone in treated animals. These results are in accordance with shcheglov and Gruzdev, (1989), Spiridon and Hebean. (1988) and Zygmunt (1987). Bedi *et al.* (2000) found that hemoglobin tended to increase, but the differences were non-significant by using level of KI. Also, Sultana *et al.* (2006) reported that the total erythrocyte count (TEC), hemoglobin percent and packed cell volume (PCV) were increased on 60 days of post-treatment in all groups (common salt, iodide salt and Lugol's iodine) compared with control group. They added that the variation might be due to effect of iodine formulation on hemopoiesis.

As shown in Table (7) total protein, T<sub>3</sub> and T<sub>4</sub> concentrations in blood plasma of I<sub>1</sub> and I<sub>2</sub> groups were significantly ( $P < 0.05$ ) greater than of I<sub>0</sub> within two stages LP and PP. Shetaewi *et al.* (1991) found that serum proteins tend to be higher in high treatment of KI than low of KI or control treatments. Vsyakikh *et al.* (1992) reported that total protein significantly increased during the second month of lactation in cows receiving KI. El-Hosseiny, *et al.* (2008) showed that serum total protein significantly increased by supplement of iodine. The increase in plasma protein with iodine addition may be due to the increase in protein synthesis, digestion of protein at the 60 and 180 days in Table (2) and increase in T<sub>3</sub> and T<sub>4</sub>. Freeman, (1983) and El-Masry and Habeeb, (1989) reported that the result of the elevation of anabolic hormone secretion that are responsible for utilization of amino acids and other physiological functions related to metabolic rate. Also, Lawrence and Fowler (1997) reported that thyroid hormones influence the function of most organs and stimulate the basic metabolic rate through regulation of the metabolism of carbohydrates, proteins and lipids. On the other hand Kobeisy and Shetaewi (1992) found that serum total proteins was lower ( $P < 0.01$ ) in I supplemented buffaloes compared with control. Pattanaik *et al.* (2001) found that T<sub>3</sub> and T<sub>4</sub> increased by I supplementation compared to control. Kobeisy and Shetaewi (1992) showed that iodine treated buffaloes had 40% higher ( $P < 0.02$ ) serum triiodothyronine than control buffalos. Khushdeep and Randhawa (2004) found that a significant elevation of plasma T<sub>4</sub> concentration in buffaloes treated with 2 ml of iodized (containing 375 mg iodine / ml) subcutaneously in the brisket region. Also, Rose *et al.*, (2007) found that supplementation with I was associated with higher levels of triiodothyronine and thyroxin in the lambs at birth. Norouzian *et al.* (2009) found that average to T<sub>3</sub> were 90.75, 91.125, 99.50 and 104.75 for control, the control diet plus 2.5, 5 and 7.5 mg Potassium Iodide/kg diet DM, respectively, while T<sub>4</sub> were 3.00, 2.675, 3.237 and 2.80 at the same treatments. In these study the increase in T<sub>3</sub> and T<sub>4</sub> concentrations with animals fed I<sub>1</sub> and I<sub>2</sub> treated animals may be due to increasing the availability of I to the thyroid to meet the increasing demand of thyroid hormone during pregnant and lactation period.

No significant difference was observed among different treatments for albumin in LP but significant difference was observed during PP period (Table7). While, significant difference was observed among treatments for globulin during LP and PP period. Also no significant difference was observed among treatments for A / G ratio during LP and PP. The increase in serum albumin and globulin with

iodine addition may be due to the increase in protein synthesis, total protein in blood and digestion of protein at the 60 and 180 days as show in Table (2). Shetaewi *et al.*, (1991) founded that serum albumin tended to be higher in high iodine treatment than low iodine or control treatment. They added that serum globulin tended to be higher in iodine treatment than control treatment. El-Hosseiny, *et al.* (2008) showed that serum albumin no significant by supplement of iodine. They added that globulin was significant by supplement of iodine. On the contrary Kobeisy and Shetaewi (1992) found that globulins was lower ( $P < 0.01$ ) and albumin tended to be lower in I<sub>2</sub> supplemented buffaloes compared with control.

Data in Table (7) illustrated that glucose was significantly ( $P < 0.05$ ) increased with groups fed I<sub>1</sub> and I<sub>2</sub> during PP period and it showed no significant difference during LP period. Bedi *et al.* (2000) reported that serum glucose level was significantly ( $P < 0.01$ ) in goats by using level of KI. Davis *et al.* (1988) found that T<sub>4</sub> injection (20 mg/d) for 4 days during successive 16 days experimental period increased mammary glucose uptake by 45%. Kobeisy and Shetaewi (1992) reported that serum glucose concentration was higher in iodine supplemented buffaloes than in control buffaloes. Draganov *et al.* (1991) found that supplemented diets (iodine, cobalt and copper) increased the concentration of blood glucose by 2.1 and 5.7 %. Vsyakikh *et al.* (1992) revealed that blood glucose significantly increased during the second month of lactating in cows receiving KI compared with control group. These results also probably attributed to the higher of blood plasma glucose and albumin concentration of animals fed iodine supplemented ration as shown in Table (7). It led to an increase in milk lactose synthesis and a consequent increase in milk production. This results may be due to the high demand for energy especially glucose as a main source of energy during late pregnancy. Manston and Allen (1981) reported that reduction in blood sugar level in the late pregnancy and 1 - 2 days after parturition indicates a heavy demand for glucose in late gestation and early lactation. Similar results were reported by El-Malky (2007) who found that Egyptian buffaloes blood glucose was decreased during late pregnancy and increased in the 3 months of postpartum. Significant Triglycerides was observed between treatments in LP and PP in Table (7). Kobeisy and Shetaewi (1992) showed that buffaloes treated with iodine in mid-lactation period had more 67% ( $P < 0.05$ ) triglycerides than control group. On the other hand, Davis, *et al.* (1988) found that was no detectable effect of T<sub>4</sub> injection on plasma triglycerides concentration. Lawrence and Fowler (1997) reported that thyroid hormones influence the function of most organs and

stimulate the basic metabolic rate through regulation of the metabolism of carbohydrates, proteins and lipids.

Also, the effect of treatment on blood urea nitrogen (BUN) was significant ( $P<0.05$ ), whereas treated groups had a higher means compared with untreated group in both pre and postpartum periods. This effect could be due to increased thyroid hormone in treated animals which in turn result in a slight increase in protein catabolism. Kobeisy and Shetaewi (1992) found that serum urea nitrogen tended to be slightly higher in buffaloes treated with I<sub>2</sub> in mid-lactation period compared with control group.

Data in Table (7) showed that cholesterol was lower in I<sub>1</sub> and I<sub>2</sub> than I<sub>0</sub> during LP and PP. It is interesting to notice the reverse relationship between T<sub>3</sub> and cholesterol concentrations that occurred during treatments. Shetaewi *et al.*, (1991) found that serum cholesterol concentration was 15% lower in lambs that received 80 mg KI/head/wk compared with controls. Also, Kobeisy and Shetaewi (1992) showed that cholesterol concentration was 20% less in treated group than that control group for buffaloes in mid lactation period supplemented with I<sub>2</sub> in diet. Kaneko (1980) reported that serum cholesterol was previously used as an index of thyroid function because hypothyroidism is generally associated with an elevation in serum cholesterol. Bergersen (1979) suggested that thyroid hormones increase cholesterol synthesis and enhance the liver's ability to excrete cholesterol in the bile. But the effect on cholesterol excretion is greater than that on cholesterol synthesis; the net result is a decrease in plasma cholesterol concentration. However, serum cholesterol varies with a variety of factors unrelated to thyroid activity such as the nature of the diet, hepatic function and other factors (Kaneko, 1980).

As shown in Table (7) EST 17 $\beta$  concentrations in blood of I<sub>1</sub> and I<sub>2</sub> groups were significantly ( $P<0.05$ ) greater than that of I<sub>0</sub> within two stages (late pregnancy LP and Postpartum PP). Data showed that level of plasma P<sub>4</sub> was lower in treated groups in prepartum period than that in the control group. Concentrations of P<sub>4</sub> tended to change slightly during the postpartum first months where the ovaries are still inactive. This may suggest that the sharp increase of EST17 $\beta$  and decrease of P<sub>4</sub> just before parturition was observed by Gordon (1996). Analysis of variance revealed that prepartum and postpartum P<sub>4</sub> concentration was affected significantly by different experimental groups. P<sub>4</sub> concentration tended to be high in I<sub>2</sub> than I<sub>1</sub> and I in postpartum period. This

finding indicated regression of corpus luteum in all cows at parturition (El-Moghazy, 2003). It is of interest to note that, average P<sub>4</sub> concentration at postpartum period was tends to be high in treated groups than control group. In general, the presented data reflected that treatment lactating buffalo cows on different iodine levels did not affect P<sub>4</sub> profile prepartum and postpartum periods. The diminished levels of P<sub>4</sub> before parturition was also stated earlier by several authors ( Kamonpatana *et al.*, 1981; Perera, 1981) working on buffaloes. Smith *et al.* (1973) reported that the decline of P<sub>4</sub> stimulates the uterus to be under estrogen dominance at a time when coordinated uterine contractions begin in cattle. In contrary, Nanda *et al.* (1981) noticed that P<sub>4</sub> levels in normal pregnant buffaloes remained almost constant from day 60 before calving to the last week of pregnancy. The importance of P<sub>4</sub> drop before onset of calving is to prevent the inhibitory effect of P<sub>4</sub> upon myometrial contraction as well as the release of oxytocin (Batra *et al.*, 1982).

Data illustrate that the level of EST17 $\beta$  increased linearly toward time of parturition in treated and untreated groups of buffalo, however clear differences were detected between both groups, whereas treated groups recorded higher EST17 $\beta$  levels than control group. After delivery, concentrations of EST17 $\beta$  decreased sharply in both groups of buffaloes in comparison with its level during late pregnancy. It seems likely that estradiol concentration in blood increases concomitantly with the decline in P<sub>4</sub> concentration (El- Wardani *et al.*, 1998). Normal expulsion of fetal membranes requires a rise in EST-17 $\beta$  before calving accompanied by a gradual and sustained fall in P<sub>4</sub> (Prakash and Madan, 1986). Moreover, normal calving requires softening and dilation of the cervix, particularly during late pregnancy due to the influence of relaxin and estrogen when P<sub>4</sub> dominance declines and uterine prostaglandin production is increasing (Taverne, 1992). This, P<sub>4</sub> decline is accompanied by a gradual increase in PGF<sub>2 $\alpha$</sub>  until 24 h before calving (Gordon, 1996).

The increase in blood studied constituents may be due to the role of iodine in improving all nutrient digestibility especially CP (Table, 2) of buffalos fed I, and also may be probably led to an increase in the absorption rate from the digestive tract, thus blood constituents of the supplemented animals reflected a corresponding increase of these values. These results are in agreement with Drebickas *et al.* (1993) who found that supplementary iodine improved chemical composition of blood in cows compared with the control diet.

**Reproductive aspects:-**

Mean values of some reproductive traits of the experimental dams are presented in table (8). Prepartum treatment with iodine reduced the period of fetal membrane expulsion compared to the other groups ( $P<0.05$ ). Buffaloes of group  $I_2$  had significant shorter intervals for uterine involution, onset of the 1<sup>st</sup> heat and days open when compared with other groups. In consequence, group  $I_2$  had the least ( $P < 0.05$ ) calving interval ( $367.2\pm24.1$  days). On the contrary to that, the control group  $I_0$  exhibited longer time to attain its uterine involution ( $52.6\pm9.16$  days) or reaching conception date ( $93.2\pm18.28$ ). Buffaloes in group  $I_2$  required only one service to conceive. Gestation periods for groups fed ration containing 0.5 mg I ( $I_2$ ) were relatively less than that of the other groups, presumably due to amelioration in fetal growth imposed by increased metabolism. Group  $I_2$  achieved a smallest value in the number of services per conception followed by groups  $I_1$  and  $I_0$ . It seems that positive impact of iodine treatments were shown on reproduction in buffaloes.

In coincidence with these findings, Sushma-Chhabra, *et al.*, (2007) found that iodine supplementation improved oestrus signs, whereas the onset of oestrus in the treatment group ranged from 4-33 days. Also, iodine treatment groups showed a better conception rate compared to the control group. Mavi and Bahga (2005) found that treatment groups improved reproductive efficiency, whereas eight heifers in the treatment group (44%) exhibited oestrus within 60 days of supplementation and four heifers (22%) conceived. In control group, only one heifer (12.5%) exhibited oestrus and none conceived in the same period. Panchal *et al.*, (1991) found that the overall pregnancy rate in the treated animals (42.17%) was significantly higher than that of the untreated controls (17.05%). Bahga and Gangwar (1989) measuring plasma protein-bound iodine in buffaloes after calving during summer and winter seasons and found that values were lower in summer than in winter, and lower in buffaloes requiring less than 30 days for uterine involution than in those requiring more than 30 days: they were lowest in buffaloes requiring more than 50 days for restoration of ovarian follicular development. Bahga (1989) reported that poor reproductive efficiency, postpartum oestrus interval, service period and number of services for conception were observed in animals in the low plasma protein bound iodine group. Gasanov (1991) reported that supplemented buffaloes with injections of tetravitamin and supplemented of trivitan, potassium iodide, sodium selenite, calcium triphosphate, proserine, cobalt

chloride or zinc sulphate reduced service interval, improved conception to first service.

Sharawy *et al.*, (1987) demonstrated that animals treated with patent thyroid preparation exhibited the highest percentage of heat appearance (86.7%) with 73.3% pregnancy rate, while group treated with supplements of crude iodinated casein revealed that 84.6% came in heat and 69.2% became pregnant and animals treated with potassium iodide revealed that 50% manifested heat while 40 conceived. In sheep, Ferri *et al.*, (2003) reported that 100% of treated ewes mated with treated rams were pregnant vs 37% of the control ewes mated with control rams, also, results demonstrated that iodine supplementation restores fertility of sheep living in iodine deficient areas.

**Pregnancy rate:-**

Table (9) showed that in control group three buffaloes came in heat after 65 days after delivery, three of them ovulated and conceived. In the second group, five animals manifested oestrus after 54 days and were ovulated while four of them got pregnant. In the third group all animals exhibited oestrus signs within 45 days, all were ovulated and all will pregnant (100%). Results revealed higher pregnancy rate in  $I_2$  (100%) than in groups  $I_1$  (66.67%) and  $I_0$  (50.0%). This may in relation to level of iodine supplementation, being better with the high (0.5 mg I per kg DM intake /h/d) than low level (0.3 mg I per kg DM intake /h/d) of supplementation. The marked improvement in pregnancy rate of buffaloes supplemented with iodine in  $I_2$  compared with  $I_0$  is in agreement with the findings of Sharawy *et al.* (1987) who found that treatment with potassium iodide revealed that 50% of the treated animals came in heat while 40% got pregnant. Also, Glotra, *et al.*, (1969) found that significant improvement in relation to heat appearance and pregnancy rate in buffaloes supplied by potassium iodide than in control.

**Health status of delivered calves:-**

As shown in Table (10) the control dams delivered only 4 calves because there was one case of abortion and one case of stillbirth, meanwhile, treated dams delivered 12 healthy calves. The calves from treated dams showed higher body weight, growth rate/day and better vitality in comparison with the control. Likewise, body weight and growth rate/day were higher in newly born calves from  $I_2$  than  $I_1$  and  $I_0$  groups. In addition, no mortality in the 1st month occurred between the newborn calves resulted from iodine treated buffaloes compared to the control group.

Moreover, the control calves showed more severe pneumonia and enteritis as a result of which

2 (33.33%) calves died. The present results support the other views (Mavi *et al.*, 2005) who found that, iodine treatment to late pregnant dams induced better state of reproductive and delivery with no retained placenta or stillbirth in comparison with the control group. The newly born calves were of heavier body weight, better healthy status and highly resistance to disease. The pre and post-partum supplementation with iodine improved the reproductive efficiency of Egyptian buffaloes (Sharawy *et al.*, 1987). Consequently, a high immunoglobulin-G concentration was observed in calves supplemented with iodine. Circulating IgG had been related to pre-weaning growth (Nocek, 1984) as well as long term performance of calves (Wittum, 1995), thus some commercial calf raisers will pay dairy producers a premium for providing calves with serum total protein that exceeds some critical threshold (usually >5.2 to 5.5 g of total protein/dl of serum). Others will reduce the amount they pay to the producer if total protein is too low. Although passive immunity has an important effect on calf health, there are a number of other factors that influence the overall cost of morbidity and mortality on a calf raising operation. These other factors include the level of exposure of calves and level of stress to which calves are exposed. Another critical control point during the calf's life is the first 24 hours. Consumption of colostrum is essential to provide animals with the antibodies and other proteins that calves need to stay

healthy. The amount of colostrum (and immunoglobulin, or IgG) consumed determines amount of passive immunity and resistance to disease. When calves consume insufficient amounts of IgG from colostrum within the first 24 hours of life, they are much more susceptible to developing disease and possibly dying. A major reason that pre-weaning mortality is higher than optimum (defined as less than 5% of calves born alive) is due to inadequate IgG intake (Quigley, 2005). Measuring a calf's level of passive immunity within the first week of life allows the producer to know the effectiveness of the colostrum management and calf feeding program. Because this is so important to the health and survival of the calf, it is an essential part of monitoring the overall heifer operation. However, the importance of achieving adequate levels of colostral immunoglobulins to protect the neonate from enteric disease and septicemia has long been recognized (Robinson, 1988).

#### 4. Conclusion

It could be recommended that using iodine supplementation at level (0.5 mg I per kg DM intake /h/d)) in female buffaloes ration tended to improve the digestion, nutritive values, immunity, milk production, milk composition and Reproductive parameters.

**Table (1).** The chemical composition of feed ingredients and the calculated composition of the Experimental rations.

Items	Chemical composition as DM basis (%)						
	DM	OM	CP	CF	EE	Ash	NFE
<b>Chemical composition of the ingredients :</b>							
CFM*	91.54	92.92	16.79	10.31	4.59	7.08	61.23
BH	90.17	84.31	14.89	24.83	1.78	15.69	42.81
RS	92.91	81.51	3.59	41.80	1.04	18.49	35.08
<b>Calculated chemical composition of tested rations:</b>							
I <sub>0</sub>	91.54	88.92	13.77	19.51	3.32	11.08	52.32
I <sub>1</sub>	91.49	89.19	14.15	18.68	3.40	10.81	52.96
I <sub>2</sub>	91.42	89.57	14.67	17.54	3.52	10.43	53.84

\*CFM; concentrate feed mix contained in percentage ; 37% yellow corn , 30% undecorticated cotton seed , 20% wheat bran, 6.5% rice bran, 3% molasses , 2.5% limestone, 1% common salt,.

**Table (2).** Effect of iodine supplementation on nutrients digestibility and nutritive value of the experimental rations fed to buffalo cows.

Items	60 Days				180 Days				SEM	
	I <sub>0</sub>	I <sub>1</sub>	I <sub>2</sub>	SEM	I <sub>0</sub>	I <sub>1</sub>	I <sub>2</sub>			
DM	65.42	65.99	66.21	0.32	66.96 <sup>c</sup>	68.50 <sup>b</sup>	71.23 <sup>a</sup>	0.53		
OM	67.12	68.08	68.69	0.39	69.84 <sup>b</sup>	71.53 <sup>ab</sup>	73.34 <sup>a</sup>	0.52		
CP	58.08 <sup>b</sup>	59.22 <sup>ab</sup>	60.08 <sup>a</sup>	0.33	59.24 <sup>c</sup>	61.99 <sup>b</sup>	64.91 <sup>a</sup>	0.66		
CF	52.31	52.50	53.30	0.27	53.11 <sup>b</sup>	56.79 <sup>a</sup>	58.50 <sup>a</sup>	0.68		

<b>EE</b>	85.66	86.26	86.89	0.34	87.54 <sup>c</sup>	89.82 <sup>b</sup>	92.38 <sup>a</sup>	060
<b>NFE</b>	70.78 <sup>b</sup>	71.61 <sup>ab</sup>	72.74 <sup>a</sup>	0.34	71.58 <sup>c</sup>	75.83 <sup>b</sup>	78.83 <sup>a</sup>	084
<b>Nutritive value (%)</b>								
<b>TDN</b>	61.63 <sup>b</sup>	62.70 <sup>b</sup>	64.21 <sup>a</sup>	0.41	62.51 <sup>c</sup>	67.08 <sup>b</sup>	69.54 <sup>a</sup>	0.95
<b>DCP</b>	8.00	8.38	8.81	0.20	8.16 <sup>b</sup>	8.77 <sup>a b</sup>	9.52 <sup>a</sup>	0.15

Means bearing different superscripts in the same raw are significantly different ( $P < 0.05$ ).

**Table (3).** Effect of iodine supplementation on Body weight of buffalo dams during pre- and post- partum and new born calves in the different experimental groups.

Items	I <sub>0</sub>	I <sub>1</sub>	I <sub>2</sub>	SEM
<b>Dam body weight LP (kg)</b>	497	495	496	2.58
<b>Dam body weight PP (kg)</b>	451 <sup>a</sup>	445 <sup>b</sup>	439 <sup>c</sup>	1.90
<b>Calf birth weight (CBW) kg</b>	35.80 <sup>b</sup>	37.00 <sup>b</sup>	41.00 <sup>a</sup>	0.70
<b>Fetal fluid, kg</b>	10.40	10.8	11.00	0.45
<b>CBW / Dam post-partum (%)</b>	7.94 <sup>b</sup>	8.31 <sup>b</sup>	9.34 <sup>a</sup>	0.48
<b>Calf weaning weight (kg)</b>	88.00 <sup>c</sup>	93.40 <sup>b</sup>	99.20 <sup>a</sup>	1.40
<b>Total gain of calf (kg)</b>	52.2 <sup>b</sup>	56.4 <sup>a</sup>	58.2 <sup>a</sup>	0.79
<b>Daily gain of calf (g/day)</b>	0.58 <sup>b</sup>	0.63 <sup>ab</sup>	0.65 <sup>a</sup>	0.07

Means bearing different superscripts in the same raw are significantly different ( $P < 0.05$ ).

**Table (4).** Immunoglobulin concentration (mg/ml) in colostrums of buffaloes as affected by iodine treatments.

<i>Ig fraction</i>	<i>Day</i>	<i>Treatments</i>			SEM
		I <sub>0</sub>	I <sub>1</sub>	I <sub>2</sub>	
<b>Ig A</b>	<b>1<sup>st</sup></b>	3.28 <sup>b</sup>	3.67 <sup>ab</sup>	4.25 <sup>a</sup>	0.14
	<b>2<sup>nd</sup></b>	3.19 <sup>b</sup>	3.26 <sup>b</sup>	4.16 <sup>a</sup>	0.19
	<b>3<sup>rd</sup></b>	2.65	2.95	3.05	0.24
<b>Ig M</b>	<b>1<sup>st</sup></b>	4.24	4.71	4.78	0.17
	<b>2<sup>nd</sup></b>	3.75 <sup>b</sup>	4.25 <sup>ab</sup>	4.50 <sup>a</sup>	0.19
	<b>3<sup>rd</sup></b>	3.36 <sup>b</sup>	3.85 <sup>ab</sup>	4.31 <sup>a</sup>	0.19
<b>Ig G</b>	<b>1<sup>st</sup></b>	34.53 <sup>c</sup>	42.50 <sup>b</sup>	46.67 <sup>a</sup>	1.03
	<b>2<sup>nd</sup></b>	33.98 <sup>b</sup>	35.75 <sup>b</sup>	40.20 <sup>a</sup>	1.02
	<b>3<sup>rd</sup></b>	32.81 <sup>b</sup>	34.00 <sup>b</sup>	39.00 <sup>a</sup>	1.05

Means bearing different superscripts in the same raw are significantly different ( $P < 0.05$ ).

**Table (5).** Effect of Iodine supplementation on milk yield and composition of lactating buffalo cows.

Item	I <sub>0</sub>	I <sub>1</sub>	I <sub>2</sub>	SEM
<b>Actual milk yield, kg / day</b>	8.30 <sup>b</sup>	9.54 <sup>ab</sup>	10.68 <sup>a</sup>	0.40
<b>7% fat correct milk yield (FCM), kg / day</b>	8.00 <sup>b</sup>	9.28 <sup>ab</sup>	11.64 <sup>a</sup>	0.58
<b>Milk composition ( % )</b>				
<b>Fat, %</b>	6.64 <sup>b</sup>	6.72 <sup>ab</sup>	7.79 <sup>a</sup>	0.23
<b>Fat yield (kg)</b>	0.55 <sup>b</sup>	0.64 <sup>ab</sup>	0.83 <sup>a</sup>	0.11
<b>Protein, %</b>	4.12 <sup>b</sup>	4.77 <sup>ab</sup>	5.65 <sup>a</sup>	0.24
<b>Protein yield (kg)</b>	0.34 <sup>b</sup>	0.46 <sup>ab</sup>	0.60 <sup>a</sup>	0.09
<b>Lactose, %</b>	3.68 <sup>b</sup>	4.37 <sup>ab</sup>	4.92 <sup>a</sup>	0.22
<b>Lactose yield (kg)</b>	0.31 <sup>b</sup>	0.42 <sup>ab</sup>	0.53 <sup>a</sup>	0.05
<b>Solid non fat (SNF) , %</b>	8.53 <sup>b</sup>	9.82 <sup>ab</sup>	11.14 <sup>a</sup>	0.41
<b>SNF yield (kg)</b>	0.71 <sup>b</sup>	0.94 <sup>ab</sup>	1.19 <sup>a</sup>	0.15
<b>Total solid (T.S) , %</b>	15.17 <sup>b</sup>	16.54 <sup>ab</sup>	18.93 <sup>a</sup>	0.57
<b>T.S yield (kg)</b>	1.26 <sup>b</sup>	1.58 <sup>b</sup>	2.02 <sup>a</sup>	0.23
<b>Ash, %</b>	0.73 <sup>a</sup>	0.68 <sup>ab</sup>	0.61 <sup>b</sup>	0.02

<b>Milk energy (Kcal / Kg milk)</b>	943 <sup>b</sup>	1064 <sup>ab</sup>	1188 <sup>a</sup>	35.69
Means bearing different superscripts in the same raw are significantly different (P < 0.05).				

**Table (6).** Feed intake and Feed efficiency of lactating buffalo cows as affected by the iodine supplementation levels.

Items	I <sub>0</sub>	I <sub>1</sub>	I <sub>2</sub>	SEM
<b>Daily feed DM intake (Kg /h/d) as:</b>				
<b>CFM</b>	6.6	6.6	6.6	-
<b>BH</b>	2.2	2.2	2.2	-
<b>R.S</b>	2.2	1.8	1.3	-
<b>Total DMI</b>	11	10.6	10.1	0.11
<b>Total TDNI</b>	6.88	7.11	7.02	0.07
<b>Total DCPI</b>	0.90	0.93	0.96	0.01
<b>7% fat correct milk yield(FCM), kg / day</b>	8.00 <sup>b</sup>	9.28 <sup>ab</sup>	11.64 <sup>a</sup>	0.58
<b>Feed efficiency:</b>				
<b>Milk yield / DMI</b>	0.75 <sup>b</sup>	0.90 <sup>ab</sup>	1.06 <sup>a</sup>	0.05
<b>7% FCM / kg DM</b>	0.73 <sup>b</sup>	0.88 <sup>a b</sup>	1.15 <sup>a</sup>	0.07
<b>7% FCM / kg TDN</b>	1.16 <sup>b</sup>	1.31 <sup>ab</sup>	1.66 <sup>a</sup>	0.06
<b>7% FCM / kg DCP</b>	8.89 <sup>b</sup>	9.98 <sup>b</sup>	12.13 <sup>a</sup>	0.10

Means bearing different superscripts in the same raw are significantly different (P < 0.05).

**Table (7).** Means concentrations of blood plasma of buffalo cows groups supplemented with dietary iodine.

Items	LP			SEM			PP		SEM
	I <sub>0</sub>	I <sub>1</sub>	I <sub>2</sub>	I <sub>0</sub>	I <sub>1</sub>	I <sub>2</sub>			
<b>RBC count x10<sup>6</sup>/mm<sup>3</sup></b>	6.45 <sup>b</sup>	6.65 <sup>b</sup>	7.94 <sup>a</sup>	0.08	7.11 <sup>c</sup>	7.84 <sup>b</sup>	8.81 <sup>a</sup>	0.11	
<b>WBC count x10<sup>3</sup>/mm<sup>3</sup></b>	5.57 <sup>b</sup>	6.11 <sup>b</sup>	6.97 <sup>a</sup>	0.11	7.44 <sup>b</sup>	7.74 <sup>a b</sup>	8.22 <sup>a</sup>	0.07	
<b>Hemoglobin (Hb) g/dl</b>	7.14 <sup>c</sup>	7.72 <sup>b</sup>	8.97 <sup>a</sup>	0.09	7.85 <sup>c</sup>	9.58 <sup>b</sup>	11.24 <sup>a</sup>	0.10	
<b>PCV (%)</b>	30.58 <sup>c</sup>	33.47 <sup>b</sup>	34.42 <sup>a</sup>	0.50	31.83 <sup>c</sup>	34.33 <sup>b</sup>	35.62 <sup>a</sup>	0.56	
<b>T. protein (g/dl)</b>	6.22 <sup>b</sup>	6.89 <sup>a</sup>	7.18 <sup>a</sup>	0.11	7.14 <sup>c</sup>	7.96 <sup>b</sup>	8.35 <sup>a</sup>	0.13	
<b>Albumin (A) (g/dl)</b>	3.06	3.20	3.21	0.02	3.35 <sup>b</sup>	3.81 <sup>ab</sup>	3.90 <sup>a</sup>	0.03	
<b>Globulin (G) (g/dl)</b>	3.16 <sup>b</sup>	3.69 <sup>ab</sup>	3.97 <sup>a</sup>	0.03	3.79 <sup>b</sup>	4.15 <sup>ab</sup>	4.45 <sup>a</sup>	0.05	
<b>A / G ratio</b>	0.97	0.87	0.81	0.02	0.88	0.92	0.88	0.01	

<b>Glucose(mg/dl)</b>	54.95	55.84	56.71	0.27	60.24 <sup>c</sup>	65.46 <sup>b</sup>	70.12 <sup>a</sup>	0.24
<b>BUN (mg/dl)</b>	30.52 <sup>c</sup>	36.47 <sup>b</sup>	43.98 <sup>a</sup>	0.91	36.06 <sup>b</sup>	45.10 <sup>a</sup>	51.21 <sup>a</sup>	0.85
<b>Triglycerides (mg/dl)</b>	21.69 <sup>b</sup>	23.47 <sup>ab</sup>	24.55 <sup>a</sup>	0.31	21.49 <sup>c</sup>	25.75 <sup>b</sup>	28.52 <sup>a</sup>	0.34
<b>Cholesterol (mg/dl)</b>	80.51 <sup>a</sup>	79.45 <sup>a</sup>	75.11 <sup>b</sup>	0.51	87.22 <sup>a</sup>	83.54 <sup>c</sup>	80.88 <sup>b</sup>	0.61
<b>T3 (ng/dl)</b>	98.56 <sup>c</sup>	110.45 <sup>b</sup>	115.55 <sup>a</sup>	2.42	103.25 <sup>c</sup>	121.36 <sup>b</sup>	130.59 <sup>a</sup>	5.22
<b>T4 (ug/dl)</b>	2.14 <sup>b</sup>	3.54 <sup>a</sup>	3.98 <sup>a</sup>	0.22	2.74 <sup>c</sup>	4.08 <sup>b</sup>	4.97 <sup>a</sup>	0.23
<b>P4 (ng/dl)</b>	3.49 <sup>a</sup>	3.03 <sup>c</sup>	3.32 <sup>b</sup>	0.16	1.39 <sup>b</sup>	1.70 <sup>a</sup>	1.76 <sup>a</sup>	0.11
<b>EST 17<math>\beta</math> (pg/ml)</b>	79.49 <sup>c</sup>	105.25 <sup>b</sup>	118.58 <sup>a</sup>	5.32	36.74 <sup>c</sup>	40.79 <sup>b</sup>	45.33 <sup>a</sup>	1.38

Means bearing different superscripts in the same raw are significantly different ( $P < 0.05$ ).

**Table (8).** Means of some postpartum reproductive traits of buffaloes as affected by iodine treatments.

Item	Treatments			
	I <sub>0</sub>	I <sub>1</sub>	I <sub>2</sub>	SEM
<b>Placental expulsion (hr)</b>	9.83 <sup>b</sup>	8.73 <sup>b</sup>	6.51 <sup>a</sup>	1.2
<b>Uterine involution (days)</b>	52.6 <sup>a</sup>	43.1 <sup>b</sup>	31.1 <sup>c</sup>	9.16
<b>Onset of 1<sup>st</sup> heat</b>	47.5 <sup>b</sup>	54.3 <sup>a</sup>	44.7 <sup>c</sup>	7.98
<b>No services / conception</b>	2.25	1.71	1.00	0.58
<b>Days open</b>	93.2 <sup>a</sup>	86.1 <sup>b</sup>	62.2 <sup>c</sup>	18.28
<b>Gestation period (days)</b>	325.6	328.6	305.0	
<b>Calving interval (days)</b>	418.8 <sup>a</sup>	414.7 <sup>a</sup>	367.2 <sup>b</sup>	24.07

Means bearing different superscripts in the same raw are significantly different ( $P < 0.05$ ).

**Table (9).** Effect of iodine administration on the incidence of estrus, ovulation and conception in buffaloes.

Groups	No. of animals	Days from delivery until appearance of 1 <sup>st</sup> heat	Response of treatment					
			Heat		Ovulation		Conception	
			No.	%	No.	%	No.	%
I <sub>0</sub>	6	65	3	50.0	3	50.0	3	50.0
I <sub>1</sub>	6	54	5	83.33	4	66.67	4	66.67
I <sub>2</sub>	6	45	6	100.0	6	100.0	6	100.0

**Table (10).** Mean values of health status of newborns of buffaloes treated with iodine supplementation.

Items	Health status of delivered calves		
	I <sub>0</sub>	I <sub>1</sub>	I <sub>2</sub>
<b>Aborted feti</b>	1	0	0
<b>Still birth</b>	1	0	0
<b>Mortality at 1<sup>st</sup> month</b>	2/6 (33.33%)	0/6 (0%)	0/6 (0%)
<b>Survival rate</b>	4/6 (66.67%)	6/6 (100%)	6/6 (100%)

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9/30/2010

# Integrated theoretical model to enhance neonatal screening for sickle hemoglobinopathies in the wake of predictive, preventive, personalized and participatory medicine

E. William Ebomoyi, Ph.D., Professor

Department of Health Studies, College of Health Sciences, Chicago State University, Chicago Illinois and he serves as a Consultant in International Health for the American Public Health Association, 9501 South King Drive, Douglas Hall 127, Chicago, Illinois 60628-1598, USA

[eebomoyi@csu.edu](mailto:eebomoyi@csu.edu), 773-995-2527

**ABSTRACT:** This study utilized the integrated theoretical model (ITM) to assess strategies to ameliorate screening for sickle hemoglobinopathies in the age of genomic medicine. Also discussed, is the relevance of predictive, preventive, personalized and participatory interventions. Comparison was made between universal and targeted screening. The international guidelines for neonatal screening were reiterated. The self-efficacy and empowerment of mothers is crucial in ensuring that they effectively participate in the treatment and follow-up of their new-born babies. We emphasized the compliance with the ethical, legal and social implications of newborn screening for genetic diseases.

[E. William Ebomoyi. Integrated theoretical model to enhance neonatal screening for sickle hemoglobinopathies in the wake of predictive, preventive, personalized and participatory medicine. Journal of American Science 2010;6(11):531-537]. (ISSN: 1545-1003).

**Keywords:** integrated theoretical model (ITM); ameliorate; sickle hemoglobinopathies; genomic medicine; neonatal screening; ethical; legal; social; genetic diseases

## Background

Sickle cell disease is a recessive hereditary disorder. "This disease involves the possession of two abnormal allelomorphic genes related to hemoglobin formation, at least one of which is the sickle cell gene, the genotypes constituting sickle disease being SS, Sc, S Thal, SE, SF 'high gene' and SD."(Konotey-Ahulu, 1974). Sickle cell is usually inherited in an autosomal recessive Mendelian pattern. The clinical abnormality caused by sickle cell anemia includes manifestations of severe pain, leg ulcers, and swellings of the joints, pains in the abdomen, arms, fatigue, and sometimes death. For pediatric age, splenic sequestration, sepsis and stroke are more common and carries a high mortality, whereas for adult cohort, eye disease and organ damage and pulmonary hypertension are quite frequent (Calloway, 1977).

The observable differences between sickle cell disease and sickle cell trait (SCT) were discovered 61 years ago. The differences lie in the quantity erythrocytes of sickle cell trait and sickle cell disease and the involvement of greater reduction in the partial pressure of oxygen which is required for a significant quantity of the trait to sickle than to sickle cell disease. In sickle cell trait, a person inherits one normal hemoglobin gene (A) from one parent and one abnormal gene (S) from the other parent. With regards to sickle cell disease two abnormal genes are inherited, one from each parent. Since sickle cell disease is a hereditary disorder, the specific mechanism for this genetic inheritance follows the Mendelian pattern (Kene, 1978).

Regarding hemoglobinopathies, these are conditions that affect the nature and proportion of hemoglobin which individuals have in their red blood cells. Hmoglobinopathies is the clinical term used to describe the presence of abnormal hemoglobin

production in the blood. Although sickle cell disease is the most common hemoglobinopathy in this century, and with innovations in genomic technologies, a litany, of sickle hemoglobinopathies will be genotyped and diagnosed. We reiterate that the success of the Human Genome Project (HGP) in mapping the entire human genome is a triumph comparable to the development of the theory of relativity, and it opened the door to a new branch of biomedical science, genomics. Genomics differs from the more established discipline of genetics in that it examines not only existing characteristics of the genome but also those related to the environment (haplotypes) and biological inheritance. In particular, genomics holds out the potential for early identification of disease and disease risk, preventive strategies, “personalized medicine” and pharmacology (Ebomoyi and Ebomoyi, 2010).

Against this background, the study described here was designed to explore adoption of integrated theoretical model to enhance screening for sickle hemoglobinopathies in the age of genomic medicine, define and accentuate the crucial nature of predictive, preventive, personalized and participatory treatment of patients, assess relevance of universal and targeted screening for sickle hemoglobinopathies, identify international guidelines for neonatal screening, succinctly discuss the ethical, legal, financial and social implications of neonatal screening; emphasizing the empowerment of women and the importance of trait counseling are valid primary preventive initiatives.

#### Integrated Theoretical Model

The two distinct models most applicable in neonatal screening for sickle hemoglobinopathies involve the integration of the Social Cognitive Theory (SCT) into the Health Belief Model (HBM)(Rosenstock,1974). The HBM hypothesizes that health-related actions depends upon individual's view of perceived susceptibility to a disease (X), such as sickle hemoglobinopathies particularly in a society where there is no premarital counseling. This situation is rampant in rural America, South America, Africa, South East Asia and the Mideast. The second issue is the belief that one is susceptible to a disease that is very severe. The classical symptoms of sickle hemoglobinopathies are many and varied. There are specific variants of sickle hemoglobinopathies that are life-threatening while very few variants confer mild distress on patients. At present, there is no known cure for sickle hemoglobinopathies (Ebomoyi, 2009).

The HBM also emphasizes the belief that compliance with health recommendation would be relevant in reducing the perceived threat. This process is dependent on both demographic and structural variables as education; that is, knowledge about the disease and prior contact with it. The cues to actions include mass media campaigns advice from others, illness of family members, magazine articles and reminder postcards from physicians (Maiman and Becker, 1974). Sickle hemoglobinopathies are quite rampant among people of African ancestry and this disease has a frequency of 1 in 8 African-Americans as carrier of the disease and 1 out of 400 carrying the autosomal recessive genes doubly homozygous (Ebomoyi, Cherry, 2010). The likelihood of complying with the recommended actions occurs once the perceived benefits of preventive actions outweigh the perceived barriers (Figure 1) Bandura (1977). (SCT) made two cogent contributions to the HBM. The first is emphasis on several sources of information for acquiring expectations particularly on the informative and motivational role of reinforcement and by observational learning through modeling. A second pertinent contribution is the introduction of the concept of self-efficacy; which means “the conviction that one can successfully execute the behavior required to produce the outcome.”(Bandura, 1977) This integrated model has been used for many screening activities involving genetic services, family health and planning and other behavioral health problems (Bandura, 1977).

In United States, neonatal screening for hemoglobinopathies has been well established in many of the urban tertiary health care centers. At present the screening for hemoglobinopathies occurs in 43 states and in the District of Columbia, Puerto Rico and the virgin island Screening is now well established by the State Department of Laboratory and Federally mandated Newborn screening program. Implementation occurs in the hospitals where all newborns are screened for hemoglobinopathies and other disorders. Annually, screening at States' level has identified over 2000 infants with sickle diseases and additional 50,000 newborn with sickle cell trait (Day et al; 1977). Once identified, children having the disease are automatically referred to their pediatrician of record or hematologist as identified by the State Newborn screening program. In Louisiana and many other states, a medical social worker develops a follow-up plan to encourage mothers of children with sickle cell to participate in health education activities to facilitate compliance with both prevention and other medical interventions.

With existing stringent budget, not many States have followed this intervention quite religiously. Day et al,(1977) remarked that national standard have not been established for follow-up of the neonates with the heterozygous condition. Besides, several factors contribute to the unsatisfactory counseling activities at state level because screening program have shoestring budget, and prompt identification and follow-up services for sickle cell patients is given low priority.<sup>12</sup> This statistics pinpoint that trait counseling and primary prevention against the sickling genes has not been intensified. Therefore, educated and affluent expectant mothers of African ancestry must utilize their self-efficacy and empowerment initiatives to encourage their counterparts to participate in neonatal screening and comply with routine medical regimen and demand that the program needs to be accorded higher priority. We must emphasize that among rural expectant mothers who use the services of traditional birth attendants, the benefits of neonatal screening could be ignored to the detriment of their newborns.

#### Predictive, Preventive, Personalized and Participatory Interventions

In the era genomic medicine these 4p's constitute precision medicine. According to Dr. Elias Zerhouni (2010), The former director of the National Institutes of Health(NIH), the new medicine must anticipate and interrupt the disease process, thereby preventing the patient from being overwhelmed by the actual disease burden. The Institute of System Medicine (ISM) (2010) defined (1) predictive approach as the development of probabilistic health projection for a person based on their DNA and protein expression. (2) preventive medicine is the creation of interventions or therapeutic that will prevent a disease that an individual is assessed to have a high probability of developing. Regarding sickle hemoglobinopathies, trait counseling of young adults of child-bearing age is one technique of making primary preventive strategy most effective. (3) Personalized medicine refers to treating individuals based on their unique human genetic variations. For example, what are their sequenced DNA and haplotype characteristics? (4) Participatory medicine implies a patient's active, informed involvement in their medical choices, treatment, and acting in partnership with their health care providers. Educated mothers of children who enroll their newborn in the neonatal screening program must ask pertinent questions about the various variances of hemoglobinopathies (FA, FAS, FS, FSC, FSA, FSD, FS OArab, FC, FAE). Questions such as the meaning of these variances of sickle

hemoglobopathies, what is the degree of severity of these diseases? At what age do the indeterminate cases become either a trait or disease? Clinically, at what time will FS convert either to SS or AS?

The mother must be able to confirm when her child can be placed on 250 milligram of oral penicillin, and what are the benefits and side effects of such prophylactic treatment and follow. Will her child be placed on this treatment plan throughout his or her pediatric age? What are the options for her regarding career choices, sporting activities and whether there could be any cognitive deficits in their newborn babies?

Many technological gadgets relevant to neonatal screening have been developed in recent times. Some of the developed technologies used to enhance personalized medical care were the 454 life sequences manufactured by Roche Diagnostics (Brandford, CT), chromatography and electrophoresis, gene amplification, capillary analysis, polymerase chain reaction tests, microarray sequencing, and iso-electric focusing. These state-of-the-science approaches and bioinformatic technologies have the potential to provide significant insights into disease manifestation in individual patients and clinical differences at the molecular level. Such knowledge will enable the physician to tailor treatment to the precise needs of patients.

DNA Vision (2010) recently created as increased technological portfolio by using next generation sequence FLX system (Roche) for genome shotgun sequencing, genome re-sequencing, transcriptome profiling and metagenomic and meta-transcriptomics. A comprehensive list of the state of the art technologies required to improve the dissemination of personalized health care services were compiled by Ebomoyi and Srinivasan (2008).

In the era of genomic medicine, the key benefits of predictive, preventive, personalized and participatory interventions to the patient include new abilities to:

- Detect disease at an earlier stage, when it is easier and less expensive to treat effectively
- Stratify patients into groups that enable the selections of optimal therapy
- Reduce adverse drug reactions by more effective early assessment of individual drug responses
- Improve the selection of new biochemical targets for drug discovery

- Reduce the time, cost, and failure rate of clinical trials for new therapies
- Shift the emphasis in medicine from reaction to prevention and from disease to wellness.<sup>17</sup>

Additional benefits of the HGP are the availability of extensive genetic map which has increased the pace by which different genes are localized in the human genome. Medical geneticists are now able to identify susceptible sections of the genome which could be responsible for many disorders as sickle cell.

#### Universal Screening

Recommendations on the issue of universal and targeted screening evolved out of an NIH Consensus Development Conference (1987), on Newborn Screening for Sickle Cell Disease and Related Hemoglobinopathies. The conference involved 400 biomedical scientists, clinicians, public health workers, parents and public representatives. From the “consensus statement” the panel recommended universal screening of all newborns for hemoglobinopathies. As enunciated by the expert panel, programs which focused their screening on specific high-risk segments of a population are fraught with missing people who are inaccurately registered. Owing to complacency among some providers, non-screening of at-risk individuals is encouraged. The Consensus panel cautioned that the health risks to children with sickle cell disease and related hemoglobinopathies are so grave that concerted efforts are required to identify and enlist every affected child. The panel therefore recommended “that most states adopt a policy of screening all newborns.”(NIH, 1987).

Many states in America have complied with the universal screening policy advocated by the consensus panel; the benefits can be appreciated from the experiences from Florida, Texas, New York and Georgia among others. The data elicited from the University of Miami/Jackson Memorial (UM/JM) Medical Center situated in a large multi-ethnic metropolitan area indicate that it is better to test all newborns for hemoglobinopathies. Because the investigators have consistently shown that it was common to find Hb S and Hb C in infants designated as White Hispanic. This study has revealed the imprecision of using the designation White or Black when screening for sickle cell disease. The need to include certain “White” populations in screening programs has become very important in situations where the possibility of detecting SCD is quite high.

#### Guidelines on neonatal screening

The guidelines recommended by the World Health Organization (2006), for most screening programs worldwide must meet a number of requirements before implementation:

- The condition in question should portray high prevalence
- The condition in question should impose a significant health and economic burden on the population
- The gene in question should be easily and inexpensively tested, the analytical validity of the test should justify screening(WHO, 2006)

#### Screening in the Age of Genomic Medicine

Newborn screening has become one of the nation’s most successful public health programs. Very well established screening centers now exist in the urban areas. However, an effective mechanism is needed to extend such services to rural areas. In centers with highly qualified medical geneticists, physicians have been able to sequence the DNA of sickle cell patients so as to determine the order of the base pairs on the globin gene of the patient with sickle cell disease and characterize how it differs from the other children without sickle cell.

In compliance with WHO (2006) guidelines, clinical epidemiologist, and Centers for Disease Control and Prevention scientists continue to advise medical laboratory technologists and the screening team to carry out inexpensive test with acceptable sensitivity and specificity. Sensitivity is when the technology indicates that the genetic disease is present, when it is actually present. Whereas, specificity is when the technology indicates the genetic disease is absent when in fact, the disease is not present in the newborn. In the first two to three months of birth there could be a few indeterminate cases of “FS”, “FA”. Sometimes, these few cases turn out to be cases of the heterozygous status of the sickling gene. One must encourage screening centers to accurately compile the results for scientific investigation and reporting.

Dr Francis Collins and others (2010) the current Director of the National Institutes of Health,

and the United States Department of Energy (2004), have cautioned clinicians and providers to be quite sensitive to analytic validity of a test, which focuses on the ability of the genetic test to measure accurately and reliably the genotype of interest. Clinical validity of a test assesses the ability of a procedure to detect or predict the presence or absence of a phenotype, clinical disease or predisposition for a disease. Clinical utility of a genetic test indicates the probability that the test will lead to an improved outcome for the patient. Screening for sickle hemoglobinopathies and other diseases in the United States major hospitals, in the wake of preventive, predictive, personalized and participatory medicine must take into cognizance the ethical, legal, financial and social implications of neonatal screening.

#### Health Education Implications Using the Integrated Model

The integrated model has all the relevant constructs to enable any expectant mother to participate in neonatal screening for sickle hemoglobinopathies. As people of African ancestry, there is the perceived susceptibility to sickle cell disease if one had not participated in premarital screening to ensure that one's partner does not have the trait for sickle cell. Since there is no known cure for the disease, the perceived seriousness of the diseases is quite authentic bearing in mind that the classical signs and symptoms of the diseases are life-threatening and costly to managed. Participation in screening is to enable people engage in primary preventive behaviors. The integration of the social cognitive theory into this model further augment the predictability of this model by insisting that we use persuasion and copious information and motivations to focus on one's self-efficacy to carry-out the preventive behavior. Therefore, among high school students of child-bearing age, health education intervention must reinforce counseling students about premarital genetic screening. Efforts must be devoted by educators to intensify trait counseling so as to reduce the frequency of the deleterious gens in the society. The education of women and their empowerment can be most useful in avoiding being impregnated by partners with the allele of any hemoglobinopathies.

#### The Ethical, Legal, Social Implications

With the availability of cutting edge biotechnology and genomic science, it is prudent to predict that genetic variation within the human genome can be characterized and genotyped for many ethnic groups. Through the use of molecular techniques, and genomic techniques scientists expect to detect increasing number of genetic diseases. Therefore, there is the urgent need to train more scientist with the capabilities to provided the relevant genomic services.

#### **Acknowledgement**

The information used in this study is a component of neonatal screening conducted with Professor Flora F Cherry (Retired) at the Tulane Medical Center, School of Public Health and Tropical medicine. I am very grateful to Dr. Cherry for serving as my preceptor and for her commitment to the success of our neonatal screening for sickle hemoglobinopathy in New Orleans Louisiana.

#### **Author:**

E. William Ebomoyi, Ph.D.

Dr. Ebomoyi is a Professor in the Department of Health Studies, College of Health Sciences, Chicago State University, Chicago Illinois and he serves as a Consultant in International Health for the American Public Health Association.

Reprint Address:

Dr. E. William Ebomoyi, Ph.D.

Dept. of Health Studies

College of Health sciences

9501 South King Drive

Douglas Hall 127

Chicago, Illinois 60628-1598

[eebomoyi@csu.edu](mailto:eebomoyi@csu.edu)

773-995-2527

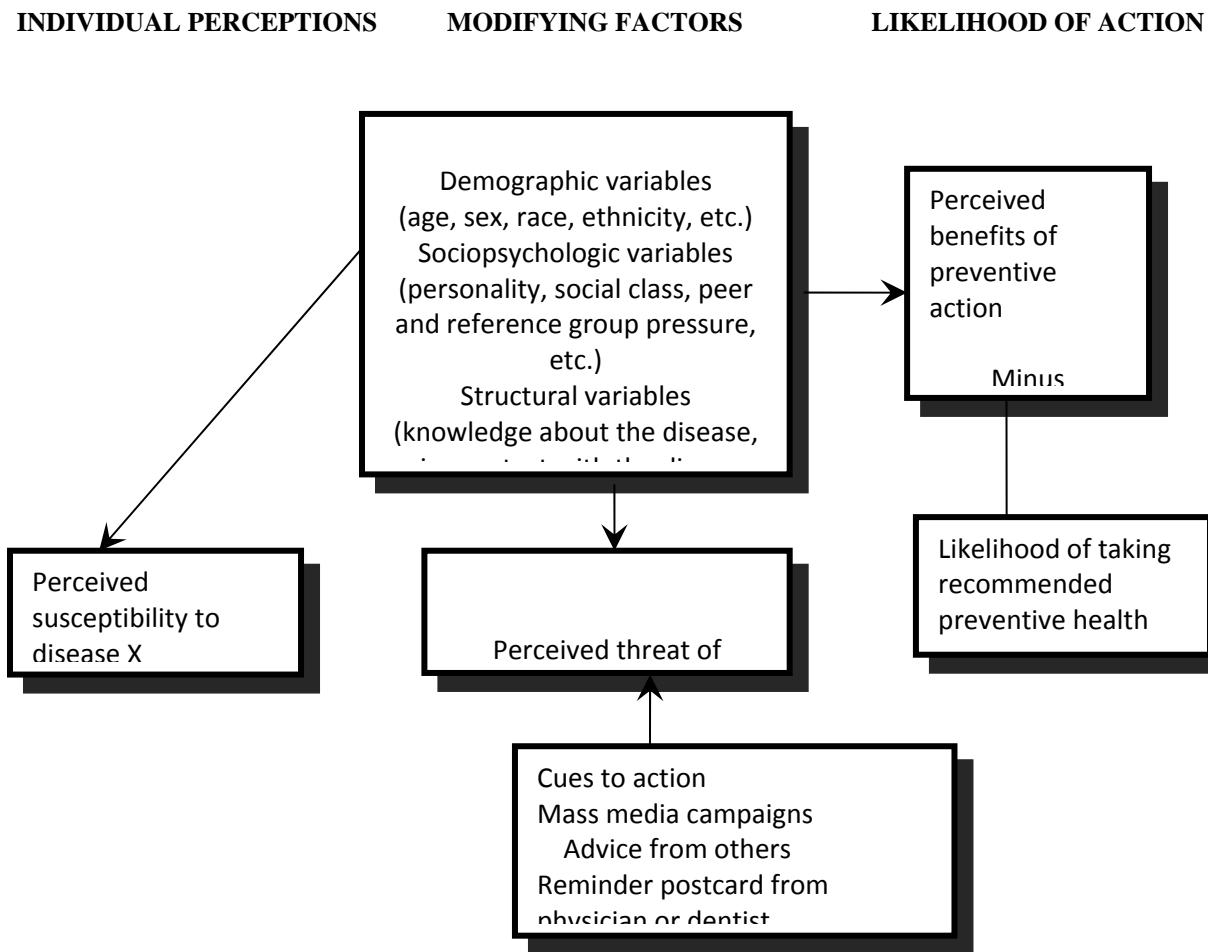


Figure 1. The Health Belief Model

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8/1/2010

# Mixed Infection of Bovine Viral Diarrhea Virus, Mycoplasma Species and Mannheimia Haemolytica in Calves Showed Chronic Pneumonia with Reference to the Histopathological Findings of the Affected Lungs

Hanaa, A. Ghoneim\*, Naglaa, I. Hassan, Hanaa, A. Elhalawany and A.M.Nabih

Animal Reproduction Research institute (ARRI) Giza, Egypt

\*hanaeg2002@yahoo.com

**Abstract:** A total of 100 nasal swabs as well as blood samples were collected from 75 diseased calves suffered from respiratory manifestations and 25 apparently healthy calves of ages ranges from 2-12 month old from three herds. Also 80 clinically pneumonic lung specimens of slaughtered calves were collected from El-warak and El- moneeb abattoir. All were examined to establish the extent of involvement of Bovine Viral Diarrhea (BVDV), *Mycoplasma* species (M. spp.) and *Mannheimia haemolytica* (M. haemolytica) in cases of chronic calf pneumonia. On virological studies, AGPT and commercial ELISA kits were rapid and accurate tests for detection of BVDV antigen. BVDV was isolated on MDBK cell line from Buffy coat, nasal swabs collected from diseased calves and lung specimens. The isolated virus was identified by IFAT using reference antisera. Also 100 serum samples collected from diseased and apparently healthy calves were tested by VNT for the detection of neutralizing antibodies against BVDV. Moreover, on bacteriological investigation, *M. haemolytica* were recovered from lung specimens of slaughtered calves as well as nasal swabs of diseased ones and apparently healthy ones. The isolated strains were biotyped as biotype A (56 isolates, 80 %) and biotype T (14 isolates , 20 %). The resistance of the isolates to most antimicrobial agents was high to ceftiofur, nalidixic acid, gentamicin, oxytetracycline, and cephalexin. While they were highly sensitive to norfloxacin, ampicillin and erythromycin. Although, *Mycoplasma* species recovery rate from the examined nasal swabs of pneumonic calves was (46.67%) relatively higher than that recovered from apparently healthy calves (32.00%), the isolation rate from the examined lung tissues reached to (25.0%). The most prevalent isolated species was *M. bovis* followed by *M. dispar*, then glucose positive, arginine negative species. Considering the mixed infection, results showed that, simultaneous isolation of the three pathogens from nasopharyngeal swabs of the examined pneumonic calves was relatively high (12.00%), followed by simultaneous isolation of BVDV& *Mycoplasma* sp as well as *M. haemolytica* & *Mycoplasma* sp. (9.33%). On the other hand, there was simultaneous isolation of both BVDV and *M. haemolytica* from nasopharyngeal swabs of (8.00%) out of the examined pneumonic calves. Examination of 80 clinically pneumonic lung tissues of slaughtered calves that were collected from abattoirs revealed that, a high percentage (17.50%) of examined lung tissues colonized both *Mycoplasma* sp. and *M. haemolytica* together. On the other hand, simultaneous isolation of the three pathogens was detected in (3.75%). However, simultaneous isolation of both BVDV and *Mycoplasma* sp. as well as BVDV and *M. haemolytica* was recorded in (2.50%) of examined lung tissues. Regarding histological studies of lung tissue specimens, there were five types of pneumonia distinguished according to types of necrosis, and cellular infiltrations in relation to microbial isolation, Caseonecrotic bronchopneumonia, 3.75%, Fibrino-necrotizing bronchopneumonia 12.5% ,Acute and chronic fibrinosuppurative bronchopneumonia 13.75%. In conclusion *M. bovis* showed two necrotic patterns, where an original focus of coagulative necrosis evolves with time into caseous necrosis ended by fibrosis.

[Hanaa, A. Ghoneim, Naglaa, I. Hassan, Hanaa, A. Elhalawany and A.M.Nabih. Mixed Infection of Bovine Viral Diarrhea Virus, Mycoplasma Species and Mannheimia Haemolytica in Calves Showed Chronic Pneumonia with Reference to the Histopathological Findings of the Affected Lungs. Journal of American Science 2010;6(11):538-555]. (ISSN: 1545-1003).

**Keywords:** Infection; Bovine Viral Diarrhea Virus; Mycoplasma Species; Mannheimia Haemolytica; Calves; Chronic Pneumonia; Lungs

## 1. Introduction:

Bovine respiratory tract disease is a multifactorial disease complex and has been one of the most serious problems due to their high mortality and economic losses in calves. This respiratory

disease complex involving several viruses one of these is the bovine viral diarrhea virus. On the other hand, severe respiratory tract disease in cattle is associated with concurrent infections of viruses and bacteria which resulted in suppress the host immune

responses leading to the disease complex. The bacteria that play prominent roles in this diseases complex are *Mannheimia haemolytica* type A, *Mycoplasma* spp as *Mycoplasma bovis*, and *Mycoplasma dispar* ( Srikumaran et al., 2007). The occurrence and severity of pneumonia may depend on a series of complex interactions between infectious agents and stress factors as adverse climatic conditions, weaning, transportation, environmental factors and immunological status of the calf (Martin et al., 1982 and Kiropes et al., 1988). Certain BVDV strains can cause primary respiratory disease and mild respiratory disorder in calves (Archambault et al., 2000 and Baule et al., 2001). The clinical presentation varied according to the age of the affected animal ( Jacob et al.2010 ). A synergistic role of BVDV in bovine respiratory disease occur by increasing pathogenicity of both viral and bacterial concomitant infection; this has been attributed to immunosuppressive effects of BVDV on the host (Potgieter, 1997). The immune suppressive effect of acute BVDV infections is due to strong affinity of the virus for immune competent cells which may be destroyed or functionally impaired which enhance the clinical disease of other pathogens and play an important role in multiple infectious diseases (potgieter, 1997).

*Mycoplasma bovis*, *M. dispar*, *M.bovirhinis* and *Ureaplasma diversum* are four species of mycoplasma that have been established as being of importance as causes of pneumonia in housed calves, based on pathogenicity studies and frequency of association with the disease (Nishimoto and Yamamoto, 2002). *M.bovis* is responsible for at least a quarter to third of calf pneumonia (Nicholas and Ayling ( 2003 )). Moreover, Haines et al. (2001) reported an increase in cases of antibiotic-resistant pneumonia and fibrinous polyarthritis in which *M. bovis* and BVDV infection were frequently detected.In addition *M.bovis* is considered as an emerging cause of mortality in feedlot cattle and is associated with bronchopneumonia and arthritis (Gagea et al , 2006 b).

*Mycoplasma dispar* is regularly isolated from pneumonic calves but is also found causing mild superficial and asymptomatic infections of the respiratory mucosa (Howard and Taylor 1983). Moreover, it is a proven cause of pneumonia and has been reported in cases of mastitis and was isolated from the lungs and nasal cavities of pneumonic cattle (Muenster et al., 1979). Nishimoto and Yamamoto (2002) reported a case of respiratory mixed infection of *Mycoplasma dispar*, *Manheimia haemolytica* and *pasteurella multocida* of a calf with nine months age. They concluded that, *M. dispar* is presented as the causative agent for pneumonia.

It has been suggested that cattle with primary mycoplasmal infection undergo immunosuppression (Potgieter, 1995), which might predispose to secondary infection with virus or bacteria (Shahriar et al., 2002). Also, Howard et al. (1978) concluded that, mixed infections of mycoplasmas and other microorganisms certainly lead to enhanced disease.

Pneumonic pasteurellosis due to *Mannheimia haemolytica* is one of the most important disease complexes causing economic loss in the cattle feedlot industry. It is responsible for the largest cause of mortality in calves farms in Egypt. Shipping fever pneumonia of calves is precipitated by stress-inducing conditions such as shipping, viral infections, inhalation of diesel fumes and overcrowding Frank (1989). Moreover, stress also resulted in nasopharyngeal overgrowth of bacteria, including *M. haemolytica* which results in more bacteria reaching the lungs via inhalation of infected droplets. Cell proliferates under stressful conditions and are eroded in large numbers into lung alveoli, where they cause the disease (Frank, & Briggs. 1992). In addition, respiratory viruses can damage the ciliated epithelium (Andrews & Kennedy, 1997) and compromising the normal defense mechanisms these allowing *M.haemolytica* to colonize the lung. Several mechanisms have been proposed to explain the phenomenon of viral-bacterial synergism in respiratory infection. These include selection of pathogenic form of bacteria, reduced efficiency of lungs in clearing bacteria, depressed phagocytosis or bactericidal potential of alveolar macrophages, depression of ciliary activities and suppression of the immune response (Sharma et al., 1990).

The present study aimed to establish the extent of involvement of BVDV, Mycoplasma species and *M.haemolytica* in cases of chronic calf pneumonia. Also, to determine the most prevalent isolated causative agent from the nasopharyngeal specimens of the examined calves in affected herds as well as from the calves' pneumonic lung tissues that collected from abattoir. Furthermore, to throw light on the most pronounced histopathological findings of the affected lung tissues accompanied by each pathogen.

## 2. Materials and methods

### Animal and samples:

A total of 100 nasopharyngeal swabs were collected from calves of ages ranged from 2 up to12 months old, from which 75 calves were suffered from respiratory manifestations, recumbency, anorexia, abdominal respiration as well as from their closely contact apparently normal calves (25 calves) from three herds at El-kalubia governorate, Misr-

Alexandria road and at the 10<sup>th</sup> of Ramadan city as shown in table (1). The samples were collected during winter (November, up to April).

**Table (1): Number of examined pneumonic as well as closely contact apparently normal calves from the three examined herds**

Herd No.	Pneumonic calves	Closely contact normal calves	Total
<b>Herd 1</b>	35	12	47
<b>Herd 2</b>	22	7	29
<b>Herd 3</b>	18	6	24
<b>Total</b>	75	25	100

Two nasopharyngeal swabs were collected aseptically from each examined calf, one sample for *Mycoplasma species* isolation in sterile screw capped tubes containing enriched heart infusion (HN) broth as a transport medium. Another swab was collected on nutrient broth for *Mannheimia haemolytica* cultivation.

More over two blood samples were collected from each examined calf one for serum separation for serodiagnostic tests and the other sample on anticoagulant and the buffy coat were separated for virus isolation and identification. Also, two blood films were freshly prepared from each examined calf for diagnosis of *Mannheimia haemolytica*. On the other hand, 80 clinically pneumonic lung tissue specimens of slaughtered calves were collected from El-warak and El-moneeb abattoirs, the gross pathological lesions were recorded and representative portions from each pneumonic lung were chosen. Lung tissue specimens were immediately immersed in 10% neutral buffered formalin solution meanwhile the other samples were packed individually in plastic bags and transferred directly to the laboratory in thermos tank with ice packs for virological, mycoplasmal and bacteriological examination. At the laboratory, the naked eye examination of the collected lungs were recorded, and a loopful of deep lung tissues after burning of its surface by very hot spatula were cultured on enriched heart infusion (HN) broth and subsequently on its corresponding agar plates for *Mycoplasma species* isolation, another loopful was inoculated in brain heart infusion agar supplemented with 5% defibrinated sheep blood, blood agar and macconkey agar for *Mannheimia haemolytica* cultivation.

#### Virological examination:

Agar gel precipitation test (AGPT) according to Hanel (1993). Samples (Buffy coat, nasal swabs and

lung tissues) were tested against standard reference positive hyper immune sera, the agar was used in concentration 1% in PBS and reaction was incubated for 24-48 h at 37°C in Co<sub>2</sub> incubator and examined for the presence of precipitin line.

#### Institute pourquier ELISA kits:

These kits were used for detection of NSP2-3 of the bovine virus diarrhea/ mucosal disease virus (BVDV).

#### Inter predation of the results:

Calculate for each sample the S/P ratio (in %) S/P = OD of sample – OD of negative control/of positive control – OD of negative control X 10 if sample S/P % < or = 25% are considered to be from animals that are not carrier to BVDV. If sample S/P % is between 25 %-30% are considered to be doubtful. If sample S/P % = or > 30% are considered from animals carrier to the BVDV.

#### 3- Cell culture:

Continues cell line of median Darby Bovine kidney cells (MDBK) was used for trials for isolation and propagation of BVDV from (Buffy coat, nasal swabs and lung tissues) which was positive in ELISA test. And also used in virus neutralization test, the cell proved to be free from non-cytopathic strains of BVDV. Monolayer cell line was grown in Eagles MEM supplemented with 10% fetal calf serum. The cell was obtained from virology department Animal Health Research Institute (AHRI). The collected samples after preparation were subjected for virus isolation via propagation on MDBK cell line according to the method described by Clark et al., (1984). Inoculated cells were incubated at 37 °C and were examined daily for 5 days post incubation for three successive blind passages, CPE changes being to appear at the fourth passage.

#### 4- Indirect immuno fluorescent technique (Indirect IFAT):

The (IFAT) was used on the inoculated cell culture with cytopathic effect (CPE) to identify the (cp BVDV), it was used also on inoculated cell culture without CPE to detect (ncp BVDV). The indirect IFAT was carried out according to OIE standers (1992).

#### 5- Enzyme conjugates:

Anti-bovine fluorescence isothiocyanate conjugate was supplied by Sigma immune chemicals used in IFAT.

#### Reference positive immune sera:

Standard reference positive bovine hyper-immune serum of BVDV was supplied by virology department AHRI.

#### 7- Virus neutralization test:

It was conducted for detection of specific BVDV neutralizing antibodies in cattle serum samples according to OIE (2004).

#### Isolation and Identification of Mycoplasma species:

The aseptically collected nasal swabs were cultured onto enriched heart infusion (HN) broth and then streaked onto the corresponding agar medium which prepared as described by Freundt (1983) for Mycoplasma species isolation. Naked eye examination of the collected lung tissue specimens were recorded, and a deep part of each lung tissue was cut into small pieces (1mm thick.), inoculated immediately in HN broth medium and shaken with a vortex. Three 10-fold dilutions were made according to the method described by Taoudi et al., (1985) and a loopful of each sample was streaked onto agar plates. All the plates were incubated at 37°C in a humid jar, under 10% CO<sub>2</sub> tension for 72 hours.

#### Identification of Mycoplasma isolates:

Genus determination: was performed using digitonin sensitivity test according to Freundt et al., (1973).

Biochemical characterization: The isolates were biochemically identified by glucose fermentation test as described by Erno and Stipkovits, (1973) and arginine hydrolysis according to Barile (1983).

Serological identification: It was carried out by growth inhibition (GI) test according to Clyde, (1964). The isolates were serologically examined against rabbit antisera of Mycoplasma bovis, M.bovigenitalium and M. dispar.

Serological test: Serum samples from all examined calves were tested for the presence of M.bovis or M.dispar antibodies by indirect haemagglutination (IHA) test according to Cho et al., (1976). The results were interpreted

#### Bacterial isolation and identification.

#### Cultural and biochemical identification:

Nasal swabs which immersed in nutrient broth and a loopful of deep lung tissues after burning of its surface by very hot spatula were cultured on brain heart infusion agar supplemented with 5% defibrinated sheep blood, blood agar and Macconkey agar. Plates were incubated at 37°C for 24 hours (Kodjo, et al. 1999). Bacterial identification was assessed by:

#### **The observation of the colonial morphology**

Gram staining and biochemical identification includes oxidase, catalase, urease tests, triple sugar iron agar, motility tests and indol tests (Atlas, 1997; Baily and Scotts, 1998 and Topley and Wilson, 1998).

#### Blood films Staining:

The freshly prepared blood films from examined calves were stained with Leishman stain and examined under oil immersion lens for detection of Gram-ve bipolar bacilli.

#### Biotyping of isolated M.haemolytica:

Biotyping of isolated M.haemolytica was applied depending on L-arabinose, trehalose, D-xylose, lactose and salicine fermentation tests (Biberstein et al., 1990).

#### Antimicrobial susceptibility test (Sensitivity test):

The susceptibilities of isolates to antimicrobial agents were determined by using the disk diffusion method according to the NCCLS Guidelines (2002). The antimicrobial disk used are Ampicillin, Amikacin, Ceftiofur, Cephaloridine, Cephalexin, Erythromycin, Norfloxacin, Oxytetracycline, Pencillin G, Streptomycin and Nalidixic acid.

#### Pathological examination:

Pneumonic lung specimens were taken immediately from the slaughtered calves and immersed in 10 % formalin. The fixed specimens were trimmed, washed and dehydrated in ascending grades of alcohol, cleared in xylene, embedded in paraffin. The embedded samples were sectioned at 3–5 µm thickness, stained with Haematoxylin-Eosin stain and, when necessary, with special stain (Giemsa stain and Periodic acid schiff (PAS) (Bancroft and Marilyn, 2002). The slides were examined using light microscope.

#### **3. Results and Discussion:**

##### Virological results and discussion:

As shown in table (2) the Buffy coat of pneumonic calves showed higher percentage of viral detection by ELISA test (92.00 %) than by AGPT (36.00 %). Also, detection of BVDV from nasal swabs by ELISA test (76.00 %) was higher than AGPT (25.33 %). Although, BVDV could not be detected by AGPT either in the buffy coat and nasal swabs of apparently healthy calves, the virus was detected in small numbers by ELISA test in buffy coat and nasal swabs (4.00 % and 12.00 % respectively). While BVDV was detected in lung tissues in higher percentage by ELISA test (25.00 %) than by AGPT (6.25 %).

In this study AGPT was used as a simple and rapid method for BVDV or antigen detection in collected samples (, nasal swabs and lungs) which reported by (Hanel, 1993; Hosny et al., 1996 and Nahed et al., 2004). Results of AGPT are low if compared with other tests due to the low sensitivity of the test or type of sample collection. Commercial Pourquier ELISA kit (antigen capture ELISA) gave rapid and accurate detection of BVDV antigen in the same original samples.

An antigen capture ELISA was developed and proved its specificity, sensitivity and accuracy (Gottschalk et al., 1992). Also, (Donis, 1995 reported that antigen detection ELISA for BVDV was usefully mainly in confirming enteric, respiratory and reproductive diseases. Many authors described the

importance of ELISA in testing program of animals BVDV (Sandvik et al., 1997 and Ferrari et al., 1999).

ELISA test was improved by the use of monoclonal antibodies which were specific to BVD viral proteins and we could detect most if not all BVDV strains (Sandvik, 1999) . In our study we use kits with specific monoclonal antibodies directed to NSP2-3 (NSP-3 associated with lytic activity of the cytopathic strain) which increased the application of ELISA for detection of BVDV strains and offered sensitivity equivalent or higher than virus isolation, this also recorded by (Ferrari et al. 1999; Cavirani et al., 2000 and OIE2000). Also, Aymen, (2002) proved that commercial ELISA kits are valuable in antigen detection.

**Table (2): Comparison AGPT and ELISA test for detection of BVD antigen in the diseased, apparently healthy and slaughtered calves.**

State of animal	Sample tested		+ve by AGPT (%)	+ ve by ELISA (%)
	Type	No .of samples		
<b>Diseased Calves</b>	Buffy Coat	75	27 (36.00%)	69 (92.00%)
	Nasal swabs	75	19 (25.33%)	57 ( 76.00%)
<b>Apparently healthy calves</b>	Buffy Coat	25	0 (0.00%)	1 (4.00%)
	Nasal swabs	25	0 (0.00%)	3 (12.00%)
<b>Slaughtered calves</b>	Lung tissues	80	5 (6.25%)	20 (25.00%)

**Table (3): Isolation and identification of BVDV from diseased, apparently healthy and slaughtered animals which were positive by ELISA test.**

State of animal	Sample tested		No. of samples with CPE	Virus identification by IFAT
	Type	No.		
<b>Diseased calves( 75 )</b>	Buffy Coat	69	24 (34.78%)	24 (34.78%)
	Nasal swabs	57	19 (33.33%)	13 (22.81%)
<b>Apparently healthy calves</b>	Buffy Coat	1	0 (0.00%)	0 (0.00%)
	Nasal swabs	5	0 (0.00%)	0 (0.00%)
<b>Slaughtered calves (80)</b>	Lung tissues	20	10 (50.00%)	7 (35.00%)

Table (3) represented the isolation of BVDV from samples positive by ELISA, out of 150 samples, 53 (35.3 %) gave signs of CPE for BVDV on the cell. These samples were then subjected for identification by indirect immunofluorescent technique (IFAT) to detect non- cytopathic BVDV, where 44 (29.3 %) were positive results (Fig. 1). MDBK cell line is considered the most common cell culture system for

virus isolation and propagation (Allam, 2000). In the present study, MDBK cells were used for three successive blind passages for samples positive by ELISA,. These samples were then subjected for identification by indirect immunofluorescent technique (IFAT) to detect non- cytopathic BVDV, where 24 (26.5%) were positive results in table (3). Edwards, (1990) and Brock (1991) reported that the

conventional diagnosis of Pestivirus is based on direct detection of the virus in the clinical samples by using cell culture method followed by immunofluorescence. Also, reported that accurate diagnosis of BVDV infection depend upon isolating the virus from blood or nasal swabs or tissue samples from affected animals in diagnostic laboratory which agrees with our results (Haines et al., 1992; Abd- El Rahim and Grunder, 1996).

The virus multiplication was detected by immunofluorescent technique that revealed diffuse or granular intracytoplasmic fluorescence in infected cells, also IFAT was used for identification of BVDV cell culture (Munoz et al., 1996; Tsuboi and Imada, 1999 and Zabal et al., 2000). the samples positive by ELISA kits and did not isolate or identify by IFAT may be due to BVDV present in those negative samples FA complexes with antibodies rendered it non infectious for cell cultures ( Palfiet et al., 1993 ). Improper handling or storage of the samples,

instability BVDV, Also ELISA can detect both BVDV and antigen.

BVDV specific antibodies in apparently healthy and diseased animals in serum samples by using VNT were shown in table ( 4 ) which revealed that , 50 serum samples ( 66.7 % ) were positive in titer range ( 1/8- 1/64 ) while 5 serum samples ( 20 %) of the apparently healthy animals were positive in a titer ( 1/4 – 1/16 ).

Serological examination of serum samples applied for detecting antibodies specific to BVDV is a useful tool for herd screening and BVDV prevalence and monitoring BVDV free herd status (Houe et al., 1995) VNT was carried out of detection of specific neutralizing antibodies for BVDV in both diseased and apparently healthy animals. Antibody tests were useful in assessing the status of animal groups as a part of disease control. VNT is the most common serologic test used as a reference method for BVDV tests (OIE, 2004).

**Table (4): Results of BVDV specific antibodies in apparently healthy and diseased calves in serum samples by VNT.**

State of animal	No. of tested sera	No. of +ve	% of +ve	Average titer
Diseased calves	75	50	66.7%	1/8-1/64
Apparently healthy calves	25	5	20%	1/4-1/16
Total No.	100	55	55%	

#### Mycoplasma results and discussion:

Several species of *Mycoplasma* may be isolated from calves with pneumonia, but only a few of these species are considered pathogenic. Respiratory pathogenic *Mycoplasma* spp. include *M. dispar*, *M. bovis*, *M. bovirhinus*, *M. bovigenitalium*, *Ureaplasma diversum*. As shown in Table (6) ,*Mycoplasma* species recovery rate from examined nasal swabs of pneumonic calves were (46.67%)

relatively higher than that recovered from apparently healthy calves (32.00%). ter-Laak et al. (1992) isolated *M.bovis* from 20% of pneumonic lungs from fattening calves but only from a small number of apparently healthy calves. Tschoop et al. (2001) confirmed the importance of *M.bovis* as an agent of respiratory disease where 50% of 400 calves introduced to infected fattening sites developed respiratory disease attributable to *M.bovis*.

**Table (5): Recovery rate and the Identified *Mycoplasma* species from examined pneumonic as well as apparently healthy calves and lung tissues**

samples	No. examined	No. +veMycoplasma (%)	Identified sp. No. (%)
*N.S of Pneumonic calves	75	35 (46.67%)	<i>M.bovis</i> 23 isolates (65.71%) <i>M.dispar</i> 7 isolates(20.00%) Glucose+ve& arginine-ve 5 isolates (14.29%)
N.S of closely contact apparently normal calves	25	8 (32.00%)	<i>M.bovis</i> 5 isolates (62.50%) <i>M.dispar</i> 2 isolates (25.00%) Glucose +ve & Arginine -ve one isolate (12.50%)
Lung tissues	80	20 (25.0%)	<i>M.bovis</i> 14 isolates (70.0%) <i>M.dispar</i> 4 isolates (20.0%) Glucose +ve& arginine-ve 2 isolates (10.0%)

\*N.S=Nasal swabs

**Table (6): Isolation rate of mycoplasma species from examined calves Correlated to the results of Indirect haemagglutination (IHA) test on their blood sera.**

Pneumonic calves n=75		closely contact apparently healthy calves n=25	
Nasal swabs	Blood serum	Nasal swabs	Blood serum
Recovered <i>Mycoplasma</i> sp. (%)	IHA antibodies ( $\geq 1/160$ )	Recovered <i>Mycoplasma</i> sp. (%)	IHA antibodies ( $\geq 1/160$ )
<b><i>M. bovis</i> 23/75(30.67%)</b> <b><i>M. dispar</i> 7/75 (9.33%)</b>	<b><i>M. bovis</i> 30/75(40.00%)</b> <b><i>M. dispar</i> 9/75(12.00%)</b>	<b><i>M. bovis</i> 5/25(20.00%)</b> <b><i>M. dispar</i> 2/25 (8.0%)</b>	<b><i>M. bovis</i> 4/25 (16.0%)</b> <b><i>M. dispar</i> 3/25 (12.00%)</b>

Serological identification of the thirty five mycoplasma isolates that were obtained from the examined pneumonic calves by growth inhibition (GI) test revealed that the most prevalent isolated species was *M. bovis* (65.71%) followed by *M. dispar* (20.00%). These results were nearly similar to that reported by Gagea *et al.* (2006a, b) who isolated *M. bovis* in a rate of 82%. Haines *et al.* (2001) detected *M. bovis* in the lungs and joints of feedlot cattle with chronic pneumonia and arthritis. They suggested that *M. bovis* should be considered as a principal pathogen in chronic unresponsive pneumonia of feedlot cattle. Unfortunately, a total of 5 isolates (14.29%) couldn't be serologically identified and showed biochemical reactivity as glucose positive and arginine negative which may be *M. bovirhinis* or *M. bovoculi*.

In the present study, only 7 isolates out of 35 mycoplasma isolates from the examined pneumonic calves were identified as *M. dispar* (20.00%). Bitsch *et al* (1976) isolated *M. dispar* from pneumonic calf lungs in a higher percentage 56%. Moreover, Shahriar *et al.* (2002) isolated *M. bovis*, *M. dispar* *M. arginini*, *M. bovirhinis*, BVDV, *Haemophilus somnus* and *pasteurella multocida* from the lungs of calves with chronic pneumonia.

On the other hand, the recovered *Mycoplasma species* from the apparently normal calves were only 8 isolates. The most prevalent identified species was *M. bovis* (62.50%), followed by 2 isolates (25.00%) as *M. dispar*. The least identified sp. (12.50%) was glucose positive and arginine negative. Mycoplasma *dispar* is a proven cause of pneumonia and has been reported in cases of mastitis but can be isolated from the lungs and nasal cavities of healthy and pneumonic cattle (Øystein *et al.*, 2009),

Mycoplasma species could be isolated from 20 out of 80 examined lungs in a percentage of (25.0%). These results were coincides with the findings of Byrne *et al.* (2001) who isolated *M. bovis* with ranges from 13 to 25% of pneumonic lungs of dairy and fattening herds and from 30% of calf herds with pneumonia. Also, with the results of Tend *et al.* (2004) who screened 34 cattle for the presence of

*Mycoplasma species* and reported that the recovery rate of *M. bovis* was 25.2 %.

In the present study, a total of 20 mycoplasma isolates were obtained from examined lung tissues. The most prevalent identified species was *M. bovis* (70.0%) followed by *M. dispar* (20.0%) and the least recovered isolate was glucose +ve and arginine negative (10.0%). This result supported by the conclusion of Nicholas *et al.*, (2000) where *M. bovis* is a major component of calf pneumonia complex. Moreover, stated that, over a third of lungs were infected with *M. bovis* while the rest contained a combination of *M. bovis* with *P. multocida* and /or *H. somnus*. Also, an alteration in the lungs were chiefly due to mycoplasma infection and the remaining bacteria contributing to complications in the pneumonic process Buchvarova and Vesselinova (1989). *M. dispar* was isolated from pneumonic calf lungs (Gourlay and Leach, 1970), which had cytopathic effect on bovine fetal tracheal organ cultures Thomas *et al.*, (1986).

Serum samples were examined by IHA test for the presence of *M. bovis* or *M. dispar* antibodies ,n= number of examined calves

As shown in table (6) showed that, the indirect haemagglutinating *M. bovis* antibodies( $\geq 1/160$ ) were detected in the blood serum of (40.00%) of pneumonic calves higher than the recovery rate (30.67%). While the blood serum of (12.00%) of the examined pneumonic calves showed high titer ( $\geq 1/160$ ) against *M. dispar*, the recovery rate of the organism from their nasal swabs reached to 9.33%. Gagea *et al.*, (2006 b) explained these findings that cases with specific antimycoplasmal antibodies in their serum but negative by mycoplasmal isolation represented acute or primary infection. Sachse *et al.* (1993) stated that the variation between mycoplasma isolation and serological response may refer to the stage or duration of infection. Pfutzner and Schimmel (1985) explained this finding that, *M. bovis* was transmitted from mastitic cows to their calves, infected their respiratory system and remained viable and infective up to sexual maturity when it could be isolated from their genital tract. Nicholas et al (2000) stated that using both mycoplasma isolation and

serological detection of the specific antibodies against the isolated species concurrently is expected to maximize the diagnosis of mycoplasma. Nicholas and Ayling, 2003 added that, serological detection of *M. bovis* antibodies is often a more reliable diagnostic method as the antibody levels remain high for many months. They concluded that the presence of specific antibodies indicates that the infection is invasive.

Considering the closely contact apparently normal calves, the results showed that, while the recovery rate of *M. bovis* from their nasal swabs reached to 20.0%, the detection of *M. bovis* antibodies in their blood sera was relatively lower (16.0%). In the present study, the detected IHA antibodies in the blood serum of apparently normal calves may be due to maternal immunity or latent infection due to the close contact with the diseased calves. This finding was discussed previously by Gagea *et al.* (2006b) who stated that, *Mycoplasmas* can be introduced in a herd by subclinical *Mycoplasma* carriers. These cattle shed the organism through nasal discharge for months to years without showing clinical signs. This finding is coincides with that of Jasper 1977) who stated that, in mycoplasma infected herds, it is usually to find animals have titer against mycoplasma in their serum without any history of illness. These animals may acquire partial immunity from prior exposure to mycoplasma infection. Moreover, Justice *et al* (2010) stated that, one of the currently documented routes of transmission of *Mycoplasma* spp. is through direct animal contact.

On the other hand, *M. dispar* specific antibodies were detected in the blood sera of (12.00%) out of the examined apparently healthy calves, whereas the organism was recovered from only 8.00% of their nasal swabs. Cho *et al.* (1976) observed that IHA results provided a more accurate diagnosis of

Mycoplasma herd infection than culture isolation and/or growth inhibition. They added that, IHA test is considered sensitive, reliable and highly specific and the titers were high in the infected or previously infected animals compared with the recently infected animals which may not show any titer. Nicholas and Ayling, 2003 added that, serological detection of *M. bovis* antibody is a true choice when antibiotics have been used extensively on examined herds. They stated that, animals in which *M. bovis* or *M. dispar* found only in the nasal passages without clinical symptoms were rarely seroconverting. Jurmanova *et al.* (1982) and Uhua *et al.* 1990) stated that the isolation of mycoplasma species from the prepuce of bull or vagina of cows without clinically apparent disease, usually didn't lead to high antibody titer in their blood sera perhaps as a result of superficial localization of the o

#### Bacteriological results and discussion :-

*Mannheimia haemolytica* (M.heamolytica) is a gram negative coccobacilli, non-motile, non-spore forming, facultative anaerobe from the family *Pasteurellaceae*. It is a normal inhabitant of the nasopharynx of healthy animals, but it is not a normal inhabitant of the bovine lung (Abdullah *et al.*, 1992 and Rice *et al.*, 2008). As shown in table (7), 29 *M.haemolytica* (38.60%) were isolated from 75 diseased calves and 37 (46.30%) isolated from 80 lung tissues of slaughtered calves hile only 4 isolates were detected from 25 apparently healthy calves (16%), under certain predisposing factors as shipping, rearing, transportation, overcrowding, mycoplasma infection and viral infection, *M.heamolytica* may shifting from being commensally to pathogen form (Confer *et al.*, 1995).

**Table ( 7 ) : Occurrence of *M.haemolytica* isolates in diseased , apparently healthy and slaughtered calves.**

State of animals	Type of samples	Total No. of sample	No. of +ve	% of +ve
Diseased calves	Nasal swabs& Blood films	75	29	38.60%
Apparently healthy calves	Nasal swabs& Blood films	25	4	16 .00%
slaughtered calves	Lung tissues	80	37	46.3%
<b>Total</b>		180	70	38.89 %

As shown in table ( 8 ), Fifty six ( 80 % ) of the isolated *M.haemolytica* were biotyped as biotype A and 14 *M.haemolytica* ( 20 % ) were biotyped as biotype T table ( 8 ) . These results revealed that *M.haemolytica* biotype A was the most frequently associated with shipping fever, adisease of beef

cattle, which characterized by fibrinous pleuropnemonia , the same results were reported by Ewer *et al.*2004 and Ilhan and Keles,2007 )

As shown in table (9), Antimicrobial susceptibility tests revealed that most of *M. haemolytica* biotype A was highly sensitive to

norfloxacin followed by ampicilline and erythromycin (75%, 65%, and 60% respectively) and highly resistant to ceftiofur, nalidixic acid, gentamicin, oxytetracycline, and streptomycin (90%, 85%, 75%, 65%, and 60% respectively). On the other hand most of *M. haemolytica* biotype T was highly sensitive to norfloxacin and erythromycin (92.86%, 71.43% respectively) and highly resistant to cephalaxin, nalidixic acid, oxytetracycline, and

streptomycin (85.71%, 78.57%, 64.29% and 64.29% respectively). These results were nearly similar to that mentioned by Esaki et al. (2005) and catry et al. (2005), and disagree with Mevius and Hartman (000) and Berge et al. (2006). The differences between our results and others may be attributed to many factors: misusing of antibiotics, individual physiological variation and differences in pathogenicity of the isolates and geographical localities.

**Table (8): Biotyping of isolated *M. haemolytica***

No. of total <i>M. haemolytica</i> isolated	Biotype A		Biotype T	
	No.	%	No.	%
70	56	80	14	20

**Table (9): Antimicrobial susceptibility tests of *M. haemolytica* biotype A. and biotype T.**

Antimicrobial Disks	Concentration Of disk	<i>M. haemolytica</i> biotype A.				<i>M. haemolytica</i> biotype T.			
		Sensitive		Resistant		Sensitive		Resistant	
		No	%	No	%	No	%	No	%
Ampicillin	10mg	13	65	9	64.30	5	35.71	7	35
Amikacin	30mg	11	55	8	57.14	6	42.86	9	45
Ceftiofur	30mg	2	10	7	50.00	7	50.00	18	90
Cephaloridine	30mg	10	50	7	50.00	7	50.00	10	50
Cephalexin	30mg	11	55	2	14.29	12	85.71	9	45
Erythromycin	15mg	12	60	10	71.13	4	28.57	8	40
Gentamycin	120mg	5	25	8	57.14	6	42.86	15	75
Norfloxacin	10mg	15	75	13	92.86	1	7.14	5	25
Oxytetracyclin	30mg	7	35	5	35.71	9	64.29	13	65
Pencillin G	10mg	9	45	7	50.00	7	50.00	11	55
Streptomycin	10mg	8	40	5	35.71	9	64.29	12	60
Nalidixic acid	30mg	3	15	3	21.43	11	78.57	17	85

Mixed infection of *BVDV*, *M. haemolytica* and *Mycoplasma species*

As shown in table (10), simultaneous isolation of the three pathogens from nasopharyngeal swabs of the examined pneumonic calves was relatively high (12.00%), followed by simultaneous isolation of *BVDV* &*Mycoplasma sp* as well as *M. haemolytica* and *Mycoplasma sp.* (9.33%). On the other hand, there was simultaneous isolation of both *BVDV* and *M. haemolytica* from nasopharyngeal swabs of (8.00%) out of the examined pneumonic calves. The present isolation data of *Mycoplasma species* from examined herds based on the previous isolation of mycoplasma from these herds which may indicate that chronic mycoplasmal infection may predispose the animals to infection by other pathogens. This explanation coincides with that of (Howard et al. (1978) who stated that, the immunosuppression resulted from mycoplasmal infection enhanced the susceptibility of the animal to infection

with other microorganisms. They added that mixed infection of mycoplasmas and other microorganisms certainly lead to enhanced disease. Additionally, Shahriar et al. (2002) reported co-infection with *BVDV* and *M. bovis* in feedlot cattle with chronic pneumonia. They concluded that cattle with primary mycoplasmal infection undergo immunosuppression which might predispose to secondary infection with other pathogens. They suggested that the synergism between *Mycoplasma* and other agents may complicate the disease condition. On the other hand, Trautwein et al. (2002) concluded that mycoplasmal infection may be able at least to exacerbate a disease condition that is probably initiated by other pathogen. Although the source of respiratory viral infection is not always obvious. It is likely that a proportion of calves acquired infection from their dams early in life. Haines et al. (2001) reported that *M. bovis* was detected in the lungs and joints of 80% of cases, while *BVDV* and *M. haemolytica* in 40 and 23%

respectively of these cases. Significant antibody titer to *M. bovis* were detected in half of 55 pneumonic examined, of which only 7 had rising titer to viral pathogens as bovine viral diarrhea virus (Nicholas and Ayling, 2003). However, *Mycoplasma sp.* only were recovered in a higher percentage (16.00%) than

the isolation rate of *M. haemolytica* only (9.33%) and the least recovered pathogen was BVDV only (2.67%). *Mycoplasma* infection might persist than other bacterial infection because many strains are resistant to antibiotics commonly used in the treatment of pneumonia (Thomas *et al.*, 2003).

**Table (10): Simultaneous recovery of BVDV, *M. haemolytica* and *Mycoplasma species* from examined nasopharyngeal swabs and lung tissues**

Infectious agent	State of calf		Lung tissues
	Diseased n= 75	Apparently normal n=25	n=80
BVDV only No.(%)	2 (2.67%)	0(0.00%)	0(0.00%)
<i>Mycoplasma sp.</i> Only No.(%)	12(16.0%)	5(20.0%)	1(1.25%)
<i>M. haemolytica</i> only No.(%)	7 (9.33%)	1(4.0%)	18(22.50%)
BVDV & <i>Mycoplasma sp.</i> No.(%)	7(9.33%)	0(0.00%)	2(2.50%)
BVDV & <i>M. haemolytica</i> No.(%)	6(8.00%)	0(0.00%)	2(2.50%)
<i>Mycoplasma sp.</i> & <i>M. haemolytica</i> No. (%)	7(9.33%)	3(12.00%)	14(17.50%)
BVDV& <i>Mycoplasma sp.</i> & <i>M. haemolytica</i> No. (%)	9(12.00%)	0(0.00%)	3(3.75%)

Regarding the closely contact apparently normal calves, there was simultaneous isolation of *Mycoplasma sp.* and *M. haemolytica* detected in (12.00%) of examined samples. Mixed infections of *M. dispar* and *M. haemolytica* have been demonstrated in field cases of calf pneumonia (George, St. *et al.*, 1973; Fatma *et al* (2008)). The frequent association of *M. bovis* infection with *M. haemolytica* or *P. multocida*, singly or in combination, suggests synergism between these pathogens (Gagea *et al.*, 2006a and Max ,2007). They added that *M. bovis* might colonize and perpetuate the lung lesions that were initiated by *M. haemolytica* or *P. multocida*, even if infection with these pasteurellaceae are cured by antibiotic therapy and host immunoinflammatory response Robert *et al* ( 2000 ).

On the other hand, the isolation pattern of each pathogen alone revealed that, while the *Mycoplasma sp.* could be detected in a higher percentage (20.0%) than the recovery rate of *M. haemolytica* (4.00%), BVDV couldn't be isolated from all examined apparently normal calves either alone or in combination with other pathogen.

Examination of 80 clinically pneumonic lung tissues of slaughtered calves that were collected from abattoirs revealed that, a high percentage (17.50%) of examined lung tissues colonized both *Mycoplasma sp.* and *M. haemolytica* together. This result coincides with the findings of Gagea *et al.* (2006a). On the other hand, Simultaneous isolation of the three pathogens together was detected in (3.75%) of examined lung tissues. However, simultaneous isolation of both BVDV and *Mycoplasma sp.* as well

as BVDV and *M. haemolytica* was recorded in (2.50%) of examined lung tissues. Shahriar *et al.* (2002) could detect *M. bovis* and BVDV antigens by immunohistochemical (IHC) staining in the lung tissues of calves with chronic Pneumonia in a percentage of 91% and 44% respectively. At the level of isolation of each pathogen alone from the examined lung tissues, the results revealed that, while *M. haemolytica* was recovered in a higher percentage (22.50%) than the recovery rate of *Mycoplasma sp.* only (1.25%), BVDV couldn't be recovered alone.

#### Pathological results & discussion:

Pneumonia is a major cause of death and economic losses to the calves industry. Recognizing the patterns of pneumonic lesions of the various types of pneumonia is important for correct diagnosis and interpretation of the lesions (Roger & Anthon, 2010). Bovine atypical interstitial pneumonia (AIP) is a multifaceted disease. It often complicated with bacterial, viral, or mycoplasmal organisms, Alan, (2010). In this work we studied the pneumonic lesions in naturally occurring bovine viral diarrhea virus (BVDV) associated bronchopneumonia and the relationship of this condition with mycoplasma spp. infection and *M. haemolytica*. Five types of pneumonia were distinguished according to etiological agents associated with (BVDV), either single or multiple they were:

#### 1-Caseonecrotic bronchopneumonia:

Represented 3.75% of tissue samples (3 of 80). Isolated organisms where as shown in table (12)

*Mycoplasma bovis* (*M.bovis*) mixed with (BVDV) with Sever ++++

**Table (11) Results of histopathological Examination demonstrate types, and percentages of pneumonia in the examined pneumonic lung tissues:**

Mixed pneumonia				Non mixed	
Type of pneumonia	Caseonecrotic bronchopneumonia	Fibrinonecrotizing bronchopneumonia	Acute and chronic fibrinosuppurative bronchopneumonia:	Interstitial pleuro-pneumonia	Serofibrinous pleuro-pneumonia
Isolated organisms	<i>M.bovis</i> ,& BVDV	<i>M.heamolytica</i> & BVDV	<i>M.dispar</i> &, <i>M.heamolytica</i> & BVDV	<i>M.bovis</i>	<i>M.heamolytica</i>
Degree.	Sever ++++	Less sever +++	Moderate ++		
No. of cases	3/80	2/80	2/80      9/80 without BVDV	6/80	25/80
%	3.75%	2.50%	2.50 %      11.25%	7.50%	31.25%

#### Gross lesions:

Dark red patches with interstitial edema and emphysema. In two cases there was multifocal sequestral formation in the form of circular, raised yellow foci of dry friable, caseous material. The intervening tissue in these areas was atelectatic. There was accumulation of fibrin on the pleural surface. Previous findings observed were nearly similar to those observed by Gagea *et al* (2006 b), and coincide with those observed by Jones *et al*. (1997); Calvin *et al.* (2008); Radaelli *et al.* (2009) and Fulton *et al.* 2009)

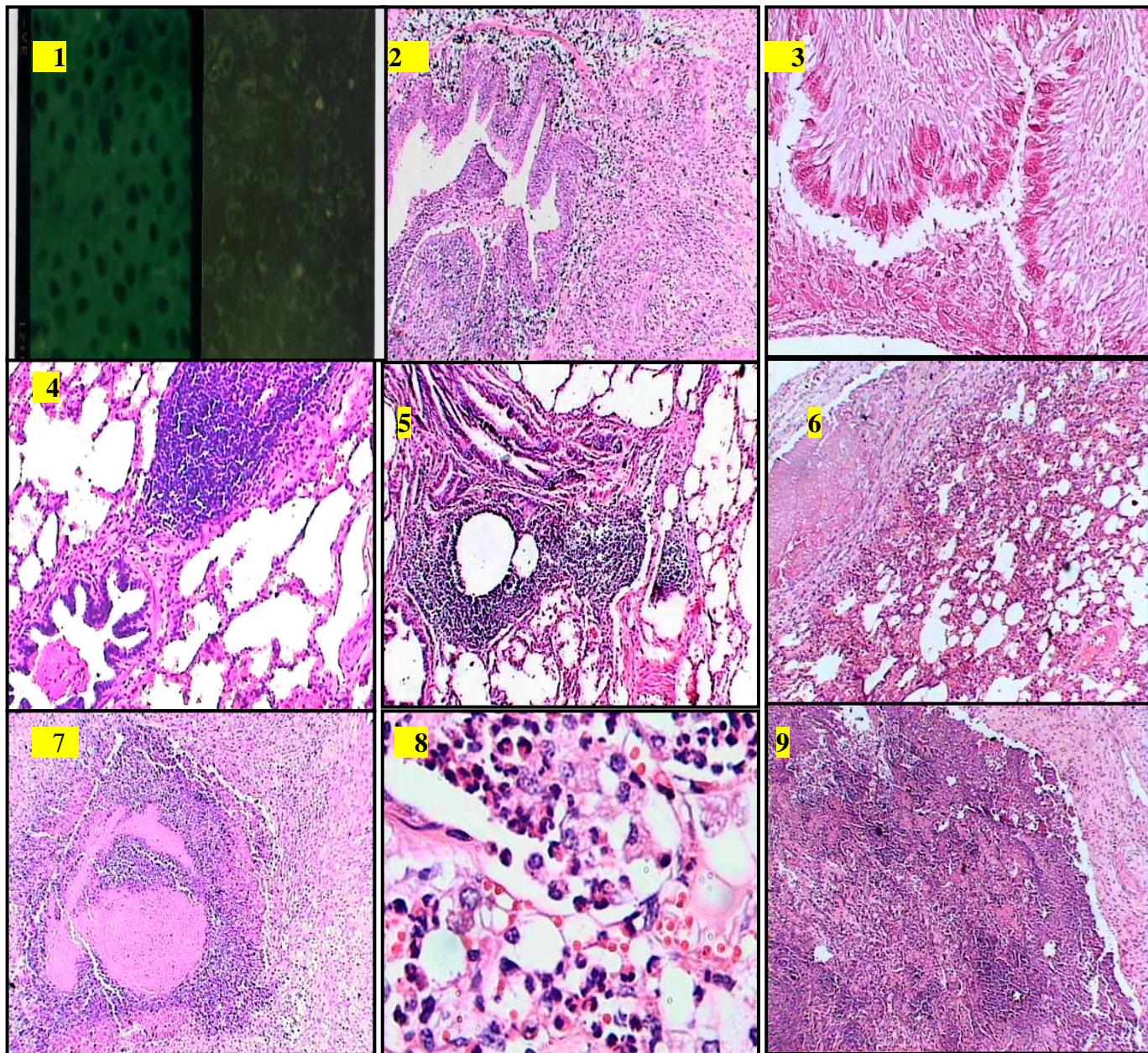
#### Histopathology:

The bronchiolar epithelium lining showed necrotic changes with loss of cilia or exfoliation of ciliated cells within bronchial lumen (Fig.2). Goblet cell hyperplasia also noticed and was confirmed with (PAS) stain (Fig. 3). This noticed hyperplasia considered as an inflammatory response to the development of mycoplasma specific antibodies and immune complexes resulted from persistent mycoplasmal infection. Such response acting on the respiratory epithelium and goblet cell precursors, (Zhang *et al.* 2006 and Aurora *et al.* 2006).

The peribronchial associated lymphoid tissue (BALT) showed marked proliferations and hyperplasia (Fig. 4). More over there were edema, per alveolar, perivasculär, peribronchial and per bronchiolar mononuclear cellular infiltrations (Fig.5). The Pulmonary blood vessels were engorged with blood, some of them showed fibrin thrombi with lymphocytic infiltrations, pleurisy, Interstitial pleuropneumonia (Fig. 6) with pronounced thickened alveolar walls. Previously mentioned findings were the remarkable findings of mycoplasmal infection,

were coincide with those described by (Mohamed & Abdelsalam, 2008, Fulton *et al.* 2009 and Alan, 2010). Such changes explained by Jubb *et al.* (1993); Macgavin & Zachary , (2007) as the infection which brings about immune suppression caused by BVD virus then ciliary dysfunction in addition to unregulated production of Tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) barrel with the development of inflammation and increased vascular congestion so widening of vascular wall pores ,which followed by fibrin escapes from blood to pulmonary tissue under the effect of bacterial toxins and increased procoagulant activity, furthermore fibrin is chemotactic to leukocytes.

Foci of coagulative necrosis with intensely eosinophilic cytoplasm also were seen (Fig.7). These foci were delineated by a zone of neutrophils and macrophages, encircled by fibroblasts (Fig.8). Other areas showed diffuse casious necrosis (Fig.9). Mycoplasma was demonstrated with Giemsa stain in the vascular wall, interstitial tissue and inside alveoli. The latter necrotic foci which noticed microscopically was explained by Hum *et al.* (2000); Edy & Joachim, (2001) and Steven *et al* .(2003) as massive presence of *M.bovis* produce necrotizing factors as protease enzyme ,hydrogen peroxide and a complex polysaccharide toxin. Necrotizing factors are resulting in destruction of the pulmonary epithelium and vascular endothelium, which leading to initiation of thrombus, ending by pulmonary infarctions with inflammatory reaction followed by severe oxidative damage of tissues. This explains the Presence of bluish green mycoplasma colonies on wall of pulmonary blood vessels.



**(Fig : 1 ) :** Normal MDBK non infected cells ( A ) and infected MDBK cells ( notice Specific intracytoplasmic fluorescent ) ( B ).

**(Fig : 2 )** bronchiole showing necrosis & exfoliation of lining cells into the lumen (H&E) x4

**(Fig : 3 )** bronchiole showing marked goblet cell hyperplasia noticed with (PAS) x4

**(Fig : 4)** hyperplasia of peribronchiolar lymphoid tissue ( H&E) x10

**(Fig :5)** perialveolar ,perivasicular , and peribronchiolar mononuclear cellular infiltrations ( H&E) x4

**(Fig : 6)** interstitial pleuropneumonia ( H&E) x4

**(Fig : 7)** caseous necrosis with calcifications (H&E) x4

**(Fig:8)** alveoli filled with neutrophils and macrophage with congested perialveolar vessels (H&E) x40

**(Fig : 9)** diffuse caseous necrosis with calcifications with in center (H&E) x4

In the present study two patterns of pulmonary necrosis were noticed, the most common pattern was caseous necrosis; the second and least common pattern was coagulative necrosis,. The previous finding were highly consistent with those the reported by (Lopez & Martinez, 2002; Khodakaram & Lopez 2004 and Mohamed & Abdelsalam 2008).They speculated that an original foci of coagulative necrosis progress with time into caseous necrosis with proliferation of granulation tissue .This variation in severity, development of disease during infections may be regarded to the environmental factors (Gulliksen *et al.* 2009) Variable surface protein antigens Vsp of *M. bovis* were demonstrated in lungs of calves by (Buchenau. *et al* 2010) . In which Vsp together with immunological factors may contribute to the chronicity of pulmonary disease. The BVDV role in those lesions were due to its stimulation of cytokines hypersecretion such as interferon TNF, (Bielefeldt *et al.* 1989;Jacob *et al.* 2010).

#### 2-Fibrino-necrotizing bronchopneumonia :

Represented 2.5% of tissue samples (2of 80) as shown in table (12) .Isolated organisms where. *M. haemolytica* and *Bovine viral diarrhea virus* BVDV. +++ With less Severity.

#### Gross lesions:

Lung tissue was pale flabby with multiple focal grayish areas of consolidation.

#### Histopathology:

Bronchiolar epithelial cells showed loss of cilia and necrosis with desquamation within the lumen (Fig.10). Marked peribronchial lymphoid tissue hyperplasia was seen. Some alveolar lumens were filled with faint eosinophilic fibrin network intermingled with mononuclear cells, mainly lymphocytes, plasma cells and histiocytes (Fig. 11). Other alveoli contained cellular exudates consisted mainly of mononuclear cells. Multifocal coagulative necrosis were detected as homogenous strongly eosinophilic areas surrounded with mononeuclear cells and neutrophils ( Fig. 12) .Oat cells and coccoid bacteria were colonized at the periphery of necrotic foci. Other foci showed congested capillaries with perivascular lymphocytic infiltration and emphysema. Pleura showed fibrinous exudate, with lymphocytic infiltrations.

The gross and microscopical findings were coordinate with (Mohamed & Abdelsalam, 2008; Jean *et al* .(2008) ; Fulton *et al.* 2009), and attributed by Jeyaseelan and Sreevatsan (2002) to the *M. haemolytica* virulence factors such as leukotoxin

which is the main virulence factor that is associated with lung lesions and secreted by all *M. haemolytica* serotypes Jeyaseelan, and Sreevatsan (2002); .At high concentrations it induces leukocyte lysis, resulting in the nuclear streaming of necrosis (Cudd *et al.*,2001 ; Bojesen *et al.*, 2007 ; Wollums *et al.*, 2009).Another important virulence factor Lipopolysaccharide,( LPS) is synergistic with leukotoxin to induces oxidative burst of pulmonary alveolar macrophages and may inhibit the production of neutrophilic granulocyte, A polysaccharide-rich capsule is resistant to phagocytosis by neutrophils and macrophages, Also it assists in attachment, and alter neutrophil function. Furthermore Neuraminidase which increases the adhesion of the bacterium to the respiratory epithelium (Wollums *et al.*, 2009). Bovine respiratory viruses increase affinity to *M. haemolytica*, and other respiratory pathogens, by infecting ciliated epithelium and reducing mucociliary clearance. Another reason for extensive pulmonary necrosis is the secretion of maximum amounts of cytokines by alveolar macrophages, Malazdrewish *et al.* (2004); Macgavin & Zachary ,(2007) .

#### 3- Acute and chronic fibrinosuppurative bronchopneumonia:

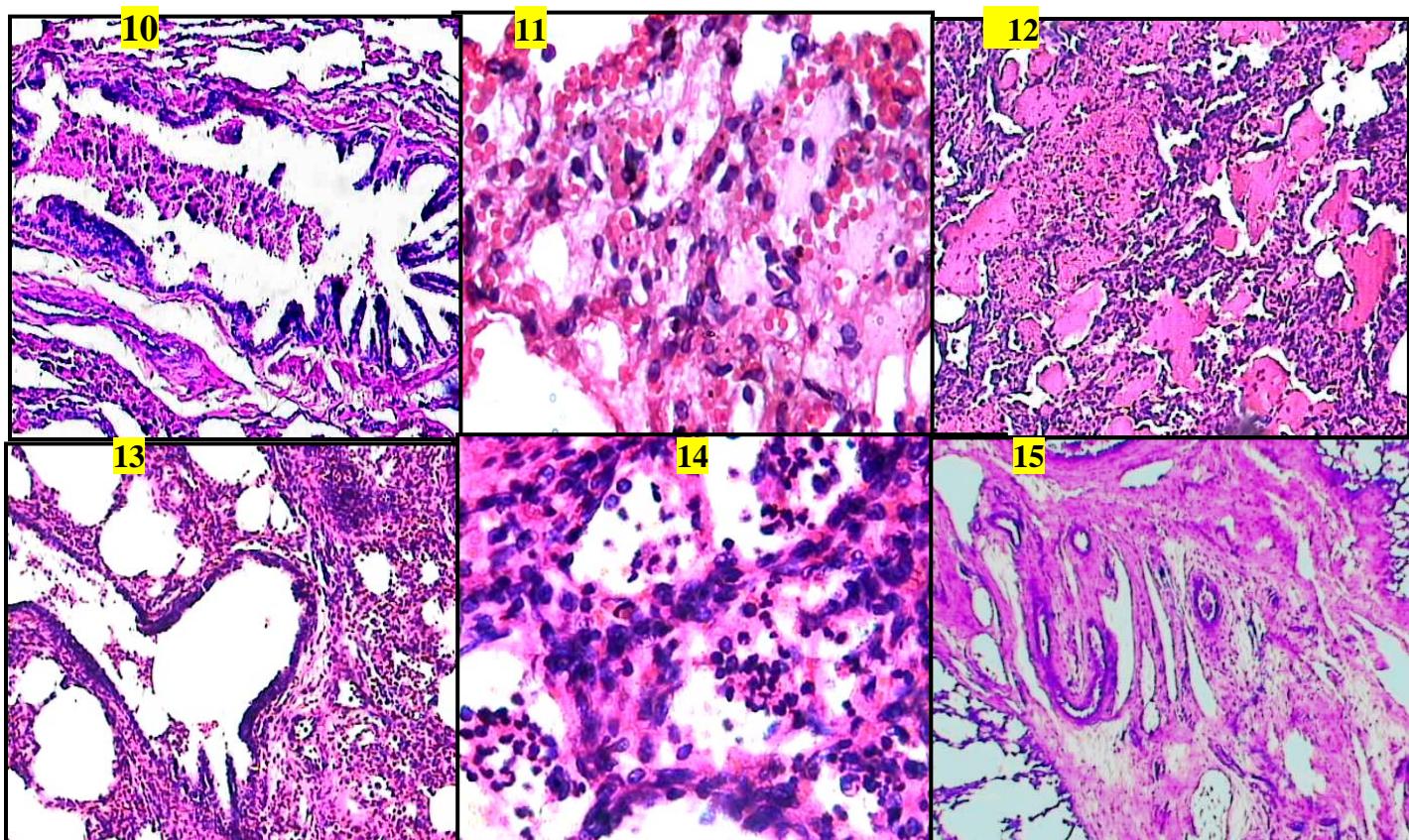
Represented 13.75% of tissue samples ( 11of 80) as shown in table (12) and isolated organisms were *M. haemolytica*, *M. dispar* and (BVD). With Moderate ++

#### Gross lesions:

The affected areas were deep red and consolidated. Pleural showed fibrosis with fibrin adhesions was evidenced. Pulmonary parenchyma showed foci of suppuration irregularly shaped and, delineated by a grayish rim.

#### Histopathology:

Bronchiolitis, bronchiectasis were seen in most cases of this category (Fig. 13) Alveoli showed atelectasis and necrosis with macrophage , neutrophils , fibrin, or both, and streaming of nuclear chromatin to form oat cells, foci of coagulative necrosis typical of *M. haemolytica* pneumonia( Fig. 14).Pleura showed aggregations of neutrophils with edema and congestion. A process of chronic fibrosis was seen with granulation tissue formation (Fig: 15). Within interstitial tissue histiocytes, plasma cells and lymphocytes were also noticed.



(Fig :10 ) Bronchiolar epithelial lining showing loss of cilia and necrosis with desquamation into the lumen (H&E) x10

(Fig :11) alveolar lumen with exudates of fibrin network intermingled with mononuclear cell mainly histiocytes ,lymphocytes , plasma cells and ,monocytes ( H&E) x40

( Fig : 12) multifocal coagulative necrosis as homogenous strongly eosinophilic areas infiltrated with mononeuclear cells ( H&E) x10

( Fig :13) Bronchiolar necrosis, intrabronchiolar neutrophils, bronchiolitis (H&E) x10

( Fig :14) Alveoli showing necrosis with neutrophils, and streaming of nuclear chromatin to form oat cells. (H&E) x40

( Fig :15) Pleura showing thickening with fibrous and granulation tissue proliferation (H&E) x10

Those neutrophil influx with alveolar necrosis and minimal bronchiolar necrosis were specific picture to *M. dispar* , and were agreed with Linda & Reggio, (2002) ; Jeyaseelan, and Sreevatsan (2002); Ajuwape *et al.* (2003) ; Ewer and Wieler 2004) ; Ilhan & Keles, 2007 and Fulton *et al.* (2009). Leukocytes within the alveoli and bronchioles undergo necrosis but retain a ghost-like outline with hyper-eosinophilic cytoplasm and fragmented nuclei (Caswell and Archambault, 2008). Studies using *M. dispar* indicate the pathogenesis of *Mycoplasma* pneumonia in calves involves degeneration and impairment of ciliated respiratory epithelial cells, thereby predisposing the lung to secondary infection with additional pathogens (Almeida & Rosenbusch,

1994). Coinfection of *M. hemolytica* with viruses resulting in complex infection after long-distance transportation and coldness (Jean *et al.* 2008).induced a moderate increase in the lesion severity ( Gourlay &Houghton, 1995 ). But only when calves were infected with *M. bovis* prior to infection with *M. hemolytica*. *M. bovis* pneumonia in calves is typically more severe when multiple pathogens, including *M. hemolytica*, (Bucharova & Vesselanova, 1989; Gregg *et al* 2010 ).

4-Non mixed pneumonia:

A- Interstitial pleura- pneumonia:

Represented 7.5 % (6 of 80) cases and isolated only mycoplasma species, lung tissue

showed interstitial lobar pneumonia, thickening interstitial tissue, desquamations of bronchiolar epithelium with in lumen. In addition to perivasculär ,perialveolar lymphocytic cuffing those findings coincide with those observed by,( Rodriguez et al., 1996).Those lesions regarded to mycoplasma toxins.

#### B- Serofibrinous pleuropneumonia :

Found in 31.25% cases, represented 25/80 and the isolated organism was only *M.haemolytica* This type represent the higher percentage, this illustrated by (Thomas et al.2002;Caswell and Williams 2007) as *M. hemolytica* are the most common organisms commensally of the bovine nasopharynx which, during stress can overcome host defense mechanisms establishing infection in the lower respiratory tract .Lung tissue showed widespread accumulation of, fibrin mash intermingled with neutrophils, macrophages . Airway Lumina and interlobular septae were distended with serofibrinous exudates. Those observations were caused by *M. hemolytica* toxins and nearly similar to those of (Larsen et al . 2001).

#### 4. Conclusion

Viral agents are usually considered the primary pulmonary pathogen, capable of destroying the respiratory epithelial lining to a degree allowing other agents to colonize. In addition to acute BVDV infections or persistent postnatal BVDV infections, undergo immunosuppression through destroying of immune competent cells of the host. The latter increases the susceptibility of these animals to secondary bacterial infection and increasing pathogenicity of concomitant diseases, that is why mycoplasmal and manhaemial infection may able to exacerbate a disease condition and initiated by other pathogen. Other theory suggested that, the viral and mycoplasmal agents are the primary infections and the bacterial gents are there as the secondary invaders.and these two suggestions supports our view.

#### Corresponding author

Hanaa, A. Ghoneim  
Animal Reproduction Research institute (ARRI)  
Giza, Egypt  
[\\*hanaeg2002@yahoo.com](mailto:*hanaeg2002@yahoo.com)

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9/1/2010

## Comparative Study between Different Denture Adhesives in Improving Phonation in Complete Denture Wearers

Essam Adel Aziz<sup>\*1</sup>, Azza Adel Aziz<sup>2</sup> Dina Essam Eldeen Ibrahim<sup>1</sup> and Ali Eldeen Mohammed Ahmed<sup>1</sup>

<sup>1</sup>Prosthodontic Department, Faculty of Oral and Dental Medicine, Cairo University, Cairo Egypt

<sup>2</sup>Phoniatric Unit, ENT Department, Cairo University, Cairo Egypt.

<sup>\*</sup>dressamaziz@msn.com

**Abstract:** Objectives: the aim of this study was to evaluate the efficiency of denture adhesives in improving phonation in complete denture wearers and to compare the efficacy of three different types of denture adhesives. Methodology: Fifteen completely edentulous patients with flat mandibular ridge shared in this study, complete denture was constructed for each patient according to the conventional method. Phonetic analysis was performed in the Phoniatric Unit via both Perceptual and Acoustic techniques to compare the efficacy of three chemically different denture adhesives (Super corega, Fittydent and Fixodent) on Arabic phonemic production. Results: a marked improvement in patients' articulation after application of the denture adhesives was reported, perceptually and acoustically, where the Fixodent denture adhesive gave the highest values. Conclusion: Whenever possible, denture adhesives should be used to improve retention and articulation. The polymethylvinyl ether malate-based adhesives (Fixodent) are strongly recommended as a highly reliable type of denture adhesives.

[Essam Adel Aziz, Azza Adel Aziz Dina Essam Eldeen Ibrahim and Ali Eldeen Mohammed Ahmed. Comparative Study between Different Denture Adhesives in Improving Phonation in Complete Denture Wearers. Journal of American Science 2010;6(11):556-561]. (ISSN: 1545-1003).

**Keywords:** Different Denture; Adhesives; Improving Phonation; Complete Denture Wearer

### 1. Introduction:

Residual ridge resorption is considered a major oral disease which could occur despite of careful prosthetic handling<sup>(1)</sup>. One of the most undesirable effects of residual ridge resorption is compromised denture retention which is considered a real challenge in complete denture therapy. There is always a question asked by the patients even in their own minds when they are seeking a prosthetic therapy which is "Is this denture going to be retentive?" Patients are asking for retention during talking, laughing, speaking, and for sure eating, regardless the condition of their remaining tissues (alveolar ridge height and soft tissue condition)<sup>(2,3)</sup>. Improving retention in cases of residual ridge resorption could be achieved via either surgical and/or prosthetic treatment. Surgical treatment may be in the form of vestibuloplasty<sup>(4)</sup>, ridge augmentation<sup>(5)</sup>, endosseous dental implants<sup>(6)</sup>. Anatomic, systemic and / or financial limitations could interfere with the surgical techniques described for flat ridge cases<sup>(7)</sup>. Denture adhesives had been used to aid in complete denture retention long time ago<sup>(8,9)</sup>. Wilson et al.<sup>(10)</sup> reported that 30% of the patients wearing dentures used denture adhesives. Another study declared that out of the 20% of the adult population in US who wear dentures at least 22% used denture adhesives<sup>(11)</sup>. Slaughter et al.<sup>(12)</sup> reported that the use of denture adhesives is considered suitable, adjunctive and effective treatment modality in removable

prosthodontics. Denture adhesives are commonly composed of three main components<sup>(13)</sup>:

a - basic adhesive substance such as methyl cellulose, sodium carboxy methyl cellulose, hydroxy methyl cellulose and/or synthetic polymers such as polyethylene oxide, acrylamides and polyvinylmethylether Maleic Anhydride.

b- Antimicrobial agent: such as hexachlorophene, sodium borate, ethanol and sodium tetraborate.

c- Preservatives, flavouring agents, wetting agents and plasticizers.

Many studies reported the effect of denture adhesives on improvement of mastication,<sup>(14-16)</sup> but their effect on Pronunciation of different phonological sounds is still lacking.

### 2. Materials and methods:

Fifteen patients (10 females and 5 males) were selected from the outpatient prosthodontic clinic, faculty of Oral and Dental Medicine, Cairo University. Their ages were ranging from 54 to 73 years with a mean age of 61.5 years. They were all completely edentulous with flat mandibular ridge, construction of complete dentures was carried out according to the conventional method. Phonetic assessment for the fifteen patients was carried out in the Phoniatric unit, faculty of medicine, Cairo University via both perceptual (subjective) and acoustic analysis (objective) methods in five phases:

Phase 1: before denture delivery while the patients were still unrestored completely edentulous.  
 Phase 2: two weeks after the final denture inspection.  
 Phase 3: two hours after application of the denture adhesive type 1 (Super corega) which is based on Carboxymethyl cellulose (CC).  
 Phase 4: this was done one week later, two hours after the application of adhesive type 2 (fittydent) which is based on Sodium Carboxymethyl cellulose and polyvinylacetate.  
 Phase 5: this was done one week later, two hours after the application of adhesive type 3 (Fixodent) which is based on sodium Carboxymethyl cellulose and polymethylvinylether maleate.

Application of each type of denture adhesive type was done on clean fitting surface according to the manufacturer recommendation (Figure 1).



**Figure (1), Application of the denture adhesive of the lower complete denture.**

#### Phonetic assessment:

I- Subjective perceptual assessment of different Arabic phonemes using the Arabic Articulatory Test (AAT).<sup>(17)</sup> Consonant sounds were sampled in initial, middle and final positions of words. The patients' products were tape-recorded and the test was transcribed on line followed by analysis of the recorded tape to verify the on-line transcription.  
 II- Objective acoustic assessment from a wide-band spectrogram display, using the Computerized Speech Lab (CSL) Kay model 4300, (Figure 2). Acoustic analysis of the perceptually-detected mostly affected phonemes (/s/, /ʃ/, /z/, /f/) was performed in the initial and the terminal-word positions. The full word was displayed on the screen and then the target sound was zoomed in. The segment was visually and auditory verified to ensure that both the beginning and the end of the sounds were included. Cursors were placed at the initial and the terminal of the target phonemes. The following acoustic parameters were analyzed: 1-Average energy in (dB), which

reflects the sound volume over a period of time. 2-Average duration in (mSec), which reflects the time required to produce a given speech sound. Subjects were instructed to repeat target words containing the consonants which appeared to be affected during perceptual assessment. The words were put in a carrier phrase (say .....again) to ensure a standard way of utterance.

Data were presented as mean and standard deviation (SD) values. Analysis of variances with repeated measures ANOVA was used and Bonferroni's test for pair-wise comparisons. The significance level was set at  $P \leq 0.05$ .



**Figure (2) Computerized speech lab (CSL).**

#### 3. Results:

Results of the perceptual assessment using Arabic Articulatory Test<sup>(17)</sup> revealed that only four fricatives were mostly affected from the whole 23 tested Arabic phonemes; they were /s/, /ʃ/, /z/ and /f/, in 100% of completely edentulous patients. The same phonemes were affected after wearing the dentures without denture adhesives in all the patients. Marked perceptual improvement in phonemes was noticed after application of every adhesive type; 100% of patients improved in /s/ sound, 95% in /ʃ/ sound, 100% in /z/ sound and 100% in /f/ sound, however, there was no perceptual difference in phonetic improvement between the three types of adhesives.

Adhesive type 1: (Super corega) which is based on Carboxymethyl cellulose (CC).

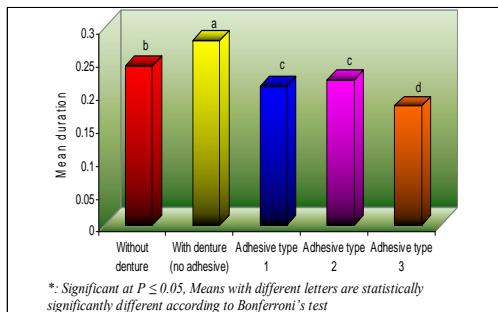
Adhesive type 2: (fittydent) which is based on Sodium Carboxymethyl cellulose and polyvinylacetate.

Adhesive type 3: (Fixodent) which is based on sodium Carboxymethyl cellulose and polymethylvinylether maleate.

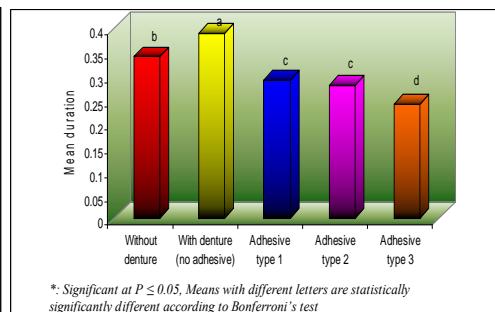
**Table (1): The total duration of the tested phonemes (/s/, /ʃ/, /z/, /f/) in initial and terminal-word positions in patients; without dentures, with dentures without adhesive and with three types of denture adhesives.**

Groups	Duration of initials in (m sec)		Duration of terminals in (m sec)	
	Mean	SD	Mean	SD
<b>Patients Without denture</b>	<b>0.24<sup>b</sup></b>	<b>0.03</b>	<b>0.34<sup>b</sup></b>	<b>0.03</b>
<b>Patients With denture (no adhesive)</b>	<b>0.28<sup>a</sup></b>	<b>0.02</b>	<b>0.39<sup>a</sup></b>	<b>0.04</b>
<b>Patients With denture With Adhesive type 1</b>	<b>0.21<sup>c</sup></b>	<b>0.03</b>	<b>0.29<sup>c</sup></b>	<b>0.04</b>
<b>Patients With denture With Adhesive type 2</b>	<b>0.22<sup>c</sup></b>	<b>0.02</b>	<b>0.28<sup>c</sup></b>	<b>0.02</b>
<b>Patients With denture With Adhesive type 3</b>	<b>0.18<sup>d</sup></b>	<b>0.04</b>	<b>0.24<sup>d</sup></b>	<b>0.03</b>

Means with different letters are statistically significantly different according to Bonferroni's test ( $P \leq 0.05$ ).



\*: Significant at  $P \leq 0.05$ . Means with different letters are statistically significantly different according to Bonferroni's test



\*: Significant at  $P \leq 0.05$ . Means with different letters are statistically significantly different according to Bonferroni's test

**Figure (3) Duration of initials.**

Results of the total duration of the acoustically tested phonemes (/s/, /ʃ/, /z/, /f/) in the initial and the terminal-word positions, as seen in Table (1), Figure (3,4) revealed a highly significant increase in the mean duration in patients with denture without adhesive (which showed the highest mean duration among the five groups) compared to patients without denture. There was a highly significant decrease in the mean duration value in patients with denture with adhesives compared to patients without denture.

**Figure (4) Duration of terminals.**

There was no significant difference in the mean duration in patients with adhesive type 1 compared to patients with adhesive type 2. There was a highly significant difference in the mean duration in patients with adhesive type 1 and adhesive type 2 compared to patients with adhesive type 3, which showed the lowest total mean duration value of the tested phonemes, both in initial and terminal-word positions.

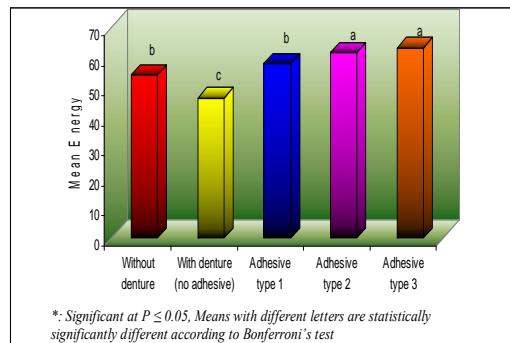
**Table (2): The total energy of the tested phonemes (/s/, /ʃ/, /z/, /f/) in initial and terminal-word positions in patients; without dentures, with dentures without adhesive, and with three types of denture adhesives.**

Groups	Energy of initials in (dB)		Energy of terminals in (dB)	
	Mean	SD	Mean	SD
<b>Patients Without denture</b>	<b>54.28<sup>b</sup></b>	<b>3.44</b>	<b>45.11<sup>d</sup></b>	<b>4.21</b>
<b>Patients With denture (no adhesive)</b>	<b>46.73<sup>c</sup></b>	<b>4.12</b>	<b>40.61<sup>e</sup></b>	<b>3.38</b>
<b>Patients With denture With Adhesive type 1</b>	<b>57.90<sup>b</sup></b>	<b>3.21</b>	<b>50.43<sup>c</sup></b>	<b>4.12</b>
<b>Patients With denture With Adhesive type 2</b>	<b>61.65<sup>a</sup></b>	<b>2.89</b>	<b>54.76<sup>b</sup></b>	<b>3.86</b>
<b>Patients With denture With Adhesive type 3</b>	<b>63.33<sup>a</sup></b>	<b>3.69</b>	<b>57.21<sup>a</sup></b>	<b>4.18</b>

Means with different letters are statistically significantly different according to Bonferroni's test ( $P \leq 0.05$ ).

Adhesive type 1: (Super corega) which is based on Carboxymethyl cellulose (CC).

Adhesive type 2: (fittydent) which is based on Sodium Carboxymethyl cellulose and polyvinylacetate.



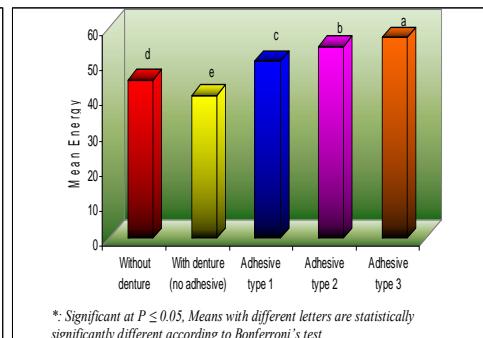
**Figure (5), Energy of initials**

The results of the total energy of the acoustically tested phonemes (/s/, /ʃ/, /z/, /ʒ/) in initial and terminal -word positions, as shown in Table (2), figure(5,6) revealed that patients wearing dentures without adhesives showed the lowest mean energy value among the five groups, both in the initial and the terminal-word positions. A highly significant difference in the mean energy values was found in patients without denture compared to patients wearing dentures without adhesives. There was insignificant difference in the mean energy in patients without denture compared to patients wearing denture with adhesive type 1 in the initial-word position. There was a significant increase in the mean energy of phonemes in patients with adhesive type 2 compared to adhesive type 1, both in the initial and the terminal-word positions. There was a further significant increase in the mean energy in patients with adhesive type 3 compared to type 2 in the terminal -word position.

#### 4. Discussion:

Perceptual Phonetic assessment of patients revealed that patients' articulation while they were edentulous was slightly deviated from normal, with defects in fricative phonemes (/s/, /ʃ/, /z/, /ʒ/), this may be attributed to a poor accommodation with the new situation. After wearing the denture without adhesives, there was a marked deterioration in the articulation. This is in accordance with Banknson and Byrne<sup>(18)</sup> who reported that loose dentures will not allow the tongue to function normally and this in turn will affect speech. This is also in agreement with Rothman<sup>(19)</sup> who clarified that pronunciation disturbance affecting fricative sounds could result from the direct influence of the artificial teeth and

Adhesive type 3: (Fixodent) which is based on sodium Carboxymethyl cellulose and polymethylvinylether maleate.



**Figure (6), Energy of terminals**

palate on the air flow as well as from different tongue positions and movements, as the tongue contacts specific parts of the teeth, alveolar ridge or the palate during each consonant pronunciation. Emily et al<sup>(20)</sup> clarified that consonant sounds are affected by the presence of poor retentive prosthetic appliance, and Ana Petrovic<sup>(21)</sup> found that unsatisfactory upper and lower dentures affect articulation markedly.

In the past, dentists used to think that the use of denture adhesives refers to poor dental skills as denture adhesives were thought as a solution for ill fitting denture<sup>(22,23)</sup>. Nowadays this philosophy was changed. The use of denture adhesives is highly recommended with patients seeking for extra retention demands that can't be achieved by the routine protocol of complete denture construction<sup>(24)</sup>. Denture retention and stability were significantly improved with the use of denture adhesives due to the bond created between the denture base and the underlying supporting tissues.<sup>(25)</sup>

Despite of the ability of the perceptual assessment to declare the improvement in the perceptually perceived phonemes in patients wearing dentures with adhesives than in those wearing dentures without adhesives, yet it couldn't differentiate the improvement between the different adhesive types. Therefore, acoustic assessment was performed as an objective assessment method for evaluating the most perceptually affected phonemes detected by the Arabic articulation test<sup>(17)</sup> which were the following fricatives; /s/, /ʃ/, /z/, /ʒ/, tested in initial and terminal- word positions.

Phonetic assessment for patients after applying adhesive type 1 revealed significant decrease in duration and significant increase in energy which reflected a better articulation, that may

be attributed to obvious improvement of lower denture retention as retention affects masticatory efficiency, phonation as well as self confidence as reported by Fujimori et al.<sup>(26)</sup>

Two hours after applying adhesive type 2(which combine both carboxy methylcellulose and polyvinylacetate), acoustic studies revealed that there was a significant decrease in the duration and a significant increase in the energy in comparison to adhesive type 1 (carboxy methylcellulose based type). This was in accordance with Panagiotouni et al<sup>(16)</sup> who concluded that fifty -dent denture adhesive gave a significant high scores on CC based adhesive type 1, when the degree of retention and retention duration were considered, due to the presence of polyvinylacetate adhesive material in combination with CC. This was almost the same results obtained by Berg et al<sup>(27)</sup> who declared that polyvinylacetate works as a powerful adhesive material but it works only on dry surface so carboxy methylcellulose acts as a sponge and absorbs saliva. Acoustic assessment for fricatives /s/, / ʃ /, / z / and / ʃ / in initial and terminal- word positions, 2 hours after applying the third denture adhesive type (which combines both polymethylvinylether maleate plus Carboxymethylcellulose) revealed a significant decrease in duration and increase in energy, which improved the articulated phonemes, in comparison to adhesive type1 and type 2. This is because polymethylvinylether maleate is a durable powerful adhesive and Carboxymethylcellulose gives a rapid initial bond. This was in accordance with Psillakis et al<sup>(28)</sup> who tested Fixodent denture adhesive and concluded that using denture adhesive (fixodent) subjectively improves speech and chewing ability. This was also in accordance with Ozcan et al,<sup>(29)</sup> who clarified that bite force until denture dislodgement was increased for both old and new dentures after the use of denture adhesive based on polymethylvinylether maleate plus carboxymethyl cellulose (kukident) and this improvement lasted for 6 hours after application of the denture adhesives.

## 5. Conclusion:

This study clarified that the use of the denture adhesives markedly improves articulation in complete denture wearers as evidenced by both subjective and objective speech assessment, mainly due to their valuable effect on denture retention. The polymethylvinylether maleate-based adhesive Compound (Fixodent) gives the best results than Carboxymethyl cellulose-based denture adhesives (Supercorega) and Carboxymethyl cellulose plus Polyvinylacetate-based adhesive (fiftydent) as evidenced by the improvement in the acoustic features of the tested Arabic phonemes.

## Corresponding author

Essam Adel Aziz

Prosthodontic Department, Faculty of Oral and Dental Medicine, Cairo University, Cairo Egypt

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9/5/2010

# Assessment of Working Memory in Normal Children and Children Who Stutter

**Hazem Aboul Oyoun<sup>1</sup>; Hossam El Dessouky<sup>2</sup>; Sahar Shohdi<sup>\*2</sup> and Aisha Fawzy<sup>2</sup>**

<sup>1</sup>Otorhinolaryngology, ENT Department, Faculty of Medicine, Cairo University. Cairo, Egypt

<sup>2</sup>Phoniatrics, Phoniatic Unit, ENT Department, Faculty of medicine, Cairo University. Cairo, Egypt    sshohdi@hotmail.com

**Abstract:** The aim of this study is to assess working memory (WM) abilities in normal children and Children Who Stutter (CWS) then to compare the results in order to detect if WM deficits have a role in the development of stuttering. 30 normal children and 30 children who stutter were subjected to WM recall abilities tests and nonword repetition tasks. The WM recall tests included recall of word sets different in length and rhyming, digit span, letter sequences and picture-number test. The nonword repetition test was used to assess phonological encoding through measuring number of phonological errors produced on repeating the task, and to measure the reaction time. The children who stutter (CWS) had performed poorly on some working memory tests compared to the control group. Conclusion: Children who stutter may have diminished ability to recall nonwords and some of working memory abilities and that further investigation into this possibility may shed light on the emergence and characteristics of childhood stuttering.

[Hazem Aboul Oyoun; Hossam El Dessouky; Sahar Shohdi and Aisha Fawzy. Assessment of Working Memory in Normal Children and Children Who Stutter. Journal of American Science 2010;6(11):562-569]. (ISSN: 1545-1003).

**Key words:** working memory; children who stutter; nonword repetition; phonological encoding, phonological errors, reaction time.

## 1. Introduction:

Stuttering has been described as a speech motor disorder that disrupts the timing and/or coordination between the respiratory, laryngeal, and vocal tract symptoms of speech (Van Lieshout et al., 2004). It is true that people who stutter suffer from some overt phenomena like tense movements of face, jaw and occasionally extremities; however it is also important to investigate where these phenomena came from (Kawai, 2008).

Recently working memory has been implicated in the development of stuttering. Working memory is universally recognized as neurocognitive system that provides temporary storage and processing of incoming information. Baddeley (2003) envisioned working memory as a multicomponent neurocognitive system that includes a central executive, visuospatial sketchpad and phonological loop. The phonological loop includes short term storage and rehearsal of incoming verbal information to enable comprehension. Phonological encoding during speech planning involves retrieving phonological material from storage to build articulatory plans (Levelt, 1989). Working memory is considered critical to phonological encoding (Gathercole and Baddeley, 1993) and vital to higher level cognition (Rosen and Engle, 1997).

One prominent theory which is the covert repair hypothesis of Postma and Kolk (1993) assumes that

stuttering arises because inefficient or slow phonological encoding leads to an increase in covert repairs to the phonological plan, particularly when the individual is intent on speaking at a rate exceeding the compliance of the phonological encoding mechanism.

Cognitive models of speech production, such as that proposed by Levelt and colleagues (1999), provide a useful framework to consider the linguistic processes that might be deficient in stuttering. A number of studies have explored the hypothesis that retrieving semantic and/or phonological information for the purposes of linguistic encoding might be a source of deficit or delay in stuttering (Newman and Bernstein-Ratner, 2007). However these studies have produced mixed findings

The aim of this study is to assess working memory (WM) abilities in normal children and Children Who Stutter (CWS) then to compare the results in order to detect if WM deficits have a role in the development of stuttering.

## 2. Subjects and methods:

### Subjects

Participants in this study were 30 normal children (group 1) and 30 children with stuttering (group 2). All children ages were between 5 and 13 years. The two groups were matched in age and gender. Each group was further subdivided according

to children's age into 2 subgroups; A) 15 Children with ages ranging between 5 and 9 years. B) 15 Children with ages ranging between 9 and 13 years. Mean age of children who stutter (CWS) was 7.51; while the mean age of the control children was 7.93. They were all selected from the outpatient clinic, Phoniatric unit of Kasr El Aini Hospital and with following exclusion criteria: No history &/or presence of delayed language development, dyslalia, mental retardation, hearing impairment, psychological problems or neurological problems. The study was done in the period from 2008-2009.

### Methods

All participants were subjected to the following protocol of assessment:

#### History taking and patient examination:

Including personal, perinatal, natal, postnatal developmental history and any history of childhood illness together with general, neurological, local and ENT examination.

#### Psychometric evaluation:

- a. Stanford Binet test (4<sup>th</sup> version) (Thorndike et al., 1986). All selected participants were of average IQ (IQ≥ 85)
- b. Illinois psycholinguistic test (Kirk et. al., 1969): Only 3 items were selected (psycholinguistic age, auditory sequential memory and visual sequential memory). These were selected to test other memory abilities rather than working memory.

The Assessment protocol of Disfluency used in Phoniatric Unit, Cairo University (Shohdi, 1999): to assess disfluency and severity of stuttering.

#### Battery of assessment of working memory (WM):

All tasks were recorded using an audiotape (National Rx- CW30F) for documentation.

#### A. WM Recall Tasks:

- 1- Recall of short versus long word sets: to assess the efficiency of retrieval of phonological sequences of different length.
- 2- Recall of similar versus dissimilar word sets: to assess the efficiency of retrieval of phonological sequences with different rhyming. The word sets were presented verbally by the experimenter. Children had to remember the words in the same order in which they were presented. For a trial to be considered correct, all words in that sequence had to be remembered in the correct order. Testing continued as long as the child managed to correctly repeat at least one of the two trials at a particular list length.
- 3-Digit span versus letter sequences: Sequences of digits and letters graded from 3 to 10 were administered verbally provided not to be in a

sequential order. Ten digits between 1 and 10 were used. Ten letters were also used (not sequential).

- 4- Picture-number test (Ekstrom et al., 1975): This test was used to address the visuospatial component of WM. Some modifications to the original test were done to be suitable for the examined age range. It was graded from 3 to 10 picture-number tests, it was presented visually for 60 seconds then the child was asked about the missing numbers associated with the presented pictures. The total score was given according to the maximum number of correct missing numbers recalled and associated with the correct presented pictures.

The purpose of assessment of WM recall abilities is to explore the role of verbal WM and its operations in the development of stuttering.

#### B. Non-Word Repetition tasks:

Non-word repetition was considered to be a phonological short term memory task, in which the phonological forms of the stimuli are unfamiliar thus requiring children to code new phonological sequences and maintain them in phonological memory. By repeating nonwords the speaker relies on the storage component of the phonological loop without the complicating effects of prior lexical knowledge (Gathercole et al., 1994). A list of 40 non-words was administered (20 bisyllabic and 20 trisyllabic non-words).

The participants were examined in a quiet setting on two sessions. Three examples of nonwords were given by the examiner and the child was asked to repeat each. Once the child appeared to be comfortable with the setting and understanding to the task, the 40 non-words were presented verbally by the examiner using the microphone of the Computerized Speech Lab. (Kay model 4300) in order to measure the reaction time using spectrographic analysis. The task was also recorded using an audiotape. Then the recording task was transcribed and analyzed for detection of (A) Phonological errors and (B) Disfluency. Reaction time (RT) was considered as the time between the end of the examiner's stimulus and the beginning of the child's response to detect if stutterers differ in their speed of phonological encoding. For the Phonological errors, the responses were scored as either correct or incorrect. All phonemes within a non-word had to be produced correctly for the response to be scored correct. Response of CWS were judged as either correct (no phonological error) or incorrect (presence of phonological error). The total number of phonological errors of the 40 non-words for all the participants was calculated. For disfluency, the response of CWS were judged as either fluent or disfluent. The number of disfluently

produced responses was then calculated for each nonword and across all the task items. Disfluency was measured in order to test its relation with phonological errors on the nonwords repetition task.

#### Statistical Analysis:

Data was analyzed by Microsoft office 2003 (excel) and Statistical Package for Social Science (SPSS) Version 10. Parametric data was expressed as mean and standard deviation while parametric data was expressed as number and percentage of the total. Student's t-test was used to compare between the 2 test groups. Measuring the mutual correspondence between two values was done using the Spearman correlation coefficient.

### 3. Results and Discussion:

#### Psychometric evaluation:

a. Stanford – Binet test (4<sup>th</sup> version) (Thorndike et al., 1986): There was no significant difference between controls and stutterers for all the test items except the *memory for sentences* was significantly better in controls (mean =84.53; SD= 8.42).

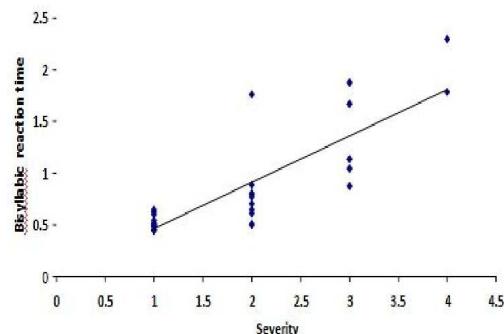
b. Illinois Psycholinguistic test (Kirk et al., 1969)

There was no significant difference between controls and stutterers regarding the tested items of the Illinois test.

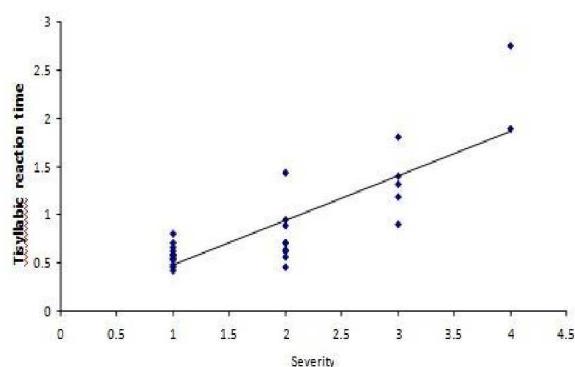
#### Disfluency:

Results showed that there were 13 children with mild stuttering, 10 children with mild to moderate stuttering, 5 children with moderate stuttering and 2 children with moderate to severe stuttering.

The correlation between severity of stuttering and reaction time of both bisyllabic and trisyllabic nonwords showed a highly significant +ve correlation ( $r$  value=0.825 and 0.827 respectively) (Figure 1 & 2).



**Figure (1): The correlation between severity of stuttering and reaction time of bisyllabic nonwords.**



between the age and recall of short word sets, long word sets, dissimilar word sets, letter sequences and

picture-number tests showed a highly significant +ve correlation (Table 1).

**Table (1): The correlation between age and WM recall tasks in controls and stutterers**

	Control age ( r value )	Significance	Stutterers age ( r value )	Significance
<b>Recall of short word sets</b>	<b>0.397</b>	S	<b>0.501</b>	<b>HS</b>
<b>Recall of long word sets</b>	<b>0.366</b>	S	<b>0.591</b>	<b>HS</b>
<b>Recall of similar words</b>	<b>0.517</b>	<b>HS</b>	<b>0.378</b>	S
<b>Recall of dissimilar words</b>	<b>0.397</b>	S	<b>0.501</b>	<b>HS</b>
<b>Digit span</b>	<b>0.401</b>	S	<b>0.382</b>	S
<b>Letter sequences recall</b>	<b>0.492</b>	<b>HS</b>	<b>0.513</b>	<b>HS</b>

WM: working memory, HS: highly significant, S=significant

Both controls and stutterers showed a highly significant better recall of short (controls: mean = 5.40; SD = 1.19, stutterers: mean = 5.00; SD = 1.08) than long word sets (controls = 3.83; SD= 0.83 and stutterers= 3.67; SD= 0.66), dissimilar (controls: mean = 5.40; SD= 1.19 and stutterers: mean= 5.00; SD= 1.08) than similar word sets (Controls: mean=3.53; SD=0.73 and stutterers: mean=3.43; SD=0.73 ), Digit span (controls: mean= 5.93; SD= 1.41 and stutterers: mean =5.03; SD=1.27) versus Letter-sequences (Controls: mean=4.57; SD=1.33 and stutterers: mean=3.97; SD= 1.10).

**Table (2): Number of phonological errors in bisyllabic nonwords and trisyllabic nonwords between controls and stutterers.**

		Controls	Stutterers	P VALUE
<b>Phonological errors</b>	<b>Bisyllabic nonword</b>	4	32	0.000002**
	<b>Trisyllabic nonword</b>	35	61	0.006**
<b>P VALUE</b>		0.000**	0.002**	

\*\* = highly significant p value ( $\leq 0.01$ )

There was no significant difference in the number of phonological errors in bisyllabic nonwords between controls (subgroup A, aged 5-9 y) and controls (subgroup B, 9-13y). While control subgroup A showed a highly significant more number of phonological errors than control subgroup B in trisyllabic nonwords.

Stutterers (subgroup A, aged 5-9 y) had a highly significant more number of phonological errors than stutterers (subgroup B, aged 9-13 y) in both bisyllabic and trisyllabic nonwords.

#### Disfluency:

There was no significant difference in the number of disfluencies between bisyllabic and trisyllabic nonwords in the stutterers ( $p=0.292$ ).

#### B) Nonword Repetition Tasks:

##### Phonological Errors (PEs):

Stutterers had a highly significant more phonological errors compared to the controls in both bisyllabic and trisyllabic nonwords repetition task.

There were a highly significant more number of phonological errors in trisyllabic nonwords than in bisyllabic nonwords in both the controls and the stutterers.

Stutterers subgroup A had a highly significant more number of disfluencies in both bisyllabic and trisyllabic nonwords compared to stutterers subgroup B ( $p=0.002$  and  $0.000$  respectively).

There was no significant difference between the number of phonological errors and the number of disfluencies in stutterers in both bisyllabic and trisyllabic nonwords ( $p=0.155$  and  $0.436$  respectively).

#### Nonwords Reaction Time (RT) (Table 3)

There was a highly significant longer reaction time in the stutterers than the controls for both the bisyllabic and trisyllabic nonwords repetition tasks (mean of bisyllabic RT in control group= $0.56$ ; SD= $0.12$ ; mean of bisyllabic RT in stuttering group= $0.85$ ; SD= $0.51$ ) and (mean of trisyllabic RT in

control group=0.58; SD= 0.14; mean of trisyllabic RT in stutterers=0.88; SD= 0.53).

Neither the controls nor the stutterers showed significant difference between reaction time

for both bisyllabic and trisyllabic nonwords repetition task.

**Table (3): Reaction time of bisyllabic and trisyllabic nonwords in controls and stutterers**

		Controls		Stutterers		<b>P VALUE</b>
		<b>Mean</b>	<b>SD</b>	<b>Mean</b>	<b>SD</b>	
<b>Non-word repetition</b>	<b>Mean BRT</b>	0.56	0.12	0.85	0.51	0.005**
	<b>Mean TRT</b>	0.58	0.14	0.88	0.53	0.006**
	<b>P value</b>	0.504	0.841			

(SD=Standard deviation; BRT= Bisyllabic Reaction Time; TRT= Trisyllabic Reaction Time)

\*\* = highly significant p value ( $\leq 0.01$ )

### 3. Discussion:

Psychometric evaluation:

Stanford-Binet test:

Testing different memory abilities showed no significant difference between controls and stutterers except for memory for sentences as controls showed a slightly better performance and that may be explained by the fact that sentences are more complex than words and convey more grammatical complexity which can increase the mental load on stuttering memory recall abilities.

Illinois Test:

There was no significant difference between controls and stutterers in visual as well as auditory sequential memories.

Severity of stuttering:

Correlation between severity of stuttering and reaction time in both bisyllabic and trisyllabic nonwords showed a significantly +ve correlation. This means that with increase in the severity of stuttering, the time needed for encoding and phonological processing increases.

Working memory recall tests:

The present study showed no significant difference between controls and stutterers in various recall tasks, however the stutterers showed poorer performance in picture-number test than controls and that could be explained that this task put more pressure and demands on their mental and recall abilities due to time pressure during the task.

There was a highly significant ability to recall short word sets than recall of long word sets in both stutterers and controls. This can be attributed to the fact that short words have less articulatory duration

and less phonological complexity and that short articulatory duration allows rapidly spoken words to be rehearsed more frequently. Words that are rehearsed more frequently are less likely to decay before an entire sequence of them can be recalled (Baddeley, 1986). And it could also be explained by what Caplen et al., 1992 had hypothesized that the word-length effect stems from speech planning rather than overt or covert articulation and that speech planning are influenced by the phonological complexity of words, as indexed by number of phonemes and syllables. Comparing the results of recall of dissimilar words versus recall of similar words, both controls and stutterers showed better performance in recall of dissimilar words than recall of similar words. Typically, sequences of phonologically similar words are remembered less well than sequences of dissimilar words (Schweickert et al., 1990). The phonological similarity effect supports the phonological loop model's assumption that verbal information is represented in a modality-specific phonological store rather than in another type of storage system. Comparing the results of digit span versus letter-sequence tests in both controls and stutterers, it was clear that both groups had better performance in digit span than in recall of letter sequences. This could be explained by the fact that children especially the younger ones are more familiar with digits than with letters.

A correlation between age and various recall parameters in both control and stuttering groups showed a significant +ve correlation in both groups. Some researchers showed that brain maturation increases with increase in age. Structural neuroimaging studies show that regions of parietal and especially frontal cortex undergo changes well

into adolescence and early adulthood. These studies suggest that the brain systems underlying WM and thus WM undergo changes well into adolescence and regions are activated when children and adults retain information in WM. Behaviorally, WM undergoes substantial growth over the course of development. For example, counting and listening span increase from 7 to 13 years of age (Siegel and Ryan, 1989), and digit span increases from 4-5 items at 4-5 years of age to 5-7 items at 14-15 years of age (Conklin et al., 2007).

#### Non-Word Repetition Task:

##### Phonological errors (PEs)

The number of phonological errors in controls was lower than stutterers. This could be attributed to the poor rehearsal and storage abilities of children with stuttering. The findings of the present study goes with the Covert Repair Hypothesis assumption that building a phonological output representation, is realized through the association of phonemes with slots in a metrically defined frame. Normally, if a slot in the frame needs to be filled, the phoneme that has the highest activation level at the critical time point is selected. In a person who stutters, however, activation spreading is slow. This means that when a specific slot needs to be filled, it is likely that competition among candidate phonemes has not settled. Consequently, a misselection may occur. Many such misselections are pre-articulatory detected and repaired, which yield interruptions and restarts in overt speech, if one couldn't do these covert repairs, the overt phonological errors would be produced.

Both controls and stutterers also produced more errors in trisyllabic than bisyllabic nonwords. The more the length of the nonwords, the more difficulty in encoding, rehearsal and storage. Present findings are also similar to those reported by Montgomery (1995), in children with specific language impairment (SLI). He found that children with SLI performed significantly worse than language-matched typically developing peers on three syllable non-words. At the 3 syllables, the CWS showed more difficulty with the task, as do many children with SLI.

On comparing the result of the number of phonological errors in bisyllabic and trisyllabic nonwords between the two control age subgroups, it was found that no significant differences between both groups as regards number of phonological errors in bisyllabic nonwords, but it showed that group (A) aged between 5-9 years produced more errors in trisyllabic nonwords than group (B) aged between 9-13 years. As regards the two stutterers' age subgroups, group (A) showed more errors than group (B) in both bisyllabic and trisyllabic nonwords. These

results suggest that phonological ability of children improves when they grow older.

#### Disfluency

Comparing the number of disfluencies between bisyllabic and trisyllabic nonwords in stuttering group, there was no significant fluctuation in fluency as nonwords increased in length. Thus, it would appear that even though CWS had greater difficulty (i.e., producing PEs) in the two and three syllable nonwords than their normally fluent peers, these difficulties did not manifest themselves in children's fluency. And thus, the poor performance of CWS in nonwords repetition task as regards the phonological errors was not related to speech production difficulties (speech disfluencies) but the results cannot be considered conclusive as the majority of the CWS were of mild and moderate degrees of severity. However, the finding is consistent with both Hakim and Ratner (2004) and Anderson et al (2006) that revealed no significant fluctuation in fluency as nonwords increased in length. On contrary, an older literature on stuttering in adults has documented that disfluencies should increase with increasing word length (Soderberg, 1966 and Wingate, 1967).

Comparing the number of disfluencies of bisyllabic and trisyllabic nonwords between the two stutterers' age subgroups, there was highly significant difference as group (A) produced more disfluencies than group (B) in both bisyllabic and trisyllabic nonwords. However there is no evidence that disfluency increases in young age.

Comparing the number of disfluencies and number of phonological errors in bisyllabic and trisyllabic nonwords in stutterers, no significant difference was found. The results were in accordance with Wolk et al (2000) who indicated that frequency of disfluency on syllables with phonological errors was similar to those produced without errors. According to them, it may have been predicted that more disfluency would occur during instances of phonological errors. One explanation for the results is that the two disorders (stuttering and disordered phonology) may indeed be separate entities. Although stuttering and phonological errors may co-occur in the same child, they may not interact in the same syllable.

#### Reaction Times (RTs)

Comparing the results of reaction time of bisyllabic and trisyllabic nonwords between the controls and the stutterers, it was found that the stutterers had longer reaction time in both bisyllabic and trisyllabic nonwords than the control group, although, that only fluent productions were selected to measure the reaction time. And that led us to the

prediction that children who stutter may take more time for phonological encoding than normal children. These results were in agreement with the results of the study by Kolk et al (1991) who suggested that individuals who stutter may demonstrate impairment in their phonological encoding mechanisms. This assumption leads to the prediction that the activation of target phonemes is somewhat delayed for people who stutter, resulting in a relatively long period of time when target phonemes are in competition with other phonemes. But the results did not match with the results of Bakhtiar et al (2008) who found no significant difference between the two groups regarding reaction time in both bisyllabic and trisyllabic nonwords. They assumed that the defect might be in the other parts of linguistic processing but not phonological ones.

The comparison of reaction time between bisyllabic and trisyllabic nonwords in both controls and stutterers showed insignificant difference. Lack of significance may be attributed to the small sample size and the difference could be significant with more increase in nonwords syllabic length. The result of the present study is in agreement with the results of Bakhtiar et al (2008).

The present study showed no difference between controls and stutterers in recall tasks, except picture-number test. This could be explained by that their prior lexical knowledge about the words and the semantic content of these words facilitated their recall.

The results of this study are in agreement with the Covert Repair Hypothesis in that the children with stuttering produced more phonological errors than normal children and they had longer reaction time. This may support the assumption of the Covert Repair Hypothesis of the presence of a phonological encoding deficit and that leads to the prediction that the activation of target phonemes is somewhat delayed for people who stutter, resulting in a relatively long period of time when target phonemes are in competition with other phonemes. The Covert Repair Hypothesis assumed that when disfluency is suppressed, overt speech errors should increase in frequency and the more overt stuttering, the less phonological errors should be observable; however, this was not proved in this study. The relation between disfluencies and phonological errors is still query waiting for further investigation.

#### **4. Conclusion:**

In accordance with several studies, it was found that performance of CWS on phonological memory tasks lag to some degree behind that of normal children. Further research in this area may shed light on the emergence and characteristics of childhood

stuttering and that can lead to new approaches for the management of stuttering.

#### **Corresponding author**

Sahar Shohdi  
Phoniatrics, Phoniatic Unit, ENT Department,  
Faculty of medicine, Cairo University. Cairo, Egypt  
sshohdi@hotmail.com

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9/2/2010

## CHEMICAL STUDIES ON 3,6-DICHLOROPYRIDAZINE

Mohamed H. Sherif, Gamal A. Ahmed, Adel A. Elbahnasawy and Eman O. Helal

Department of Chemistry, Faculty of Science, Zagazig University, Egypt  
[meahsherif@hotmail.com](mailto:meahsherif@hotmail.com)

**ABSTRACT:** Reaction of 3,6-dichloropyridazine (**1**) with acid hydrazides, p-toluene sulfonylhydrazine, anthranilic acid derivatives and ammonium hydroxide afforded the compounds (**2a,b**), (**3**), (**4a,b**) and (**5**) respectively. Compound (**5**) reacted with aromatic aldehydes yielded the Schiff's bases (**6**) and (**7**). Compound (**6**) reacted with anthranilic acid derivatives and gave (**8**). Also, compound (**1**) easily reacted with 2-chlorobenzylamine, sodium azide and thiosemicarbazide afforded the compounds (**9**), (**10**) and (**11**) respectively.

[Mohamed H. Sherif, Gamal A. Ahmed, Adel A. Elbahnasawy and Eman O. Helal. CHEMICAL STUDIES ON 3,6-DICHLOROPYRIDAZINE. Journal of American Science 2010;6(11):570-574]. (ISSN: 1545-1003).

**Keywords:** 3,6-dichloropyridazine; acid hydrazides; p-toluene sulfonylhydrazine; anthranilic acid derivative; ammonium hydroxide

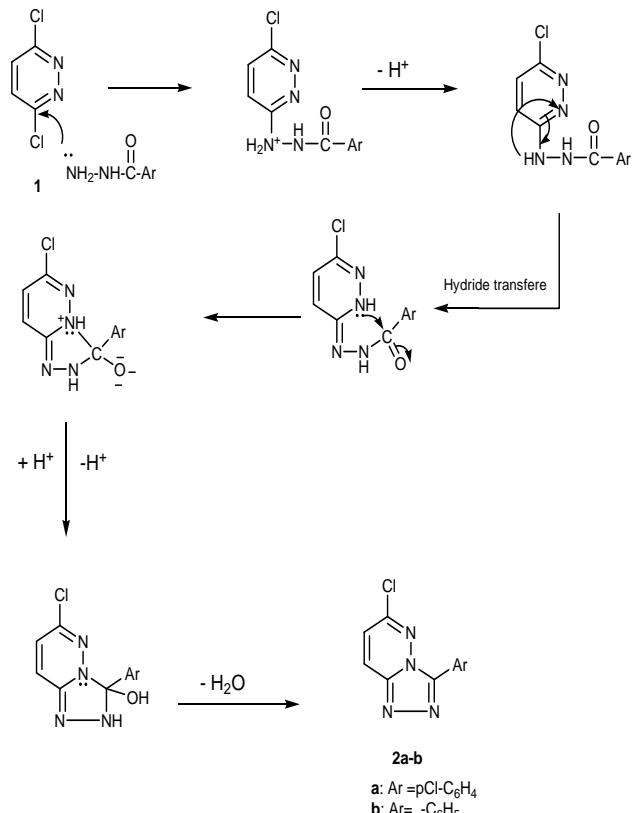
### 1. Introduction

Pyridazine derivatives continue to attract considerable attention due to the wide range of their biological activity (Yamada et al., 1981) and (Easmon et al., 2001). 3,6-disubstituted pyridazine (**1**) had a bioactive effect and was considered to be an appropriate starting material for pyridazine derivatives. It was well known that selective mono-substitution of single chlorine atom in (**1**) can be achieved when (**1**) was allowed to react with oxygen (Parrot et al., 1999) and (Huang et al., 2003), sulfur (Parrot et al., 1999), nitrogen (Rabisson et al., 2003) and (Ding et al., 2002) and o-halogen nucleophiles (Goodman et al., 1999), (Hamdouchi et al., 2003) and (Tye et al., 2006). Heterocyclic thiosemicarboxamide (pyridine and diazines) have shown an inhibitory effect on the acidity of gastric secretions (Van Hoeven et al., 1975), (Yamanoto et al., 1995) and (Pagni et al., 2000). The second important pharmaceutical property was an action against mycobacteria in particular against mycobacterium tuberculosis (Luo et al., 2004), (Caumul et al., 2005) and (Al-Awadi et al., 2007).

### 2. Results and discussion

When 3,6-dichloropyridazine (**1**) was allowed to react with acid hydrazides, namely (p-chlorobenzoylhydrazine and benzoylhydrazine) it gave the triazolopyridazine derivatives (**2a,b**) which were elucidated from their correct spectral data (Table 1).

The mechanism of reaction could be as:

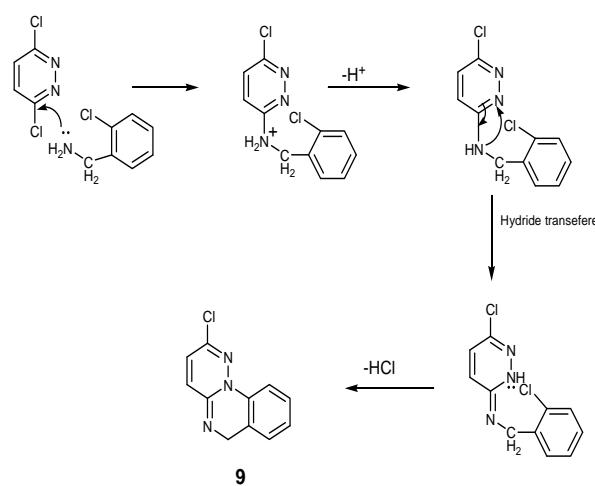


Also, compound (**1**) easily reacted with p-toluenesulfonylhydrazine afforded the compound (**3**). On the other hand, when compound (**1**) was reacted with anthranilic acid derivatives yielded the tricyclic compounds (**4a,b**).

Compound (**1**) easily reacted with ammonium hydroxide solution which formed the 3-amino-6-

chloropyridazine (5) which condensed with aromatic aldehydes, (namely, 3,4,5-trimethoxy benzaldehyde and m-nitrobenzaldehyde), and gave the Schiff's bases (6) and (7) respectively. Reaction of compounds (6) and (7) with anthranilic acid derivatives gave compounds (8a,b) (scheme 1).

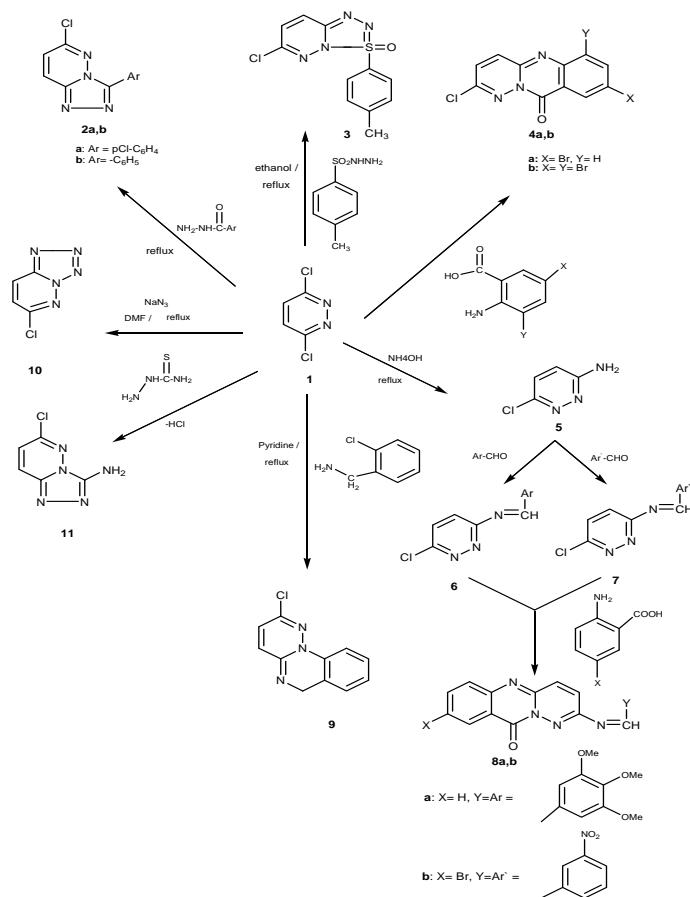
Reaction of compound (1) with O-chlorobenzylamine in pyridine afforded the tricyclic compound (9). The structure of (9) was confirmed from (1) of correct weight ( $M/e = 217$ ) and  $^1H$  NMR spectrum (Table 1). The mechanism of reaction could be as:



On the other hand, when compound (1) was allowed to react with sodium azide afforded the chlorotetrazolopyridazine derivative (10) (scheme 1). The structure of (10) was elucidated from the correct spectral data (table 1), and the mass spectrum showed a base peak at ( $M/e = 151$ ). Reaction of compound (1) with thiosemicarbazide in ethanol afforded compound (11). The chemical structure of compound (11) was confirmed from the correct spectral data (Table 1).

### 3. Experimental

All melting points were uncorrected and were determined on Gallenkamp electric melting point apparatus. IR spectra (KBr discs) were recorded on a FT/IR-400 spectrophotometer (Perkin Elmer).  $^1H$  NMR spectra were recorded on a varian-300 (DMS-d6) solution. Chemical shifts were reported as  $\delta$  values relative to tetramethylsilane (TMS) as internal reference. The mass spectra were run at 70 ev on a varian MAT 711 mass spectrometer.



**Scheme 1**

#### 1. Reaction of compound (1) with acid hydrazides; formation of compounds (2a,b).

A mixture of compound (1) (0.01 mole) and acid hydrazides, namely (p-chlorobenzoylhydrazine and benzoylhydrazine) in absolute ethanol (20 ml) was heated under reflux for 7 hours. The solid product obtained upon cooling, was filtered off, dried, and crystallized from ethanol to give compounds (2a,b) as white crystals (Table 1).

#### 2. Reaction of compound (1) with p-toluenesulfonylhydrazine; formation of compound (3).

A mixture of compound (1) (0.01 mole) and  $p$ -toluenesulfonylhydrazine in absolute ethanol (20 ml) was heated under reflux for 7 hours. The solid product obtained upon cooling, was filtered off, dried, and crystallized from ethanol to give compound (3) as white crystals (Table 1).

**Table (1): Physical and spectral data of the prepared compounds.**

Compd. No.	m.p. °C	Yield (%)	Solvent of cryst.	IR cm <sup>-1</sup>	<sup>1</sup> H NMR ppm
<b>2a</b>	170	60	Et-OH	1593(C=N) 1557(C=N)	7.90(d,1H, CH-) 8.10(d,1H, CH-) 6.50-7.00(m, 4H ar)
<b>2b</b>	230	60	Et-OH	1569(C=N) 1584(C=N)	7.60(d,1H, CH-) 7.30(d,1H, CH-) 6.70-7.00(m,5H ar)
<b>3</b>	180	80	Et-OH	1572(C=N) 1340(S=O)	2.30(d,1H, CH-) 6.90(d, 1H, CH-) 7.40(d, 1H, CH-) 6.20-6.80(m,4H ar)
<b>4a</b>	260	76	Et-OH	1769(C=O) 1629(C=N)	8.12(d, 1H, CH-) 8.41( d, 1H, CH-) 7.39-7.72(m,3H ar)
<b>4b</b>	280	65	Et-OH	1740(C=O) 1641(C=N)	8.27(d, 1H, CH-) 8.51(d, 1H, CH-) 7.58-7.61(2H ar)
<b>5</b>	240	65	Et-OH	3320, 3214 (NH <sub>2</sub> )	6.40-6.60(broad,2H, NH <sub>2</sub> ) 7.50(d,1H, CH-) 7.30(d, 1H, CH-)
<b>6</b>	>360	70	Et-OH	1587(C=N)	7.10(s,1H, CH=N-) 7.30(d, 1H, CH-) 7.50(d, 1H, CH-) 3.80-4.00(s, 9H, 3 OCH <sub>3</sub> )
<b>7</b>	>360	78	Et-OH	1590 (CH=N) 1450 (NO <sub>2</sub> )	8.30(d,1H, CH-) 8.50(d, 1H, CH-) 7.50(s, 1H, CH=N-) 7.80-8.00(m, 4H ar)
<b>8a</b>	190	76	Et-OH	1688(C=O) 1586(C=N)	3.40-4.00(s,9H, 3 OCH <sub>3</sub> ) 7.40(d, 1H, CH-) 7.60(d, 1H, CH-) 7.50(s, CH=N) 6.30-7.20(m,6H ar)
<b>8b</b>	205	72	Et-OH	1690(C=O) 1560(C=N) 1460(NO <sub>2</sub> )	7.80(d,1H, CH-) 7.60(d, 1H, CH-) 7.50(s, CH=N-) 6.50-7.40(m, 7H ar)
<b>9</b>	160	65	Et-OH	1600(C=N)	7.80(d,1H, CH-) 7.60(d, 1H, CH-) 4.62(s, 2H,CH <sub>2</sub> -) 6.90-7.40(m, 4H ar)
<b>10</b>	130	65	Benzene	1564(C=N)	7.50(d,1H, CH-) 7.75(d, 1H, CH-)
<b>11</b>	235	65	Et-OH	1610(C=N) 1549(C=N) 3380,3290(NH <sub>2</sub> )	7.40(d, 1H, CH-) 7.60(d, 1H, CH-) 5.60(broad,2H, NH <sub>2</sub> )

### 3. Reaction of compound (1) with anthranilic acid derivatives; formation of compounds (4a,b).

A mixture of compound (1) (0.01 mole) and anthranilic acid derivatives (namely 5-bromoanthranilic acid and 3,5 dibromoanthranilic acid) (0.01 mole) in absolute ethanol (20 ml) was heated under reflux for 7 hours. The solid product obtained upon cooling, was filtered off, dried, and crystallized from ethanol to give compounds (4a,b) as white crystals (**Table 1**).

### 4. Reaction of compound (5) with aromatic aldehydes; formation of schiff's bases (6) and (7).

A mixture of compound (5) (0.01 mole) and aromatic aldehydes (namely, 3-Nitrobenzaldehyde and 3,4,5-trimethoxy benzaldehyde) (0.01 mole), in glacial acetic acid (20 ml) was heated under reflux for 7 hours. The solid product obtained upon cooling, poured on water, filtered off and crystallized from ethanol to give compounds (6) and (7) respectively as white crystals (**Table 1**).

### 5. Reaction of compound (6) with anthranilic acid derivatives; formation of compounds (8a,b).

A mixture of compound (6 and / or 7) (0.01 mole) and anthranilic acid derivatives in absolute ethanol (20 ml) was heated under reflux for 7 hours. The solid product obtained upon cooling, was filtered off, dried, and crystallized from ethanol to give compounds (8a,b) as white crystals (**Table 1**).

### 6. Reaction of compound (1) with o-chlorobenzylamine; formation of compound (9).

A mixture of compound (1) (0.01 mole) and o-chlorobenzylamine (0.01 mole) in pyridine (20 ml) was heated under reflux for 7 hours. The solid product obtained upon cooling, poured on ice , neutralized with HCl, and the precipitated solid was filtered off, washed with water, dried and crystallized from ethanol to give compounds (9) as brown crystals (**Table 1**).

### 7. Reaction of compound (1) with sodium azide; formation of compound (10).

A mixture of compound (1) (0.01 mole) and sodium azide (0.01 mole) in DMF (20 ml) was heated under reflux for 7 hours. The solid obtained upon dilution with water, filtered off and crystallized from benzene to give compounds (10) as white crystals (**Table 1**).

### 8. Reaction of compound (1) with thiosemicarbazide; formation of compound (11).

A mixture of compound (1) (0.01 mole) and thiosemicarbazide hydrochloride (0.01 mole) in absolute ethanol (20 ml) was heated under reflux for 7 hours. The solid product obtained upon cooling, was filtered off, dried, and crystallized from ethanol to give

compounds (11) as white crystals (**Table 1**).

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9/29/2010

# Citizens' Attitude toward's Local Government and Citizen's Participation in Local Government

Seyed Hamid Mohammadi

Department of Social and Development Sciences  
Faculty of Human Ecology, Putra University, Malaysia  
Tel: 60-17-2118806 E-mail: [hmd\\_mohamadi@yahoo.com](mailto:hmd_mohamadi@yahoo.com)

Sharifah Norazizan

Department of Social and Development Sciences  
Faculty of Human Ecology, Putra University, Malaysia  
E-mail: [sharifah@putra.upm.edu.my](mailto:sharifah@putra.upm.edu.my)

Nobaya Ahmad

Department of Social and Development Sciences  
Faculty of Human Ecology, Putra University, Malaysia  
E-mail: [nobaya@putra.upm.edu.my](mailto:nobaya@putra.upm.edu.my)

**Abstract:** The purpose of this paper is to describe the citizen's attitude toward local government and its relationship with level of participation. Participation in local government issues, requires a favorable attitude towards local government, councilors and councils' performance in terms of efficiency. The paper is based on the study of citizens' attitude towards local government, which was carried out in Torbat Hedarieh city, Iran. The analysis of data uses Pearson correlation to determine the relationship between variables involved. The findings revealed that two level of ladder participation (Tokenism and Citizen-power) have positive and significant relationship with attitude, while Non-participation level of ladder participation has negatively significant relationship with attitude. The findings of the study imply that those respondents who have positive attitude toward local government, councilors and council performance, would have the higher tendency to be actively involved in higher levels of participation, whereas who have negative attitude toward local government, would put less effort in higher levels of participation.

[Seyed Hamid Mohammadi. Citizens' Attitude toward's Local Government and Citizen's Participation in Local Government. Journal of American Science 2010;6(11):575-583]. (ISSN: 1545-1003).

**Keywords:** Citizen attitude, Citizen participation, Local government, social exchange.

## 1. Introduction

There is consensus among scholars, which attitude toward local government is regarded as effective factor to people's participation in local government. Some scholars generally agree that positive attitude toward local government encourage citizens participation in council matters (Kosecik & Sagbas, 2004, Suzanne et al, 2007). As local governments become increasingly important in citizens' everyday lives, the investigation of public attitude toward local government is vital for success of future council programs and reforms. Without regular and systematic analysis of public attitude, viable local government policies will become more difficult to design and implement in the future.

The development of local government is ensure people's involvement in local government matters, thus, promoting people's participation in local government is important. Aldashev (2003) considers participation as a social behavior, and Rishi (2003) added attitude as a central element in social behaviors and argued that attitude is imperative for making change of the behaviors. According to Rishi (2003) social actions of people or their personal program are directed by their attitudes, if the attitude of people is positive toward an event or an action, it is more likely, that they divert their behavior in more meaningful way (Rishi, 2003). Similarly, if local people have positive attitude towards local government, it is more likely that they support the local government issues as well as participating more

in local government matters. Thus, understanding the citizen attitude can help to access the degree/level of citizen participation in council issues. The main objective of this study is to describe the relationship of citizen's attitude toward local government and level of people's participation in local government, on the basis of social exchange theory. The paper attempts to establish a relationship between social exchange theory, attitude of citizens and level of people's participation. This research is important because up to present moment, there has been very little research or discussion focusing upon the attitude toward local government and its relationship with level of participation in council matters. And also in this study, the participation of people in local government is linked with theory of social exchange. The theory of social exchange has been given only limited attention in participation in local government literature, even though it has been extensively used in other areas such as; tourism (Aref, et al, 2009), and agriculture (Bagherian, et al, 2009).

## 2. Literature Review

Citizen participation is considered as an important factor for successful and prosperity of local government. Citizen's participation in local government produce more efficiency in programs as well as promote good governance (Lowndes et al, 2001, According to Aref et al, (2009), without community participation, there are obviously no accountability, no development, and no program. Ashley & Roe, (1998) describe community participation as a spectrum from passive to active involvement to full local participation, where there is active community participation and venture ownership. Meanwhile, some scholars such as; Pretty (1995), Oakley (1991), Johnson (1982), and Wandersman (1987), provided a typology of participation, but the most suitable typology adopted in urban issues is Arnstein ladder. According to Arnstein; participation is a process that enables "have-nots" citizens, those who are excluded from decision-making process, to be included in future. It is the strategy that have-notes involve in sharing-information, and join to set priorities and goals. The Arnstein's ladder has eight rungs and each rung corresponding to the extent of citizens' power in determining the plan and/or program. The eight rungs are categorized into three categories. The bottom rungs of the ladder are manipulation and therapy. These two rungs describe level of non-participation, which the real objective is not to enable people to participate in planning, but to enable power-holders to educate the participants. The following categorization involves three levels of tokenism;

informing, consultation, and placation. In this level citizens may indeed hear or be heard, but under these conditions they lack power to influence decisions (Arstein 1969). It is the illusion of a voice without the voice itself. The highest level of ladder is citizen-power, which include; partnership, delegated power and citizen control. In this level, citizen control all issues and win the majority of decision-making seats.

Todays, Local government are well placed to play a crucial role in enhancing citizen's participation and enabling local communities to participate in decision-making process (Mariana, 2008).

Local government is an essential component of administrative systems of all modern societies, which look for the improvement of public services and provide the situation for reaching/achievement good governance values at local level (Kosecik and Sagbas, 2004). Local government provides opportunities for public participation, and ensures effective and efficient public service delivery (Stoker, 1996: 6). Public participation at local level is achieved only if local people have an interest in local government affairs. The efficiency and effectiveness of local government is ensured when local people or citizens participate in the decision-making process of local government and keep local government under control (Kosecik and Sagbas, 2004, Jerry and David 1996). Local government will make better decisions and will have greater impact on their communities, when they increase the frequency, diversity and level of engagement of local people. Citizen involvement in local government will produce more public-preference decision making on part of administrators and better appreciation by the larger community among the public (Stivers 1990, Oldfield 1990, Box 1998).

These attitudes about the benefits of participation in local government are evaluated by citizens. The evaluation of the benefits and costs of local government by citizens link this study with social exchange theory.

This theory asserts that people develop attitudes toward others and things based on the benefits they could obtain while those activities assuming to increase benefits will be positive support and activities assuming to be costly will tend to be perceived negatively (Napier & Napier, 1991). Social exchange theory argues that all human relations are formed by the use of a subjective cost-benefit analysis. For social exchange when the costs and benefits of a relationship are equal, the relationship is defined as equitable. The concept of equity is fundamental in social exchange theory.

In Homans' (1958) view, who is the initiator of the theory, the social behavior is an exchange of goods, material goods and also non-material goods, this can be symbols of approval or prestige. Blau,

(1964) also notes that individuals can also enter into and maintain relationships, they can satisfy their own interest as long as they are sure that the benefits outweigh the costs.

**Tbale 1: level of citizen participation in local government**

Levels	Types	Characteristics
Citizen-power	Citizen control	This range is the highest level. Citizens have the degree of power (or control) which guarantees the participation in governing a program from citizens (Arnstein, 1969).
	Delegated power	Citizen participation is performed through negotiations between citizens and authorities, this results in positive role the citizens played in partial decision making with the authority over a particular plan or project (Arnstein, 1969).
	Partnership	power is in fact redistributed through negotiation between citizens and power-holders (Arnstein, 1969).
Tokenism	Placation	<i>Placation</i> is a stage that citizens begin to have some degree of influence though tokenism is still apparent (Arnstein, 1969).
	Consultation	people are invited to give their suggestions, this rung of the ladder is still a sham since no assurance is offered. Concerns and ideas of citizens will not be taken into consideration (Arnstein, 1969).
	Informing	authorities inform citizens of their rights, However, more emphasis is put on a one-way flow of information (Arnstein, 1969).
Nonparticipation	Therapy	With respect to group therapy, masked as citizen participation, should be on the lowest rung of the ladder because it is both dishonest and arrogant (Arnstein, 1969).
	Manipulation	Based on so-called citizen participation, people are placed on rubber stamp advisory committees (Arnstein, 1969).

**Source:** Sherry Arnstein, 1969.

Prekumar and Ramamurthy (1995) declared, social exchange theory has been used by some researchers as a theoretical framework to investigate the community relationships that are not based on the economical aspects, rather than they are based on non-economical aspects, such as power, trust, interdependency, and the like. Therefore, this theory is not only for exploration of the economic relations, rather it can be used for exploration of non-economic and social relationships. Prekumar and Ramamurthy (1995) stated social exchange theory provides the base for the study of relationships between groups

and organizations in community (Prekumar and Ramamurthy 1995, p. 306). According to social exchange theory, the outcomes of an collective/organization's behavior will be related to the responsive behavior of the other participants within the relationship (Son et al. 2000). The main issue of this viewpoint is that the relationship between community groups does not necessarily need to be directly related to any economic outcomes (Hallen et al. 1991, Humphreys et al. 2001). Humphreys et al. (2001) postulate that social exchange theory is a appropriate base for studying

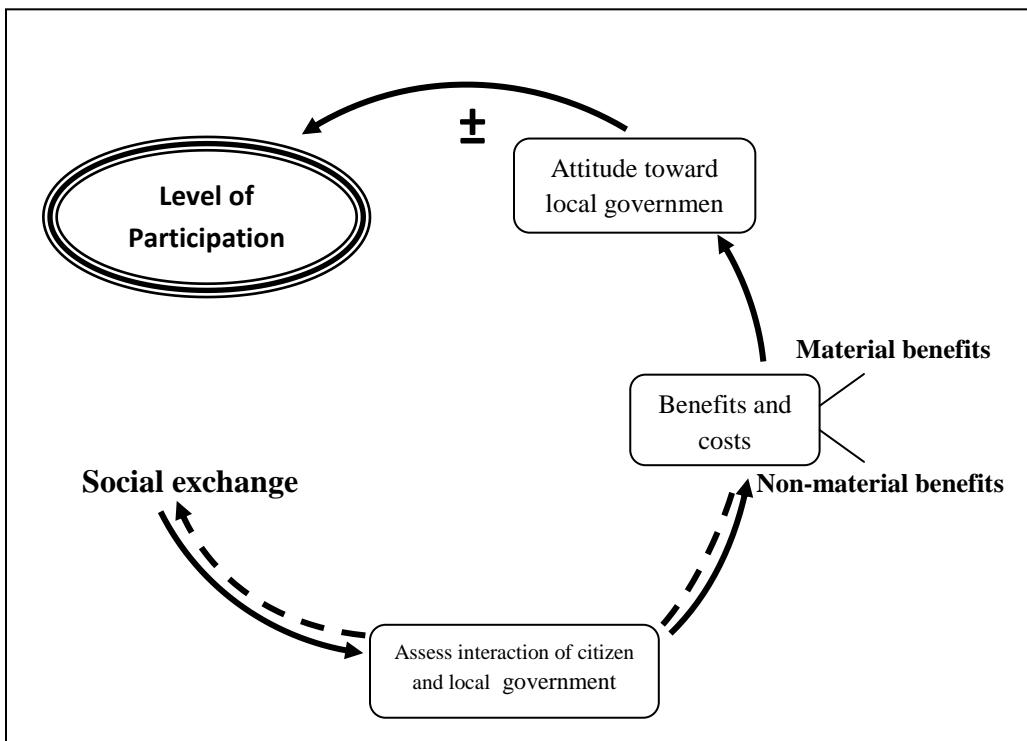
non-profit relationships. So, in terms of people's participation in local government, the social exchange theory is adopted to investigate the social aspects of relationships between citizens and local government. According to Ledingham (2001) Citizens tended to participate in local government, when they perceive that local government is providing benefits for local people, acting in the best interest of local people, and dedicating resources to support matters of importance of citizens in the exchange relationship between people and local government. He added Citizens expect mutual interactions with local government, they seek a balance between social costs of interaction with their local government. Ledingham and Bruning (2001) concluded: "To be effective, relationships need to be seen as mutually beneficial, based on mutual interest between an organization and its significant publics". People cannot be expected to present positive attitude toward local government if the benefits and costs of local government is not equal for them.

An attitude is a hypothetical construct that represents an individual's degree of like or dislike for an item. Attitudes are generally positive or negative views of a person, place, thing, or event- this is often referred to as the attitude object (Bagherian, et al., 2009). Attitudes are generally viewed as a kind of disposition toward various aspects of the world including persons, events and subjects. It has been generally believed that attitude change is necessary before other behavioral modifications can be effected (Zainuddin, 1977).

Kosecik & Sagbas (2004) found that there is a relationship between citizens' attitude toward local government and level of participation in local government affairs. Stevenson (2007) found that people with positive attitude toward local government are more likely to participate in local government. Rishi (2003) outlined that understanding of attitudes is one of the central concerns in social life and is relatively crucial in bringing the desired change in the behavior. Collective and personal actions of people are directed by their attitudes. Attitudes make it possible to predict and control the behavior, which ultimately is useful to implement program successfully. Kosecik & Sagbas (2004) in

their study found, public attitude affects public participation and that is an essential element for increasing participation in local government. The positive attitude toward local government can make local people more active and eager to participate in activities that are related to local government. According to Hiskey and Seligson (2003) there is link between performance of local government and citizen attitude, he demonstrates as a first step, performance of local government affect citizen attitude toward council. It is unlikely that performance of local government affect citizen attitude but does not influence their level of participation (Hiskey and Seligson, 2003). Aspden and Brich (2005) demonstrated that there are a number of factors and issues that affect the publics' attitude towards participation in local affairs and decision-making. Their demonstration consist of the; citizen interest and understanding of local government, citizens satisfaction for their involvement, citizen trust to local government and its members, and previous experience of voluntary participation (Aspden and Brich, 2005). A better understanding of citizen attitude is necessary if councilors are to address the very real problems of apathy of citizens that hinder public participation, and if they are maximize the impact and effectiveness of participation (Lowndes, et.al, 2001). Public attitude toward local government might differ according to certain variables such as, age sex, education, occupation, and income (Kosecik & Sagbas, 2004). These variables have considerable influence in citizen attitude toward local government and consequently in their participation in council affairs.

According to the above figure, the social exchange theory is used to illustrate that citizens assess their interaction and cooperation with local government based on benefits and costs that its brings to them. Participation occurs when the cost of participation is low and the benefit of participation is high, and consequently it makes positive attitude toward participation in local government. But if the costs outweigh the likely benefits, no interest to participation.



**Figure 1; Cycle of social exchange theory, attitude toward local government and level of participation**

### 3. Research Design

The study was carried out on 400 citizens in Torbat Heydarieh. Torbat Heydarieh is located in the east north of Iran in Khorasan Razavi province; it is 1005km far from Tehran (capital of Iran). In Iran, the size of council range is between five and fifteen depending on population. Since the population of Torbat Heydarieh is less than 200'000, so, the members of local government are seven (the Constitution of Islamic Republic of Iran). In some big cities, local government has established neighborhood councils due to increase in public participation for achievement of its targets. Neighborhood councils are subset of local government, which try to close the members of local government to local people and recognize their problems. In Iran among the cities with less than 200,000 populations, Torbat Hedarieh was the first city that established neighborhood council, for increasing public participation and improving the issues. According to Ghanizadeh (1999) "in Iran, neighborhood councils by attraction of public participation have could given assistance to local government in executive issues".

The study used survey design in which questionnaire was used to collect the data. Questionnaires are well-established methods of collecting data within social science research (Dillman, 2000). Questionnaire survey is a useful tool of research that are related to community participation (Shin, 2004). A questionnaire is a data instrument that each respondent fills out as part of participating in research study (Johnson & Christensen, 2004). The respondents were 400 citizens, where each citizen as respondents was chosen randomly.

In this study Cluster Sampling was used. This is type of random sample that use multi stages and is often used to cover wide geographic areas. Cluster sampling was chosen because we believe through that, we can select a proxy for community that they represent the voice of people. The population of this research will be all of the inhabitants include men and women and 17 above years who live in Torbat Hedarieh. Eleven questions were developed based on the literature review of the measurement of attitude toward local government. The respondents were asked to insure these questions which were constructed to gauge their attitude

toward local government, councilors and its efficiency. The questionnaire was piloted tested to have its content validated by several reviewers of Persian background. Statements for citizen attitude were tested for their validity using Cronbach's alpha. The respondents in pilot study had diverse demographic characteristics, especially with regards to community.

To test the proposed objective, this research was used statistical statics such as Pearson correlation and descriptive statistic. Pearson correlation was employed to measure the degree of relationship between variables involved (the attitude toward local government and levels of participation). Pearson correlation statistic is a statistical technique to measure the strength of the association that exist between two quantitative variables (Ary et al., 1996). In statistics, correlation (often measured as a correlation coefficient) points to the strength and direction of a linear relationship between two variables that has been determined randomly (Aref and Redzuan, 2009). And, Descriptive analysis was employed to determine level of people participation. In this study participation is a composite variable, consisting of three level, namely, nonparticipation (5 items), tokenism (7 items), and citizen power (9 items). The study used Likert-scale to measure every item. In the analysis, the citizen attitude was correlated with the three levels of participation in order to determine the strength of their relationships. Meanwhile, means and standard deviations are the descriptive statistics that were used to describe the basic features of these variables.

#### **4. Result and Discussion**

As have been mentioned above, the main objective of the study is to determine the relationship between citizens' attitude toward local government and level of participation. Pearson correlation was used to identify these relationships. Table 1 shows the findings of the study in relations to means and standard deviations of studied variables. For the three variables related to level of participation (nonparticipation, tokenism and citizen power), the data reveal that generally, the mean scores of nonparticipation level is higher than tokenism and citizen-power. This is reflected by the means of every level – nonparticipation level ( $M = 20.26$ ,  $SD = 3.12$ ), tokenism level ( $M = 17.27$ ,  $SD = 3.93$ ), citizen-power level ( $M = 15.91$ ,  $SD = 4.8$ ). These findings imply that participation in nonparticipation level is more frequent than tokenism and citizen-power. Moreover, the standard deviations show that there are relatively small deviations (differences)

between respondents (citizens) in terms of their participation in each level. Meanwhile, the mean of citizens' attitude in regard to local government is relatively moderate ( $M = 27.03$ ,  $SD = 7.90$ ). It implies the majority of citizen have relatively moderate attitude toward local government, councilors and its performances.

The second analysis for this paper focuses on the relationships between the attitude toward local government and the level of participation based on Arnstein ladder. The results of the analysis are shown in table 2. The correlation between citizen attitude and nonparticipation level is found to be negatively significant ( $r = -.414$ ,  $N = 400$ ,  $P = 0.000$ ). Meanwhile, the correlation between citizen attitude and the level of tokenism is found to be positively significant ( $r = .323$ ,  $N = 400$ ,  $P = 0.000$ ), as well as the correlation between citizen attitude and citizen power is positive and significant ( $r = .385$ ,  $N = 400$ ,  $P = 0.000$ ).

When comparing the nonparticipation, tokenism and citizen-power levels of participation with attitude toward local government descriptively, Non-participation level has a negative relationship with attitude. - Since this level is unrealistic and superficial and its real objective is not to enable citizens to participate in planning (Arnstein, 19969), this relationship is understandable. Because whatever attitude towards local government is positive, Participation will be increased. And it can surpass non-participation level to reach higher levels, i.e Tokenism and Citizen-Power - . However, Tokenism and Citizen-Power levels have positive relationship with attitude. It confirmed the assumption that participation will be increased when the attitude of local people be positive toward local government, its efficiency, and its services (Aspden and Brich, 2005).

The findings from this study supported the previous studies in terms of citizen attitude toward local government and their participation in local government. The findings also are consistent with the past studies that have been concluded by Stevenson (2007) and Suzanne et al (2007). Most of these studies evaluated citizen attitude toward the local government and services that are provided by it, and the role of citizen attitude in participation.

In addition study indicated that those people who had positive attitude toward program more likely to participate in program. These findings also are consistent with results of Rishi (2003), Shahroudi and Chizari (2008), Vicente (2008) and Asadi *et al.* (2009) in their research that found significant

relationship between attitude and level of participation.

**Table 2: Means and Standard Deviation of the studied variables**

Variables	Means	Standard Deviation
Level of nonparticipation	20.26	3.12
Level of tokenism	17.22	3.93
Level of citizen-power	15.91	4.8
Attitude toward local government	27.03	7.90

**Table 3: Correlation Matrix Analysis of Attitude toward Local government and Levels of Participation (n=400)**

Variable	X	Y <sub>1</sub>	Y <sub>2</sub>	Y <sub>3</sub>
X- Attitude toward local government	1.00			
Y <sub>1</sub> - Non-Participation level	-.414**	1.00		
Y <sub>2</sub> - Tokenism level	.323**	.418**	1.00	
Y <sub>3</sub> - Citizen-Power level	.385**	-.491**	.493**	1.00

\*\* Correlation is significant at 0.01 level

## 5. Conclusion

By using social exchange theory framework, this paper attempts to illustrate the relationship between attitude towards local government and the level of participation. This theory helps to create a clear understanding about the relationship between perceived attitude and support for local government matters.

This study aimed at examining citizen attitude to local government and its relationship with level of participation, based on the findings of the questionnaire carried out in Torbat Hedarieh city, Iran. The basic argument about this study was that citizen's participation is the most important part of local government, which enhanced efficiency and effectiveness of council issues. The efficiency of the performance of local government is ensured if citizens participate in decision-making process and hold local government accountable. If citizens are not interested in local government and remain passive, it is not expected that the responsibilities of local government to the citizens will be undertaken.

From the study, it is found that there is linear relationship between level of participation and citizen attitude. It can be concluded that, if citizen attitude toward local government is positive, it is more likely that citizens are willing to participate in council affairs. Also, according to literature and other researches, attitude is one of the most important and effective factors for participation in local

government, which play a significant role in encouraging citizens to participate. Therefore the result of this study indicates main recommendation, that is; focus on measures which are believed to improve citizen attitudes to local government. The findings of this study have an implication on understanding the role of attitude in participation in local government. It is suggested that the good performance of local government make a positive attitude toward local government among citizens or local people. Consequently local government can receive a support from local communities. As, local government is important to citizens in daily lives, positive attitude toward local government can encourage them to engage in council issues. However the creation of such attitude toward local government in Iran has always been challenged, because, people would rather consider local government as a governmental organ than public and non-governmental organ, level of public knowledge about the functions of local government, and lack of efficient and expertise among councilors.

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9/30/2010

# Algal Abundances and Growth Performances of Nile Tilapia (*Oreochromis niloticus*) as Affected by Different Fertilizer Sources

M.A. Elnady\*, H.A. Hassanien, M.A. Salem and H. Marian Samir

Department of Animal Production, Faculty of Agriculture, Cairo University, Giza , Egypt .

\*[melnadyahmed@yahoo.com](mailto:melnadyahmed@yahoo.com)

**Abstract:** The experiment was designed to study the effect of different fertilizer sources (chemical fertilizer, organic fertilizer or combined chemical +organic fertilization) on plankton abundances , growth performances of Nile tilapia juveniles and water quality parameters in concrete tanks compared to feeding fish at satiation .The average secchi disk readings were shallower in the chemical and combined fertilizer treatments compared to those of the ration and organic fertilizer treatments as a result of increased algal density and abundances. Ammonia and orthophosphate concentrations in the chemical and combined fertilizer treatments were higher with an increase in algal growth, abundance. Within fertilizer treatments, the daily weight gains of Nile tilapia reared in the chemical and combined fertilizer treatments (0.43 and 0.5 g/fish/day, respectively) were significantly higher than those reared in the organic fertilizer treatment (0.32g/ fish/ day). This indicated that the use of chemical fertilizer in a fertilization program is superior in increasing fish growth compared to that of the organic fertilizer .It can be concluded that Nile tilapia juveniles can obtain major nutritional requirements for growth( 48% of its total feed requirements) from feeding only on algae during this stage of growth. Results of the current experiment recommended that organic fertilizer should not be used as sole source in fertilizer programs and should be combined with chemical fertilizer in order to produce good algal growth necessary for the nourishment of farmed fish.

[M.A. Elnady, H.A. Hassanien, M.A. Salem and H. Marian Samir. Algal Abundances and Growth Performances of Nile Tilapia (*Oreochromis niloticus*) as Affected by Different Fertilizer Sources. Journal of American Science 2010;6(11):584-393]. (ISSN: 1545-1003).

**Key words:** Fertilizers, manure, algae, plankton, Nile tilapia.

## 1. Introduction:

Pond fertilization through organic and inorganic sources has become a management protocol in aquaculture (Bhakta et al., 2006). Almost all extensive and the majority of semi- intensive aquaculture operations in Asia are dependent on the use of chemical fertilizers and organic manures (De Silva and Hasan, 2007). The purpose of pond fertilization is to augment fish production through autotrophic and heterotrophic pathways (Jha et al., 2008). Natural food supply is enhanced by using organic and inorganic fertilizers and low-cost supplemental feeds derived agricultural by-products (Halwart et al., 2002). It is well known that high fish yield can be achieved by higher abundance of plankton in culture system (Jha et al., 2004). Teferi et al.(2000) found that Nile tilapia was essentially planktivorous. Moreover, small tilapia filtered significantly more phytoplankton than larger individuals (Turker et al., 2003).

Chemical fertilization of ponds effectively stimulated primary productivity and mean chlorophyll a concentration (Green et al .,2002) and application of medium amount of chemical fertilizers ( urea and TSP) as pond inputs has been proven to

produce up to a three- fold increase in fish yield (Pant et al., 2002). Pond fertilization practices using animal wastes are widely used in many countries to sustain productivity at low costs (Gupta and Noble, 2001; Majumder et al., 2002) since soluble organic matter supplied to ponds by using manure stimulate phytoplankton growth (Sevilleja et al., 2001). Moreover, it increases biomass of zooplankton and benthic organisms (Atay and Demir, 1998). Consequently, animal wastes lead to increased biological productivity of ponds through various pathways, which result in an increase in fish production (Dhawan and kaur, 2002).

The study was designed to explore whether chemical fertilizer (nitrate and phosphate) is superior to organic fertilizer (pigeon manure) in promoting natural food abundance and fish growth compared to the combined use of both chemical and organic fertilizer in rearing tanks.

## 2. Materials and methods

The current experiment on growth performances of Nile tilapia (*Oreochromis niloticus*) was conducted at the Fish Culture Research Unit, Faculty of Agric.,

Cairo Univ., Egypt. The experiment was performed in outdoor concrete tanks (2.0 x 1.2 x 1.0m each).

The experiment consisted of four treatments with three replicate tanks per treatment as follows:

1-The chemical fertilizer treatment included a weekly application of ammonium nitrate ( $\text{NH}_4\text{NO}_3$ , 33%N) at 2 g / $\text{m}^2$ /week and superphosphate ( $\text{P}_2\text{O}_5$ , 15.5%P) at 2 g / $\text{m}^2$ /week.

2-The organic fertilizer treatment included a weekly application of dry pigeon manure at a rate of 16 grams dry matter/ $\text{m}^2$ / week (40 grams/ tank/ week).

3-The combined fertilizer treatment included the application of both chemical fertilizers (ammonium nitrate at 2 grams/  $\text{m}^2$ / week + superphosphate at 2 grams/  $\text{m}^2$ /week) and pigeon manure fertilizer (16 grams dry matter/  $\text{m}^2$ /week) in the same tank.

4-The ration treatment included the application of commercial diet (30% crude protein) as fish feed administrated to satiation, six day per week. The experiment lasted 90 days.

The growth performance parameters of juveniles were measured in terms of total length, body weight, specific growth rate, weight gain, daily weight gain and condition factor .Fish growth data were estimated using average individual weight and length to the nearest 0.1 g and 0.1 cm, respectively. The daily weight gain was determined using the formula  $(\text{DWG}) = (\text{WT}_F - \text{WT}_I) / T$  where  $\text{WT}_F$  = final fish weight (g),  $\text{WT}_I$  = initial fish weight (g) and  $T$ = growing period (days) between initial and final weight. Specific growth rate (% body weight day $^{-1}$ ) was calculated using the formula:  $\text{SGR}=(\ln \text{WT}_F - \ln \text{WT}_I) \times 100 / T$ . The condition factor of fish was calculated from the formula  $K= \text{WT}_F / L_F^3 \times 100$  where  $\text{WT}_F$ = final fish weight (g),  $L_F$  =the final total length (cm).

Water from each tank was tested once a month for temperature ( $\text{C}^\circ$ ), secchi disk visibility (SD), PH, ammonium ( $\text{NH}_4$ ), and orthophosphate – phosphorus ( $\text{PO}_4\text{-P}$ ). All determinations were carried out in the Water Research Laboratory, Ministry of Irrigation, Qanater Khayria, Egypt .Chemical analyses were carried-out according to the Standard Methods, American Public Health Association (APHA, 1993).

Statistical analyses were performed using one way analysis of variance (ANOVA) and Duncan's multiple range test to determine differences among treatment means at a significance level of 0.05. Means (X) and standard deviations (S.D.) were calculated accordingly. Statistical tests on parameter

values were conducted using (SAS INC, 1992) computer software.

### 3. Results and Discussion:

As shown in table (1), the average water temperatures during the experimental period were 28.6, 29.1, 29.7 and 28.7  $\text{C}$  in chemical, organic, combined and ration treatments, respectively. This indicated that average water temperatures in all treatments were optimal for fish growth and algal production. Tilapia is known to increase their growth rate when water temperature is above 25  $\text{C}^\circ$ , with optimal growth rate taken place when water temperature is 25-30  $\text{C}^\circ$  (Boyd, 1990).

The overall average of total ammonia concentration in tank water during the experimental period indicated that the chemical fertilizer treatment had an over abundance of ammonia (0.708 mg / l ) followed by combined fertilizer (0.623 mg / l ), ration (0.475 mg / l ) and organic fertilizer (0.381 mg / l ) treatments, respectively. The shallow secchi disk readings during the experiment in the chemical fertilizer and combined fertilizer treatments ( 22.9 and 27.1 cm, respectively) were due to the effect of over- abundance of ammonia in water which increased algae growth. This resulted in an increase algae turbidity (concentration) and shallower secchi disk readings in those treatments.

Although organic fertilizer may be consumed directly or as manure- derived detritus after heterotrophic microbial activity, the role of manure or manure – derived detritus as a source of food for fish is not universally known (Knud-Hansen et al., 1993). Manure is a costless fertilizer that stimulate the development of natural foods especially phytoplankton and zooplankton (Lan et al.,2000). Animal wastes lead to increased biological productivity of ponds through various autotrophic (algae production) and heterotrophic (bacterial production) path-ways, which result in an increase in fish production (Orhibhabor and Ansa, 2006).

Plankton levels ( phyto- and zooplankton) were significantly higher and fish growth was significantly more in manured ponds ( Dhawan and kaur,2002). When comparing different treatments, zooplankton was significantly higher in manured ponds (Kumar et al., 2005) and the highest density of zooplankton was obtained from pond water that had high concentration of nitrogen and phosphorus salts (Lan et al., 2000). Organic manures contain almost all the essential nutrient elements (Jana et al.,2001) that stimulate the growth of plankton ( Wurts,2000 ; Ansa & Jiya,2002; Kadri & Emmanuel,2003) and consequently, manure contains considerable quantities of nutrients for fish production (Gabriel et al., 2007).

Ammonia production in tank water of the three fertilizer treatments was mainly through the decomposition of dead algal by bacterial activity and the mineralization of algal protein into ammonia by fish and aquatic animals. According to Ludwig(1999) when organic fertilizers are added to pond they are decomposed by bacteria , releasing ammonia and phosphates which are rapidly utilized by phytoplankton and other bacteria (Jha etal., 2008). The three fertilizer treatments enhanced algal production and decomposition in their tanks and led to the accumulation of ammonia over time in the fertilized tanks .The high build up of ammonia concentration in the chemical fertilizer and combined fertilizer treatments was due to the increase in the intensity of algal production, followed by bacterial decomposition of dead algae in the sediment. Dead algae are mineralized to ammonia by bacterial action in the presence of aerobic conditions (Hargreaves, 1998).

Mineralization of organic matter in sediment and the consequent regeneration of nutrients at the sediment-water interface in aquaculture ponds is important as a source of ammonia to the water column (Hargreaves, 1998). A simulation model describing ammonia dynamics in commercial catfish ponds estimated that 25 to 33% of the ammonia supplied to the water column was derived from the sediment (Hargreaves, 1997). As shown in Table (1), the chemical fertilizers stimulate the natural productivity, through photosynthesis, whereas animal manures provide, upon decomposition, nutrients for both autotrophs and heterotrophs (Nguenga el al., 1997).

The high ammonia concentration in the ration treatment (0.475 mg/l) was caused by the mineralization of dietary protein into ammonia during metabolism by fish and aquatic animals. Normally 40-90% of the nitrogenous wastes, resulting from the metabolism of dietary proteins is excreted by fish and shrimp as ammonia from the gills and in urine (Goddard, 1996).Most of this ammonia is excreted passively in the unionized form from the gills.

Phytoplankton is known to absorb ammonia readily at higher rate compared with nitrate (Boyd, 1990).Ammonia is known to be utilized by phytoplankton during the process of protein synthesis (Kumar et al., 2005). The major sources of ammonia in pond water are the direct excretion of ammonia by fish (Tucker and Boyd, 1985) and microbial decomposition in the sediments .In catfish ponds, daily feed addition during the warmer months results in inputs of 200 – 400 mg N/m<sup>3</sup>/day and 30-60 mg P/m<sup>3</sup>/ day (Massout, 1999). Nitrogen and phosphorus salts are known to be major nutrients required for the primary production of plankton in pond water (Kumar et al. 2005).

Total ammonia concentration (TAN) was a major factor that affected algae concentration and production in water during the experimental period.

This is in agreement with Padmavathi and Ptasad (2007) who indicated that high PH and ammonia levels are associated with algal blooms. Higher total ammonia concentrations in the chemical fertilizer and combined fertilizer treatments were correlated positively with higher algal abundances (shallower secchi disk readings).As shown in table (1), the lower total ammonia concentrations in the organic fertilizer and ration treatments produced lower algal abundances compared to other treatments. This indicated a lower efficiency of organic fertilizer in respect to ammonia production in water .The present results are in accordance with Green et al. (2002). Organic fertilizer should not be used as sole source in fertilizer programs and should be combined with chemical fertilizer in order to produce good algal growth necessary for the nourishment of farmed fish.

Orthophosphate (PO<sub>4</sub> – P) was significantly higher ( $p < 0.05$ ) in the chemical and combined fertilizer treatments compared to those of the organic fertilizer and ration treatments (table 1). The overall mean of orthophosphate concentrations were 0.575 and 0.527 mg – P /l for the chemical and combined fertilizer treatments, respectively, while those of the organic fertilizer and ration treatments were 0.295 and 0.18 mg - P/l , respectively. The increased algal abundances in the chemical and combined fertilizer treatments (secchi disk readings = 22.9 and 27.1 cm, respectively) were also due to the higher concentrations of orthophosphates in those treatments.

Fluctuations of environmental factors in relation to growth of *Tilapia mossambica* exposed to single superphosphate indicated that fish growth was dependent upon the plankton organisms .The harvest weight of fish was significantly positively correlated with the phosphate, available phosphorus and calcium carbonate (Sarkar and Konar, 1989). Soluble reactive phosphorus and total phosphorus concentrations were both correlated with fish production (Wudtisin and Boyd, 2005).

Averages orthophosphate (PO<sub>4</sub> – P) concentrations were 0.295 and 0.187 mg/l PO<sub>4</sub> – P in the organic fertilizer and ration treatments, respectively, which were much lower than those of other treatments (table 1). This lowered the photosynthetic rate and algal abundance in those treatments (secchi disk readings were 39.8 and 36.1 cm, respectively) and negatively affected growth rate of fish in the organic fertilizer treatment since algae is food material for fish. These results were consistent with Wudtisin and Boyd (2005).

Sources of orthophosphate ( $\text{PO}_4^{3-}$ -P) in ration treatment were primarily a product of dietary phosphorus metabolism by fish through excretory processes .Kund-Hansen et al. (1993) evaluated the functional role of chicken manure for Nile tilapia *Oreochromis niloticus* production in central Thailand. Regression analysis suggested that chicken manure P was about 10% effective as TSP-P at increasing net fish yield (NFY). Simple economic comparisons discourage the purchase of chicken manure as a source of soluble N and P for increasing algal productivity in Thailand (Knud- Hansen et al., 1993).

Because of low N and P content and high oxygen consumption, organic fertilizer alone is unlikely to provide adequate nutrients for algae and sufficient oxygen for fish. To stimulate the growth of

food organisms for fish in aquaculture ponds, a combined use of inorganic and organic fertilizer is recommended, but the amount of organic fertilizer should be determined with care to avoid water quality deterioration ( Qin et al., 1995).

As shown in table (1), early morning pH in concrete tanks were significantly high ( $p<0.05$ ) in the chemical fertilizer treatment (8.5) than those of the combined fertilizer (7.86) or ration (7.63) treatments. This may be due to the higher algal growth and production in the chemical fertilizer treatment compared to other treatments. The pH of water increases when plants are rapidly removing carbon dioxide from water for use in photosynthesis (Boyd, 1990).

**Table (1). Averages of water quality parameters in experimental tanks under different fertilizer treatments.**

Parameter Treatment	chemical fertilizer	organic fertilizer	combined fertilizer	ration treatment
Water temperature (C°)	28.6 <sup>a</sup> ± 0.8	29.1 <sup>a</sup> ± 1.2	29.7 <sup>a</sup> ± 1.5	28.7 <sup>a</sup> ± 0.7
Total ammonia (mg-N/l)	0.708 <sup>a</sup> ± 0.18	0.381 <sup>b</sup> ± 0.16	0.623 <sup>ab</sup> ± 0.19	0.475 <sup>ab</sup> ± 0.09
Early morning pH	8.5 <sup>a</sup> ± 0.4	7.46 <sup>b</sup> ± 0.51	7.86 <sup>ab</sup> ± 0.25	7.63 <sup>b</sup> ± 0.45
Orthophosphate (mg-P/l)	0.575 <sup>a</sup> ± 0.03	0.295 <sup>b</sup> ± 0.04	0.527 <sup>a</sup> ± 0.1	0.18 <sup>b</sup> ± 0.03
Secchi disc depth (cm)	22.9 <sup>a</sup> ± 6.3	39.8 <sup>c</sup> ± 5.6	27.1 <sup>ab</sup> ± 2.6	36.1 <sup>bc</sup> ± 4.7

-a,b,c means with different superscripts among treatments are significantly different( $p < 0.05$ ).

The least early morning pH (7.46) was observed in the organic fertilizer treatment ( $p<0.05$ ). This was due to the negative effect of the organic manure on the pH value of water. In this case, heterotrophic activities of aerobic bacteria reduces pH through respiration (Boyd, 1990). Early morning pH in the combined fertilizer and ration treatments were significantly lower ( $p<0.05$ ) compared to that of the chemical fertilizer treatment. This may be due to their higher organic inputs (diet or manure inputs) and the increased heterotrophic activities by aerobic bacteria on these inputs .Organic manure and dietary wastes decompose and serve as a continuous source of carbon dioxide (Boyd, 1990) and when carbon dioxide accumulates in water ,water pH declines.

The higher early morning pH reflected higher algal production and increased photosynthetic activities that resulted in higher algal concentration and abundances .These results are in accordance with

Padmavathi and Ptasad (2007)who elucidated the fact that high PH levels were associated with algal blooms

.In intensive aquaculture, high density algal blooms can lead to high water pH (Pote et al., 1990) and the relationship of photosynthesis and respiration to pond pH has been well documented by Tucker & Boyd (1985) and Boyd (1990) who reported that autotrophic activity increases water pH through  $\text{CO}_2$  absorption, while heterotrophic activity decreases water pH through respiration.

The average secchi disk readings were significantly lower ( $p<0.05$ ) in the chemical and combined fertilizer treatments (22.9 and 27.1cm, respectively) compared to those of the ration and organic fertilizer treatments (36.1 and 39.8 cm, respectively).This was due to the increased algal density and abundance in the chemical and combined fertilizer treatments compared to other treatments. The ammonia and orthophosphate concentrations in

the chemical and combined fertilizer treatments were significantly higher ( $p < 0.05$ ), increasing algal growth, abundances and biological turbidity of water reducing water visibility in those treatments.

The secchi disk visibility data indicated that the chemical and combined fertilizer treatments had good algal abundances and were better considering sound water quality management applied in fish farming. Semi-intensive aquaculture ponds often develop dense phytoplankton population ( chlorophyll A  $> 250 \text{ mg/m}^3$  and secchi disk visibility  $< 20 \text{ cm}$ ) in response to a high rate of nutrient input (Hargreaves, 1998). In addition, monthly net fish yield of *Oreochromis niloticus* was strongly correlated to secchi disk depth, total phosphorus and water temperature (Diana et al., 1988).

Concentrations of both ammonia and orthophosphate salts required to increase algal growth were least in the organic and ration treatments. This lowered algal abundances and increased secchi disk visibility depth in those treatments. The ration treatment did not receive any fertilizers consequently, algal abundance (turbidity) was lower and secchi disk reading was higher in that treatment.

Algae were the major source for Nile tilapia nutrition in the fertilized tanks, and the decreased secchi disk visibilities in the fertilizer treatments indicated a good potential for fish nutrition. Jamu et al (1999) reported that secchi reading is commonly used by aquaculture pond managers as an indicator of algae concentration in pond water. Almazan and Boyd (1978) found a high degree of correlation between phytoplankton abundances, secchi disk visibility, gross productivity and chlorophyll "a" content.

During this investigation, an over-abundance of algae (required for fish nutrition) in the chemical and combined fertilizer treatments over those of organic and ration treatments was detected. Algae or manure or both were the major sources of fish nutrition in the fertilizer treatments, while both diet (30% crude protein) and algae were the sources of fish nutrition in the ration treatment.

In fish farming practices where fish nutrition depend largely or entirely on natural food produced by fertilization programs, levels of algal abundances (reflected by secchi disk readings) are positively correlated with fish yield (Dhawan and Kaur, 2002). Ponds used for intensive fish culture are normally turbid with algae which grows in response to additions of fertilizer or fish feed (Boyd, 1990; Green et al.,2002). Ponds which received applications of fish feed also had abundant phytoplankton growth because roughly 75 percent of the nutrients in feed are excretory products during the process of metabolism (Boyd, 1979).

When secchi disk visibility is shallower, this reflects a high algal abundance in the photic zone which is positively correlated with fish growth and nutrition (Abbas and Hafeez-Ur-Rehman, 2005). Secchi disk visibility is commonly used by aquaculture pond managers as an indicator of phytoplankton concentration (Jamu et al. , 1999 ; Wudtisin & Boyd ,2005) . It is well known that Nile tilapia could obtain more than 50% of its nutritional requirements from feeding only on algae and zooplankton, especially during the Juvenile stage of growth (Turker et al., 2003). Consequently, using low cost fertilization programs can highly reduce nutritional requirements for dietary ration (Abbas and Hafeez – Ur-Rehman, 2005).

Kumar et al. (2004) demonstrated that a mix of manure and organic fertilizers was successful in fish culture. Such a combination of fertilizers promotes both autotrophic and heterotrophic organisms in the pond and enables better nutrient management as well as maintenance of water quality (Kumar et al.,2002).Recent studies have shown that the combined use of inorganic and organic fertilizers is effective in productivity improvement in earthen ponds ( Grozev et al., 2001 ; Kumar et al. ,2004 ; Afzal et al. ,2007 ; Jha et al. , 2008).Moreover, the combined use of inorganic and organic fertilizers is effective in maintaining phytoplankton and zooplankton population in rearing ponds (Qin & Culver ,1992 ; Afzal et al. , 2007) .

The least algal abundances ( $p < 0.05$  ) was observed in the organic fertilizer treatment .This was due to the decreased efficiency ( $p< 0.05$ ) of organic fertilizer in promoting algal growth and abundances compared to other fertilizer treatments. The decreased organic fertilizer efficiency in promoting algal abundances was due to its lower dose (  $2.3 \text{ gm/m}^2/\text{day}$  ) in the present study and lower content of phosphorus compared to that of chemical fertilizers. Moreover, bacterial activity on decomposable organic fertilizer ( manure ) consumes a high proportion of ammonia (TAN) and phosphate released in water during the process of manure decomposition .Those ammonia and phosphate are necessary components for bacterial growth on manure particles. This process, in addition to the lower phosphate content of manure, lowered the organic fertilizer (manure) efficiency in promoting algal growth and abundances compared to those of other fertilizer treatments. It is well known that phosphorus play a major role in promoting algal growth and abundances in water of aquaculture ponds ( Wudtisin and Boyd,2005).

Algal abundances in the ration treatment (artificial diet to satiation) were of low magnitude and were enhanced by the direct excretion of ammonia and phosphate by fish as a result of dietary

protein metabolism (Boyd, 1990). Nile tilapia juveniles were feeding on both diet and plankton in the ration treatment.

Because of low N and P content and high oxygen consumption, organic fertilizer alone is unlikely to provide adequate nutrients for algae and sufficient oxygen for fish (Kumar et al., 2004) , consequently, to stimulate the growth of food organisms for fish in aquaculture ponds, a combined use of inorganic and organic fertilizer is recommended ( Jha et al ., 2008).

It is concluded that the use of combined (chemical + organic) fertilizer promoted algal growth and abundances necessary for the nutrition of Nile tilapia. These fertilizer programs are of low costs and can enhance fish production in semi – intensive farming systems.

As shown in table (2) , average body weight of Nile tilapia Juveniles increased from 38.6- 42.6 grams at the start of the experiment to 81.3, 71.0 , 85.5 and 119.0 grams at the end of the experiment for the chemical fertilizer, organic fertilizer, combined fertilizer and ration treatments, respectively.

Final weight of Nile tilapia fed at satiation was heavier than those raised in the combined fertilizer treatment by 39.2%. Better growth performances of Nile tilapia in the ration treatment was achieved as a result of feeding on both algae and prepared diet compared to those reared in the fertilizer treatments which were feeding mainly on algae or manure or both.

Fish growth was influenced by the presence of natural food (Pant et al., 2002). Fish growth is slower on plankton feeding alone because larger fish lack the capacity to acquire sufficient ration even in ponds with high plankton stocks. Moreover, feeding is begun as a supplement to plankton forage, and soon becomes the dominant nutritional source, and rapid, near optimal growth is attained on a ration of approximately 50% of satiation amounts ( Szyper et al .,1996).

The present results indicated that Nile tilapia juveniles can obtain major nutritional requirements for growth (more than 48% of its total feed requirements) from feeding only on algae during the juvenile stage of growth compared to that of ration treatment where dietary inputs were offered. Based on analyses of stomach content, up to half the food intake of tilapia in intensively fed ponds was natural food, which indicated its substantial contribution to tilapia growth (Lim, 1989). Evaluation of the growth performance of major carps in fertilized ponds supplemented with feed, indicated that primary productivity (i.e. plankton ) contributed 57.4 % towards the increase in fish yield (Aziz et al .,2002) .

As Nile tilapia derives most of their nutrition from phytoplankton (Colman and Edwards, 1987), a strong correlation between algae production and net fish yield was expected (Knud- Hansen and Batterson, 1994; Green et al ., 2002).Most often natural food forms the basis of fish nutrition with artificial supplement (diet) given to increase fish production (O' Grady and Spillett, 1985).

The results confirmed that the best growth of *Oreochromis niloticus* was in the ration treatment (1.25% per day), followed by fish in the combined fertilizer treatment (0.81% per day).Comparing different fertilizer treatments ,the chemical fertilizer and combined treatments had significantly higher specific growth rates (0.81 and 0.71% per day, respectively) than that of organic fertilizer treatment (0.57% per day ).

Growth of fish was significantly increased by the increases in level of natural food (Pant et al., 2002 and 2004). In addition, some fish derive a high proportion of their food from plankton organisms, rather form supplement (i.e. artificial diet) and these fish maintain rapid growth even though they compete poorly for supplement (Wahlam and Shephard, 1988). Consequently, organic and inorganic fertilizations can produce high plankton abundances to be capable of supporting fish growth ( Jha et al., 2008).

As shown in table (2) , net fish yield increments in fertilizer treatments ranged 0.64- 1.0 gram /m<sup>2</sup>/day (equivalent to 6.4-10.0 kg fish weight/ ha/day) and were significantly lower (p<0.05) than that of the ration treatment fed at satiation which averaged 1.78 gram / m<sup>2</sup>/ day( equivalent to 17.8 kg fish weight/ ha/ day). The lowest (p< 0.05) net fish yield increment (0.64 gram / m<sup>2</sup>/ day) was observed in tanks fertilized with organic manure which was equivalent to 6.4 kg fish weight/ ha/ day.

Maximum manuring rate recommended in earthen ponds are 50 grams dry manure per square meter per week (Nyandat, 2007).Ponds fertilized with four levels of chicken manure (12.5, 50 and 100 g dry weight/ m<sup>2</sup>/ week) during 149 day experiment for Nile tilapia, yielded from 4.9 to 15.7 kg fish weight/ ha/ day (Kund – Hansen et al., 1991). Net fish yield was correlated to both net primary productivity and chicken manure fertilization .This is agreement with Dhawan and Kaur (2002) and Jha et al. (2008). Zhu et al. (1990) reported that the average net fish yield (different species of carp) in many experiments, was 10.2 kg/ha/day over a growing period of 150-200 days.

A significant increase in Nile tilapia production attained in the combined fertilizer treatment indicated that the combined use of chemical and organic fertilizers promoted fish

production (10.0 kg/ha/day) above that attained either by single use of chemical fertilizer (8.6 kg/ha/day) or

organic manure (6.4 kg/ha/day). These results are in

**Table (2) .Averages of growth performance parameters of Nile tilapia juveniles under different fertilizer treatments.**

Parameter Treatment	chemical fertilizer	organic fertilizer	combined fertilizer	ration treatment
Initial weight (grams/fish)	42.6 <sup>a</sup> ± 2.9	42.6 <sup>a</sup> ± 4.1	40.6 <sup>a</sup> ± 2.9	38.6 <sup>a</sup> ± 0.4
Final weight (grams/fish)	81.3 <sup>b</sup> ± 10.1	71.0 <sup>c</sup> ± 6.3	85.5 <sup>b</sup> ± 15.6	119.0 <sup>a</sup> ± 10.5
Weight gain (grams/fish)	38.7 <sup>b</sup> ± 8.3	28.4 <sup>c</sup> ± 4.4	44.9 <sup>b</sup> ± 13.6	80.4 <sup>a</sup> ± 10.5
Daily weight gain (g/fish/day)	0.43 <sup>b</sup> ± 0.09	0.32 <sup>c</sup> ± 0.4	0.5 <sup>b</sup> ± 0.15	0.89 <sup>a</sup> ± 0.12
Condition factor	1.66 <sup>bc</sup> ± 0.19	1.48 <sup>c</sup> ± 0.22	1.74 <sup>b</sup> ± 0.39	2.12 <sup>a</sup> ± 0.13
Specific growth rate (% per day)	0.71 <sup>c</sup> ± 0.1	0.57 <sup>d</sup> ± 0.07	0.81 <sup>b</sup> ± 0.15	1.25 <sup>a</sup> ± 0.1
Net yield (gram/ m <sup>2</sup> /day)	0.86	0.64	1.0	1.78

-a,b,c means with different superscripts among treatments are significantly different( p < 0.05).

accordance with Kumar (2004), Terziyski et al.(2007) and Afzal et al . (2007).

High nutrient inputs led to a two - fold increase in monthly net adult yield (9.0 kg/ha/d) over low input ponds (4.5 kg/ha/d) and there was a significant difference in net adult yield and biomass between organically (11.2 kg/ha/day) and inorganically (9.0 kg/ha/day) fertilized ponds at high nutrient loading ( Diana et al. ,1991).Increased fertilization rate resulted in larger fish yields and higher primary production (Dhawan and Kaur, 2002; Kumar et al., 2002; Jha et al.,2004).

Green et al. (1989) compared yields of tilapia (*O. niloticus*) in sets of ponds receiving chicken litter, cow manure or chemical fertilization. The added nitrogen was equal in all three treatments. Chemical fertilization and cow manure supported similar fish yields, 8.0 and 8.6 kg/ha per day, respectively. The yield with chicken manure was 11.7 kg/ha per day.

When net fish yield increment in the ration treatment was compared to those of the fertilizer treatments, the importance of algae as a source for tilapia nutrition during medium size stages was evident. Increasing algal growth and abundances in the chemical and combined fertilizer treatments caused an increase in growth of Nile tilapia at a proportional rate parallel to algae production. There was a positive correlation between net fish yield increment and algal concentration (secchi readings) in rearing tanks.

Organic and inorganic fertilizers are often used in fish ponds to increase pond fertility and to improve fish production (Shrestha & Lin, 1996; Grozev et al, 2001; Terziyski et al., 2007). Popma et al. (1995) described pond management practices, and fish yields for Guatemalan farmers with less than two hectares of land: average total fish yield form non-integrated ponds receiving approximately 500 kg of nutrients /ha- month was 8.4 kg /ha/day. At nutrient loading rates near 1500 kg /ha- month, net fish yields averaged 12.7 kg/ha /day in 6 months. Fish production cycles were usually 4 to 9 months.

In conclusion, the most productive treatment of the experiment was that of tanks fed at satiation, followed by that of the combined fertilizer treatment (17.8 and 10.0 kg/ha /day, respectively). Moreover, when the chemical fertilizer and ration treatments were compared, it was evident that Nile tilapia at medium size range can obtain at least 48% of their nutritional requirements from feeding only on algae.

#### Corresponding author

M.A. Elnady

Department of Animal Production, Faculty of Agriculture, Cairo University, Giza , Egypt.  
[melnadyahmed@yahoo.com](mailto:melnadyahmed@yahoo.com)

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9/6/2010

# Age as Moderated Influence on the Link of Spiritual and Emotional Intelligence with Mental Health in High School Students

Jafar Shabani\*, Siti Aishah Hassan, Aminah Ahmad, Maznah Baba

*Faculty of Educational Studies, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia  
jshabani@yahoo.com*

**Abstract:** This study examined whether, spiritual intelligence (SI) and emotional intelligence (EI) can be considered as predictor for mental health. The present investigation was also to test the moderating effects of age on the relationship of SI and EI with mental health among high school students. The participants in the study were 247 High school students (124 male and 123 female) in the age range of 14-17 years old, at the Gorgan City, north of Iran. Three valid and reliable instruments were used to assess SI, EI and mental health. Descriptive statistics, multiple and moderated regression analysis were used to analyses the data. The result demonstrated that mental health can be influences by SI and EI. In addition, the moderated effect of age on the relationship of SI and EI with mental health was not found.

[Jafar Shabani\*, Siti Aishah Hassan, Aminah Ahmad, Maznah Baba. Age as Moderated Influence on the Link of Spiritual and Emotional Intelligence with Mental Health in High School Students. Journal of American Science 2010;6(11):394-400]. (ISSN: 1545-1003).

**Keywords:** Psychology, education, high school students, spiritual and emotional intelligence, mental health

## 1. Introduction

Mental health is vital to the overall health and well-being of adolescents (World Health Organization [WHO], 2004). The WHO conceptualized mental health separate from mental ill-health and defined the concept as: a state of well-being in which the individual realizes his or her own abilities, can cope with the normal stresses of life, can work productively and fruitfully, and is able to make a contribution to his or her own community. (WHO, 2007, p. 1) Previous studies are clear on the influence of better mental health versus mental ill-health for the individual and society. Individually, mental health affects our expressive, cognitive, perceptive, relational, and coping abilities, undergirding our general health and wellbeing and capacity to integrate into and become productive members of society (Dwivedi & Harper, 2004). Better mental health outcomes in adolescents are characterized by greater adaptation in family, school, and society environment, improved quality of life, and reduced symptoms of psychological disorders (Hoagwood et al., 1996; USDHHS, 1999). Positive mental health is also link to better physical health, increased pro-social behaviors, and participation in less adverse behaviors in adolescence (Resnick, 2000). On a societal level, mental health is perceived as a positive source contributing to asset development individually, socially, and economically (WHO, 2004). Conversely, poor mental health and well-being (i.e. depression, low self-esteem) during the adolescent years can lead to adolescent health risk

behaviors, school failure, physical ill-health, suicide, involvement in juvenile and criminal justice systems, negative life choices, and mental disorders in adulthood (Lewinsohn et al., 1993; Canals, et al., 2002; Trzesniewski et al., 2006; Hjemdal et al., 2007).

There is some evidence that spiritual and emotional intelligence development and spiritual and emotional experiences are helpful for health. At the same time, there is a significant relationship between awareness of spiritual and emotional experiences and health (Hay & Morisy, 1990; Ioannis & Ioannis, 2005). As whole, it seems spiritual and emotional functions including SI, EI and its components can be used as an instrument in relates with individual mental health.

Many authors claimed and reported that there existed a significant relationship between EI and mental health (Goleman, 1995; Salovey & Mayer, 1990; Ioannis and Ioannis, 2005), and SI and mental health (Emmonce 2000; Nobel, 2000). Also, spiritual including SI can be used as a possible instrument to increase individual's mental health (West, 2004). Therefore, the aim of this study was to investigate the link of SI and EI with mental health. In addition, the current study aims at providing more evidence regarding the relationship of SI and EI with mental health condition. In particular, this study examines whether SI and EI affects on mental health functioning.

Emotional intelligence (EI) was originally recognized as having its roots in the concept of social

intelligence (Thorndike, 1920; Salovey & Mayer, 1990; Goleman, 1995). Later, researches provided evidence that the two concepts actually represent interrelated components of the same construct (Salovey & Mayer, 1990; Bar-On et al., 2003; Lane & McRae, 2004). Consequently, this broad construct was accurately referred to as "emotional-social intelligence" (Bar-On, 2006). Based on historical reference, traits such as the capacity to navigate through and to adapt to one's own environment and the possession of social and emotional "skills" are important not only to basic survival, but have implications in the areas of relationships, work, school, and emotional and mental health (Goleman, 1995; Salovey & Mayer, 1990).

The popularity of the concept for the past decades has led researchers to examine its potency in various areas of human functioning. Among the areas with the strongest connections to EI is developmental, educational, clinical and counselling, industrial and organizational psychology. Hence, characteristic or ability EI were related to life success (Bar-On, 2001; Goleman, 1995), life satisfaction and well-being (Martinez-Pons, 1997; Palmer et al., 2002), physical and mental health (Ioannis and Ioannis, 2005), interpersonal relationships (Fitness, 2001; Flury & Ickes, 2001), academic achievement (Van der Zee et al., 2002; Parker et al., 2004), and more.

Today, there has been an increasing interest in how emotional reactions and experiences affect on mental health. For example, it has been claimed that negative emotional states are associated with unhealthy patterns of physiological functioning, whereas positive emotional states are associated with healthier patterns of respond in both cardiovascular activity and immune system (BoothKewley & Friedman, 1987; Herbert & Choen, 1993).

According to Salovey (2001) although suppressing negative feelings is neither a healthy strategy, he suggested that emotions' manifestation has a positive impact on physical health when people are confident about their abilities to regulate them. Moreover, Taylor (2001) argued that if you are emotionally intelligent then you can cope better with life's challenges and control your emotions more effectively, both of which contribute to good physical and mental health. Furthermore, Dulewicz, et al., (2003), examined the role emotional self-management such as stress, distress, morale and poor quality of working life play in everyday life. They demonstrated that EI was strongly correlated with both, physical and psychological health.

Also, considering Gardner's theory, existential intelligence can be define as an ability to find and realize meaning in life (Halama & Strizenec 2004).

Based on this definition, Halama & Strizenec (2004) suggested that the ability to find and realize meaning in life is an important element of SI. Since, SI involves a set of abilities that draw on spiritual resources, it can be concluded that existential and SI is non-identical but mutually related and overlapping construct (Halama & Strizenec 2004). Drawing on Gardner's definition of intelligence, Emmons (2000b) argued that spirituality can be viewed as a form of intelligence because it predicts functioning and adaptation and offers capabilities that enable people to solve problems and attain goals. Earlier, Emmons (1999) defined spirituality as the search for, and the experience of elements of sacred meaning, higher consciousness, and transcendence, SI entails the abilities that draw on such spiritual themes to predict functioning and adaptation and to produce valuable products or outcomes. Zohar & Marshall (2000) stress the utility of SI in solving problems of meaning, value, and those of an existential nature, concurring with Vaughan (2002) and Wolman (2001). Looking at spirituality through the lens of intelligence, Emmons (1999) writes, "SI is a framework for identifying and organizing skills and abilities needed for the adaptive use of spirituality" (p. 163). Hence, SI can be differentiated from spirituality in general, spiritual experience, (e.g. a unitary state), or spiritual belief, (e.g. a belief in God), (Amram, 2007). However, the theory and research of the spirituality and SI were well reviewed by many authors and researchers (Emmons, 1999; MacHovec, 2002; Mark, 2004; Schuller, 2005; Sisk & Torrance 2001; Wolman, 2001; Zohar & Marshall, 2000; Nasel, 2004; Amram, 2009).

## 2. Method

### 2.1. Sample

Two hundred and forty seven Iranian high school students in Gorgan city, north of Iran (124 females and 123 males) were recruited as respondents in this study. Their ages ranged from 14 – 18 years. They were selected by using cluster sampling technique, and their participation was voluntary and anonymously.

### 2.2. Measures

All participants responded to of the three instruments that have been translated to Persian language:

#### 2.2.1. The Integrated Spiritual Intelligence Scale (ISIS, Amram & Dryer, 2008)

Amram & Dryer's Integrated Spiritual Intelligence Scale (ISIS) was reviewed and chosen for measure of SI due to its comprehensive nature and strong psychometric properties (Amram & Dryer,

2008). ISIS is an 83-item long form, and a 45-item short form, self-report and observer-rated instrument containing 22 subscales assessing separate capabilities that are grouped into five main domain scales of spiritual intelligence. Responses are answered a six-point scale ranging from "never or almost never" to "always or almost always". For this study the simple Likert method (1–2–3–4–5–6) was chosen. The measure yields an overall SI scores (range 0–270). The scale has a Cronbach alpha of 0.76.

#### 2.2.2. Emotional Intelligence Inventory, Youth Version (EQ-i YV, Bar-On & Parker, 2000)

Utilized to measure emotional intelligence, the Bar-On Emotional Quotient Inventory: Youth Version (EQ-i: YV) was developed by Reuven Bar-On, Ph.D. and James D.A. Parker, Ph.D., and published by Multi-Health Systems, Inc. (2000). The EQ-i: YV was developed to measure emotional intelligence in adolescent populations, based on the theoretical basis of the Bar-On model of social and emotional intelligence. This 60-item inventory is a self-report instrument designed to measure emotional intelligence in young people age 7 to 18 years. The instrument measures a cross-section of abilities and competencies that constitute the core features of emotional intelligence. Responses are invited on a four-point scale ranging from "very seldom true of me" to "very often true of me". For this study the simple Likert method (1–2–3–4) was chosen. The measure yields an overall EI scores (range 0–240). The scale has a Cronbach alpha of 0.74.

#### 2.2.3. General Health Questionnaire (GHQ 28, Goldberg, 1972; Goldberg & Williams, 1998)

In 1972, Goldberg developed a simple questionnaire, the General Health Questionnaire (GHQ), which is the most widely used instrument for detecting non-psychotic psychiatric "Cases". The GHQ is a self-administered screening questionnaire used to diagnose psychiatric disorders both in primary care and in the community. The main benefits of GHQ are that it is easy to administer, brief, and objective. Several versions of GHQ are available: there is a 60-item version, and shorter versions (comprising 30, 28 and 12 items). The 28-item version (GHQ-28) developed by Goldberg and Hillier (1979) is constructed on a different basis when compared with the other versions. Responses are responded on a four-point scale ranging from "less than usual", to "much more than usual". Of the four possible ways of scoring this instrument (Goldberg & Williams, 1998), for this study the simple Likert method (0–1–2–3) was chosen. The measure yields an overall health scores (range 0–84)

and is composed of four subscales described as somatic symptoms, anxiety and insomnia, social dysfunction and depression. High scores indicate high levels of psychological strain. The measure was found to have an acceptable level of internal consistency reliability ( $\alpha = 0.92$ ). High score on this scale indicate poor general health.

### 3. Results

To attain the main objectives of the present study, the collected data were subjected to a number of statistical analyses by using statistical package for social sciences (SPSS 17.0). Besides, descriptive statistics, multiple and moderated regression analyses was also used in this study.

*3.1. Descriptive statistics;* Table 1 indicates the mean and standard deviations of all the observed variables. Descriptive statistics is worked out to know the pattern of score distribution. A perusal of table 1 reveals that the mean score on SI is 3.93 with the SD of .36, EI is 2.90 with the SD of .29, and on total mental health the mean score was .91 with the SD of .43. (See table 1)

*3.2. Multiple Regression Analysis (MRA);* MRA was conducted to assess the strength of relationship between dependent variable and independent variables. MRA provides an opportunity with little ambiguity to assess the importance of each of the predictors to the overall relationship. The results of regression analysis for the dependent variable (mental health) are presented in table 2. It is clear from the results that the regression analysis indicated both the variables (SI and EI) as a significant predictor of mental health. This table shows that  $R = .640$ ,  $R^2 = .409$ , and  $\{F (2,244) = 48.50 p < .05\}$ . This  $R^2$  value means that 40.9% of the variance in mental health increase is explained by SI and EI. Based on the values reported in the table, the beta coefficient for spiritual intelligence was -.293, and for emotional intelligence was -.413. This means that emotional intelligence is the strongest predictor followed by spiritual intelligence to explain the criterion variable (mental health).

*3.3. Moderated Multiple Regression (MMR);* (MMR) was employed in examining the effects of moderator variable (Age) on the relationships between the independent variable (SI and EI) and dependent variable (mental health). MMR involves two steps. First, it is needed to form two regression equations, one includes the first-order only and a second model include the first-order effects as well as a product term including the moderator variable. In this research, the product term is age. The following

are the two equations formed that derived from the regression procedure by entering independent variables and product term block by block in order to create two models.

Table 3 shows that for model 1,  $R = .640$ ,  $R^2 = .409$ , adjusted  $R^2 = .404$  and  $\{F (2,244) = 48.50 p < .05\}$ . This  $R^2$  means that 40.9% of the variance in mental health increase is explained by SI and EI. Model 1 does not include the product term and, thus, ignores a possible moderating effect of age. To find out whether the potential moderating effect of age on the SI and EI with mental health relationship, we need to interpret the model 2 in table 3.

Model 2 shows results after the product term has entered the equation. As shown in table 3, the addition of the product term resulted in an  $R^2$  change of .000, F change (1,243) = .132, Sig. F change = .717 at the  $p < .05$ . This result do not supported the presence of a moderating effect. In other words, the moderating effect of age explains .0% of variance in mental health above and beyond the variance explained by spiritual intelligence and emotional intelligence. The results suggest that the age is not important moderating factors on relationship between SI, EI and mental health.

#### 4. Discussion

The results in this study found emotional intelligence was significantly and negatively correlated with mental health scores. This finding is in line with (Bar-On, 2002; Palmer et al, 2002; Ioannis and Ioannis, 2005). Also between spiritual intelligence and mental health scores the finding of this study provides evidence to the claims of the previous researchers (Hay and Morisy, 1990; Emmonce 2000; Nobel, 2000; Zohar and Marshall (2000); West, 2004). The results of the Multiple Regression Analyses (MRA) revealed the overall scores of the emotional and spiritual intelligence are statistically significant predictors of mental health in the study. Emotional Intelligence wad found to be the strongest predictor followed by spiritual intelligence for mental health scores. So, the findings of this study supported a positive effect of spiritual and emotional intelligence on students' mental health scores. The overall regression model was successful in explaining approximately 40.9% of the proportion variance explained in mental health scores. Finally, the findings of the results failed to provide evidence for the hypothesis that age has moderating effect on the relationship between two independent variables (spiritual and emotional intelligence) and dependent variable (mental health).

Table 1: Descriptive Statistics of the Independent and Dependent Variable

Variables	N	Minimum	Maximum	Mean	Std. Deviation
Spiritual Intelligence (SI)	247	3.02	4.87	3.9340	.35637
Emotional Intelligence (EI)	247	2.15	3.67	2.9028	.29031
Total Mental Health	247	.04	2.04	.9110	.42770

Table 2: Multiple Regression Analysis of Total Mental Health

Variables	Summary of Regression	Un-std Coefficient B	Un-std Coefficient Std. Error	Std. Coefficient Beta	t	Sig. Value
(constant)		4.063	.248			
Spiritual intelligence		-.352	.076	-.293	- 4.638	.000
Emotional intelligence		-.609	.093	-.413	-6.533	.000
Multiple R	.640					
R Square	.409					
Adjusted R Square	.404					
F-Statistics	84.504					

Note. Predictors: SI & EI. Dependent Variable: Total Mental Health. \*  $p < .05$ .

Table 3: Result of MMR Analysis for the Moderated Effect of Age. Dependent Variable Total Mental health

Model	R	R Square	Adjusted R Square	Std. Error of the estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.640a	.409	.404	.33009	.409	84.504	2	244	.000
2	.646b	.410	.402	.33068	.000	.132	1	243	.717

Note. Predictors step 1: Total SI & EI; step 2: Total SI & EI, Students Age. \* p < .05.

## 5. Conclusion

The main purpose of the present study is conducted to explain the role of SI and EI on mental health (somatic symptom, anxiety, social dysfunction and depression) of high school students. The present investigation also was to test the moderating effects of age on the relationship of SI and EI with mental health. In this research, we found that student's mental health can be predicted by SI and EI. In other words, The R-squared of .409 implies that the two predictor variables (SI and EI) explain about 40.9% of the variance in the mental health (dependent variable). Also, this study does not support the presence of a moderating effect of age on link of SI and EI with mental health. In other words, the moderating effect of age explains .0% of variance in mental health above and beyond the variance explained by SI and EI. The result suggests that the age is not important moderating factors on relationship between SI and EI with mental health. These findings suggest that SI and EI are important and should be encouraged in school and students mental health life. Since, combining the concept of spiritual and emotional intelligence in the analyses of multiple regression and moderated regression, a new understanding emerged in this area of psychology. Therefore, this information will be valuable to community counsellors, teachers, school counsellors, and parents, all of whom are concerned with spiritual-emotional development and mental health of the school students, especially those of Iranian population.

## Acknowledgment

We thank the administration officers at all schools of this research sample for giving us information about students in their schools. We also appreciate the contribution of high schools students by participating in this research, thus allowing us to collect the necessary data for the study.

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9/13/2010

# Morphometrical, Histopathological, and Cytogenetical ameliorating Effects of Green tea Extract on Nicotine Toxicity of the Testis of Rats

<sup>1\*</sup>Azza M. Gawish, <sup>2</sup>Aliaa M. Issa, <sup>3</sup>Aziza M. A., and Sherin Ramadan

<sup>1</sup>Department of Zoology, Faculty of Science, Cairo University, Cairo, Egypt

<sup>2</sup> Cell biology Department, National Research Centre, Dokki, Cairo, Egypt

<sup>3</sup> National Organization for Drug control and Research Dokki – Giza, Cairo, Egypt

\*[azzagawish@ymail.com](mailto:azzagawish@ymail.com)

**Abstract:** Nicotine is a major toxic component of cigarette smoke and it is a major risk factor in the development of functional disorder of several organ systems. The natural diet contains a variety of compounds that exhibit protective effects towards different toxicities of nicotine as green tea. Four groups of male Swiss albino mice were divided: untreated control group; Nicotine-treated group (2.5 mg/kg/day); Green tea-treated group (40 mg/kg./day); and Nicotine and green tea treated group interperitoneal administration for successive 28 days. Results showed that disorganization of the seminiferous tubules associated with reduction of spermatogenic cells, leading to widening of lumen of tubules upon nicotine toxicity. Many of seminiferous tubules exhibited degenerative phases of spermatocytes and spermatides as well as missing of sperms and hypo-spermatogenesis. The recorded data in nicotine intoxicated group showed significant and gradual decrease of number of leydig cells throughout all intervals of experiment. In the last, cytogenetically examination demonstrated significant increased in the number of nucleated polychromatic erythrocytes (MnPCE) and decreased in number of polychromatic erythrocytes (PCE) in bone marrow of nicotine-treated animals using micronucleus assay. Green tea treatment reduced number of nucleated polychromatic erythrocytes (MnPCE) and restored number of polychromatic erythrocytes (PCE) to nearly normal. In conclusions, intake of green tea might suppress the toxicity and mutagenic activity of nicotine.

[Azza M. Gawish, Aliaa M. Issa, Aziza M. A., and Sherin Ramadan. Morphometrical, Histopathological, and Cytogenetical ameliorating Effects of Green tea Extract on Nicotine Toxicity of the Testis of Rats. Journal of American Science 2010;6(11):401-411]. (ISSN: 1545-1003).

**Keywords:** Smoking - Nicotine – Fertility – Antioxidants – Green tea

## 1. Introduction:

Smoking has enormous negative health consequences worldwide. Nicotine is a naturally occurring alkaloid found in tobacco plant (*Nicotiana tabacum*) and it is the major constituent of tobacco responsible for the compulsive use of tobacco (Wu *et al.*, 2002). Nicotine and its metabolites are also being investigated and researched for the treatment of a number of disorders as Alzheimer's diseases (Hecht, 2003). Okamoto *et al.*, (1994) recorded the LD<sub>50</sub> of nicotine as (50 mg/kg) for rats and (3 mg/kg) for mice.

Nicotine administration induces changes in gonadal functions and deficiency in sperm maturation and spermatogenesis and has a detrimental effect on the sperm-fertilizing potential of male rats (Reddy *et al.*, 1998 and Yamamoto *et al.*, 1998). Also nicotine was reported to have toxic effects on gonadal functions in males in addition to its role in the lowering testosterone and estradiol levels in the serum (Kavitharaj *et al.*, 1999). Nicotine has been associated with decrease in number of germ cells, germinal cells and increased

chromosomal abnormalities in sperm and with increased the risks of birth defects and neonatal death and genetic mutations in sperm, reduced sperm fertilizing capacity and decreased embryonic implantation rates (Polyzos *et al.*, 2009). Nicotine has a deleterious effect of nicotine on sperm membrane intactness and DNA and apoptosis in mouse leydig cells treated with nicotine were recorded by (Arabi, 2004 and Kim *et al.*, 2005). From human studies; nicotine smoke contains harmful mutagens and carcinogens metabolites; that may induce defective semen quality, nuclear DNA damage of spermatozoa (Arif *et al.*, 2000, De Flora *et al.*, 2003 and Elshal *et al.*, 2009).

Nicotine had been shown to increase the frequency of micronuclei in human gingival fibroblasts and induce DNA strand breaks in human spermatozoa (Arabi *et al.*, 2004). Information on the *in vivo* genotoxicity of nicotine have shown that nicotine induces aneuploidy and polyploidy (Bishun *et al.*, 1972), sister chromatid exchange and chromosome aberrations in bone-marrow cells of mice (Sen *et al.*, 1991). High doses of nicotine

increase the frequencies of premature centromere separation and premature anaphase and reduce the number of oocytes ovulated; however, the results of this study suggested that nicotine does not elevate aneuploidy levels in mouse oocytes (Mailhes *et al.*, 2000). The increased generation of ROS can produce a condition of oxidative stress that can result in the oxidation of lipids, induction of DNA single-strand breakage, inactivation of certain proteins, and disruption of biological membranes (Durak *et al.*, 2002). Increased oxidative stress has been suggested to play a major role in the pathogenesis of several smoking-related diseases such as cancer, cardiovascular and oral diseases (Reibel, 2003 and Sudheer *et al.*, 2007)

Natural antioxidants as polyphenols of green tea extracts have received much attention for treatments of oxidative-stress-related pathological conditions (Park *et al.*, 1998 and Yokozawa *et al.*, 2004). Green tea can suppress the DNA adduction, and hence act as inhibitors of cancer and it is a rich source of polyphenols, which are antioxidants in natural and their ameliorating effect on genital organs were recorded (Ogura *et al.*, 2008). Study on acute effect of green tea extract and its polyphenols constituents, *in vitro* showed stimulated testosterone production by rat Leydig cells and reduced the testicular tissue content of total cholesterol (TC), triglycerides (TG), phospholipids (Fabiano *et al.*, 2009).

Tea polyphenol could inhibit the mutagenicity of chemical mutagens and chromosome aberrations (Shim *et al.*, 1995). The frequencies of sister-chromatid exchange (SCE) in peripheral lymphocytes were study were significantly higher among smokers who were non tea drinkers, than those of non-smokers and smokers who consumed green tea at least two to three cups per day during the past 6 months (Lee *et al.*, 1997). It is possible that consuming high levels of green tea over a long period may reduce the DNA damage caused by tobacco smoking (Liang *et al.*, 2007). Our aim of work is the evaluation of the role of green tea in the protection from the effects of cigarette smoking.

## 2. Materials and methods

### 1-Experimental animals:

The experimental animals used in this study were male Swiss albino mice. Eighty Male Swiss albino mice aged 9 – 12 weeks and weighing 25 -30 gm were used throughout the study. Animals were fed a commercially prepared diet and had free access to tap water. All mice were kept under the same experimental condition, feeding standard diet, and water was available *Adlibitum*.

After one-week acclimatization period, the selected animals of nearly a similar weight were divided into 4 experimental groups so as to keep more or less the same mean body weight within the individual groups. The selected animal groups treated as follows:

1-Nicotine – Treated group: Each mouse in this group was given intraperitoneally (i.p.) dose of 3 nicotine 2.5mg/ kg / day for successive 28 days.

2-Green Tea – Treated group: For successive 28 days each mouse in this group was injected i.p. with freshly prepared green tea extract (40 mg / kg body weight / day).

3-Nicotine-Green Tea -Treated group: Each animal in this group was given i.p. of nicotine (2.5mg/kg/day) at the same dose as group1 concomitantly with green tea extract (40 mg / kg body weight) at the dose as second group for successive 28 days.

4-Control -Treated group: Each animal in this group was injected i.p. with distilled water (1ml/ day) for successive 28 days and handling on the same conditions exactly similar to that of the previously mentioned groups.

### II-Chemicals:

The treated elements in the experiment were nicotine ((*S*)-3-(1-Methyl-2-pyrroli-dinyl) pyridine) and green tea extract. Nicotine was supplied as colorless liquid, obtained from faculty of pharmacy, Cairo University, Egypt. The mean LD<sub>50</sub> for intraperitoneal administration of nicotine to 8-week-old mice was reported as 12.5 mg (Favarro *et al.*, 2003). Green tea extract was supplied in form of tablets obtained from Technomad Groups Company, Egypt, they were soluble in water.

### III- Experimental design:

Five mice from each group were scarified by cervical dislocation at end of 1, 2, 3, and 4 weeks (7<sup>th</sup>, 14<sup>th</sup>, 21<sup>th</sup>, 28<sup>th</sup> days) of the experimental period and decapitation, two femurs are removed and stripped clean of muscle for cytogenetically examination. Also, lung, liver and testes were also sampled and kept in aqueous Bouin for histological, histochemical and morphometrical examinations.

### Experimental analysis:

#### 1--Histopathological examination:

For histopathology and morphometric evaluation, testes of all groups were collected deceiption and fast dissection. They were fixed in aqueous Bouin solution, dehydrated through alcohols, cleared in xylene and then embedded in paraffin wax. According to the method described by

Bancroft and Stevens, (2002). 5 $\mu$  thickness paraffin sections were prepared and mount on clean slides. For histopathological study sections were stained with Ehrlich's haematoxylin and counterstained with eosin. A number of photomicrographs were taken at known magnification.

#### 2-Image analysis:

The data were obtained using Leica Qwin 500 image analyzer computer system (England). The image analyzer consisted of a colored video camera, colored monitor, hard disc of IBM personal computer connected to the microscope, and controlled by Leica Qwin 500 software. The image analyzer was first calibrated automatically to convert the measurement units (pixels) produced by the image analyzer program into actual micrometer units. These measurements were done using an objective lens of magnification 40, i.e. of total magnification 400. Ten readings were obtained in each specimen. Regarding the no of Leydig cells using an objective lens of magnification 40, i.e. of total magnification 400, they were counted using interactive measurement in 10 fields in each specimen. The data obtained were subjected to statistical analysis using Duncan's Test and ONE WAY ANOVA.

#### 3- Micronucleus assay

The most attractive features of the micronucleus assay are rapidly and easily with which in vivo genetic activity can be demonstrated. The first test to be developed and the most commonly used is carried out in mouse bone marrow by scoring micronuclei polychromatic erythrocytes (Heddle, 1973). In this study the micronucleus polychromatic erythrocyte were prepared according to an improved method adopted by (Salamone *et al.*, 1980).

##### a. Harvesting Marrow.

Animals are killed by cervical dislocation, and two femurs are removed and stripped clean of muscle. The marrow is removed by making a small opening at the iliac end of the femur and introducing the pointed shaft of a 2.5-cm safety pin into the femur at the epiphysial end. As the pin is slowly pushed and twisted into the marrow canal, the marrow exudes out the hole at the iliac end. The marrow is placed directly on a slide, and then a drop of fetal calf serum is added. With the aid of the edge of a clean slide, the marrow is mixed with the serum until homogeneous and then is spread as a smear; additional slides from a given animal can be prepared by simply transferring some of the mixed preparation onto other slides. Prepared slides are air-dried, fixed for 5 min in absolute methanol.

##### b- Slide Staining:

After air-drying, the smear were stained for 20 min in May-Grunwald stain in conjunction with the Gimsa stain (May-Grunwald/Gimsa (Schmid, 1975)), this combination consisted of: 3 ml Gimsa (stock), 12 ml May-Grunwald (0.25gm MayGrunwald/100ml methanol), 2 ml 0.1M Na<sub>2</sub>HPO<sub>4</sub>, 4.5 ml 0.1M KH<sub>2</sub>PO<sub>4</sub>, and 51 ml D. Stain was freshly prepared and mixing well before used. After the staining, the slides were washed thoroughly in distal water and left to dry overnight. All glass slides were coded before observation. Examination under oil lense.

##### c- Scoring

1000 polychromatic erythrocytes (PCEs) were scored per animal and the numbers of micronucleated PCEs were recorded. The results were expressed as the average number of micronucleated PCEs / 1000 PCEs. For each sampling time, bone marrow smears from five animals per four / group were used for evaluation. From each animal, 1000 polychromatic erythrocytes (PCE) were scored under the microscope (1000-1250x; Optech, Germany) for the incidence of micronucleated polychromatic erythrocytes (MnPCEs). In addition, the number of PCEs among 1000 total erythrocytes (PCE + NCE) per animal was recorded to evaluate bone marrow cytotoxicity. The ratio of polychromatic to normochromatic erythrocytes (PCE / NCE) was calculated, based on 1000 erythrocytes (PCE / NCE) scored per slide. These ratios were used as a measure of toxicity of test materials.

#### IV- Statistical analysis:

Statistical analyses were carried out using analysis of variance, Duncan's Test and One Way ANOVA (Snedecor and Cochran, 1980). To evaluate the effect in between groups and give a change for multiple comparison in between.

### 3. Results:

#### 1-Histopathological results:

The control untreated male mice testes tissues showed an outer capsule of fibroblastic connective tissue bound normal seminiferous tubules microscopy (Fig 1).

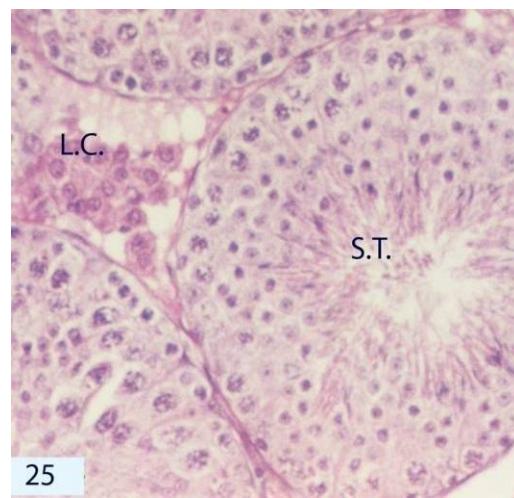
Testis of Swiss albino mice due to interperitoneal and daily injected nicotine treatment at the dose level 2.5mg/kg showed more or less normal testis architecture with complete spermatogenesis and focal decreased in population of leydig cells at the first week of nicotine treatment (Fig2). Along three weeks of nicotine treatment, some seminiferous tubules shows disorganization and atrophy of

seminiferous tubules with widening of its lumen and reduction in sperm count. Other seminiferous tubules had arrest in spermatogenesis. Some spermatogenic cells showed apoptosis especially in 1ry and 2ry spermatocytes. Also, few vacuoles were appeared in some somniferous tubules. Leydig cells were more reduction compared with previous stages and control stage (Fig. 3). Compared with normal group, the animal treated with nicotine for four weeks revealed most of seminiferous tubules had reduction in more than one stage of spermatogenesis such as reduced in



**Fig (1):** Micrograph of testis section of mice in control group showing normal architecture of somniferous tubules (S.T.) .Atypical appearance of mice seminiferous tubules with different cellular association .The tubules had normal progression cells and Leydig cells have normal disruption (L.C.) (H&E., 200X)

number of spermatocytes and spermatid (marked arrest in spermatogenesis) and marked degeneration of the leydig cells and decrease in this cell population (Fig. 4). Histological examination of testis sections of mice treated with green tea at different interval of experimental showed normal architecture (Fig. 7), complete spermatogenesis and maturation of germinal epithelium. Also, animals treated with green tea exhibited normal appearance, regarding size and arrangement of the tubules (Fig8).



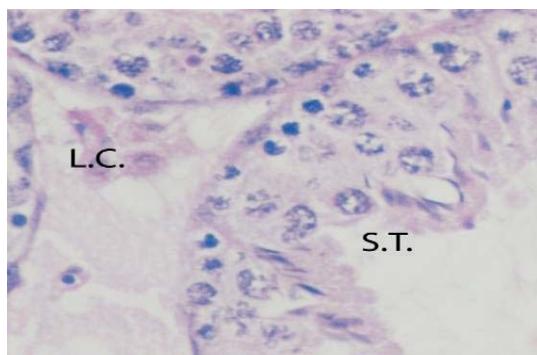
**Fig (2):** Micrograph of testis section treated with nicotine for a week showing complete spermatogenesis (S.T.) and nearly normal testis structure with lower decreased in population of Leydig cells (L.C.). (H&E., 400X)

## 2-Morphometric results:

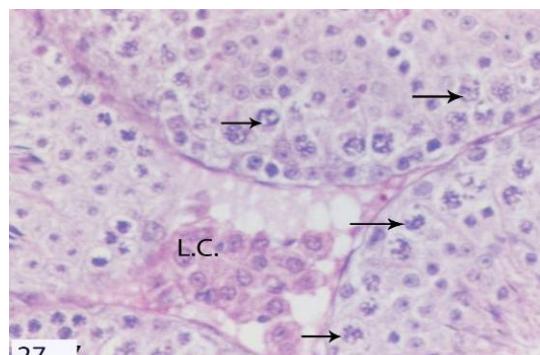
### Number of leydig cells in testis:

Table (1) and Figure (9) represent the variation in the number of leydig cells of testis due to nicotine intoxication and / or green tea protection. In normal group, all Swiss albino mice had normal distribution in nucleus area of liver and no significant changed presented along all interval of experimental.(  $23.40 \pm 1.784$ ;  $23.70 \pm 1.726$ ;  $23.90 \pm 1.940$ ;  $24.60 \pm 2.242$  );. The recorded data in nicotine intoxicated group showed significant ( $P < 0.05$ ) and gradual decrease of number of leydig cells throughout all intervals of treatment of experimental ( $18.10 \pm 2.282$ ), ( $15.30 \pm 1.783$ ), ( $12.40 \pm 1.979$ ) and ( $9.50 \pm 1.462$ ). As compared to control level, nicotine intoxicated group obtained insignificant ( $P < 0.05$ ) decrease of number of leydig cells after first week of treatment ( $18.10 \pm 2.282$  vs.,  $23.40 \pm 1.784$ ) but the

value recorded being highly significant decreased till reached to minimal value at last week of experimental ( $15.30 \pm 1.783$  vs.,  $23.70 \pm 1.726$ ); ( $12.40 \pm 1.979$  vs.,  $23.90 \pm 1.940$ ); ( $9.50 \pm 1.462$  vs.,  $24.60 \pm 2.242$ ). Treatment with green tea prevented the decrease in number of leydig cells in nicotine treated mice with different degree and showed general increase in these cells. As compared to nicotine intoxicated group, the recorded data showed insignificant increased in number of leydig cells after first and second weeks of treatment With continues injection of green tea, till four weeks of experimental showed significant increased in number of leydig cells and highly significant ( $P < 0.05$ ) increased at three and last week of experimental ( $19.50 \pm 2.591$  vs.  $12.40 \pm 1.979$  and  $21.60 \pm 2.390$  vs.,  $9.50 \pm 1.462$ ) were observed compared to control values.



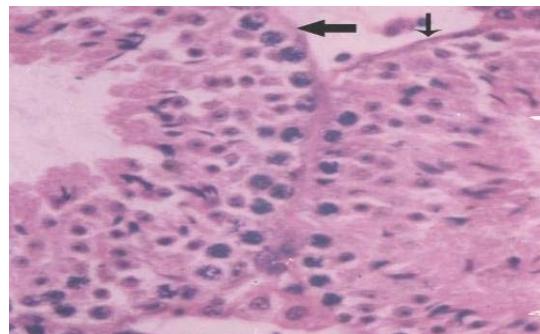
**Fig (3):** Micrograph of testis section treated with nicotine for three weeks showing apyknosis among spermatogenic cells especially necrosis in 1<sup>try</sup> & 2<sup>nd</sup> spermatocytes (thin arrow). (H&E., 400X)



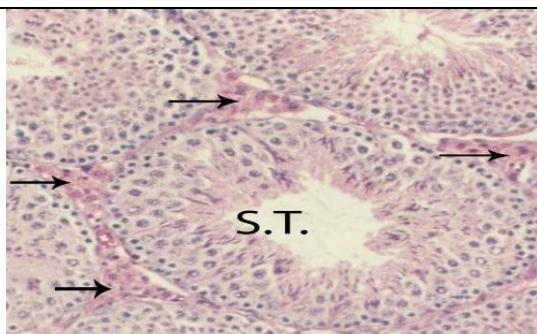
**Fig (4):** Micrograph of testis section treated with nicotine for four weeks showing different degree of damage of seminiferous tubules such as a disorder of systemic arrangement of the stages of spermatogenesis, loss of one or more stages of spermatogenesis (S.T.) and few number of Leydig cells are appeared(L.C.). (H&E., 400X)



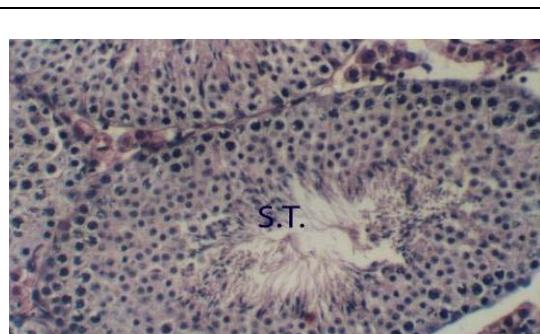
**Fig (5):** Micrograph of testis section treated with green tea concomitantly with nicotine for a week showing almost normal appearance of seminiferous tubules (S.T.) with normal spermatogenic cells and arrangement Leydig cells are more increased, (H&E., 200X)



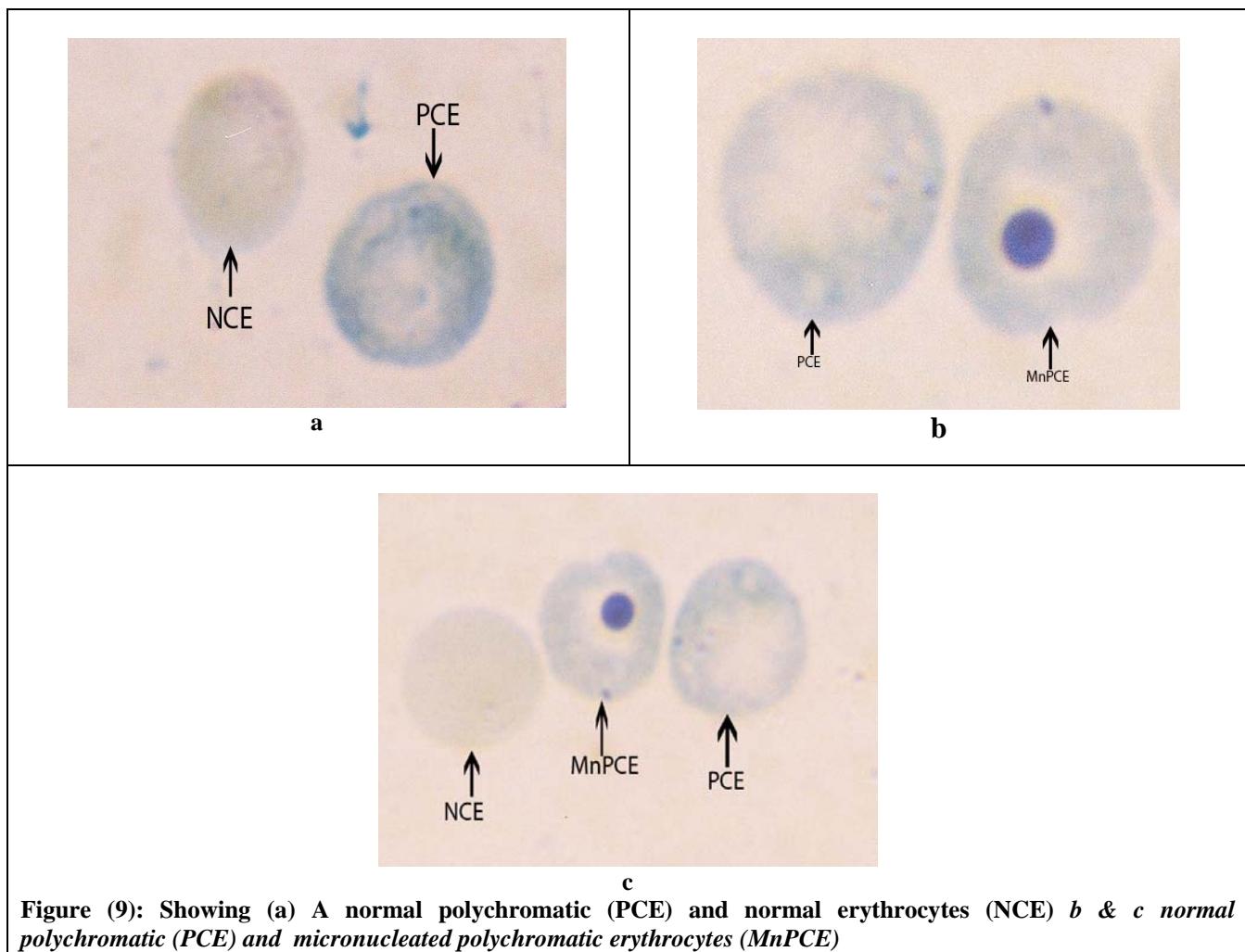
**Fig (6):** Micrograph of testis section treated with green tea concomitantly with nicotine for three weeks showing most of sommiferous tubules



**Fig (7):** Micrograph of testis section treated with green tea concomitantly with nicotine for four weeks showing a little change in architecture of sommiferous tubules with normal and order spermatogenesis (S.T.). (H&E, 400X)



**Fig (8):** Micrograph of testis section treated with green tea showing a normal testis structure, complete spermatogenesis (S.T.) and normal appearance of Leydig cells (arrow). (H & E , 400 X).



**Figure (9): Showing (a) A normal polychromatic (PCE) and normal erythrocytes (NCE) b & c normal polychromatic (PCE) and micronucleated polychromatoc erythrocytes (MnPCE)**

Improvement and protective effect of green tea increased significant and gradually with increased time of administration on nicotine intoxicated group. Individual green tea injection to albino mice showed no significant difference in the number of Leydig cells throughout all the tested experimental period or over all intervals of experimental were attained compared to control level. In conclusion, green tea had obviously improved on nicotine toxicity and Leydig cells back to normal number in male albino mice. As well as, it is showed an amelioration and obviously improvement to the nicotine toxicity effect on area of nucleus in liver, elastic fiber and leydig cells of testis of male albino mice.

### 3-Cytogenetical Results:

The cytogenetic damage induced by intraperitoneal injection of nicotine at ( 1/5 LD<sub>50</sub> = 0.6 mg /kg to mice ) as well as the antimutagenic effects of green tea at ( 40 mg /kg ) treated

intraperitoneal for one, two, three and four weeks with or without nicotine were investigated in bone marrow of male mice utilizing micronucleus assay. Micronucleus test were performed with nicotine and green tea. The % MnPCE in each treatment group as well as the PCE / NCE ratio are shown in tables 1 and 2. Normal polychromatic erythrocytes (PCE), normal normochromatoc erythrocytes (NCE) and micronucleated polychromatoc erythrocytes (MnPCE) showing in photos (1) a, b, and c.

In the present study, The ameliorative effect of green tea is gradual, increased with increased the time of administration of green tea with nicotine when study polychromatic erythrocytes (PCE), PCE/ NCE ratio and Mn-PCEs as shown in Tables 1&2. Treatment of green tea with nicotine was effective in reduction the frequencies of MnPCE in response to the time treatment. When green tea treated with nicotine for one, two or three weeks, the reduction in MnPCE come down to the same range of control

group and the percent of the reduction in group 1, 2, 3 and 4 was 62.5%, 57.1 %, 77.35 % and 81.8 % respectively. Whereas treatment of green tea with nicotine, significantly ( $p<0.05$ ) improved the number

of PCEs and the ratio of PCEs to NCEs were significant ( $p<0.05$ ) enhanced in compared to the control group.

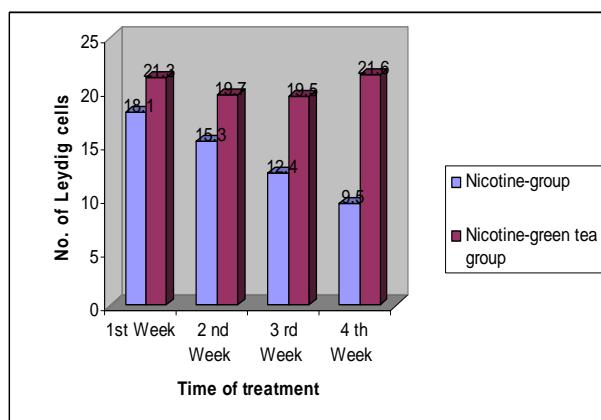
**Table (1): Effect of nicotine and / or green tea on the number of Leydig cells of testis section of Swiss albino mice.**

Groups of experiment Time Of Treatment	Control groups (Mean $\pm$ S.E.)	Nicotine groups (Mean $\pm$ S.E.)	Green Tea groups (Mean $\pm$ S.E.)	Nicotine -Green Tea groups (Mean $\pm$ S.E.)
<b>Week 1</b>	23.40 $\pm$ 1.784 <sup>BAa</sup>	18.10 $\pm$ 2.282 <sup>Ba</sup>	24.80 $\pm$ 1.836 <sup>Aa</sup>	21.30 $\pm$ 1.880 <sup>BAa</sup>
<b>Week 2</b>	23.70 $\pm$ 1.726 <sup>BAa</sup>	15.30 $\pm$ 1.783 <sup>Ca</sup>	25.40 $\pm$ 1.507 <sup>Aa</sup>	19.70 $\pm$ 2.290 <sup>BCa</sup>
<b>Week 3</b>	23.90 $\pm$ 1.940 <sup>Aa</sup>	12.40 $\pm$ 1.979 <sup>Bba</sup>	25.50 $\pm$ 1.607 <sup>Aa</sup>	19.50 $\pm$ 2.591 <sup>Aa</sup>
<b>Week 4</b>	24.60 $\pm$ 2.242 <sup>AA</sup>	9.50 $\pm$ 1.462 <sup>Bb</sup>	25.80 $\pm$ 1.576 <sup>Aa</sup>	21.60 $\pm$ 2.390 <sup>Aa</sup>

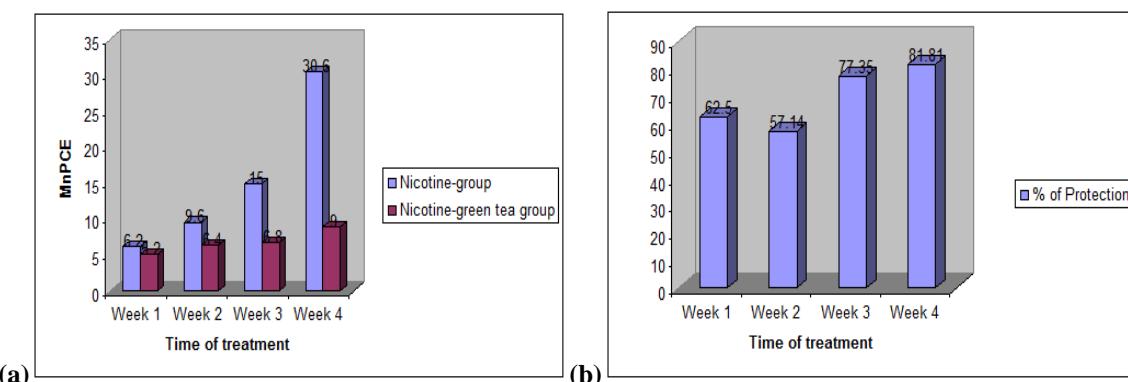
( $P<0.05$ ) is significant; ( $P<0.01$ ) is highly significant

Numbers of experimental animals were 5 in all groups

Compared between control treated group, nicotine treated group, nicotine-green tea treated group and green tea treated group tacked capitals latter but compared within group of each group tacked small latter.



**Fig (10): Effect of green tea administration on number of leydig cells in testis of nicotine intoxicated male albino mice.**



**Figur (11): Histogram showing: (a) frequency of MnPCEs in nicotine and nicotine-green tea groups  
(b) Percentage of the reduction in MnPCEs in different treatments**

**Table (2): Represent the effect of Nicotine and Green Tea Treatment on Frequencies of MnPCE.**

TOTAL NO. OF MnPCE / 5000 PCE					
Groups of Experimental	Control Groups	Nicotine groups	Green Groups	Tea	Nicotine -Green Tea Groups
	(Mean ± S.E.)	(Mean ± S.E.)	(Mean ± S.E.)	(Mean ± S.E.)	(Mean ± S.E.)
<b>Week 1</b>	<b>23</b> 4.60 ± 0.748 <sup>Aa</sup>	<b>31</b> 6.20 ± 0.860 <sup>Ac</sup>	<b>20</b> 4.00 ± 0.707 <sup>Aa</sup>	<b>26</b> 5.20 ± 0.663 <sup>Ab</sup>	
<b>Week 2</b>	<b>4.00 ± 0.837<sup>Ba</sup></b>	<b>9.60 ± 1.166<sup>Ab</sup></b>	<b>3.80 ± 0.860<sup>Ba</sup></b>	<b>6.40 ± 1.208<sup>Bba</sup></b>	
<b>Week 3</b>	<b>4.40± 0.678<sup>CBa</sup></b>	<b>15.00± 1.224<sup>Ab</sup></b>	<b>3.60 ± 0.509<sup>Ca</sup></b>	<b>6.80 ± 0.663<sup>Bba</sup></b>	
<b>Week 4</b>	<b>4.20 ± 0.583<sup>Ba</sup></b>	<b>30.60± 4.523<sup>Aa</sup></b>	<b>3.20 ± 0.583<sup>Ba</sup></b>	<b>9.00 ± 1.140<sup>Ba</sup></b>	

Statistical analysis of results were done according to Duncan's multiple rang test. Means with different letters within each column (small letters) or each raw (capital letters) are significant at 5 % level. = Micronucleated polychromatic erythrocytes PCE Mn. Five animals /group; 1000 cells /animal.

**Table (3): Represent the effect of Nicotine and Green Tea Treatment on the ratio of normochromatric erythrocytes (NCEs) to polychromatic erythrocytes (PCEs)**

Parameter	Groups of Experimental	Control groups	Nicotine groups	Green Tea groups	Nicotine -Green Tea groups
		(Mean ± S.E.)	(Mean ± S.E.)	(Mean ± S.E.)	(Mean ± S.E.)
%PCE	<b>Week 1</b>	45.40 ± 0.953 <sup>Aa</sup>	42.50± 1.580 <sup>Aa</sup>	46.30 ± 1.028 <sup>Aa</sup>	42.96 ± 2.26 <sup>Aa</sup>
	<b>Week 2</b>	46.34 ± 0.668 <sup>Aa</sup>	37.94± 0.624 <sup>Ba</sup>	46.90 ± 1.492 <sup>Aa</sup>	38.18 ± 0.85 <sup>Bb</sup>
	<b>Week 3</b>	47.28 ± 0.435 <sup>Aa</sup>	29.38± 3.919 <sup>Cb</sup>	47.60 ± 0.646 <sup>Aa</sup>	37.84 ± 0.79 <sup>Bb</sup>
	<b>Week 4</b>	47.70 ± 0.750 <sup>Aa</sup>	20.60± 2.454 <sup>Cc</sup>	48.26 ± 0.483 <sup>Aa</sup>	34.32 ± 1.17 <sup>Bb</sup>
%NCE	<b>Week 1</b>	54.60 ± 0.953 <sup>Aa</sup>	57.50± 1.580 <sup>Ac</sup>	53.70 ± 1.028 <sup>Aa</sup>	57.04 ± 2.26 <sup>Ab</sup>
	<b>Week 2</b>	53.66 ± 0.668 <sup>Ba</sup>	62.06± 0.624 <sup>Ac</sup>	53.10 ± 1.492 <sup>Ba</sup>	61.82 ± 0.85 <sup>Aa</sup>
	<b>Week 3</b>	52.72 ± 0.435 <sup>Ca</sup>	70.62± 3.919 <sup>Ab</sup>	52.40 ± 0.646 <sup>Ca</sup>	62.16 ± 0.79 <sup>Ba</sup>
	<b>Week 4</b>	52.30 ± 0.750 <sup>Ca</sup>	79.40± 2.454 <sup>Aa</sup>	51.74 ± 0.483 <sup>Ca</sup>	65.68 ± 1.17 <sup>Ba</sup>

#### 4. Discussion:

Our result showed that disorganization of the seminiferous tubules associated with reduction of spermatogenic cells, leading to widening of lumen of seminiferous tubules upon nicotine toxicity. Many of seminiferous tubules exhibited degenerative phases of spermatocytes and spermatides as well as missing of sperms and hypo-spermatogenesis.

Fávaro *et al.* (2006); Ahmadnia *et al.* (2007) and Yamamoto *et al.* (2007) reported many alterations attributed to the direct cytotoxic effects of nicotine leading to the inhibition of prostaglandin's synthesis and decrease of testosterone which play a functional role in reproduction system of the male mice. Other investigation displayed that nicotine was a CNS depressor that can inhibit the neural stimulus essential for the release of pituitary gonadotrophine (Reddy *et al.*, 1998), which lead to a lack of pituitary gonadotrophins essential for initiating and completing spermatogenesis and steroidogenesis in the testis (Aydos *et al.*, 2001). Destruction of Leydig cells may cause testicular atrophy; gonadal dysfunction, erectile dysfunction, and male factor

infertility were showed by Kim *et al.* (2005). Other studies explained that metabolites of nicotine as cotinine produced dose response inhibition of luteinizing hormone and 3 $\alpha$ -hydroxysteroid dehydrogenase, enzyme stimulated testosterone production (50-70%) (Fávaro *et al.*, 2006).

Aydos *et al.*, (2001) and Ahmadnia *et al.*, (2007) explained that nicotine toxicity may be due to change in the proportion of collagen fibers and contractile myofibroblastic cells, which may prevent the appropriate release of spermatozoa from the germinal epithelium into the lumen.

The oxidative stress of nicotine may cause a peroxidant/antioxidant imbalance in blood cells, blood plasma, and tissues resulting in a decrease in the activity of endogenous antioxidant enzymes (Suleyman *et al.*, 2002). Other researchers reported disruption of spermatogenesis in nicotine treated animals' testes tissues may be modulated by free radical toxicity (El-Sweedy *et al.*, 2007). Also nicotine metabolite cotinine generates free radicals / ROS in tissues in the liver and testes (Kalpane and Menon, 2004), and induces oxidative tissue injury

(Husain *et al.*, 2001 and Argentine & Cicchetti, 2004).

Direct genotoxic effects have been shown in human gingival fibroblasts (Sassen *et al.*, 2005 and Kleinsasser *et al.* (2006) and DNA strand breaks in human spermatozoa in nicotine aneuploidy and polyploidy, sister chromatid exchanges and chromosome aberrations in bone marrow cells of mice (Sen and Sharma, 1991 and Yauk *et al.*, 2007). High doses of nicotine increase the frequencies of premature centromere separation and premature anaphase and reduce the number of oocytes ovulated (Attia, 2007 and Sudheer *et al.*, 2007)

Results concerning the mutagenicity of nicotine in several test systems are contradictory. In the present study, nicotine intoxicated group obtained highly significant and gradual increased of MnPCEs throughout all the tested experimental periods. The statistical analysis of data is also provided. Nicotine treated mice, for one, two, three and four weeks resulted in highly significant alterations of all monitored parameters, but with different intensity and distinctive time trends. Nicotine intoxicated group obtained highly significant and gradual decreased of PCEs and PCEs /NCEs but gradual increased in NCEs throughout all the tested experimental periods.

The differences between the results can be attributed to different drug concentrations and the different genotoxic endpoints considered in the test systems. Different repair capacities of the various cell types used may also explain the discrepancies. The micronucleus technique has been proposed as a useful tool for measurement of genotoxicity (Madhumita *et al.*, 2003) induced by nicotine as it is able to assess both the clastogenic and aneugenic properties of a test compound. The genotoxic effects of nicotine have been shown in human gingival fibroblasts and spermatozoa and damage to DNA may result in mutations and altered cell functions (Kleinsasser *et al.*, 2005 and Ogura *et al.*, 2008).

In the present investigation a significant increase in the levels of NCE, and increased micronuclei frequency in nicotine-treated groups, as indicative of DNA damage, when compared with control group. The results were in a gradient with those reported by Villard *et al.* (1998) who mentioned that nicotine causes *in vivo* DNA single-strand breaks (SSB) in lung and liver of mice and those reported by Sassen *et al.* (2005) who reported that nicotine increases the DNA fragmentation in mini organ cultures. Hassan and Ahmed 2004, proved that green tea were inhibit the induction of Mn-PCEs in bone marrow of mice by 66% and 90%

when treated for one and two weeks respectively. Ayako (2004) suggests that tea catechins are not genotoxic but rather have a preventive effect against reactive oxygen species ROS -induced chromosomal damage at their physiological condition. These useful effects of tea catechins against ROS-induced chromosomal damage may support the cancer-preventing effects of tea constituents (Fujiki *et al.*, 2002).

#### **Corresponding author:**

Azza M. Gawish

Department of Zoology, Faculty of Science, Cairo University, Cairo, Egypt

[azzagawish@gmail.com](mailto:azzagawish@gmail.com)

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9/19/2010

# Tourism as an Economic Development Tool

Mohammad Taleghani

Assistant professor, Islamic Azad University - Rasht Branch, IRAN.

[Taleghani@iaurasht.ac.ir](mailto:Taleghani@iaurasht.ac.ir)

**ABSTRACT** - Probably, the greatest single deterrent to tourism development is the lack of appreciation and enthusiasm for tourism by civic and business leaders. When tourism is not understood and its benefits are unclear, planning and implementation of measures to improve the industry are often lacking. Notably, global tourism has become the largest industry in the world, with nearly 500 million consumers of tourism services per year spending hundreds of billions of dollars. The industry provides employment to over 100 million people worldwide. Thus, in view of tourism's increasing role in economic activity, the factors affecting its performance should be analyzed. An understanding of these factors is crucial to determine the ways in which national and international financial institutions, NGOs and other entities can play the most value-adding role. This paper provides a brief profile of key factors and trends in tourism and their economic effects at the global, national and regional levels.

[Mohammad Taleghani. Tourism as an Economic Development Tool. Journal of American Science 2010;6(11):412-416]. (ISSN: 1545-1003).

**Keywords:** Tourism, Economic Development, Supply and Demand, GDP.

## 1. INTRODUCTION

The most important economic feature of activities related to the tourism sector is that they contribute to three high-priority goals of developing countries: the generation of income, employment and foreign-exchange earnings. In this respect, the tourism sector can play an important role as a driving force of economic development. The impact this industry can have in different stages of economic development depends on the specific characteristics of each country. "Given the complexity of tourism consumption, its economic impact is felt widely in other production sectors, contributing in each case towards achieving the aims of accelerated development" (Davidson, 2004).

A major difficulty in defining the boundaries of the tourism sector is to ascertain what investment costs should be ascribed to the development of tourism. Although heretofore not treated by international agencies as a "sector" in national accounting terms, tourism entails a collection of goods and services that are provided specifically for visitors and would not have been provided otherwise. Because of its interdependence with other sectors of the economy, it is difficult to analyze and plan for tourism. Furthermore, the lack of reliable statistical data hampers identification of the mechanisms by which tourism generates growth, as well as its potential for development.

Yet, in those instances where analysis has been carried out and research has preceded planning, tourism's priority in competing for scarce investment funds has been established. In these cases, long-term programmes for tourism development have been designed.

Nature and heritage tourism development has investment needs that differ, in certain respects, from traditional hotel development. There may be a greater need to improve access to the attraction site or facility, and for a mode of development that does not interfere with a sensitive habitat or historic area (Eadington & Redman, 2001).

## 2. LITERATURE REVIEW

### 2.1 Elements of Supply and Demand

There are primary factors that influence the level of nature and heritage tourism demand, namely; the overall tourism growth, the growth in specialty travel and the increasing awareness and concern for the environment. Each of these factors is in turn influenced by a number of elements. Overall tourism, for instance, is expected to continue to grow more rapidly than the world's economic output as a result of factors, such as population growth, rising incomes and employment, shorter working weeks in many parts of the world and the increasing integration of the world's economies and societies. The rapid growth of specialty travel is fuelled by some of the same factors, but there are a number of additional explanations, such as the boom in outdoor recreation and the new interest in health and fitness. Additionally, environmentalism is one of the elements that has changed people's attitudes on how they should spend their vacations.

### 2.2 The Importance of Location

People with incomes high enough for foreign travel are concentrated in a few countries. Most developing countries are far from the key points of origin. In this regards, countries like Mexico and

some Caribbean islands that are close to the United States and Canada benefit from a comparative advantage. These tourist destinations have reaped early success in promoting their attractions (Murphy, 2003).

### **2.3 Income Elasticity**

In several countries, travel receipts have been the fastest growing export item. The tourism sector represents over two-third of the value of total exports of goods and services from the Bahamas, three-fifth of those from Barbados and over one-third from the Dominican Republic and Jamaica. As national incomes increase, expenditure on travel also increases even faster<sup>1</sup>. As a result of this trend, international receipts from foreign travel have been increasing by nearly 11 percent a year (over eight percent in constant prices) or more than twice the rate of national incomes (Hogan & Mcpheters, 2003).

### **2.4 Decreasing Travel Costs**

There is evidence that tourism demand is also price-elastic, particularly below certain price levels. The two major costs of a trip abroad are transport charges and expenditures in the destination country. For long-distance traffic, air transport is predominant, and the average air transport costs have been declining as well. Where such transport costs constitute a high potential of the total costs of a trip, this decline is of great significance for potential long-distance travel growth.

### **2.5 Public and Private Sector Involvement**

Tourism is mainly a private sector enterprise, but the timely provision of hotel and other visitor services, such as entertainment, food and sport facilities, requires the public-sector participation in the form of infrastructure, promotional support, as well as fiscal and financial incentives, so as to attract private investment to the sector.

### **2.6 Availability of Credit**

Another important factor directly related to tourism facilities is the availability of credit on suitable terms, which is an essential catalyst for sound tourism investment. In some countries, when the private financial system does not provide this credit availability, the public sector has established credit lines for tourism investment.

### **2.7 Tourist Destination Attributes**

<sup>1</sup>- A model developed by the IDB for 17 major source countries gave a weighted average of 1.7 for the income elasticity of tourism demand.

Tourist demand is spurred by innovation in the type of holidays offered (new commodities) and by improvements in transport, accommodations and attractions (quality changes). The tourism sector offers multi-dimensional product that if vigorously promoted, is likely to lead to changes in the pattern of demand and general new demand for services. Yet, as in any other sectors of economic activity, a minimum set of parameters needs to be in place in order to make an investment viable. In this sense, it is important to identify those attributes of a destination area that are necessary to attract tourism projects and make them viable. Such attributes relate to at least six different categories, namely; climate, natural resources, infrastructure, amenities, culture, as well as socioeconomic and political factors. The table below presents these categories with their related attributes (Inskeep, 2008).

An ideal combination of these attributes should result in the form of tourism development that maximizes returns to the economy, the investors and the consumers of tourism services.

### **2.8 Tourism and GDP**

The tourism sector in the Latin American and Caribbean countries contributes significantly to GDP earnings, though this contribution is not reflected in the domestic income and product accounts of most countries. In the Bahamas, tourism accounts for about one-third of GDP and most sectors of economic activity are directly or indirectly linked to it. In Barbados, tourism was the leading economic sector, accounting for 15 percent of the GDP in 2002. In Jamaica, the tourism contribution to GDP was 13.4 percent in 2002, while in Mexico, it was only 4 percent, and as for Iran, it was accounted for about 1.3 percent.

Not all tourism receipts are retained within the economy. In fact, there is an outflow of foreign exchange for some of the goods and services consumed by visitors, as well as for capital goods invested in tourism and for payments abroad. The import needs depend on the level of development and the degree of diversification of substitutes for imported products and on the qualitative level of the tourism supply in each country (World Tourism Organization, 2000a).

### **2.7 Tourist Income Multiplier and Value-Added**

The tourist income multiplier (time) is a coefficient that expresses the amount of income

generated by a unit of tourism expenditure<sup>2</sup>. In Jamaica, a stopover visitor spending one dollar creates a ripple effect of US\$1.60 within the local economy, while a dollar spent by a cruise-ship visitor generates US\$1.20. In the Dominican Republic, the time has been estimated at US\$1.70.

The value-added concept is particularly important when considering the impact of tourism in the Caribbean region. Value is added when a product is developed, processed, refined, or remarked in a manner that allows it to be sold at a higher price than the prices of the raw materials, services and agricultural sectors supplying tourist consumption that are well positioned to achieve higher levels of value-added in the tourism sector.

When a country's natural resources are packaged by foreign tour operators and sold through sophisticated marketing techniques, a substantial portion of the value-added is created and captured by those tour operators, and therefore not returned to the country (Murphy, 2005). To increase the value-added of tourism, the host-country businesses and residents must offer travel services such as packaged tours ("land services"), offering locally owned accommodations and providing the necessary means for tourists to visit natural areas.

## **2.8 Income Distribution Effect (IDE) and Employment**

The IDE offers one of the strongest socioeconomic arguments in favour of tourism development. It describes how the income generated by the sector is distributed. The analysis can be undertaken at spatial and functional levels.

At the spatial level, tourists prefer to travel in regions with little industrial development. They also tend towards areas of little agricultural value. For these reasons, tourism can become a dynamic force in regional economies. Within a country, tourism demand originates in urban concentrations where the highest incomes are found. A percentage of such incomes is normally set aside for tourism in areas that are geographically different from the visitors' home base, thus, reinforcing the process of internal income redistribution. Internationally, a portion of the tourism consumption by developed countries occurs in the developing countries, favouring the process of international income redistribution (Williams & Shaw, 2009). On the other hand, at the functional

level, the income generated tends to favour employment, which is estimated to contribute more to the total value-added of the industry than other factors do, because so much of tourism involves personal services. In addition, it has been estimated that, worldwide, tourism directly or indirectly supports sixty-five million jobs, including hotel managers and staff, taxi drivers, tour operators and shop attendants, among others. Conversely, secondary employment is generated in agriculture, industry, handicrafts and services.

Tourism compares favourably with other economic activities as a generator of both employment and income, toothy directly and diffused through the economy. An OAS study on new hotel development in the Caribbean estimates that every investment of US\$80,000 in the tourism industry in the region generates forty-one jobs<sup>3</sup> (Organization of American States, 2005). The same investment would create only sixteen new jobs in the petroleum industry and fifteen in metallurgy. Moreover, according to the CTO, the 77,319 hotel rooms in fifteen Caribbean countries equal 88,697 jobs, or almost 1.15 per room<sup>4</sup> (Caribbean Tourism Organization, 2008).

Hotels account for about 75 percent of tourism employment, i.e. distribution, transport, finance, insurance and entertainment that make up the other 25 percent. Every room in a three- or four-star hotel in Venezuela generates one job. According to the IDB, for five-star hotels, each room creates 1.3 jobs. Moreover, based on the OAS study, one job generated by a hotel generates one more job elsewhere in the tourism trade and two in the rest of the economy; thus, one job generates an estimated three others. The tourism sector, particularly hotels, can play an important role in attracting foreign investment and providing training for employees. Many tourism ventures include foreign equity participation and technical knowledge about the construction and operation of hotels. The former represents a mobilization of international financial resources, which can be regarded as a desirable substitute for foreign borrowing. Outside management can be used to train large numbers of employees who would not otherwise have access to training. Furthermore, business provides a stimulus for the development of other ancillary businesses catering to tourists (WTO & IHRA, 2009).

<sup>2</sup> - The calculation is based on the familiar Keynesian multiplier  $K = 1/MPS + MPM$ , where MPS is the marginal propensity to save and MPM is the marginal propensity to import (or to spend on tourism abroad).

<http://www.americanscience.org/journals>

<sup>3</sup> - Organization of American States. The Optimum size and Nature of New Hotel Development in the Caribbean, Washington, D.C., 2005.

<sup>4</sup> - Caribbean Tourism Organization, Caribbean Tourism Statistical Report, 2008.

## 2.9 Tourism and Balance of Payments

Tourism can make an important contribution to a country's balance of payments. It offers the developing countries the possibility of diversifying their export earnings, particularly given that; (i) traditional exports are subject to price fluctuations, and (ii) there is a trend towards reducing the administrative, monetary and border formalities that affect international tourism mobility.

With only a few exceptions, the terms of trade for developing countries, i.e. the ratio between the prices that a country receives for its exports and the prices it pays for its imports have traditionally been unfavourable because of the fluctuations in the prices of raw material exports. But in the case of international tourism, if the index of average international tourist expenditure is taken as the expression of the price of the international product, the prices received have enjoyed greater stability than the prices of raw materials. In fact, prices have tended to increase in a stable manner due to several reasons, such as the demand for holidays, the growth of business travel and the rigidity of destination

supply in short and medium terms (Kottke, 2008). Conversely, the prices of other products are affected by the speculative or strategic offers; however, this is generally not the case with tourism. It is therefore a sector that tends to improve the terms of trade of an economy in the medium as well as the short terms.

## 2.10 Tourism the Most Important Economic Activity

For many third world countries, tourism has become the most important economic activity, especially as the major earner of foreign exchange. This is in part the result of the declining importance of sugar, bananas, bauxite and oil as the engines of growth. But it is also a reflection of the increasing importance given to recreation and leisure as a result of world's rising income levels. In addition, unlike many goods and services, tourism has no exact substitutes, meaning that the demand for holidays will grow rather than be raided for something else. Political boundaries that split regions, metropolitan areas or other natural market areas may also be an issue.

**Table (1): Tourist Destination Attributes**

CLIMATE	NATURAL RESOURCES	INFRASTRUCTURE	AMENITIES	CULTURAL	SOCIOECONOMIC POLITICAL
Temperature	Beaches	Water/energy Supply	Accommodations	Historic Features	Industrial Structure
Rainfall	Lakes	Drainage	Tourism Organizations	Theaters	Government Structure
Humidity	Rivers	Telecommunications	Restaurants	Concert Halls	Planning System
Sunshine	Forests	Roads	Shopping	Art galleries	Language
	Mountains	Railways	Sport facilities	Museums	Religion
	Flora	Ports	Recreational	Architecture	Gastronomy
	Fauna	Airports	Parks	Exhibitions	Hospitality
		Waste removal	Zoos		
			Entertainment		

**Table(2): What 100 Tourists a Day Mean to a Community (Jobs Created).**

Industry	Transportation	Retail	Hotels and Amusement	Eating and Drinking Places
Construction	0.19	0.41	0.52	0.19
Manufacturing	0.84	1.91	2.43	1.87
Transportation	8.21	0.68	0.87	0.37
Communication & Utilities	0.19	0.55	0.70	0.28
Wholesale Trade	0.39	0.68	0.87	0.94
Retail Trade	0.97	34.24	2.09	1.12
Finance Insurance & Real Estate	0.65	1.36	1.22	0.75
Hotel Amusements	0.13	0.27	22.08	0.19
Eating & Drinking	0.78	2.05	1.74	24.84
Services	2.13	5.73	6.26	2.72
Other	0.06	0.00	0.17	0.09
<b>Total Jobs Created</b>	<b>14.45</b>	<b>47.87</b>	<b>38.95</b>	<b>33.37=134.75</b>

Differences in local values and laws can have a dramatic effect on the apparent relative attractiveness of two otherwise comparable areas. The tourism effects appear most often when two adjacent jurisdictions have differing policies on such controversial recreational and entertainment activities as legalized gambling or the sale and consumption of alcoholic beverages. Often, however, in these cases, customers are drawn from the local or regional markets (Beckhuis, 2001). Thus, less of an "export" effect is created from spending, and therefore, little economic stimulus is added to the region. Nonetheless, in comparison with nearby jurisdictions which do not permit these activities, the counties or communities permitting such activities can be tremendously successful as measured by the revenues and jobs created at the local level.

The following table summarizes the economic impacts and demonstrates the multiplier effects of adding 100 tourists on job creation in a community.

**Table (3): Jobs Created from \$1 Million in New Demand for Final Output for Hotels and Amusements.**

Industry	County	Metro Area
Construction	0.3	0.4
Manufacturing	1.4	1.9
Transportation, Communication & Utilities	0.9	1.1
Wholesale Trade and Retail Trade	1.7	2.4
Finance Insurance & Real Estate	0.7	1.0
Hotel & Amusements	12.7	14.5
Services	4.6	5.9
Other	0.1	0.2
<b>Total Jobs Created</b>	<b>22.4</b>	<b>27.4</b>

### 3. Conclusion

Research found that an average of 22.4 direct and indirect jobs is created in non-metropolitan counties for each \$1 million of new demand for final output from the hotel and amusement industries. The impact on metropolitan areas is slightly higher at 27.4 new jobs created. The increase in demand for hotel and amusement services also affects almost all major sectors of the business community as shown in the following table. As may be expected, the greatest impact is on direct employment in the hotel and amusement industries, where 12.7 and 14.5 jobs are created in rural counties and metro areas, respectively

(World Tourism Organization, 2006).

There are other local revenues that are not easily quantified, as not all tourist expenditures are formally registered in the macro-economic statistics. Money is earned from tourism through informal employment, such as street vendors, informal guides, rickshaw drivers, etc. The positive side of informal or unreported employment is that, the money is returned to the local economy and has a great multiplier effect as it is spent over and over again. The World Travel and Tourism Council estimates that tourism generates an indirect contribution that is equivalent to 100% of direct tourism expenditure.

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## Impact of Metformin on Immunity and Male Fertility in Rabbits with Alloxan- Induced Diabetes

Naglaa, Z.H. Eleiwa \* ; Hesham, A.M.; Hosny Abdel Fadil and Abdel Motal, S.M.

Dept. of Pharmacology, Faculty of Veterinary Medicine, Zagazig University, Egypt.  
\* [eleiwa02@yahoo.com](mailto:eleiwa02@yahoo.com)

**Abstract:** A study was designed to explore the possible side effects of metformin on immunity and fertility of male rabbits with alloxan- induced diabetes. Sixteen adult male rabbits were used in this study, they were classified into four equal groups as follows: the first group received neither alloxan nor metformin and remained as control group. Rabbits in the 2<sup>nd</sup> group were orally treated with metformin at a dose of 120 mg/kg b.wt once a day for 3 months .Rabbits in the 3<sup>rd</sup> group were administered alloxan, I/V, at a single dose of 100 mg/kg b.wt.Rabbits in the 4<sup>th</sup> group were administered alloxan ( 100 mg/kg b.wt, single I/Vdose ) then treated orally with metformin (120 mg/kg b.wt.) once daily for 3 months. Rabbits in all groups were subcutaneously injected with 2 ml polyvalent rabbit pasteurellosis vaccine after two months from the beginning of experiment for studying the immunological profile of the drug. Treatment of diabetic and non-diabetic rabbits with metformin evoked a significant decrease ( $P < 0.05$ ) in nitric oxide production on the 1<sup>st</sup> and the 2<sup>nd</sup> day post vaccination .In response to treatment with metformin, rabbits demonstrated a significant decrease ( $P < 0.05$ ) in serum lysozyme activity on the 1<sup>st</sup>, 2<sup>nd</sup> , 3<sup>rd</sup> day and in the 1<sup>st</sup> week post vaccination while diabetic rabbits treated with metformin showed a significant decrease ( $P < 0.05$ ) in serum lysozyme activity on the 3<sup>rd</sup> day and on the 1<sup>st</sup> , 3<sup>rd</sup> and 4<sup>th</sup> week post vaccination . In addition, treatment with metformin of diabetic and non-diabetic rabbits resulted in a significant decrease( $P < 0.05$ ) in testicular weight , sperm cell count, sperm motility and serum testosterone with a significant increase in sperm abnormalities and dead sperm %. Summing up our observations, the present study calls into question the justification for the use of metformin in a frame of a therapeutic strategy for diabetes due to its resulting negative impact on immunity and male fertility.

[Naglaa, Z.H. Eleiwa; Hesham, A.M.; Hosny Abdel Fadil and Abdel Motal, S.M. Impact of Metformin on Immunity and Male Fertility in Rabbits with Alloxan- Induced Diabetes. Journal of American Science 2010;6(11):417-426]. (ISSN: 1545-1003).

**Key words:** Metformin – alloxan – diabetes - rabbits

### 1. Introduction:

Diabetes mellitus is a syndrome characterized by disturbed metabolism and inappropriately high blood sugar (hyperglycaemia) resulting from either low levels of insulin or abnormal resistance to insulin's effects coupled with inadequate levels of insulin secretion to compensate (Tierney et al., 2002).

Treatment of type II diabetes has greatly improved due to the availability of new classes of oral antidiabetic drugs (OADs) and new insulin analogs (Rosak, 2002).

Metformin is one of antidiabetic drugs which belongs to the biguanide class of oral antihyperglycemic agents. It was first synthesized in 1929 and was shown to be a potent hypoglycemic agent, it was rediscovered in 1957 and widely used in Europe to treat type II obese patients. Metformin resurfaced in the 1980s and it was shown to increase insulin sensitivity; this encouraged its introduction to clinical practice in the United States for the first time (Bell and Hadden., 1997)

Metformin acts in the presence of insulin to increase glucose utilization and reduce glucose production, thereby countering insulin resistance. The effects of metformin include increased glucose uptake, oxidation and muscle glycogenesis, increased glucose metabolism to lactate by the intestine, reduced hepatic gluconeogenesis and possibly a reduced rate of intestinal glucose absorption (Clifford, 1993)..

On the other hand, many investigations reported some side effects of metformin therapy as lactic acidosis (Brassøe et al.,2005) ,vitamin B12-malabsorption that was recorded in about 1/3 of the diabetic cases (Ting et al.,2006) and high incidence of gastrointestinal side effects (Hoffman et al.,2003) as well as higher homocysteine levels(Wulffele et al., 2003).

It is extremely true and fitting to mention that the clinical effectiveness of a given drug would be in jeopardy if its adverse effects outweigh its efficacy. Therefore, the present work was designed to

explore the possible side effects of metformin on immunity and male fertility in rabbits with alloxan-induced diabetes.

## 2. Materials and methods

Materials:

1-Drug:

Metformin hydrochloride (Glucophage®), a product of Minapharm company, Egypt. Metformin is present in the form of tablet containing 1500mg of active drug.

Chemical name:

1,1-Dimethylbiguanide hydrochloride.

2-Alloxan:

Alloxan was purchased from El-Gomhoria Company, Egypt. It was given for induction of diabetes

3-Vaccine:

Formalized polyvalent rabbit pasteurellosis vaccine (VET. SER., VACC. RES. INST.-CAIRO-EGYPT) was used at a dose of 2ml s/c after two months from the beginning of experiment for immunological investigation

4- Experimental animals:

Sixteen adult male rabbits, weighing 2kg each, were obtained from Salsabil company, Fakous, Sharkeya, Egypt and allowed to acclimatize for a week at the animal house at the faculty of Veterinary Medicine . Animals were randomly assigned into four equal groups, four rabbits each and kept in a cage of four separate divisions , maintained at a 12-hour light dark cycle and a constant temperature of  $23 \pm 2^{\circ}\text{C}$  , received regular rabbit chow (Standard laboratory chow) and water was provided ad-libitum.

Methods:

Induction of diabetes:

Diabetes was induced in two groups of animals (The third and the fourth ones) by single intra-venous injection of alloxan with a concentration of 10% solution in 0.9% NaCl, at the dose of 100 mg/kg. b.wt. Diabetic status was confirmed when the fasting blood sugar value was above 200 mg/dl (Nammi et al.,2003).

Experimental design:

Sixteen adult male rabbits were used in this study, they were kept under hygienic conditions and fed on basic ration free from any medications or chemical additives and water was provided ad-lib.

Rrabbits were classified into four equal groups, as follows:

The first group (Control group ,Non diabetic non treated,):

Rabbits in the first group received neither alloxan nor metformin and remained as control group.

The second group (Non diabetic treated with metformin):

Rabbits in the second group were treated with metformin at a dose of 120 mg/kg b.wt per os through stomach tube after morning meal once a day for 3 months (Marquie, 1983).

The third group (Diabetic non treated, diabetic control):

Rabbits in the third group were injected I/V with alloxan at a single dose of 100 mg/kg b.wt.

The fourth group (Diabetic treated with metformin):

Rabbits in the fourth group were administered alloxan at a single dose of 100 mg/kg b.wt.(I/V), then treated with metformin at a dose of 120 mg/kg b.wt. Orally. The drug was given to rabbits early in the morning after meal once daily for 3 months

Rabbits in all groups were subcutaneously injected with 2 ml polyvalent rabbit pasteurellosis vaccine after two months from the beginning of experiment for immunological studies.

Laboratory assay:

A- Collection of samples:

5ml of venous blood samples were collected from the ear vein of all rabbits after 24, 48, 72hrs and 1<sup>st</sup> , 2<sup>nd</sup> , 3<sup>rd</sup> and 4<sup>th</sup> weeks of vaccine administration. The collected blood samples were allowed to clot. Clear serum samples were obtained by centrifugation of blood samples at 3000 rpm for 20 min. One part of serum samples was used immediately for blood glucose level estimation using spectrophotometer by glucose oxidase method (Barham and Trinder, 1972). The other part was stored at -20°C for immunological evaluation and measurement of serum nitric oxide production, lysozyme activity and testosterone level.

At the end of experimental period (3 months), rabbits were slaughtered, testes were dissected and weighed, tails of epididymis were collected for semen analysis.

B- Immunological evaluation:

1-Measurement of serum nitric oxide production:

Nitric oxide level in the serum was measured by spectrophotometer according to Rajaraman et al.,(1998) .

2-Measurement of lysozyme activity by agarose gel cell lysis assay:

The lysozyme activity in the serum was measured according to Schultz (1987).

The areas under the curve (AUCs) , representing lysozyme activity and nitric oxide release through the 30<sup>th</sup> days post vaccination , were evaluated as shown previously (Abdel Motal et al., 1987 ) . The areas for the control were expressed as 100%, the others relative to it.

#### C- Male fertility evaluation:

##### I-Testicular weight:

Was done by using digital electrical balance.

##### II- Semen analysis:

Was done according to method described by Williams et al., (1990) for examination of both sperm cell concentration, sperm motility, abnormalities and live/dead ratio.

##### III- Measurement of serum testosterone level:

Serum testosterone levels were estimated according to Burtis and Ashwood, (1994) using active testosterone enzyme immunoassay (EIA) DSL-10-4000 kit obtained from Diagnostic Systems Laboratories Inc.

#### D- Statistical analysis:

The data were analyzed using SPSS program. Results were reported as mean  $\pm$  S.E .The total variations were analyzed by performing the statistical design T-test. Probability levels of less than 0.05 were considered significant (SPSS, WIN.2003).

### 3. Results

#### I. Effect of metformin on blood glucose levels

As shown in table (1) ; treatment with metformin revealed a non significant decrease in blood glucose level on the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> day post vaccination and a significant decrease ( $P<0.05$ ) in its level in the 1<sup>st</sup> , 2<sup>nd</sup> ,3<sup>rd</sup> and 4<sup>th</sup> week post vaccination compared with the control group.

All diabetic rabbits revealed a highly significant increase ( $P<0.01$ ) in blood glucose levels 24 hour post vaccination and then after compared with the control group while medication of diabetic rabbits with metformin evoked a highly significant decrease( $P<0.01$ ) in blood glucose level in all samples collected post vaccination compared with diabetic rabbits.

**Table (1): Effect of metformin (120 mg/kg b.wt. per os) on serum glucose level (mg/dl) in non diabetic and alloxan induced diabetic rabbits. (Mean  $\pm$  S.E) (n = 4)**

Groups	Serum glucose level (mg/dl)						
	Time post vaccination						
	1 <sup>st</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day	1 <sup>st</sup> week	2 <sup>nd</sup> week	3 <sup>rd</sup> week	4 <sup>th</sup> week
control	108.33 $\pm$ 6.56	101.0 $\pm$ 0.57	102.66 $\pm$ 4.33	98.0 $\pm$ 3.00	102.00 $\pm$ 4.33	104.0 $\pm$ 3.60	101.66 $\pm$ 6.38
Treated with metformin	90.33 $\pm$ 6.56	88.88 $\pm$ 6.02	87.0 $\pm$ 4.35	84.66 $\pm$ 3.17*	87.0 $\pm$ 4.04*	85.00 $\pm$ 3.13*	78.66 $\pm$ 2.96*
Diabetic control	443.33 $\pm$ 20.88**	426.66 $\pm$ 8.871**	414.66 $\pm$ 18.27**	415.0 $\pm$ 13.01**	411.0 $\pm$ 17.15**	417.33 $\pm$ 12.38**	425.00 $\pm$ 11.71**
Diabetic treated with metformin	175.33 $\pm$ 20.95++	162.66 $\pm$ 15.45++	188.0 $\pm$ 5.13++	175.0 $\pm$ 2.51++	182.33 $\pm$ 2.96++	180.33 $\pm$ 20.69++	166.0 $\pm$ 21.45++

\*  $P \leq 0.05$       \*\*  $P \leq 0.01$       \* Compared with non diabetic- non treated

+  $P \leq 0.05$       ++  $P \leq 0.01$       + Compared with diabetic- non treated

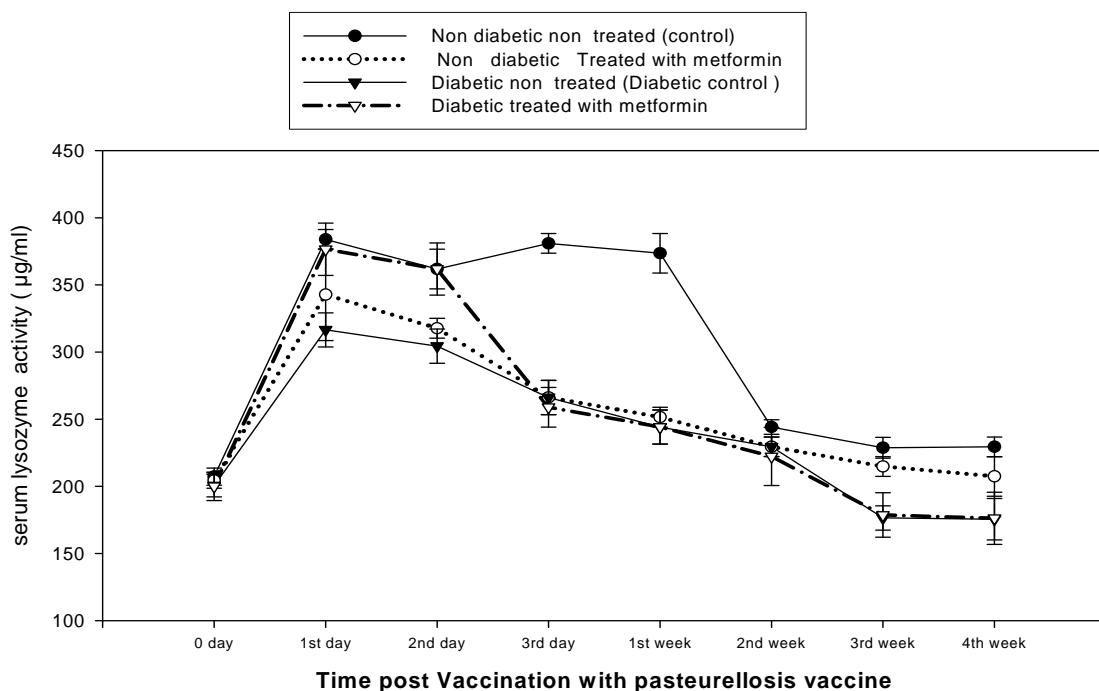
#### II. Effect of metformin on the immunological response:

##### A. Effect on nitric oxide production:

As shown in Fig. (1) and table (2) ; treatment with metformin exhibited a significant decrease ( $P<0.05$ ) in nitric oxide production on the 1<sup>st</sup> and 2<sup>nd</sup> day post vaccination and non significant decrease ( $P<0.05$ ) in nitric oxide production on the 3<sup>rd</sup> day, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> weeks post vaccination compared with the control group.

Diabetic rabbits showed a highly significant decrease ( $P<0.01$ ) in nitric oxide production on the 1<sup>st</sup> and 2<sup>nd</sup> day post vaccination and non significant decrease in nitric oxide production on the 3<sup>rd</sup> day, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> weeks post vaccination compared with the control group.

Diabetic rabbits showed a highly significant decrease ( $P<0.01$ ) in nitric oxide production on the 1<sup>st</sup> and 2<sup>nd</sup> day post vaccination and non significant decrease in nitric oxide production on the 3<sup>rd</sup> day, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> weeks post vaccination compared with the control group.



**Fig. (1): Effect of metformin (120 mg/kg b.wt. per os) on nitric oxide production (ng/ml) in non diabetic and alloxan induced diabetic rabbits.**

Treatment of diabetic rabbits with metformin demonstrated a significant decrease in nitric oxide production on the 1<sup>st</sup> and the 2<sup>nd</sup> day post vaccination and non significant decrease in nitric oxide production on the 3<sup>rd</sup> day, 1<sup>st</sup> week, 2<sup>nd</sup> week, 3<sup>rd</sup> week and 4<sup>th</sup> week post vaccination compared with the control group.

Rabbits treated with metformin displayed 27.27% decrease in nitric oxide release allover the 30<sup>th</sup> days post vaccination. Diabetic rabbits demonstrated 45.45% decline, diabetic rabbits treated with metformin showed 27.27% decrement.

#### B. Effect of metformin on serum lysozyme activity.

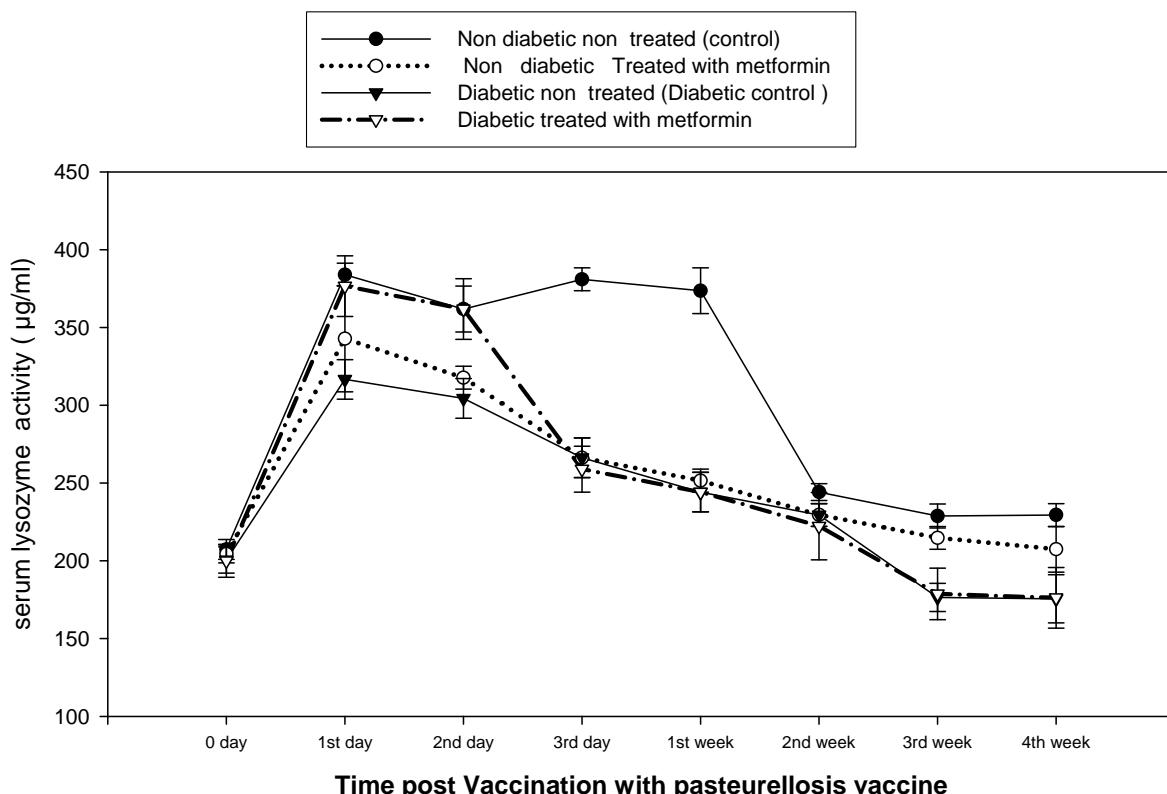
As shown in Fig. (2) and table (2); treatment with metformin demonstrated a significant decrease ( $P<0.05$ ) in serum lysozyme activity on the 1<sup>st</sup>, 2<sup>nd</sup> and a highly significant decrease ( $p<0.01$ ) on the 3<sup>rd</sup> day and in the 1<sup>st</sup> week post vaccination and non significant decrease in serum lysozyme activity in the 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> week post vaccination compared with the control group.

**Table (2): Effect of metformin (120 mg/kg b.wt. per os) given on nitric oxide production and lysozyme activity (represented by AUC and expressed as %) in rabbits with alloxan - induced diabetes.**

GROUPS	NITRIC OXIDE (%)	Lysozyme (%)
<b>Control</b>	100	100
<b>Treated with metformin</b>	72.73	80
<b>Diabetic control</b>	54.55	66.66
<b>Diabetic treated with metformin</b>	72.73	80

Diabetic rabbits revealed a significant decrease ( $P<0.05$ ) in serum lysozyme activity on the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> week and a highly significant decrease ( $p<0.01$ ) on the 3<sup>rd</sup> day and in the 1<sup>st</sup> week post vaccination compared with the control group.

Diabetic rabbits treated with metformin showed non significant decrease ( $P<0.05$ ) in serum lysozyme activity on the 1<sup>st</sup> day, 2<sup>nd</sup> day and 2<sup>nd</sup> week post vaccination and a significant decrease in serum lysozyme activity on the 3<sup>rd</sup> day and on the 1<sup>st</sup>, 3<sup>rd</sup> and 4<sup>th</sup> week post vaccination compared with the control group.



**Fig. (2): Effect of metformin (120 mg/kg b.wt. per os) on serum lysozyme activity ( $\mu\text{g}/\text{ml}$ ) in non diabetic and alloxan induced diabetic rabbits.**

During the 30<sup>th</sup> days post challenge with polyvalent rabbit pasteurellosis vaccine, rabbits treated with metformin displayed 20% decrease in lysozyme activity. Diabetic rabbits showed 33.34% decline, diabetic rabbits treated with metformin displayed 20% decrease.

### III. Effect of metformin on male rabbit fertility:

#### 1) Effect on testicular weight

In response to treatment with metformin, rabbits demonstrated a significant decrease ( $P<0.05$ ) in testicular weight compared with the control group, while, diabetic rabbits exhibited non significant decrease in testicular weight compared with the control group (table 3).

Treatment of diabetic rabbits with metformin evoked a highly significant decrease ( $P<0.01$ ) in testicular weight compared with the diabetic rabbits.

#### 2) Semen analysis

##### a) Effect on sperm cell count

Treatment of rabbits with metformin showed a highly significant decrease ( $P<0.01$ ) in sperm cell count compared with the control group.

Diabetic rabbits demonstrated a significant decrease in sperm cell count compared with the control group.

Medication of diabetic rabbits with metformin elicited a highly significant decrease ( $P<0.01$ ) in sperm cell count compared with the diabetic rabbits.

##### b) Effect on sperm motility

Treatment of rabbits with metformin, displayed a significant decrease in sperm motility compared with the control group.

Diabetic rabbits displayed non significant decrease in sperm motility compared with the control group.

Treatment of diabetic rabbits with metformin induced a highly significant decrease ( $P<0.01$ ) in sperm motility compared with the diabetic rabbits.

**Table (3): Effect of metformin (120 mg/kg b.wt. per os) on male fertility in non diabetic and alloxan induced diabetic rabbits. (Mean  $\pm$  SE) (n = 4)**

Parameters Groups	Testicular weight (gm)	Sperm cell count (sp.c.c $\times 10^6$ /ml)	Sperm motility (%)	Dead (%)	Sperm abnormalities (%)	Serum testosterone Level (ng/ml)
control	20.33 $\pm$ 1.45	160.83 $\pm$ 5.83	91.66 $\pm$ 1.66	7.00 $\pm$ 0.28	9.56 $\pm$ 0.34	0.58 $\pm$ 0.30
Treated with metformin	15.33 $\pm$ 0.33*	110.00 $\pm$ 1.44**	80.00 $\pm$ 2.88*	13.26 $\pm$ 0.43**	13.50 $\pm$ 0.76**	0.11 $\pm$ 0.01*
Diabetic control	17.66 $\pm$ 0.33	135.83 $\pm$ 5.83*	88.33 $\pm$ 1.66	12.33 $\pm$ 0.88**	15.76 $\pm$ 0.39**	0.13 $\pm$ 0.03*
Diabetic treated with metformin	9.33 $\pm$ 0.88++	17.33 $\pm$ 1.45++	55.33 $\pm$ 2.88++	14.40 $\pm$ 0.30	24.16 $\pm$ 0.72++	0.08 $\pm$ 0.00+

\* P  $\leq$  0.05      \*\* P  $\leq$  0.01  
+ P  $\leq$  0.05      ++ P  $\leq$  0.01

\* Compared with non diabetic- non treated

+ Compared with diabetic- non treated

#### c) Effect on dead sperm%

Rabbits treated with metformin exhibited a highly significant increase (P<0.01) in dead sperm % compared with the control group.

Diabetic rabbits demonstrated a highly significant increase (P<0.01) in dead % compared with the control group.

Medication of diabetic rabbits with metformin showed non significant increase in dead % compared with the diabetic rabbits.

#### d) Effect on sperm abnormalities

In response to treatment of normal rabbits with metformin, rabbits demonstrated a highly significant increase (P<0.01) in sperm abnormalities (coiled tail sperm and detached tail sperm) compared with the control group.

Diabetic rabbits displayed a highly significant increase (P<0.01) in sperm abnormalities (detached tail sperm and stunted growth sperm) compared with the control group.

Treatment of diabetic rabbits with metformin induced a highly significant increase (P<0.01) in sperm abnormalities (coiled tail sperm and detached head and tail sperm) compared with the diabetic rabbits.

#### 3) Effect on serum testosterone level

Rabbits treated with metformin displayed a significant decrease (P<0.05) in serum testosterone compared with the control group.

Diabetic rabbits demonstrated a significant decrease (P<0.05) in serum testosterone compared with the control group.

Medication of diabetic rabbits with metformin revealed a significant decrease (P<0.05) in serum testosterone compared with the diabetic rabbits.

#### 4. Discussion:

Metformin (dimethylbiguanide) is an antihyperglycaemic drug used to treat non-insulin dependent diabetes mellitus. It acts in the presence of insulin to increase glucose utilization and reduce glucose production, thereby countering insulin resistance. The effects of metformin include increased glucose uptake, oxidation and muscle glycogenesis, increased glucose metabolism to lactate by the intestine, reduced hepatic gluconeogenesis and possibly a reduced rate of intestinal glucose absorption. Metformin appears to facilitate steps in the post receptor pathways of insulin action, and may exert effects that are independent of insulin. In muscles, metformin increases translocation into the plasma membrane of certain isoforms of the glucose transporter (Clifford, 1993).

Nitric oxide (NO), has a wide variety of effects on cells. It has been found to affect different kinds of cells, with particularly striking effects in the control of blood pressure and the immune system (Rang et al., 1999).

In all sites, where NO is active, it is produced by the reaction of arginine with molecular oxygen to give citrulline as well as NO. The reaction is catalyzed by the enzyme nitric oxide synthase (NOS). NOS exist in several slightly different forms, depending on the kind of cell in which is found (Campbell, 1995).

Acknowledging the differences, it could be reasoned that a similar effect of NOS in the immune system might pertain.

In the current study, healthy rabbits treated with metformin demonstrated a significant decrease in nitric oxide production 24 and 48 hour post vaccination compared with the control.

Regrettably enough, our data are by no means sufficient to provide us with a ready clarification for the previous effect.

Nevertheless, going through literature could provide us with all right clue.

Convincing evidence is accumulating for the ability of vitamin B12 to modulate cellular immunity especially lymphocyte counts, CD8 cells and the natural killer (NK) cells. (Tamura et al., 1999).

Similarly, Erkurt et al., (2008) reported that vitamin B12 had important immunomodulatory effects on cellular immunity, and abnormalities in the immune system in pernicious anemia are restored by vitamin B(12) replacement therapy.

In vitamin B12-deficient patients, numbers of CD4 and CD8 lymphocytes decreased, CD4/CD8 ratio increased, and NK cell activity was depressed. After cyanocobalamin treatment, absolute numbers and percentage of lymphocyte subgroups were elevated. Increased CD4/CD8 ratio and depressed natural killer (NK) cell activity were restored and levels of C3, C4, and immunoglobulins were elevated (Erkurt et al., 2008).

The use of a multinutrient containing optimum amounts of essential trace elements and vitamins (as beta-carotene, Vitamins B6, B12, C, D and E, and folic acid) result in enhanced immune responses and reduction in the occurrence of common infections (Chandra , 2004).

Vitamins (A, B6, B12, C, D, E and folic acid) and the trace elements (iron, zinc, copper and selenium) work in synergy to support the protective activities of the immune cells. All these micronutrients are essential for antibody production. Overall, inadequate intake and status of these vitamins and trace elements may lead to suppressed immunity, which predisposes to infections and aggravates malnutrition. Thus, supplementation with these selected micronutrients can support the body's natural defense system by enhancing all three levels of immunity (physical barriers (skin/mucosa), cellular immunity and antibody production) (Maggini et al., 2007).

Kaplan and Basford (1976) noted that morphological and quantitative neutrophil abnormalities are common in the megaloblastic anemias of vitamin B12 and folic acid deficiency. The authors recorded that vitamin B12 has specific role in the production of intermediates necessary for normal cell function.

Patients receiving metformin have diminished vitamin B12(Bauman et al., 2000 and Ting et al., 2006) .

Sahin et al.,(2007) reported that patients with type 2 diabetes, metformin reduced levels of folate and vitamin B12 and increases homocysteine (Hcy). This deficiency was occurred through interfering with its absorption in the distal ileum (Quillen et al., 1999 and Rufenacht et al., 2008) .

Given the previous tapestry, it might be tenable to point out that vitamin B12 deficiency induced by metformin could account for the significant decrease in NO production.

In the current study, alloxan- induced diabetes elicited a significant decrease in serum lysozyme activity 24 and 48 hour and in the 3<sup>rd</sup> and 4<sup>th</sup> week post vaccination.

Lysozyme is bactericidal for almost all bacteria, hydrolyzing its cell wall resulting in its lysis. It activates the complement system and phagocytes (Jolles and Jolles, 1984).

The lysozyme protein (N-acetylmuramidase) is a major component of the specific granules of the polymorphonuclear leucocytes (PMN)and is found in high concentrations in the mucosal secretions in the eyes, oropharynx, respiratory tract and vagina. Several lines of evidence suggest that its localization at these sites is related to its role in the defense system. It is actively secreted by (PMN) cells during inflammatory response into the external environment as it has antimicrobial activity by degrading the glycosidic linkage of bacterial peptidoglycan (Richard and Theodore, 1991).

Hyperglycemic environment may enhance the virulence of various microorganisms. Candida albicans shows competitive binding and inhibition of complement mediated phagocytosis in a hyperglycemic environment (Hostetter, 1990).

Glucosuria enhances Escherichia coli growth and may be a reason for the increased incidence of urinary tract infections in diabetics (Geerlings et al., 1999).

Oxidative stress which is the metabolic response to hyperglycemia in patients with DM, may affect neutrophil lifespan, and phagocytic cell function resulting in a decrease in their ability to prevent or eliminate infection (Geerlings and Hoepelman, 1999 and Watson, 2002).

In general, patients with diabetes are at high risk of infections, which are more serious and prolonged. It is notable that the circulating levels of proinflammatory cytokines are elevated in diabetic patients and it has been suggested that the impaired functions of neutrophils contribute to the increased susceptibility to infections observed in these patients (Pickup et al., 2000).

The neutrophils of diabetic patients display increased necrosis and enhanced production of reactive oxygen species (Shurtz-Swirski et al., 2001). In the present experiment, healthy rabbits treated with metformin showed a significant decrease in serum lysozyme activity 24 and 48 hour post vaccination.

As pointed out previously with nitric oxide finding, the reduction in lysozyme activity, another

representative of cellular immunity could be further rationalized by vitamin B12 deficiency.

In the present study, alloxan induced diabetes in rabbits elicited a significant decline in sperm cell count and serum testosterone, and increase in dead % and sperm abnormalities.

In like manner, Naziroğlu, (2003) reported that streptozotocin (STZ) induced diabetes in rats was associated with impairment of testicular function leading to reduced fertility. Its etiology may involve oxidative damage by reactive oxygen species, and protection against this damage can be offered by antioxidant supplementation.

Similarly, Scarano et al. (2006) recorded that diabetes resulted in decreased body and reproductive organ weights, as well as diminished sperm counts in the testis and epididymis, associated with diminution of plasmatic testosterone levels, after natural mating, there was a decrease in the fertility in the diabetic adult male rats (25.5%) compared with control animals (81.5%).

Likewise, Shrilatha and Muralidhara, (2007) observed oxidative impairments in testis of STZ-induced diabetes in adult rats developed over time might at least in part contribute towards the development of testicular dysfunction through testicular degeneration leading to reduced fertility. Interestingly enough, diabetic rabbits medicated with metformin revealed a significant decrease in testicular weight, sperm cell count, sperm motility and serum testosterone, as well as an increase in dead % and sperm abnormalities.

Given the evidence, one is intrigued to surmise that the reproductive dysfunction seen in the shade of metformin treatment may be imputed to vitamin B12 deficiency.

The previous contention is commendable by the notion that vitamin B12 deficiency is induced by long-term use of metformin (Lin et al., 2007)

In this frame of reference, it is interesting to note that Vitamin B<sub>12</sub> deficiencies in men can lead to reduced sperm counts and lowered sperm motility. For this reason, B<sub>12</sub> supplements have been tried for improving fertility in men with abnormal sperm production (Kumamoto et al., 1988).

The concept is further boosted by the fact that mecabalamin (Me-B 12) enhances testicular function, resulting in an increased output of mature sperm. By oral administration of Me B 12 (1.0 mg/kg/day) to the oligozoospermic mice for 10 weeks, the sperm count, sperm motility, motile sperm count, diameter of seminiferous tubules and the percentage of good motile sperm were increased as compared with those of the control (Oshio et al., 1989).

On similar grounds, Sinclair, (2000) noted that number of nutritional therapies have been shown to improve sperm counts and sperm motility, including carnitine, arginine, zinc, selenium, and vitamin B12.

## 5. Conclusion:

Summing up our observations, the present study calls into question the justification for the use of metformin in a frame of a therapeutic strategy for diabetes due to its resulting negative impact on immunity and male fertility.

## Corresponding author

Naglaa ,Z.H. Elewa  
Dept. of Pharmacology, Faculty of Veterinary Medicine, Zagazig University, Egypt.  
[elewa02@yahoo.com](mailto:elewa02@yahoo.com)

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9/25/2010

# Amniotic Membrane Extract for Acute Ocular Chemical Burns

Hosam Sheha, Hisham Hashem, Lingyi Liang, Mohamed Ramzy, Ahmed ZaKi,

**Abstract:** Background: Ocular chemical burns induce devastating and permanent damage to the ocular surface. Rapid intervention is required for maximal visual rehabilitation. Amniotic membrane transplantation (AMT) may save the ocular surface, however it introduces a potentially unnecessary surgical trauma in such compromised eyes. Amniotic membrane extracts (AME) could be a practical substitute of AMT in acute chemical burn. Aim: To evaluate the efficacy of topical AME in the management of acute ocular chemical burn. Methods: Non-comparative interventional case series. Six eyes of 4 consecutive patients with mild to moderate acute chemical burn, exhibiting persistent epithelial defect, inflammation and haze despite extensive conventional therapy were recruited. Topical AME was prepared and added to the conventional treatment within 2 days of the injury. Pain relief, inflammation, haze, and corneal epithelial healing were monitored. Results: Pain was significantly relieved, and inflammation was markedly reduced in all cases. The corneal epithelial defects rapidly healed while visual acuity improved within 11 (range 4-23) days. During an average follow-up period of 6 months (range, 3-8 months), all eyes retained stable surface with improved corneal clarity without neovascularization or symblepharon. Conclusions: Topical application of AME could be an effective adjunct in the treatment of mild to moderate cases of acute chemical burns. It allows non-traumatic and economic early intervention to promote epithelialization, reduce pain, haze and inflammation in acute phase, and prevent cicatricial complications in chronic phase. This result justifies additional large series controlled studies in the future.

[Hosam Sheha, Hisham Hashem, Lingyi Liang, Mohamed Ramzy, Ahmed ZaKi. Amniotic Membrane Extract for Acute Ocular Chemical Burns. Journal of American Science 2010;6(11):427-433]. (ISSN: 1545-1003).

**Key words:** Acute chemical burn, amniotic membrane extract, corneal epithelial defect

## 1. Introduction:

Ocular chemical burns injury is a serious ocular emergency in which rapid, devastating, and permanent damage can occur. The severity of the injury correlates directly to exposure duration and the causative agent. Treatment of such injuries requires medical and surgical intervention, both acutely and in the long term. Regardless of the underlying chemical involved, the common goals of management include removing the offending agent, controlling inflammation and promoting ocular surface healing with maximal visual rehabilitation. [1]

Various medical therapies have been used to achieve these objectives, including topical and systemic ascorbate, citrate, tetracycline, progesterone and steroids.[2-7] Previous studies revealed that early intervention with amniotic membrane transplantation (AMT) in mild and moderate chemical burns results in a marked reduction of symptoms, rapid restoration of the ocular surface, and improved visual acuities while preventing cicatricial complications in the chronic stage. [8-17]. However, surgically performed AMT renders a relatively high cost and potentially unnecessary surgical trauma in such compromised eyes. Furthermore, the membrane patch usually dissolves within several days so that multiple sessions of AMT may be required. [8; 16; 17] Recent studies have shown that topical amniotic membrane extracts (AME) has a comparable effect to AMT in promoting epithelialization, decreasing inflammation,

and suppressing corneal neovascularization.[18-21] Therefore, we hypothesized that AME could be a practical substitute for AMT in acute chemical burns. Herein we reported our experience in preparing and using AME as a rapid, economic, non-traumatic alternative modality in the treatment of acute chemical burns.

## 2. Methods

Patients:

This study was conducted, according to the tenets of the Declaration of Helsinki, to evaluate the effect of AME as an adjunct in the treatment of mild to moderate acute ocular chemical burns. This small series included 6 eyes of 4 patients, all males, with a mean age of  $34.5 \pm 15.8$  (range, 19-56 years). Their demographic data and clinical characteristics are summarized in Table 1. After obtaining a written informed consent, all patients received topical AME in combination with conventional treatment within 2 days following the onset of chemical burn. The injury was bilateral in 2 patients (Cases 1 and 2) and unilateral in the other two (Cases 3 and 4). The causative agents were alkali in 3 patients and acidic substance in one (Case 4).

Upon presentation, all patients complained of significant ocular pain, light sensitivity and blurred vision. Corneal epithelial defects were partial [Cases 1, 2 and 4 (Fig 1A, 1B)] or total [Cases 2 and 3 (Fig 2A, 2B)] and with various degrees of limbal and

conjunctival involvement. In addition, 4 eyes had limbal ischemia ranging from 2 to 6 clock hours. The severity was classified as Grade, I (2 eye), Grade II (2 eyes) and Grade III (2 eyes) based on the criteria defined by Roper-Hall.[22] Microorganism culture of all ulcer smears was negative and intraocular pressure (IOP) was normal in all cases.

All patients were initially treated with conventional medical therapies including saline/water irrigation to normalize the ocular surface pH, removal of remaining particulate materials, topical antibiotics, lubricants, 10% ascorbic acid and 6% citrate, antibiotic-steroids and cycloplegics, oral vitamin C or a combination thereof for the first 2 days after injury. When improvement was not apparent, all patients were detailed with information about the clinical course of chemical burns of the eye, alternative treatments, and advantages and disadvantages of AME and AMT. After a written consent was obtained, AME eye drops were added to the regimen.

Preparation of AM and extraction was carried out under sterile conditions. Each placenta was rinsed with sterile saline solution containing 1% penicillin-streptomycin-neomycin (PSN) antibiotic mixture (Invitrogen/Gibco, Grand Island, NY). After peeling off from the attached chorion, AM was submerged in liquid nitrogen. Under cold conditions, the membrane was cut into small pieces, manually ground into fine powder, and homogenized with normal saline. The homogenate was then centrifuged twice at 15,000 rpm for 30 minutes at 4°C. The supernatant was collected and the protein concentration was measured by DC protein assay (Bio-Rad, Hercules, CA). Non preserved 100 µg/ml eye drops were prepared and kept frozen at -20°C.

AME was instilled hourly for the first week, once every two hours until complete re-epithelialization were achieved, then 4 times daily for 2 weeks and was tapered off thereafter.

Subjective symptoms were scaled at each visit as 0 (No discomfort, No haze), 1 (Mild

discomfort and/or Mild haze), 2 (Moderate discomfort and/or Moderate haze) and 3 (Severe discomfort and/or Dense haze). Ocular surface inflammation was graded as 0 (absent), 1 (mild), 2 (moderate), 3 (severe). Fluorescein staining was conducted to evaluate epithelialization.

### 3. Results:

The average follow-up period was 6±2.3 months (range, 3-8 months). The results were summarized in Table 1. All patients reported a significant relief of pain and light sensitivity within the first week after AME treatment with overall symptomatic scores reduced from 2-3 to 0-1. Inflammation scores significantly decreased from 2-3 at first visit to 0-1 at the second week.

Rapid and progressive epithelialization was also observed in all eyes depending on the severity; for Grade I injury (Case 4, Fig 1) with less limbal involvement the epithelialization was completed in a centripetal manner. However, for Grade III injuries (Cases 3, Fig 2) with extensive limbal involvement the epithelialization started circumferentially to close the limbal defect before moving centripetally to close the corneal defect. After AME treatment, complete epithelialization was obtained in all eyes within a mean period of 11 days (range, 4-23 days, Table 1).

Accompanied with re-epithelialization, corneas, which were initially presented with edema and haze became clear (Fig 1E), or left with mild haze without edema (Fig 2E).

Impression cytology analysis was then performed after the ocular surface had been stable for more than three months, where no limbal stem cell deficiency was noted. There were no cicatricial complications such as symblepharon at the final visit (Fig 1F, 2F). Best corrected visual acuities (BCVA) improved to 20/20 in 5 eyes (83%) while Case 3 had BCVA of 20/60 due to residual corneal haze (Table 1). The ocular surface remained stable in all eyes during the follow up period.

**Table 1. Results of AME in treating acute chemical burns.**

Case No.	Eye No.	Agent	Grade	Limbal Ischemia (CH)	Symptoms Score		BCVA		Inflammation Score		Epithelialization (Days)
					Before	After	Before	After	Before	After	
1	1	Alkali	I	0	2	0	20/40	20/20	2	0	6
	2		II	2	3	0	20/50	20/20	3	0	8
2	3	Alkali	III	6	3	1	20/60	20/20	3	0	21
	4		II	4	2	0	20/400	20/20	3	1	12
3	5	Alkali	III	6	3	1	20/400	20/60	3	1	15
4	6	Acid	I	0	2	0	20/200	20/20	2	0	4

Note: CH: Clock Hours, BCVA: best corrected visual acuity

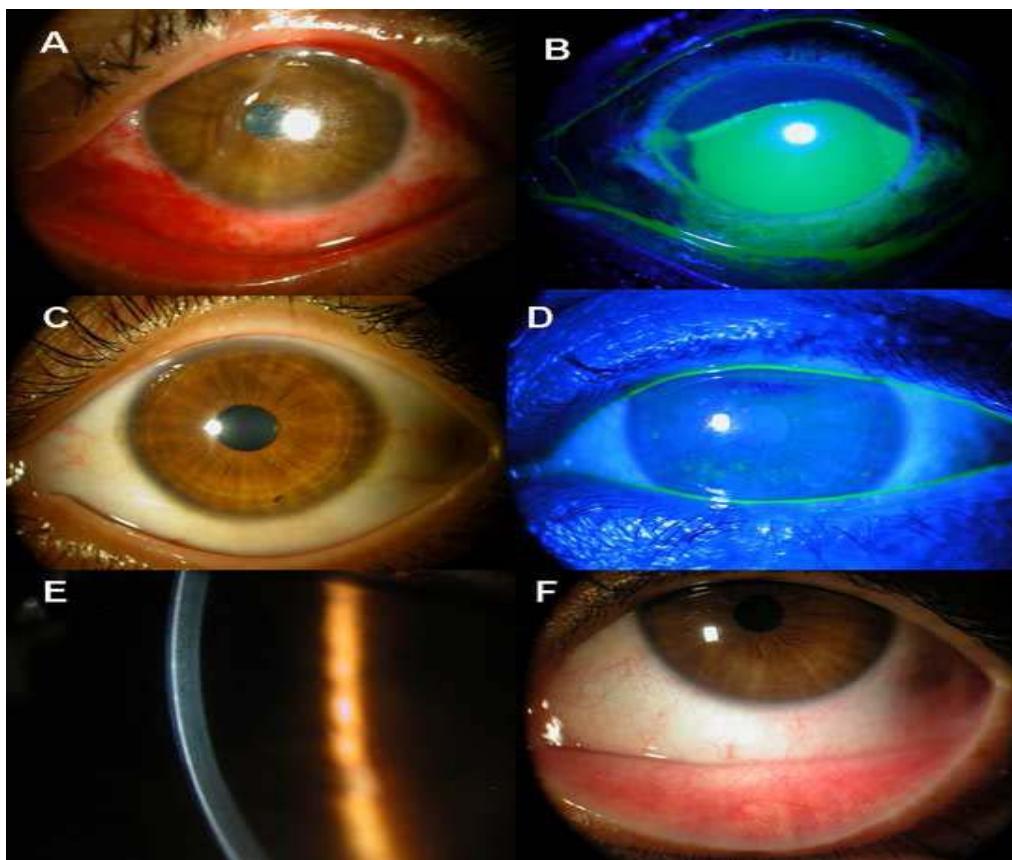


Fig. 1

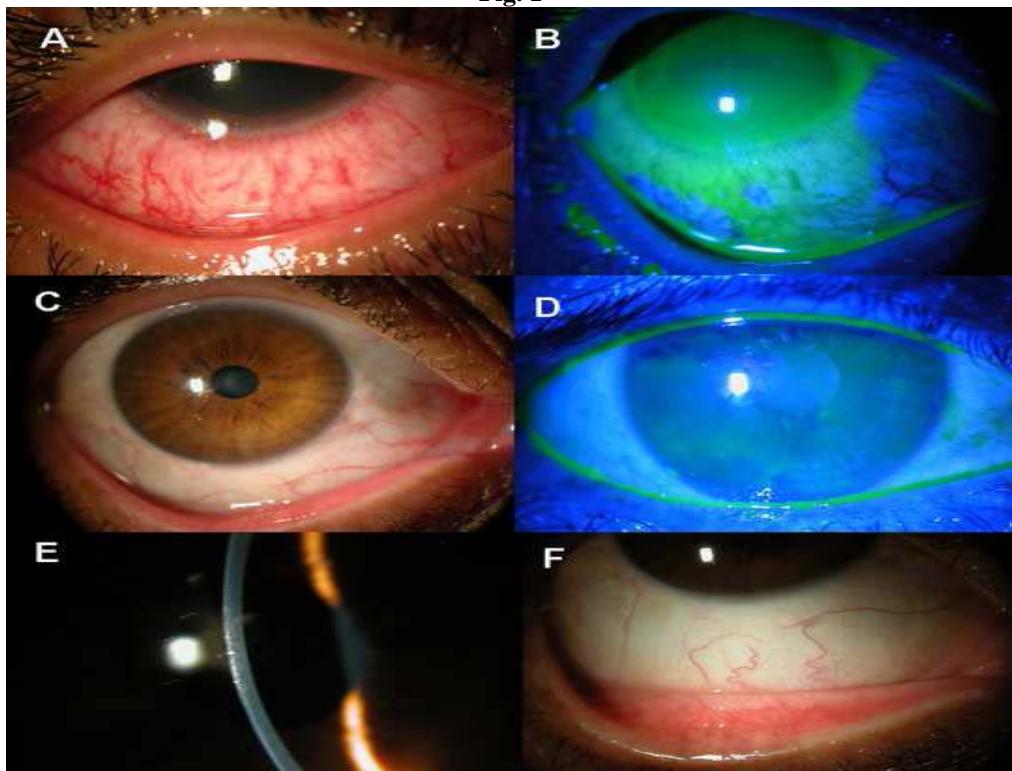


Fig. 2

#### 4. Discussion:

Inflammatory mediators released from the ocular surface at the time of chemical burns induce tissue necrosis and attract further inflammatory reaction. This vigorous inflammatory response not only inhibits re-epithelialization but also increases the risk of corneal ulceration and perforation. In this non-comparative case series, AME was prepared and applied simultaneously with the conventional treatment to help break this inflammatory cycle and promote healing in patients with acute chemical burn with and without limbal involvement. The results of the present study revealed that early application of AME could be effective in rapidly relieving symptoms, reducing inflammation, and promoting epithelialization in mild to moderate acute chemical burn and consequently. It may also thwart limbal stem cell deficiency (LSCD) and symblepharon at the chronic stage.

Human amniotic membrane transplantation has been widely performed as a therapy for a variety of ocular surface disorders, and has been known to be highly effective not only in promoting re-epithelialization but also in suppression of inflammation. The mechanisms of action of the amniotic membrane transplantation are considered to include prolongation and clonogenic maintenance of epithelial progenitor cells, promotion of goblet and non-goblet cell differentiation, suppression of Transforming Growth Factor beta signaling, myoblastic differentiation of normal fibroblasts, and exclusion of inflammatory cells.[23] Based on the inherent biological actions known to amniotic membrane,[24-26] several investigators have explored the clinical efficacy of deploying AMT as a temporary graft to reduce inflammation and to promote healing in acute chemical burns.[9;12-14;17] Application of AME as eye drops is a different approach for the treatment of alkali injuries; Bonci et al [21] prepared a suspension containing homogenized amniotic membrane to investigate its beneficial effect on ocular surface diseases. They used this suspension to treat 21 patients with different ocular diseases exhibiting inflammation and epithelial defects; re-epithelialization was completed after 15–30 days with no side effects. However, they focused on the beneficial effects of the amniotic stromal matrix, rather than on the function of viable amniotic cells. It has been reported that amniotic cells have multiple functions, such as the synthesis and release of neurotransmitters [27-32] and neurotrophic factors [33; 34] and are a source of soluble anti-inflammatory factors. [20] In our study we modified the technique described by Jiang et al, [18] to ensure keeping all the active ingredients of AM by avoiding heat and filtration.

Despite variable extents of ocular surface epithelial defects, all patients reported significant relief of pain and light sensitivity within the first week after AME treatment.

Although one may attribute AME's effect in relieving pain to its anti-inflammatory action, we suspect that such a rapid action in pain relief might be mediated by a yet unknown anti-pain action that deserves further investigation.

Ocular surface inflammation was markedly reduced in all cases after treatment. Although the exact action mechanism remains to be determined, the aforementioned effect may be associated with early delivery of AM's anti-inflammatory active ingredients, which are retained in AME.[18-21] This anti-inflammatory effect is crucial in the treatment of chemical burns, as inflammation severely threatens stem cell survival[35], aggravates neovascularization, and induces apoptosis of keratocytes and stromal melting[36]. While topical corticosteroids in chemical burn are debatable, AME can function as an inflammation inhibitor without side effects.

When AM was used as a temporary patch, polymorphonuclear cells rapidly adhere to its stromal side in rabbit models of alkali burns [10] and in human patients with chemical burns, [37; 38] where these adherent cells underwent rapid apoptosis. [38, 39] Similarly, mononuclear cells, including lymphocytes and macrophages also underwent rapid apoptosis when adherent onto AM stroma in a murine model of HSV-induced necrotizing stromal keratitis. [40; 41] Such a unique anti-inflammatory action of the AM by promoting cellular apoptosis has been recapitulated in an in vitro culturing system using murine macrophages, [42] and recently He et al, reported that such an activity could be retained in AME. [43]

Although AME lacked the bandage effect of AMT, epithelialization started as early as four days in cases with nearly intact limbus; however, it took longer when there was nearly total limbal involvement with/without regional ischemia. It has to be noted that no impression cytology was performed at the initial visits, and limbal stem cell dysfunction was assumed clinically in cases with limbal ischemia. All eyes maintained corneal integrity without stromal melting. We speculate AME might be responsible for inhibiting such a stromal melting through multiple mechanisms: *first*, by maintaining the balance between the matrix metalloproteinase (MMP) and tissue inhibitor of matrix metalloproteinase (TIMP),[44] as TIMPs were found in epithelial and mesenchymal cells as well as in the compact layer of the amniotic membrane stroma, [45] and *second*, through reducing keratocyte apoptosis or modifying

the proliferation and migration of stromal fibroblasts.[39;46]

Interestingly, there was no abnormal limbal or peripheral corneal vascularization during the follow-up period. We further observed that the healed surface did not show any evidence of conjunctivalization, clinically or by impression cytology. We believe that limbal healing resulted from rapid recovery of limbal epithelial stem cells after the acute insult. Further investigation is needed to confirm the ability of AME to resurrect and promote *in vivo* expansion of limbal stem cells.

Collectively, our results showed that AME could be an effective adjunct in the treatment of mild and moderate cases of acute chemical burn by promoting healing, reducing inflammation, and restoring vision. Although we noted that instillation of AME in combination with the conventional therapy could successfully deliver AM's desirable actions, our series was still too small to determine its efficacy in managing different types of chemical burns. Additional controlled studies are needed to confirm that AME is a safe, non-traumatic, convenient, and economical alternative therapy to enhance corneal wound healing in acute chemical burn as well as other inflammatory ocular surface diseases.

#### Acknowledgements:

This paper was presented at annual meeting of the American Academy of Ophthalmology in San Francisco 2009. The study was supported in part by an unrestricted grant from the Eye Foundation of America, Morgantown, WV, USA. Shunsuke R. Sakurai assisted in editing the text.

#### Corresponding author

Hisham Hashem

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9/21/2010

## Phylogenetic Diversity and Relationships of Some Tomato Varieties by Electrophoretic Protein and RAPD analysis

Elham A. A. Abd El-Hady\*, Atef A. A. Haiba , Nagwa R. Abd El-Hamid, and Aida A. Rizkalla

Department of Genetics and Cytology, National Research Center, Dokki, Giza, Egypt.

\*[elhamabdelhady@hotmail.com](mailto:elhamabdelhady@hotmail.com)

**Abstract:** Biochemical and molecular characterization of eight tomato varieties were carried out based on seed storage proteins electrophoresis and RAPD markers. The electrophoretic pattern of water soluble protein produced 4 monomorphic bands, 6 polymorphic band and 3 unique bands .The pattern of non soluble protein produced 9 bands, one band is unique and considered a positive specific band of tomaten cartago variety and the others are polymorphic bands. RAPD results revealed a high level of polymorphism among the studied genotypes. All of the seven random primers screened gave reproducible polymorphic DNA bands. A total number of 81 amplified DNA bands were generated across the studied genotypes with average of 11.57 bands /primer. 37 bands out of the total number were polymorphic and 19 were unique. Combination of the all data derived from the SDS-protein markers of both water soluble and non soluble proteins produced a dendrogram almost similar to that obtained by the RAPD analysis. It could be concluded that, both of SDS-Protein and RAPD markers are equally important for genetic analysis and indicate a considerable amount of genetic diversity between the different studied varieties of *Lycopersicon esculentum* L.

[Elham A. A. Abd El-Hady\*, Atef A. A. Haiba , Nagwa R. Abd El-Hamid, and Aida A. Rizkalla. Phylogenetic Diversity and Relationships of Some Tomato Varieties by Electrophoretic Protein and RAPD analysis. Journal of American Science 2010;6(11):434-441]. (ISSN: 1545-1003).

**Keywords:** Tomato, Genetic diversity, SDS-protein, RAPD-PCR.

### 1. Introduction:

Tomato (*Lycopersicon esculentum* L.) is a member of the family Solanaceae and significant vegetable crop of special economic importance in the horticultural industry worldwide (He *et al.*, 2003 and Wang *et. al.*, 2005). Although the genus *Lycopersicon* includes a few species, its taxonomy is still questionable and phylogeny has not been completely established (Warnock, 1988)

The classification between various subgenera, species and subspecies is based primarily on morphological attributes. However, these morphological characters may be unstable and influenced by environmental conditions (Goodrich *et al.*, 1985). Over the years, the methods for detecting and assessing genetic diversity have extended from analysis of discrete morphological traits to biochemical and molecular trait. Therefore, the advent of the electrophoresis as an analytical tool provides indirect methods for genome probing by exposing structural variations of enzymes or other protein genome (Cook, 1984 and Gilliland, 1989).

The electrophoresis of proteins is a method to investigate genetic variation and to classify plant varieties (Isemura *et al.*, 2001). Its banding pattern is very stable which advocated for cultivars identification purpose in crops. It has been widely suggested that such banding patterns could be

important supplemental method for cultivars identification (Tanksley and Jones, 1981; and Thanh and Hirata, 2002). Analyses of SDS-PAGE are simple and inexpensive, which are added advantages for use in practical plant breeding.

DNA molecular markers technology, which are based on sequence variation of specific genomic regions, provide powerful tools for cultivar identification and seed quality control in various crops with the advantages of time-saving, less labor-consumption and more efficiency (Hu and Quiros, 1991; Mongkolporn *et al.*, 2004; Dongre and Parkhi, 2005; Garg *et al.*, 2006 and Liu *et al.*, 2007).

Random Amplified Polymorphic DNA (RAPD) is based on *in vitro* amplification of randomly selected oligonucleotide sequences. Amplification takes place by simultaneous primer extension of complementary strands of DNA; the primers use the plant DNA as a template for PCR amplification. RAPD is very useful in the study of biodiversity, hybridization, gene mapping and genetic map construction (Sharma and Sharma, 1999).

Generally, molecular markers have proven to be useful tools for characterizing genetic diversity in agricultural crops. Researchers have studied genetic variation in tomato landrace and cultivar collections using various molecular techniques, including restricted fragment length polymorphism

(RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD) and simple sequence repeats (SSR) (Miller and Tanksley, 1990, Rus- Kortekaas *et al.*, 1994, Bredemeijer *et al.*, 1998, Villand *et al.*, 1998, Mazzucato *et al.*, 2003, Park *et al.*, 2004, Carelli *et al.*, 2006 Garcia-Martinez *et al.*, 2006). The aim of the present study was to find out the phylogenetic relationships of eight tomato varieties using protein profiles and random amplified polymorphic DNA (RAPD) analysis.

## 2. Materials and methods

Seeds of eight tomato cultivars of diverse origins grown in Egypt (Tomaten cartago GC781, Karnak, Fac-68, Floradid, Jack pot, Casel rock, Packmor and Petto 86) were used for the present Study and kindly supplied by Horticulture Research Institute, Agricultural Research Center, Giza, Egypt.

### SDS-protein electrophoresis:

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for water soluble and non soluble proteins was performed according to the method of Laemmli (1970), as modified by Studier (1973). Molecular weights of different bands were calibrated with a mixture of standard protein markers include myosin (212 KDa), B-galactosidase (120KDa), phosphorylase b (97KDa), bovinserumalbumin (66.2KDa), ovalbumin (45KDa), carbonic anhydrase (31KDa), soybean trypsin inhibitor (20KDa), lysozyme (14.4KDa) and aprotinin (6.5KDa) from Bio Basic Inc. The banding profile was photographed and scored.

### DNA isolation:

Genomic DNA was isolated from the young leaves by using Bio Basic Kits. The quality of isolated genome DNA was checked by agarose gel electrophoresis.

### DNA amplification:

Seven decamer oligonucleotide primers obtained from (metabion international AG) were used for the polymorphism survey. Amplification reactions were carried out in 25 $\mu$ L volumes, containing (5  $\mu$ L of 5x buffer, 3.0  $\mu$ L of dNTPs (2.5mM) 3  $\mu$ L of Mg cl2 (25 m M), 3.0  $\mu$ L primer (2.5  $\mu$  L) ,0.3  $\mu$  L of Taq polymerase( 5U/  $\mu$ L) and 2.0  $\mu$ L of genomic DNA (50 ng/  $\mu$ L). Amplification was performed in PTC-100 PCR version 9.0 from M J Research-USA. Programmed for an initial denaturation at 94 °C 5 min, 40 cycles of 1 min denaturation at 94 °C, 1 min annealing at 40°C and 2 min extension at 72°C followed by final extension for 5 min at 72°C.

Amplified products from the RAPD reactions were separated by horizontal gel electrophoresis unit using 1.5% agarose gel in TAE buffer and stained with ethidium bromide .The run was performed at 95 volt for 55 min. DNA ladder 1Kb was used from fermentas with lengths ranged from 264 to 11507 bp and then the gel was visualized by UV-transilluminator to examine the reproducibility of banding patterns, then photographed by gel documentation system, Biometra – Bio Doc Analyze. Each PCR reaction was repeated twice in order to ensure that RAPD banding patterns were consistent and reproducible and only stable products were scored.

### Statistical analysis:

The electrophoretic patterns of water soluble and non soluble proteins, and the reproducible banding patterns of each primer which produced by RAPD were chosen for analysis. Each gel was scored as present (1) or absent (0), and pair wise comparisons between individuals were made to calculate the Jaccard's coefficient of genetic similarity matrix using SPSS program (statistical Package for Social Scientists) version-10 (Norman *et al.*, 1975). Cluster analysis was performed to produce a dendrogram using unweighted pair-group method with arithmetical average (UPGMA).

## 3. Results and Discussion:

### 1. SDS protein analysis

#### 1-1. Water soluble protein:

SDS-electrophoretic patterns of water soluble and water non soluble protein fractions were used to find out the phylogenetic relationships among some varieties of tomato. Figure (1) demonstrates the water soluble protein banding patterns of the eight tomato varieties belonging to (*Lycopersicon esculentum* L.).

The SDS banding pattern of water soluble protein produced 13 bands distributed in all varieties with molecular weights ranging from 6.50 KDa to 130.24 KDa. with polymorphism percentage reached to 86.231% between the eight tomato varieties. The pattern of these bands is as follows, 4 bands are monomorphic with molecular weights of 42.55, 18.14, 11.08 and 9.63 KDa., while 6 bands are Polymorphic with molecular weights of 103.09, 46.89, 26.67, 12.62, 8.31 and 7.15 KDa., in addition to three unique bands which have been observed in three varieties, Jack Pot had one of these positive specific band with molecular weight of 130.24 KDa. Also, Casel Rock had one positive specific band with molecular weight of 16.33 KDa, and tomaten Cartago had the third positive specific bands at molecular weight of 6.50 KDa. These bands could be

considered as specific markers for distinguishing these varieties from each others.

#### 1-2. Water non soluble protein:

The SDS banding pattern of water non soluble protein produced 9 bands distributed in all varieties with molecular weights ranging from 6.50 to 69.02 KDa. The polymorphism percentage reached to 100% between the studied tomato varieties. Figure (1) shows that water non soluble protein bands distributed as follows, only one band is unique with molecular weight of 14.37KDa. and considered a positive specific band of tomaten cartago variety, while the others are polymorphic bands.

The UPGMA method was used to calculate the similarity coefficient among the studied tomato varieties of both water soluble and water non soluble proteins individually, based on existence of the bands (presence or absence) and their average was used as an approximate value for recognizing groups of varieties in dendrogram (Fig.2 a&b), which showed the same relationship between tomato varieties, when the data of water soluble proteins and water non soluble proteins were combined for UPGMA cluster analysis, the obtained dendrogram (Fig.2c) revealed almost the same cluster pattern of the eight studied tomato varieties.

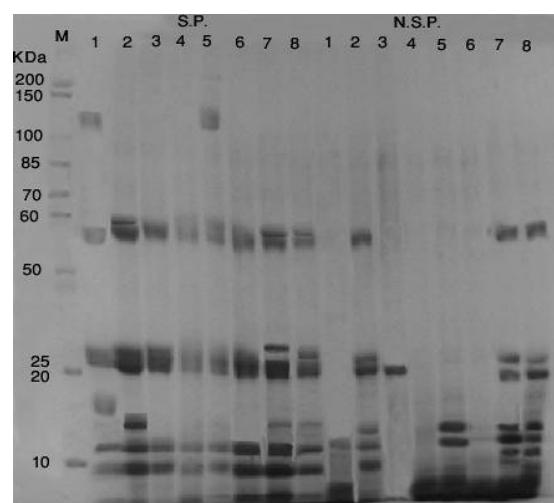
The similarity coefficient based on water soluble and non soluble proteins markers ranged from 0.771 to 0.135. Table (1) showed that the highest similarity value (0.771) was between Cartago and Jac-pot. While the lowest value of (0.135) was between Cartago and Betto. The relationship between Casel rock and Floradid was 0.574, and between Casel rock and Fac-68 was 0.646. Betto is equally closely related to both Karnak and Pack-mor (0.733).

The clusters obtained from the dendrogram Fig. (2c). showed that the studied tomato varieties are grouped in three main groups. The first one consists of tomaten cartago and jack-pot which were the most related varieties to each other. The second group includes Casel rock, Floradid and Fac-68 .while, the third group consists of karnak , Betto and Pack-more ,therefore , it could be concluded that the results of water soluble and non soluble protein could differentiate between the studied tomato varieties producing some specific bands that can be used to distinguish such variety from each others. These specific variations were analyzed to assess the protein polymorphisms between different varieties of tomato and clarify the genetic nature of polymorphic bands. Similary, different cultivars of cultivated chickpea were examined by Ahmad and Slinkard (1992), they concluded that seed protein was a very conservative trait in chickpea. Also, Raymond *et al.* (1991), and De vries (1996), reported similar electrophoretic

patterns of protein among the cultivars of sunflower and lettuce. Munazza *et al.* (2009) studied the electrophoretic characterization in different genotypes of oilseed Brassica based on analysis of seed storage proteins to assess the protein polymorphisms within and different cultivated species and clarify the genetic nature of polymorphic bands to differentiate the yellow and brown seeded varieties of Brassica.

Furthermore, This electrophoretical proteins can detect genetic purity test in case of vegetables such as tomato by several studies using isozyme and protein polymorphism (Thanh *et al.*, 2006, Tanksley and Jones, 1981; Wang *et al.*, 2005).The reduction of genetic variation in tomato (*Solanum lycopersicum L.*) through domestication and breeding (Tanksley and McCouch,1997; Barrero and Tanksley,2004) has resulted in the need for conservation, characterization, and utilization of genetic resources.

The high stability of protein profile makes protein electrophoresis a powerful tool in elucidating the origin and the evolution of cultivated plants (Ladizinsky and Hymowitz,1979), so it seems to say that SDS-PAGE technique has proven to be a useful in supporting classical taxonomy studies (Thanh *et al.*, 2003).



**Fig. (1)** SDS-protein banding pattern of water soluble and water non soluble proteins of the eight tomato genotypes. 1. cartago GC781 2. Karnak 3. Fac-68 4. Floradid 5. Jack pot 6.Casel rock 7. Pack-mor 8.Petto 86.

**Table (1):** Similarity coefficients of the eight tomato varieties based on water soluble and non soluble proteins markers.

Case	Matrix file input							
	Car.	Kar.	Fac.	Flo.	Jac.	Cas.	Pac.	Bett.
Car.	1.00							
Kar.	0.142	1.00						
Fac.	0.443	0.541	1.00					
Flo.	0.357	0.357	0.746	1.00				
Jac.	<b>0.771</b>	0.255	0.357	0.341	1.00			
Cas.	0.535	0.357	<b>0.646</b>	<b>0.574</b>	<b>0.357</b>	1.00		
Pac.	0.142	<b>0.711</b>	0.355	0.257	0.264	0.243	1.00	
Bett.	0.135	<b>0.733</b>	0.341	0.357	0.205	0.443	<b>0.733</b>	1.00
	Car.	Kar.	Fac.	Flo.	Jac.	Cas.	Pac.	Bett.

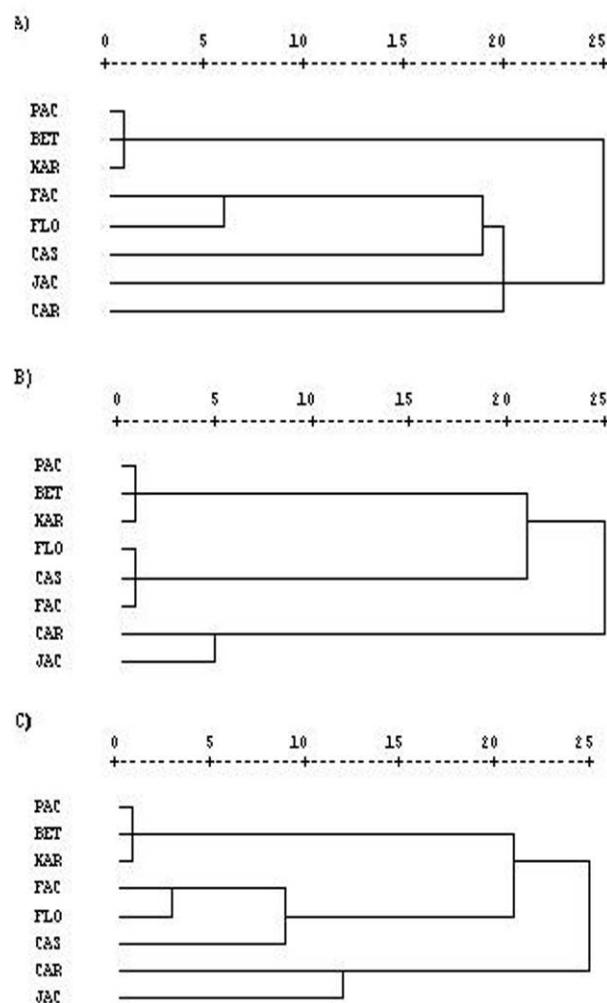


Fig. (2): UPGMA dendrogram indicating the genetic relationships among the eight tomato varieties based on:

- water soluble proteins markers
- water non soluble proteins markers
- combination of water soluble and non soluble proteins

## 2- Molecular studies:

Seven RAPD primers were tested against the eight tomato varieties. The sequences of these primers are listed in Table (2). The RAPD profiles of the amplified products are shown in Fig. (3). The number of bands and the degree of polymorphism revealed by each primer are given in Table (2). Generally, the levels of polymorphism were varied with different primers among the different tomato varieties. The percentage of polymorphism produced by each primer differed from one primer to the other, the maximum value of polymorphism was 85.714% produced by primer OPD-13. While, the minimum value of polymorphism was 45.455% by primer OPX-17, with an average polymorphism of 66.479 % across all the genotypes.

A total number of 81 amplified DNA bands were generated across the studied genotypes with average of 11.57 bands/ primer. Out of the total band, 37 polymorphic and 19 unique ones were detected. The RAPD profiles of the amplified products of each primer are shown in Fig.3 (a, b, c, d, e, f and g). A maximum number of 17 bands were amplified with primer opc-19 and a minimum of 7 bands with primer OPD-13. The number of monomorphic bands was primer dependent and ranged from 1 band by primer D-13 to 6 bands by primer OPN-06 and primer OPX-17.

The genetic similarity coefficient, recognized the eight studied tomato variety, the highest similarity value was 0.891 which recorded between Cartago and Jac-Pot, while the lowest similarity value was 0.401 between cartago and Karnak Table (3).

Similarity coefficient matrices were used to generate a dendrogram of tomato genotypes based on UPGMA analysis Fig (4), the analysis divided the eight genotypes into three distinct clusters .The first cluster includes Cartago and Jac-Pot with the highest similarity value (0.891), while the second cluster contains three genotypes, Floradid and Casel Rock which are moderately related (0.673) then come Fac-

68 which is highly related to Casel Rock (0.810). The third cluster comprises of Betto, Pack-more and Karnak. In which Pack-mor and Karnak are highly related to each other (0.864) and Betto was closer to Pack-more (0.795) than Karnak (0.699).

From the previous results it could be concluded that, the dendrogram on the basis of RAPD revealed almost the same phylogenetic relationships between the eight studied tomato varieties that obtained by combining the data from the markers of water soluble and non soluble proteins.

Some earlier researchers stated that the application of both biochemical and molecular genetics techniques have an important potential to provide a new tool for the study of both wild and domesticated species in respect to investigation of evolution and migration of species from their gene pool centers (Badr *et al.*, 2000 and Fregonezi *et al.*, 2006 ).

The identification and characterization of species become possible through fingerprinting for each species since DNA is a source of informative polymorphism (El-Rabey, 2008), consequently, techniques of molecular genetic markers have an important potential for the detection of genetic differences among species (Benmoussa and Achouch, 2005). Many investigations reported that, RAPD analysis is revealed high genetic polymorphism of the tomato genome and established the phylogenetic relationships among members of the genus *Lycopersicon* Mill. The resulting dendrogram was consistent with *Lycopersicon* phylogeny based on the molecular data of RFLP, ISSR, microsatellite analysis and with the classification based on morphological characters (Ruck, 1979; Palmer and Zamir, 1982; Miller and Tanksley 1990; Khrapalova, 1999 and Lingxia *et al.*, 2009). Therefore, the use of molecular markers in the applied breeding programs can facilitate appropriate choice of parents involved

for crosses. Munazza *et al.*, (2009) reported that the assessment of genetic diversity within and between landraces should have priority for varieties improvement. At the same time it is necessary to develop better methods of characterization and evaluation of germplasm collections, to improve strategies for conservation and collection of germplasm and to increase the utilization of plant genetic resources.

#### 4. Conclusion:

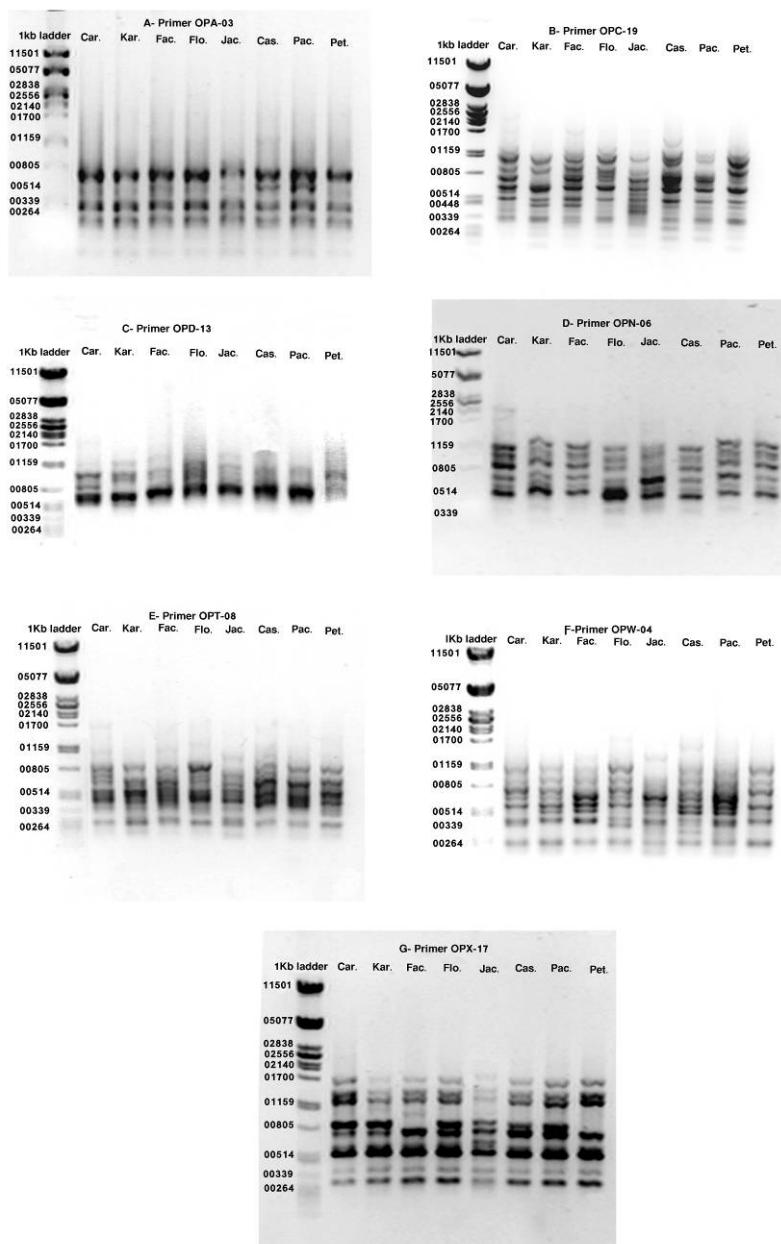
Eight tomato varieties were used in order to elucidate their genetic diversity by using SDS-proteins and RAPD-PCR analysis. It could be concluded that the present biochemical results, water soluble and non soluble protein, can differentiate between the studied tomato varieties by producing some specific bands that could be used to distinguish any variety from each others. These specific variations were analyzed to assess the protein polymorphisms between different varieties of tomato and clarify the genetic nature of polymorphic bands. On the molecular level, seven primers were used to differentiate between these varieties and gave reproducible results with wide variations in their band numbers. The molecular markers obtained by the RAPD technique revealed a remarkable molecular discrimination between the eight tomato varieties under the study. The phylogenetic analysis on the basis of RAPD derived a dendrogram revealed almost the same cluster pattern that obtained from the combined markers of water soluble and non soluble proteins and confirm the phylogenetic relationship between the eight studied tomato varieties. It could be concluded that, both of SDS-Protein and RAPD markers are equally important for genetic analysis and indicate a considerable amount of genetic diversity between the different studied varieties of *Lycopersicon esculentum* L.

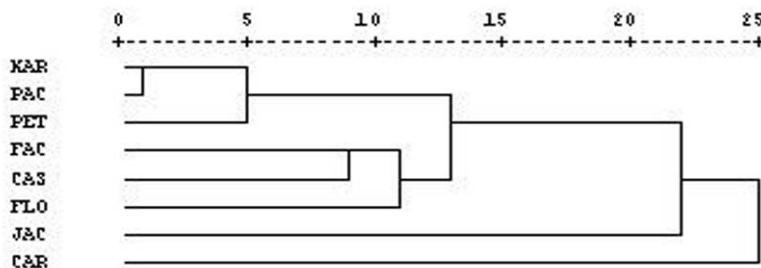
**Table (2):** Code and sequence of the seven DNA random primers used for identifying the tomato varieties and types of the amplified DNA bands.

Primers Cod	Sequence	Total No. of bands	No. of Monomorphic bands	unique band	No. of polymorphic bands	% of Polymorphic loci.
OPA-03	5'-AGTCAGCCAC-3'	13	2	6	5	84.615
OPC-19	5'-GTTGCCAGCC-3'	17	3	4	10	82.353
OPD-13	5'-GGGGTGACGA-3'	7	1	3	3	85.714
OPN-06	5'-GAGACGCACA-3'	8	6	1	1	25.000
OPT-08	5'-AACGGCGACA-3'	11	4	1	6	63.636
OPW-04	5'-CAGAAGCGGA-3'	14	3	2	9	78.571
OPX-17	5'-GACACGGACC-3'	11	6	2	3	45.455
Total	-	81	25	19	37	-
Average/primer	-	11.57	-	-	-	66.479%

**Table (3):** Similarity coefficients of the eight tomato varieties based on RAPD markers.

Case	Matrix File Input							
	Car.	Kar.	Fac.	Flo.	Jac.	Cas.	Pac.	Bett.
Car.	1.000							
Kar.	0.401	1.000						
Fac.	0.612	0.482	1.000					
Flo.	0.686	0.517	0.673	1.000				
Jac.	0.891	0.545	0.591	0.545	1.000			
Cas.	0.479	0.673	0.810	0.673	0.509	1.000		
Pac.	0.455	0.864	0.533	0.427	0.445	0.471	1.000	
Bett.	0.469	0.699	0.682	0.582	0.500	0.536	0.795	1.000
	Car.	Kar.	Fac.	Flo.	Jac.	Cas.	Pac.	Bett.

**Fig. (3):** RAPD fingerprints of the eight obtained tomato varieties generated by the seven primers a) OPA-03    b) OPC-19    c) OPD-13    d) OPN-06    e) OPT-08    f) OPW-04    g) OPX-17



**Fig (4):** UPGMA dendrogram indicating the genetic relationships among the eight tomato varieties based on RAPD markers.

#### Corresponding author

Elham A. A. Abd El-Hady  
Department of Genetics and Cytology, National Research Center, Dokki, Giza, Egypt.  
[elhamabdelhady@hotmail.com](mailto:elhamabdelhady@hotmail.com)

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9/22/2010

# Protective effect of three different fluoride pretreatments on artificially induced dental erosion in primary and permanent teeth

Sherine B Y Badr<sup>1</sup>, Mohamed A Ibrahim<sup>2</sup>

<sup>1</sup> Pediatric dentistry Department, Faculty of Oral and Dental Medicine, Cairo University, Cairo, Egypt

<sup>2</sup> Restorative Dentistry Departments, Collage or Oral and Dental surgery, Misr University for Science and Technology, 6<sup>th</sup> of October, Egypt

\*[shbadr5@hotmail.com](mailto:shbadr5@hotmail.com)

**Abstract:** **Objective:** To assess the effect of acidulated phosphate fluoride gel (APF), sodium fluoride varnish (NaF) and casein phosphopeptide-amorphous calcium phosphate fluoride paste (CPP-ACPF) on the dental erosion produced by coca cola in primary and permanent teeth. **Design:** Sixty extracted human primary molars ( $n = 30$ ) and young permanent premolars ( $n = 30$ ) were used in this study. The coronal portion of each tooth was sectioned mesio-distally. Specimens were prepared by embedding the crown sections in acrylic resin blocks leaving the enamel surfaces exposed. Specimens were ground, polished and randomly assigned to one of three groups each of 10 according to the protective agent used: APF gel (1.23% F), NaF varnish (0.1%F), and CPP-ACPF paste (0.2%F). Half of the exposed enamel surface was protected with adhesive tape during the treatment of the remaining surface according to their group. Six daily demineralization–remineralization cycles of 5 minutes of immersion in a cola drink (pH 2.3) and 30 minutes in artificial saliva were conducted for 14 days. Surface Vickers Micro-hardness readings were recorded at baseline and 14 days later for both halves. Percentage surface microhardness reduction (%SMHR) was then calculated. Data were analyzed using ANOVA and Duncan's post-hoc test ( $p < 0.05$ ). **Results:** All of the tested fluoride treatments were able to reduce erosive enamel loss in both primary and permanent groups. In primary teeth only APF gel showed significantly higher anti-erosive effect than both fluoride varnish and CPP-ACPF paste. In permanent teeth both CPP-ACPF paste and APF gel showed significantly higher protective anti-erosive effect than fluoride varnish. **Conclusions:** under the conditions of this study, all of the tested fluoride treatments were able to reduce erosive enamel loss in both primary and permanent teeth. Primary and permanent enamel substrates reacted differently to different fluoridated compounds. CPP-ACPF paste is a promising remineralizing material.

[Sherine B Y Badr<sup>1</sup>, Mohamed A Ibrahim. Protective effect of three different fluoride pretreatments on artificially induced dental erosion in primary and permanent teeth. Journal of American Science 2010;6(11):442-451]. (ISSN: 1545-1003).

**Keywords:** dental erosion, fluoride varnish, fluoride gel, CPP-ACPF paste, microhardness, primary, permanent.

## 1. Introduction

Erosive tooth wear or dental erosion has gained more attention from the dental profession since the decline in dental caries in many industrialized countries. Dental erosion is a localized loss of the tooth surface by a chemical process of acidic dissolution of nonbacterial origin.<sup>1</sup> This process may be caused by extrinsic or intrinsic agents. Extrinsic agents include acidic foodstuffs, beverages, snacks and may also occur following environmental exposure to acidic agents.<sup>2,3</sup> One of the most common extrinsic factors that cause dental erosion is the excessive consumption of acidic food and beverages.<sup>4,5</sup> Intrinsic erosion is associated with gastric acid which may be present intra-orally

following vomiting, regurgitation, gastro-oesophageal reflux.<sup>6</sup>

Lifestyle changes and a rise in the consumption of acidic foods and beverages have led to an increase in the prevalence of dental erosion around the world in recent years. High prevalence numbers ranging from 30%<sup>7</sup> to 68%<sup>8</sup> have been reported, especially among children and adolescents,<sup>9</sup> while the prevalence and etiology of dental erosion have been the focus of innumerable papers in the last two decades, studies regarding the prevention of this disease by chemical substances are still needed.

Several studies have reported the effectiveness of topical fluoride as a cariostatic agent in enhancing enamel remineralization.<sup>10-14</sup> A similar

anti-erosive capability of different topical fluorides was tested.<sup>15-17</sup>

In recent years casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) nanocomplexes have also been demonstrated to have anticariogenic<sup>16-21</sup> as well as anti- erosive properties.<sup>22-24</sup>

Current recommendations in many recent studies for management of demineralized lesions include the use of oral products containing CPP-ACP (e.g., Tooth Mousse and Recaldent) and fluoride,<sup>18,19,22,24</sup> thus MI paste plus was recently introduced in the market combining both. MI paste plus is water based, lactose free cream containing casein phosphopeptide and amorphous calcium phosphate fluoride (CPP-ACPF). The level of fluoride is 0.2% w/w (900 ppm) which approximates that of adult toothpastes. When CPP-ACPF is applied in the oral environment, it will bind to biofilms, plaque, bacteria, hydroxyapatite and soft tissue localizing bio-available calcium, phosphate and fluoride.

A controversial issue in dental erosion literature involves differences in erosion progression rates between primary and permanent teeth. Some authors stated that primary enamel is more susceptible to erosion than permanent enamel<sup>25-27</sup> while others found no differences between these two types of substrates.<sup>28-30</sup> However, the protective effect of different remineralizing agents especially CPP-ACPF on both primary and permanent enamel did not receive much attention.

Softening of the enamel surface is an early manifestation of the erosion process. Reduced surface hardness which accompanies erosion of the enamel surface by acidic beverages can be assessed using a physical measurement such as the hardness test.<sup>17, 23, 31</sup>

In view of the above considerations, the present paper aimed to investigate the protective effect of single application of 0.1% NaF varnish, 1.23% APF gel and CPP-ACPF on artificially induced dental erosion in human primary and permanent enamel. Thus the null hypothesis tested was that the different fluoride treatments will not exhibit different protective potential on enamel erosion.

## 2. Material and Methods

### Sample preparation

Sixty human primary molars ( $n = 30$ ) extracted from children 10- 12 years old and young permanent premolars ( $n = 30$ ) extracted for orthodontic purpose from children 12-14 years old were used in this study. Enamel specimens were prepared by sectioning the coronal portion from the

radicular portion of each tooth using a diamond bur in a high-speed handpiece with an airwater spray. The crowns were then transversely sectioned from the mesial to distal surface through the center of the crown using a high-speed saw (Buehler Int., Evanston, IL) cooled with water. The enamel sections that were free of any caries or any enamel defects were embedded in acrylic resin with the outer buccal or lingual enamel surface exposed. The enamel surfaces were ground wet using 600-2000 grit silicon carbide abrasive paper (Buehler, Lake Bluff, IL) and polished with 1.0 and 0.05 mm alumina suspension (Buehler) to expose flat enamel for microhardness measurements. Test specimens were randomly assigned to one of three groups each of 10 according to the protective agent used: 1.23% APF gel (dentsply professional 1301 Smile Way, York PA 17404), 0.1 % NaF varnish (flour protector, Ivoclar Vivadent AG, FL - 9494 Schaan Liechtenstein), and 0.2% CPP-ACPF paste (Prospec™ MI Paste, GC Corporation, Tokyo, Japan).

### Fluoride treatment

Before topical fluoride application, the specimens were thoroughly rinsed with deionized water and delicately dried using a paper towel. Half of the exposed enamel surface of each specimen was protected with adhesive tape while the remaining surface was treated according to their group. APF gel and CPP-ACPF paste were applied for 4 minutes with the aid of a cotton tip and were later removed by squirting deionized water to rinse thoroughly. The NaF varnish was applied using a microbrush, left to act at the enamel surface for 12 hours to simulate clinical topical fluoride application<sup>10</sup>, and then delicately removed using cotton tips immersed in deionized water. No chemical substances were used for the removal of the varnish in order not to alter the enamel surface. All specimens were then stored in artificial saliva overnight.

### pH cycling

Six daily demineralization-remineralization cycles of 5 minutes of immersion in a cola drink (pH 2.3) and 30 minutes in artificial saliva were conducted for 14 days. All specimens were stored in artificial saliva between and after cycles. During demineralization cycles, the specimens were immersed in cola drink (Coca-Cola) of pH 2.3; at room temperature for 5 minutes in separate containers (15 ml/specimen) hermetically sealed.<sup>32</sup> The cola drink was changed every cycle. After thorough rinsing with deionized water and careful drying, the specimens were stored in artificial saliva solution during the 30 minutes resting intervals and

overnight. The artificial saliva solution was changed every 2 days.

### **Hardness assessment**

Enamel demineralization was measured as surface softening. The surface Micro-hardness (SMH) of the specimens was determined using Digital Display Vickers Micro-hardness Tester (Model HVS-50, Laizhou Huayin Testing Instrument Co., Ltd. China) with a Vickers diamond indenter and a 20X objective lens. A load of 200 g was applied to the surface of the specimens for 15 seconds. Five indentations were equally placed over a circle of 1-mm diameter at the middle third of the specimens. The diagonal length of the indentations was measured by built in scaled microscope and Vickers values were converted into micro-hardness values. SMH was obtained using the following equation:  
 $HV=1.854 P/d^2$  where, HV is Vickers hardness in Kgf/mm<sup>2</sup>, P is the load in Kgf and d is the length of the diagonals in mm. The surface Micro-hardness of the specimens was measured once at baseline, and after 14 days of the six daily demineralization-remineralization cycles for both treated and untreated teeth halves. Additionally, the percentage reduction in surface microhardness (% SMHR) was calculated as:

$$\frac{\text{Microhardness (at base line)} - \text{Microhardness (After)}}{\text{Microhardness (at base line)}} \times 100$$

### **Statistical analysis**

Data were presented as means and standard deviation values. Analysis of Variance (ANOVA) was used to compare between means of the three groups. Duncan's post-hoc test was used to determine significant differences between the means when ANOVA test result is significant. Paired t-test was used to compare between mean microhardness values before and after treatment within each group.

The significance level was set at  $P \leq 0.05$ . Statistical analysis was performed with Statistical Package for Scientific Studies (SPSS 16.0, SPSS, Inc., Chicago, IL, USA.) for Windows.

## **3. Results**

### **Primary teeth:**

The mean ( $\pm SD$ ) values of SMH in the different groups at base line and after 14 days of demineralization-remineralization cycles for treated and untreated teeth halves were respectively (263.4 $\pm$  2.6, 213.5 $\pm$ 6.6 and 184.8 $\pm$  19.5), (277.7 $\pm$ 73, 191.3 $\pm$ 23.4 and 176.9 $\pm$ 11.5) and

(285.5 $\pm$ 37.9, 196.9 $\pm$ 28 and 184.9 $\pm$ 32.5) for the gel, varnish and paste groups respectively, table (1). As regards the mean ( $\pm SD$ ) %SMHR of treated and untreated teeth halves, were (18.6  $\pm$ 3.3 and 29.6 $\pm$ 6.7), (29.3  $\pm$ 9.6 and 33.9 $\pm$ 12.2), and (29.8  $\pm$  15.4 and 34.8 $\pm$ 12.2) for the gel, varnish and paste respectively, table (2) and fig. (1).

Statistical analysis showed that all of the three treatments were able to diminish the amount of enamel hardness loss and provide a significant protective effect against erosive enamel loss than the untreated teeth halves with no statistically significant difference between untreated halves. Moreover, APF gel group showed the statistically significantly lowest mean % SMHR (i.e. APF gel showed the highest protective effect against erosive enamel loss) compared to fluoride varnish and CPP-ACPF paste with no statistical significant difference between them.

### **Permanent teeth:**

The mean ( $\pm SD$ ) values of SMH in the different groups at base line and after 14 days of demineralization-remineralization cycles for treated and untreated teeth halves were respectively (268.4 $\pm$ 41.4, 187.4 $\pm$ 18.7 and 170.8 $\pm$ 22.62), (336.5 $\pm$ 25.7, 191.9 $\pm$ 9 and 169.8 $\pm$ 15.9), and (297.5 $\pm$  32.3, 225.9 $\pm$ 1.6 and 185.7 $\pm$ 28.1) for the gel, varnish and paste group respectively, table (1). As regards the mean ( $\pm SD$ ) %SMHR of treated and untreated teeth halves, were (28.7  $\pm$ 16.3 and 35 $\pm$ 16), (42.9 $\pm$ 2 and 49.6 $\pm$ 1.6) and (23.6  $\pm$ 8.8 and 36.7 $\pm$ 16.3) for the gel, varnish and paste respectively, table (2) and fig. (1).

Similar to primary teeth statistical analysis showed that all of the three treatments were able to diminish the amount of enamel hardness loss and provide a significant protective effect against erosive enamel loss than the untreated teeth halves with no statistically significant difference between untreated halves. Moreover, there was no statistically significant difference between APF gel and CPP-ACPF paste groups which showed lower means % SMHR (i.e. highest anti-erosive protective effect) than the fluoride varnish group.

Comparing primary and permanent teeth, in primary teeth only APF gel showed statistically significantly the lowest mean % SMHR compared to the CPP-ACPF paste and varnish groups. However, in the permanent teeth both APF gel and CPP-ACPF paste showed statistically significantly the lowest mean % SMHR compared to the fluoride varnish group.

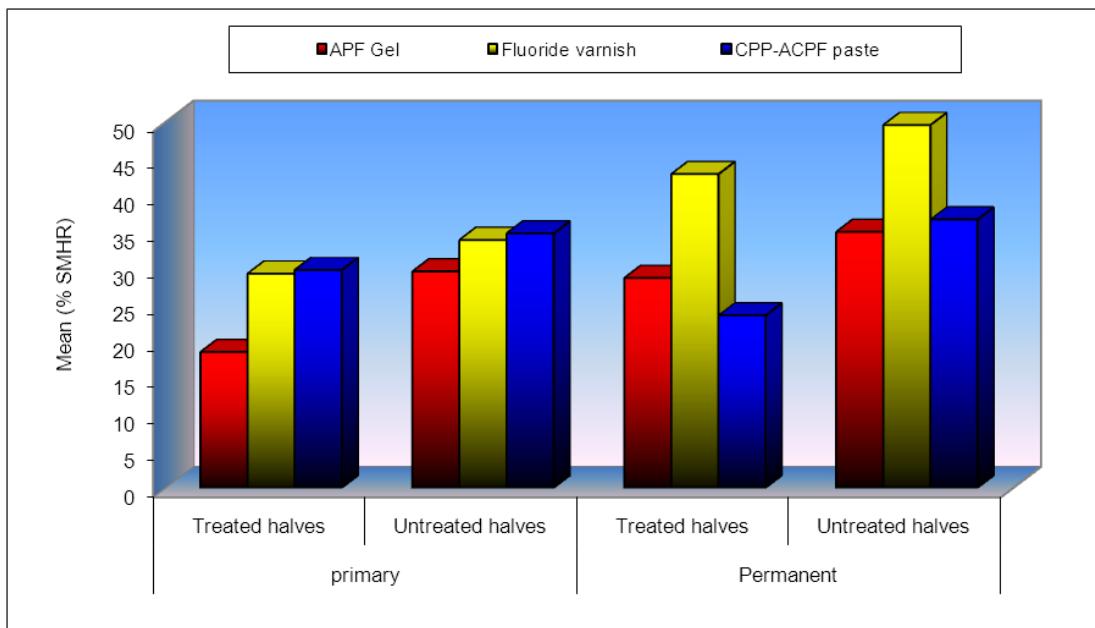
**Table (1):** Means and standard deviation (SD) values of surface microhardness (SMH) in the different groups at base line and after 14 days of demineralization-remineralization cycles for both treated and untreated teeth halves in primary and permanent teeth.

Teeth	Treatment	Group		APF gel		fluoride varnish		CPP-ACPF paste	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
primary	Before (at base line)	262.4	2.6	277.7	73	285.5	37.9		
	After (Treated half)	213.5	6.6	191.3	23.4	196.9	28		
	After (Untreated half)	184.8	19.5	176.9	11.5	184.9	32.5		
Permanent	Before (at base line)	268.4	41.4	336.5	25.7	297.5	32.3		
	After (Treated half)	187.4	18.7	191.9	9	225.9	1.6		
	After (Untreated half)	170.8	22.6	169.8	15.9	185.7	28.1		

**Table (2):** Means (+SD) values and results of ANOVA test for the comparison between (% SMHR) of the treated and untreated teeth halves in primary and permanent teeth.

Teeth	Teeth halves	Group		APF gel		fluoride varnish		CPP-ACPF paste		P-value
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	
primary	Treated halves	18.6 <sup>b</sup>	3.3	29.3 <sup>a</sup>	9.6	29.8 <sup>a</sup>	15.4			<b>0.034*</b>
	Untreated halves	29.6	6.7	33.9	12.2	34.8	12.2			<b>0.872</b>
Permanent	Treated halves	28.7 <sup>b</sup>	16.3	42.9 <sup>a</sup>	2	23.6 <sup>b</sup>	8.8			<b>0.045*</b>
	Untreated halves	35	16	49.6	1.6	36.7	16.3			<b>0.386</b>

\*: Significant at  $P \leq 0.05$ , Means with different letters are statistically significantly different according to Duncan's test



**Fig. (1):** Means (% SMHR) of both treated and untreated teeth halves in primary and permanent teeth

#### 4. Discussion:

Epidemiological studies have reported that dental erosion is common in adolescents, 37% in the UK and 41% in the US,<sup>33</sup> and that its incidence is increasing with time.<sup>34</sup> Erosion may not only cause direct loss of surface enamel or dentin, but also renders tooth structures more susceptible to caries.<sup>35</sup> The 1993 UK Child Dental Health survey<sup>36</sup> reported that over half of five and six years-old children had eroded surfaces on one or more primary incisors. Among children aged 11 years or older, a quarter or more were found to have some erosion of the palatal surfaces of the upper permanent incisors. Erosion appears to have increased in children particularly from the higher socioeconomic groups.<sup>37, 38</sup>

Recently a new material CPP-ACPF paste was introduced to the market combining both CPP-ACP and fluoride. None of the studies up to our knowledge studied the protective anti-erosive effect of this material on both primary and permanent teeth. Moreover, in this study this effect was compared to two of the most commonly used fluoride products in dental practice nowadays as 2.26% NaF varnish and 1.23% APF gel.

Cola was used in this study to induce artificial erosive effect as in other studies<sup>23, 31</sup> since; it is one of the most commonly consumed acidic beverages. The cola drink was changed every cycle to ensure that it was carbonated and to reduce the buffering effect from ions dissolved from the enamel surface. Cola containers were hermetically sealed because removal of gas from the drink may increase its pH and decrease its potential of dissolving hydroxyapatite

Indentation hardness testing with either Knoop or Vickers indenter has been used for the measurement of initial enamel hardness, enamel softening as an early manifestation of the erosion process, as well as enamel hardening after remineralization. Both indenters are suitable for hardness testing of non-metallic materials.<sup>10, 17, 23, 39, 40, 41</sup> Vickers hardness test was chosen in this study with the 200 g load because it provided the appropriate size of indentations for accurate measurement with the available equipment and the present experimental design. SMH readings were recorded at baseline, and after 14 days of the six daily demineralization–remineralization cycles for both treated and untreated teeth halves to determine minor changes due to erosive enamel loss. Additionally, the % SMHR was then calculated to refer this minor reduction in SMH to the initial readings at base line of the same teeth.

The results of our study showed that all of the three fluoride treatments were able to diminish the amount of enamel hardness loss and provide protective effect against erosive enamel loss in the treated teeth halves than the untreated halves in both primary and permanent teeth. These results are in accordance with those of previous studies that investigated the effect of APF gel<sup>17, 42</sup> and NaF varnish<sup>17, 43, 44</sup> on dental erosion. Moreover similar to our study, these studies showed a reduction of hardness in all treated groups indicating that, although fluoride products can inhibit dental erosion, they do not completely prevent it. Contradictory results ranging from no or limited protection of topical fluoride against dental erosion<sup>38, 45, 46</sup> up to

almost complete protection<sup>47</sup> are found in literature. This may be due to differences in study design, particularly regarding the type of dental substrate, the frequency of application, the pH and concentration of the different fluoridated substances used.<sup>17,48</sup>

In primary teeth our results showed that APF gel provided the highest protective effect against erosive enamel loss compared to fluoride varnish and CPP-ACPF. This can be explained on bases that the acidic pH of APF gel may have etched the enamel surface and helped to increase the incorporation of fluoride into enamel. Another explanation may be that the free negative fluoride ions become more reactive in acidic media, thus enhance the formation of CaF<sub>2</sub>. Several studies agreed with the enhancement of the formation of the CaF<sub>2</sub> layer under acidic conditions when comparing neutral to acidic fluoride solutions<sup>49</sup> and when comparing neutral to acidic fluoride gels.<sup>50-51</sup> limited studies compared the APF gel to the varnish due to difference in application techniques.<sup>10,17</sup> However, our result that the APF gel offer higher protective anti-erosive effect than the fluoride varnish comes in agreement with Lee et al., 2010<sup>10</sup> who found that APF gel showed the better effect in terms of fluoride uptake. Moreover, Murakami et al., 2009<sup>17</sup> stated that although the fluoride varnish used in his study had a greater concentration of fluoride (2.26%F) and was left to act in contact with the teeth for a longer period of time, the APF gel showed similar protective anti-erosive effect. However, in our study the significantly higher anti-erosive effect of the APF gel over the varnish can be attributed to the lower concentration of fluoride in the varnish used in our study (0.1%F).

In the permanent teeth, although all of the three treatments were able to diminish the amount of enamel hardness loss and provide protective effect against erosive enamel loss than the untreated teeth halves similar to primary teeth. Yet, the protective effect offered not only by APF gel but also by the CPP-ACPF paste was significantly higher than that of the fluoride varnish. Thus CPP-ACPF paste showed significantly high erosive protection potential in permanent teeth. This can be attributed to the formation of a stabilized amorphous calcium fluoride phosphate phase. These results come in agreement with several studies that proved that the combined effect of CPP-ACP and fluoride as two separate products was beneficial in enhancing remineralization and anticariogenic effect,<sup>19, 52</sup> as well as improving acid-resisting effect.<sup>52, 53</sup>

Our results can be justified on bases that this recently introduced material combines both fluoride and CPP-ACP in one product thus offering the protective effect of both of them.

The protective effect of fluoride is mainly attributed to the formation of a CaF<sub>2</sub> like layer on the tooth surface, which acts as a fluoride reservoir. During an acidic attack, fluoride released from the CaF<sub>2</sub> deposit can be incorporated into the mineral by forming fluoroapatite or fluorohydroxyapatite resulting in a decreased susceptibility to further dissolution. A similar mode of action is assumed for the anti-erosive capability of fluorides. Additionally, the CaF<sub>2</sub> layer might act as a mechanical barrier hampering the contact of the acid with the underlying enamel or as a mineral reservoir, which is attacked by the erosive challenge, thus leading to a buffering or depletion of hydrogen ions from the acid. The formation of the CaF<sub>2</sub> layer depends on the pH and the concentration of the fluoride agent and the duration of application.<sup>54</sup> As high concentrated fluoride agents or a prolonged application time might lead to a thicker and more stable CaF<sub>2</sub> precipitate, an intensive fluoridation is considered as most effective for prevention of erosive enamel loss.<sup>55, 56</sup>

The protective effect of CPP-ACP lies in the fact that it provides a reservoir of neutral ion pair that inhibits enamel demineralization and promotes remineralization.<sup>18, 57</sup> Calcium and phosphate ions are building blocks for the remineralization process, and are found in saliva. Casein phosphopeptide amorphous calcium phosphate complex (CPP-ACP) has been introduced as a supplemental source of calcium and phosphate ions in the oral environment. The amorphous calcium phosphate is biologically active, and is able to release calcium and phosphate ions to maintain saturation levels of calcium and phosphate at the tooth surface. It is hypothesized that, in addition to the prevention of erosive demineralization, CPP-ACP also remineralizes (repairs) eroded enamel and dentine crystals. This hypothesis is supported by an observation that superficial granular structures, probably representing remineralized enamel crystals were formed on the enamel surface after exposure to a sports drink containing CPP-ACP.<sup>58</sup>

Comparing primary and permanent teeth, in primary teeth only APF gel showed statistically significantly the lowest mean % SMHR compared to the CPP-ACPF paste and varnish groups. However, in the permanent teeth both APF gel and CPP-ACPF paste showed statistically significantly the lowest mean % SMHR compared to the fluoride varnish group. This can be attributed to two factors, first to the structural difference between both primary and permanent enamel. Deciduous teeth demonstrate a higher degree of enamel porosity<sup>59</sup> and a lower degree of mineralization<sup>60</sup> than permanent teeth. This was attributed to greater density of the interprismatic fraction and the prism-junction in deciduous enamel

than its permanent analogue.<sup>61</sup> This difference in porosity might contribute, at least in part, to the observed variation to the response to various protective agents. Other differences between deciduous and permanent tissues may also be of importance. For example deciduous enamel has a higher content of carbon dioxide and carbonate, as well as a lower content of phosphorous and calcium phosphate than the permanent tissue in its composition.<sup>29,62,63</sup> Primary enamel has less organized microcrystals<sup>64</sup> and a greater diffusion coefficient.<sup>28</sup> Furthermore, primary teeth possesses an aprismatic layer on its outer surface, which erodes in a highly irregular manner and is probably not as liable to erosive destruction when compared to prismatic enamel.<sup>24, 65</sup>

A second explanation to the significantly high anti-erosive effect of CPP-ACPF paste on permanent enamel and not on primary one may be the increased reactivity of the permanent teeth. In this study different primary and permanent substrates, at different developmental stages and with different post-eruptive ages were used. Thus, an "older" tooth that has been exposed to the oral environment and in contact with the acids and fluoride for longer periods of time during its life cycle than a newly erupted "young" tooth is expected to be more mineralized and more acid resistant.<sup>66, 67</sup> In the present study, exfoliated human primary molars and young premolars extracted for orthodontic purpose from children aged 12-14 years old were used. The exfoliated primary molar specimens had been exposed to the oral environment for a much longer period of time, adding more acid-resistant fluoridated crystals to its enamel's composition when compared to young permanent premolars. Thus it can be anticipated that it may differ than permanent teeth in its response to different remineralizing agents.

Our results that CPP-ACPF paste showed statistical significant low mean % SMHR as APF gel in the permanent teeth has shown that CPP-ACPF is a very promising remineralizing material. In study by Schupbach et al., 1996<sup>68</sup> it was demonstrated that CPP-ACPF could be incorporated into the pellicle in exchange for albumin and that it inhibits the adherence of *S. mutans* and *S. sobrinus*. Therefore, CPP-ACPF can be expected to be effective intraorally on both permanent and primary teeth in high-risk patients. The fact that this paste can be self-applied as recommended by the manufacturer renders it available in the oral cavity for longer periods, thus saliva will enhance the effectiveness of CPP-ACPF and the flavor will stimulate the saliva flow. The longer CPP-ACPF and saliva are maintained in the mouth, the more effective the expected results. Although our study could not simulate the complex

oral environment, it showed the potential of CPP-ACPF paste for reversing the harmful effect of a cola drink on tooth surfaces especially in permanent teeth. It is speculated that the effect of CPP-ACPF paste will be enhanced under oral conditions in the presence of biofilm which can bind to casein phosphopeptide and act as a reservoir for calcium and phosphate ions, thus enhance remineralization. It also neutralizes the acid challenges from acidogenic bacteria in plaque and from other external and internal acid source.<sup>23</sup>

### 5. Conclusion:

Under the conditions of this study, these conclusions can be derived:

1. All of the tested fluoride treatments positively reduced erosive enamel loss in both primary and permanent teeth.
2. In primary teeth only APF gel showed effective anti-erosive effect.
3. In permanent teeth both CPP-ACPF paste and APF gel showed effective protective effect against dental erosion.
4. Primary and permanent enamel substrates reacted differently to different fluoridated compounds.
5. CPP-ACPF paste is a promising remineralizing material.

### Recommendations:

Future studies are needed to determine which topical fluoride agent exerts the most protective anti-erosive effect intraorally.

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9/28/2010

## Diatoms of Tropical Eutrophic Lagoon

<sup>1</sup>Paul. Chuks. Onuoha, <sup>2</sup>Dike Ikeagwu Nwankwo and <sup>3</sup>Vyverman, Wim

<sup>1</sup>Department of Fisheries and Marine Biology, Federal College of Fisheries and Marine Technology, Bar-beach Victoria Island, Lagos Nigeria.

<sup>2</sup>Department of Marine Sciences University of Lagos, Akoka, Lagos, Nigeria

<sup>3</sup>Protistology and Aquatic Ecology Research Laboratory, University of Ghent, Belgium  
[hydro\\_vision@yahoo.com](mailto:hydro_vision@yahoo.com)

**Abstract:** The diatoms of Oloe lagoon for the first time were studied at monthly intervals for two years (February 2002-January 2004). A total of forty-eight species belonging to eighteen genera was found in diatoms, with pennate forms being more diverse and less abundance than the centric forms. *Aulacoseira granulata*, *A. granulata var. angustissima*, *A. granulata var. angustissima f. spiralis*, *A. granulata var. angustissima f. curvata*, *A. granulata var. muzzaensis*, *A. islandica* and *Cyclotella meneghiniana* were the more abundant and frequently occurring centric species throughout the study period. More frequently occurring pennate diatoms include: *Synedra ulna*, *Nitzschia closterium*, *Pinnularia major*, *Navicula oblonga*, *Cymbella minuta*, *Nitzschia palea*, *Surirella elegans* and *Gomphonema parvulum*. Rarely occurring diatoms at this station included *Biddulphia laevis*, *Melosira varians*, *Nitzschia accicularis*, *Pinnularia laevis*, *Cocconeis placentula* and *Eunotia gracilis*. In this study, six new diatoms species were recorded for Lagos lagoon complex. Community structure analysis shows a highly diverse environment.

[Paul Chuks Onuoha, Dike Ikeagwu Nwankwo and Vyverman, Wim. Diatoms of Tropical Eutrophic Lagoon. Journal of American Science 2010;6(11):452-456]. (ISSN: 1545-1003).

**Keywords:** diatom; Oloe lagoon; genera; Lagos lagoon complex; diverse environment

### INTRODUCTION

Diatoms are valuable indicators of environmental conditions, since they respond directly and sensitively to many physical, chemical and biological changes that occur in the aquatic environment. Among unicellular microalgae, diatoms probably represent one of the most diverse groups, with a number of species estimated to be between 10000 and 100000 (Battarbee et al, 1999) hence, they constitute an ideal group to study its biodiversity. The diversity, abundance and distribution of phytoplankton within any lagoon have a direct correlation with the water quality and consequently the whole community structure. This is due to the fact that phytoplankton forms the base of any aquatic food chain and organic production in the lagoon and coastal ecosystem (Carson and Pan 1999). According to Van Den et al 1998, the composition of diatom communities reflects an entire complex of ecological parameters at a particular site. Lagoon and coastal ecosystems are the most productive zone of any marine environment due to the high anthropogenic inputs and shallowness of this zone which allows effective light penetration for photosynthesis by phytoplankton. The bulk of local fish production comes from the artisanal sector operating within this zone in Nigeria. Yet, the lagoonal environments are being highly influenced by ecological factors and human actions mainly from refuse and sewage dump,

as well as agricultural wastes coming from river discharges and industrial effluents. The Nigerian coastal zone experiences a tropical climate consisting of rainy season (April to October) and dry season (November to March), it is low lying with heights of not more than 3.0 m above sea level and is generally covered by fresh water swamp, mangrove swamp, lagoonal marshes, tidal channels, beach ridges and sand bars. Lorhurst (1964) reported that the Nigerian coastal surface water is uniformly warm (about 28°C) and of low salinity (<32%). The vegetation is also characterized by Mangrove forests, brackish swamp forests and Rain forests. At present, there is no such checklist for Oloe lagoon diatoms hence this study lists diatoms species of this lagoon and also mentioned the species that is first record to Nigeria coastal waters.

### DESCRIPTION OF STUDY AREA

Oloe lagoon (Fig. 1) is an expanse of shallow freshwater which extends between Lagos and Ogun States. It is presumably the smallest of the lagoons in South Western Nigeria with a surface area of 9.4km<sup>2</sup>, and lies at the distal end of Badagry creek between longitudes 6° 26'N to 6° 30'N and latitudes 3° 01'E to 3° 07'E. The main body of the lagoon lies within Badagry Local Government Area and it opens up to the Atlantic ocean via the Lagos Harbour and Dahomey in the Republic of Benin. The major

source of water are River Owo with a source in a town called Toto Owo where River Ore and Illo form a confluent with River Oponu in Ogun State (Akanni, 1992). Seventeen stations were chosen for sampling within the lagoon. The lagoon is shallow at most points and is open all year round via the Lagos harbour to the sea (Hill and Webb, 1958; Sandison, 1966; Sandison and Hill, 1966). Like all parts of South-western Nigeria, the Ologe lagoon is exposed to two distinct seasons namely the wet (May – October) and the dry (November – April) (Nwankwo, 2004b). Like all parts of South-western Nigeria, the Ologe lagoon is exposed to two distinct seasons namely the wet (May – October) and the dry season (November – April) (Nwankwo, 2004b; Sandison and Hill, 1966). The harmattan, a short season of dry, dusty North-East Trade winds experienced sometimes between November and January in the

region reducing visibility and lowering assemblages is the common macrofloral assemblages especially in areas with reduced anthropogenic influence. The lagoon deposits are varied, and are reflected in the pattern and type of vegetation in the region. Most parts of the Ologe lagoon are colonized by recognizable riparian dense swamp rainforest community dominated by raphia palms especially *Raphia hookeri*, *Elaeis guineensis*, *Acrotiscum aureum* and *Cocos nucifera* (Nwankwo, et al 1999). Very few mangrove communities are recognizable around the Badagry creek end. Notable fauna of the area includes amphipods, Oligochaetes, few polychaetes, isopods, barnacles, oysters, periwinkle, nematodes, fiddler crabs, crabs, among others (Sandison and Hill, 1966; Onyema, et al 2007). The mainstay of communities that live around this environment is artisanal fishing.

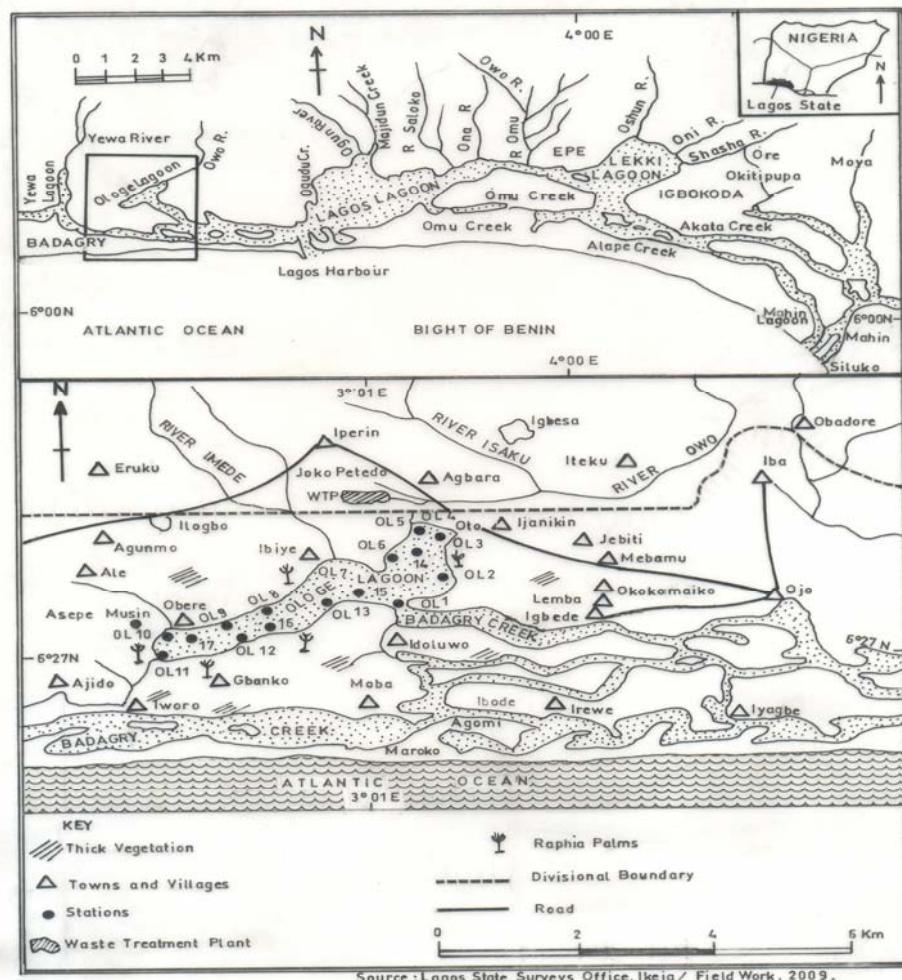


Fig 1: Ologe lagoon showing sampling stations

## MATERIALS AND METHODS

A motorized boat and a Global Positioning System (GPS) were used in seventeen sampling stations during the 24 months sampling period (February 2002-January 2004). These stations were chosen to reflect differences in environmental gradients which exist in the same body of water. All samples were taken using standard plankton net of 55 µm mesh size towed steadily for 10 min at low speed and preserved in 4% unbuffered formalin in appropriately labeled plastic container.

All samples were collected during the hours of daylight to minimize variations due to diurnal migration. To enhance diatom identification sub samples of the original samples were acid-cleaned using nitric acid and investigation was made using Olympus BX51 photomicroscope at the Protistology and Aquatic Ecology Research Laboratory, University of Ghent, Belgium. Taxonomic keys employed in the identification included Hustedt (1930, 1937, 1942, 1971), Patrick and Reimer (1966, 1975), Prescott (1973, 1982), Round (1991), and Van-Den et al (1998).

## RESULTS

As shown in table 1, out of a total of forty-five diatom taxa belonging to eighteen genera were observed in this study, twenty-nine were pennates while sixteen are centric forms. Species indicated in asterisk are first reports for Lagos lagoon complex.

**Table (1): A checklist of diatom species of the Ologe lagoon**

DIVISION: BACILLARIOPHYTA

CLASS: BACILLARIOPHYCEAE

ORDER 1: CENTRALES

*Aulacoseira granulata* (Ehr.) Sim.

*A. granulata* var.*angustissima* (O.F.Muller) Sim.

*A. granulata* var.*angustissima* f.*spiralis* Hust.

*A. granulata* var.*angustissima* f.*curvata* (Hust.) Sim.

*A. granulata* var.*muzzaensis* (Meist.) Hust

*A. islandica* (O. Muller)

*Stephanocyclus* sp

*Cyclotella meneghiniana* (Kutzing)

*C. striata* (Kutz.) Grunow

*C. stelligera* Cleve ex Grunow

*Coscinodiscus centralis* Ehrenberg

*C. eccentricus* Ehrenberg

*Melosira varians* Agardh

*Actinoptychus* sp

*Biddulphia laevis* Ehrenberg

*Terpsinoe musica* Ehrenberg

ORDER 11: PENNALES

*Synedra ulna* (Nitzschia) Ehr

*S. acus* Kutzing

*Nitzschia palea* (Kutz) W.M.Smith

*N. closterium* (Ehr.) W.M.Smith

*N. acicularis* (Kutz.) W.M.Smith

*N. vermicularis* Hantzsch

*Pinnularia major* (Kutz.) Cleve

*P. interrupta* W.M.Smith

*P. laevis* (Ehr.) Compere

*P. hemiptera* (Kutz.) Rabenh.

*P. ambigua* Cleve

*Pinnularia* sp

*Navicula oblonga* Ehrenberg

*N. radiosa* Kutzing

*N. gracilis* Ehrenberg

*N. mutica* Kutzing

N. *cuspidata* Meist  
*Cocconeis placentula* (Ehr.) Cleve  
 C. *Disculum* (Schum) Cleve  
*Epithemia* sp  
*Cymbella affinis* Kutzing  
 C. *minuta* Hisle ex.Rabenh  
*Eunotia gracilis* Meister  
 E.*lunar*is (Ehr.) Grunow  
 E.*monodon* Ehrenberg  
*Surirella elegans* Ehrenberg  
 S. *ovata* Kutzing  
*Fragilaria construens* Ehrenberg  
*Gomphonema parvulum* Kutzing

## DISCUSSION

The number of diatoms taxa (45) observed in Ologe lagoon is very low compared to other published study from other lagoons and rivers so far. This checklist is the first that will record such low number of diatoms possibly due to high eutrophication in the lagoon. The pennales recorded higher number of taxa followed by centrals. This is in conformity with the species composition and phytoplankton abundance density found for some diatom species of Lagos lagoon (Nwankwo, 1990), some coastal waters of Nigeria (Kadiri, 1999) and Bonny River during complex research study concerning ecosystem of Niger Delta (RPI Report, 1985). The pennales were more prevalent and could be as a result of rainfall which introduces flooding thereby mixing up the water, boat navigation since artisanal fishing is the mainstay of the people living around the area or may be due to their possession of raphe with which they adhere to suitable substrate. From the checklist it can be stated that Ologe lagoon was dominated by single floristic grouping with *Aulacoseira granulata* and its varieties being more abundant species all through the stations.

### Correspondence to:

Dr Onuoha Chuks Paul  
 Department of Fisheries and Marine Biology, Federal College of Fisheries and Marine Technology, Barbeach Victoria Island, Lagos Nigeria  
 Mobile Phone: 234-8023011048  
 E-mail: [hydro\\_vision@yahoo.com](mailto:hydro_vision@yahoo.com)

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8/21/2010

# Can Dermatoglyphics be used as an Anatomical Marker in Egyptian Rheumatoid Patients?

Hanan M. Elsaadany<sup>1</sup>, Elham Kassem<sup>1</sup>, Mervat El-Sergany<sup>\*1</sup> and Abdel -Razek A. Sheta<sup>2</sup>,

<sup>1</sup>Rheumatology & Rehabilitation, <sup>2</sup>Anatomy Departments, Faculty of Medicine, Tanta University, Tanta, Egypt  
<sup>\*</sup>elahm77@hotmail.com

**Abstract:** Background/aim: Rheumatoid arthritis (RA) is supposed to be influenced by genetic and environmental factors and so also dermatoglyphics. Therefore, the present study was undertaken to find out a possible correlation of some quantitative and qualitative dermatoglyphic variables with rheumatoid arthritis (RA) and its radiological grading. Materials and methods: This study was conducted on 60 clinically confirmed RA patients and an equal number of controls. Different qualitative dermatoglyphic patterns (ulnar& radial loops, whorls and arches) and quantitative dermatoglyphic measures (total finger ridge count, pattern intensity and a-b ridge count) in addition to palmar creases were studied on rheumatoid arthritis patients and controls. Comparison between patients and controls in both sexes was done and recorded. Also, correlation between significant dermatoglyphic changes in RA patients and radiological changes were studied. Results: Loops were the most common type of the qualitative dermatoglyphic patterns of the fingers, followed by whorls then arches. In both male and female patients, there was significant marked decrease in ulnar loops and significant increase in arches. Total ridge count and pattern intensity of patients were decreased in both hands of both sexes; however, this decrease was significant in the left hand of males and right hand of females. Moreover, the a-b ridge count was significantly decreased in both hands of female and left hand of male patients. Regarding the unusual palmer flexion creases, there was significant increase only in the Sydney line in female right hands. Significant inverse correlation was noted between total ridge count of the fingers and the radiological erosion in both males and females. Conclusion: The findings of the present work demonstrate the association between some dermatoglyphic patterns and RA suggesting that dermatoglyphics can represent an anatomical, non-invasive, inexpensive tool for screening high-risk population, and thus facilitate early detection and management. Also the relationship between total ridge count and the aggressive type of RA indicate that this dermatoglyphic variable might play a significant role not only for screening but also for studying the behavior of the disease.

[Hanan M. Elsaadany, Elham Kassem, Mervat El-Sergany and Abdel-Razek A. Sheta. Can Dermatoglyphics be used as an Anatomical Marker in Egyptian Rheumatoid Patients. Journal of American Science 2010;6(11):457-466]. (ISSN: 1545-1003).

**Keywords:** dermatoglyphics, fingerprints, rheumatoid arthritis.

## 1. Introduction:

Rheumatoid arthritis is a chronic inflammatory disease that predominantly manifests as persistent synovial inflammation of peripheral joints. Severity and prognosis of RA are influenced by a variety of demographic factors, such as race, gender, age, profession and educational level (Landewe, 2007).

The clinical course of Rheumatoid arthritis (RA) fluctuates and its prognosis is difficult to predict. In many patients the disease was severe resulting in progressive joint erosion, destruction and severe disability which are not capable of recovery (Dubucquoi et al., 2004). Early treatment with the currently available anti-rheumatic drugs may stop or delay such erosions. To avoid the potentially serious side effects, diagnostic tests of high specificity for RA are desirable (De Vries Bouwstra et al., 2005).

The prognostic markers that could identify patients with the aggressive rapidly progressing type of the disease would help in early diagnosis and treatment. They would also protect patients with less aggressive disease from possible over treatment and toxicities (Landewe, 2007).

Dermatoglyphics came from ancient Greek derma (skin) and glyph (Carving). It is the scientific study of the pattern configurations of finger and palm prints. They have a significant genetic component and its development begins during the second month of intrauterine life. They also reflect the non genetic environment of early pregnancy, an important time window for tissue differentiation and organogenesis. Fingerprints and the number of epidermal ridges observed in postnatal life provide a measure of fingertip growth activity during the early fetal period and may be useful in the study of metabolic or

anatomic programming related to the early prenatal environment. Also, the presence of abnormalities in dermatoglyphics constitutes an evidence of a prenatal insult that has occurred in early prenatal life (Zhou et al., 2002 & Kahn et al., 2008).

The finger and palmar print patterns have already been studied with respect to various genetic diseases like Down, Turner and Klinefelter syndromes (Richards and Mandasescu, 1997 & Kobyliansky et al., 1999). In one study, dermatoglyphics were used as markers of early prenatal stress in children with idiopathic intellectual disability (Rosa et al., 2001). Also, several studies have reported its alterations in gastric cancer (Zivanović et al., 2003), bronchial asthma (Gupta and Prakash, 2003), schizophrenia (Chok et al., 2005), open angle glaucoma (Novak-Laus et al., 2005) and breast cancer (Chintamani et al., 2007).

Few studies are available on the association between dermatoglyphics and RA. Both genetic and environmental factors can contribute to the susceptibility and severity of RA disease (Reveille, 1998). Since ridge patterns are formed early in fetal development and remain unchanged throughout life, unusual dermatoglyphics may indicate gene or chromosomal abnormalities consistent with a disease such as RA (Rajangam et al., 2008).

Therefore, the present study was undertaken to find out a possible correlation of some quantitative and qualitative dermatoglyphic variables with rheumatoid arthritis (RA) and its radiological grading.

## 2. Materials and methods

This study was carried out on 60 RA patients diagnosed according to the American College of Rheumatology (ACR) revised criteria for the classification of RA (Arnet et al., 1988). They were 12 males and 48 females and their ages ranged from 23-63 years with a mean of  $41.3 \pm 8.2$ . The duration of the disease ranged from 5 months to 10 years with a mean of  $4.2 \pm 2.3$  years. These patients were recruited from the attending outpatient clinic of the Physical Medicine and Rheumatology of Tanta University Hospital in addition to 60 apparently healthy persons (12 males and 48 females) that had no self or familial history of RA and served as a control group. It may be noted that, for any study on dermatoglyphics, age similarity may not be required because the dermatoglyphics, once formed usually do not change, unless affected by occupational hazards. Consent was taken from all participants and detailed clinical examination was done to exclude patients with congenital diseases, hand burn, deformity or injury.

The following parameters were obtained: different patterns of finger tips (finger prints), total finger ridge count (number of epidermal ridges), pattern intensity (total number of tri-radial of the fingers), types of palmar creases and the a-b ridge count of the palm (number of ridges between the bases of index and middle fingers). The prints of all participants were taken by the ink and paper method. The ends of the fingers were inked and then pressed or rolled one by one on a glossy paper. Also, the palm of clean dried hands were smeared with the ink and pressed against the paper to show palmar creases and the interdigital areas. The prints were then scanned and analyzed. Counting of palmar and finger ridges were done by magnification of the prints by computer or a magnifying hand lens. All parameters were studied on both hands together and separately. Comparison between patients and controls in both males and females was also done.

### Dermatoglyphic Patterns:

Fingerprint patterns in the present study included three main types: whorls, loops, and arches. Under these major types, other subtypes were also noticed in the present study and considered normal anatomical variations (Figs. 1, 2, 3). The whorl is distinguished by its concentric design. The majority of the ridges make circuits around the core (Fig. 1). In arches, the dermal ridges pass from one margin of the digit to the other with a gentle, distally bowed sweep (Fig. 2). In loops, the ridges curve around only one extremity of the pattern forming the head of the loop. Loops can be further divided into ulnar and radial types: ulnar loop when the loop opens to the ulnar margin of the hand and radial loop when the loop opens to the radial margin (Fig. 3).

A triradius is located at the meeting point of three opposing ridge system (Fig 4). This marks the edge of the loop pattern as it possesses only one triradius. In arch pattern, there is no triradius while whorls usually have two triradii.

**Ridge Count of the fingers:** After locating the triradii and the core, as the outer and inner points of the count, a line is set in position to connect them (Fig. 3). Ridge counts for each fingertip were calculated from the number of primary epidermal ridges that intersected or touched the straight line drawn from the central core of the fingerprint pattern to the bifurcation of the triradius. The count on the ten fingers of each individual is then summed up to give a single value, the total ridge count. Consistent with standard methods, fingertips with an arch pattern were assigned a ridge count of zero. Fingertips with a loop pattern have a ridge count equal to the number of ridges crossing the single straight line (Fig. 3). For fingertip patterns with two triradial points e.g.,

whorls, the following ridge-counting protocol was used according to Kahn et al., (2001) ridge count = ridges crossing the longer line + half of ridges crossing the shorter line (Fig. 3).

The a-b ridge count is a measure of the size of the second interdigital area of the palm of the hand, between the bases of the index and middle fingers. It is made by the count of the number of ridges between the triradius a, at the base of the index finger, and the triradius b, at the base of the middle finger (Fig. 4).

**Pattern Intensity:** Pattern intensity refers to the complexity of ridge configurations. It can be expressed by counting the number of triradii present. According to the number of triradii, a digit can have a pattern intensity that ranges from zero to two. Considering the two hands together, the number of triradii in all ten fingers of an individual ranges from zero to twenty. The simple arch, which lacks a triradius, is assigned the number 0, the tented arch and the loop are both assigned 1, as each has one triradius and the whorls typically possess two triradii (Figs.1,2,3).

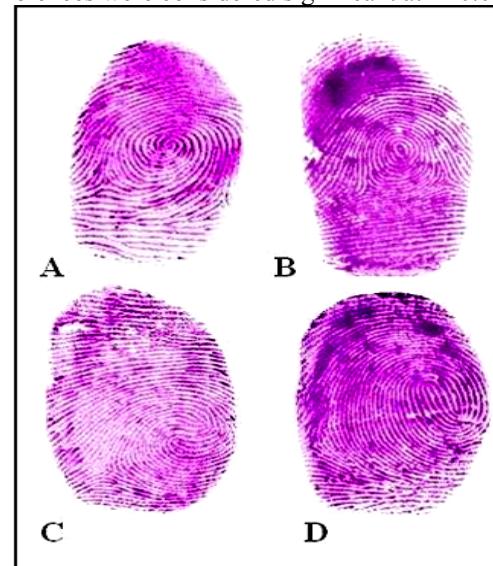
**Flexion creases:** Normally there are three main flexion creases in the palm of the hand, distal transverse, proximal transverse and longitudinal. The abnormal palmer flexion creases considered in the present study were the Simian line, partial Simian line and the Sydney line (Figs. 5,6). In Simian Line, the two distal horizontal creases are fused to form a single horizontal crease. Sometimes there will be traces of the original lines which attempt to fuse as they move across the palm but instead of creating a fully formed Simian, they form what is called partial simian line. Sydney line means the continuation of the proximal transverse crease until the ulnar border of the palm. Interpretation of all parameters was done according to the previous dermatoglyphic studies of Rosa et al., (2001); Zhou et al., (2002); Ravindranath et al., (2003); Corona Rivera et al., (2005) and Karmakara et al., (2008).

**Radiological assessment:** Postero-anterior radiographs of the hands of the patients were obtained and the degree of RA progression was assessed according to Larsen scoring (Larsen, 1995).

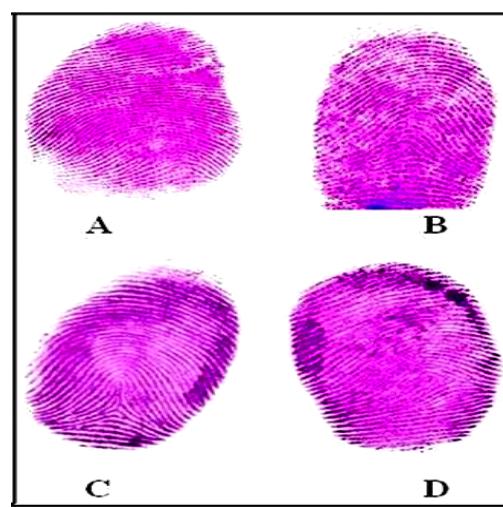
#### Statistical Analysis:

The collected data were organized, tabulated and statistically analyzed using SPSS software statistical computer package, version 12. The differences of qualitative data of finger tips (whorls, loops and arches) and that of the quantitative data (total finger ridge count, pattern intensity and a-b ridge count) as well as palmar creases were recorded. As regards to the qualitative data, the number and percentage of distribution were calculated. For quantitative data, the range, mean and standard

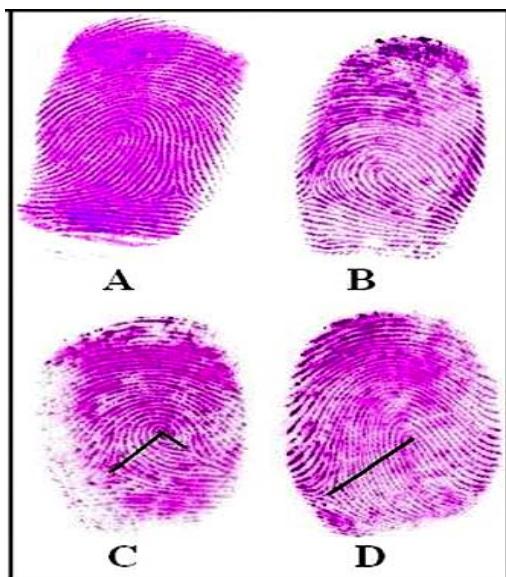
deviation (SD) were measured. In addition, the correlation between radiographic erosion and the significant dermatoglyphic variables detected in this study was evaluated by Pearson's correlation coefficient. The difference between two means was statistically analyzed using the student t-test and the differences were considered significant at  $P<0.05$ .



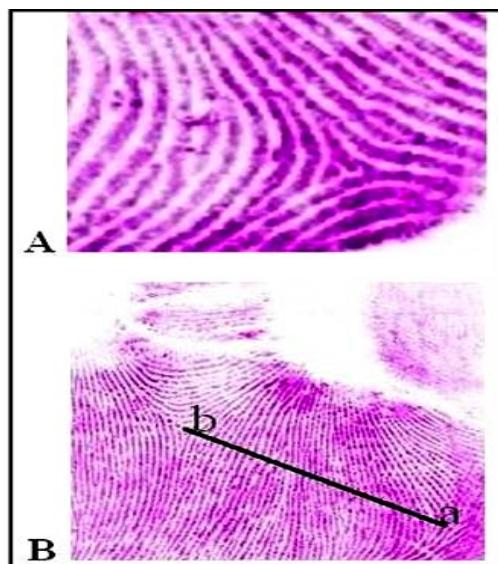
**Fig. (1):** A photograph showing the different types of whorls observed in the present study.  
**(A) Shell or snail whorl (B) plain or simple whorl  
(C) Double loop whorl (D) Elongated whorl**



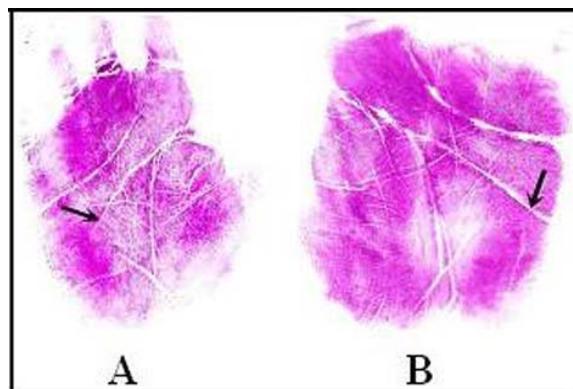
**Fig. (2):** A photograph showing the different types of arches observed in the present study.  
**(A) Simple arch. (B) High arch.  
(C) Tented arch. (D) Arch with a loop.**



**Fig. (3):** A photograph showing: (A) Ulnar loop. (B) Radial loop. (C) Measurement of total ridge count in whorl pattern. Notice the long line between the core of the whorl and the triradius, and the short line between the core and the second triradius. (D) Measurement of total ridge count in a loop pattern. Notice the presence of only one line between the core of the loop and the only one triradius.



**Fig. (4):** A photograph showing: (A) Magnification of the triradius. (B) Measurement of a-b ridge count. Notice the line between the triradius a at the base of index finger and triradius b at the base of the middle finger.



**Fig. (5):** A photograph showing: (A) Normal palmar flexion creases. Notice the proximal palmar crease (arrow). (B) Sydney line. Notice the continuation of the proximal palmar crease until the ulnar border of the palm (arrow).

### 3. Results

In the present study, loops were the most common type of finger ridge patterns, accounting for 48.5% of finger prints of normal individuals, followed by whorls (40.83%), and lastly the arches (10.67%).

The distribution of finger ridge patterns in patients and controls are shown in tables (1, 2). It was found that ulnar loops were significantly decreased in both hands of male and female patients. In males, the P value was 0.021 in right hand and 0.004 in left hand, while in females P value was 0.011 in right hand and 0.001 in left hand. The radial loops showed non significant increase in male left hand ( $P= 0.863$ ) and non significant decrease in female right and left hands ( $P= 0.0352, 0.896$ ) respectively. Arches were significantly increased in both right and left hands of males ( $P=0.009, 0.007$ ) respectively and of females ( $P= 0.003, 0.028$ ) respectively. As regards to whorls, non significant decrease was noticed in male right and left hands ( $P= 0.078, 0.089$ ) respectively, while in females there was non significant decrease in right hand ( $P= 0.0236$ ) and non significant increase in the left hand ( $P= 0.058$ ). (Tables 1, 2).

The total ridge count of fingers is presented in table 3. The mean ridge count of the RA patients was decreased in both hands of males and females when compared to the controls. This decrease was statistically significant in the left hand of males ( $P= 0.002$ ) and right hand of females ( $P= 0.001$ ).

The main pattern intensity (number of triradii in finger tips) included in the present study is shown in table 4. It was decreased in RA patients in both males and females. It was statistically significant in left hand of males ( $P= 0.019$ ) and in right hand of females ( $P= 0.012$ ).

The a-b ridge count (number of ridges between the triradii a&b at the bases of index and middle fingers) is considered in table 5. The mean a-b ridge count of RA patients was decreased in both hands of males and females in comparison to the control. This decrease was statistically significant in left hand of males ( $P= 0.002$ ) and both hands of females ( $P= 0.001, 0.001$ ).

The distribution of unusual palmar flexion creases in the RA patients and controls is shown in tables 6,7. The most frequent unusual creases were

Sydney line, Simian and partial Simian creases. The only significant increase was in the Sydney line in female right hands ( $P=0.050$ ).

Comparison between patients with radiological erosion detected by x-ray and the significant dermatoglyphic variables observed in this study was done. Considering both hands together, significant inverse correlation was noted between total ridge count of the fingers and radiological erosion in both males and females ( $P=0.023, 0.039$ ) respectively (Tables 8, 9 and figures 7,8).

**Table 1: Pattern distribution on fingertips of males included in the study:**

Type of pattern	Control (n=12)		RA (n=12)		P1	P2
	Right hand	Left hand	Right hand	Left hand		
<b>Whorls</b>	<b>28</b>	<b>24</b>	<b>24</b>	<b>20</b>	<b>0.078</b>	<b>0.089</b>
<b>Radial Loop</b>	<b>2</b>	<b>2</b>	<b>2</b>	<b>3</b>	<b>0.963</b>	<b>0.863</b>
<b>Ulnar Loop</b>	<b>27</b>	<b>27</b>	<b>16</b>	<b>6</b>	<b>0.021*</b>	<b>0.004*</b>
<b>Arches</b>	<b>5</b>	<b>5</b>	<b>19</b>	<b>31</b>	<b>0.009*</b>	<b>0.007*</b>

\* significant P1 comparison between right hands of control and RA patients

P2 comparison between left hands of control and RA patients.

**Table 2: Pattern distribution on fingertips of females included in the study:**

Type of pattern	Control (n=48)		RA (n=48)		P1	P2
	Right hand	Left hand	Right hand	Left hand		
<b>Whorls</b>	<b>99</b>	<b>94</b>	<b>97</b>	<b>102</b>	<b>0.236</b>	<b>0.058</b>
<b>Radial Loop</b>	<b>27</b>	<b>13</b>	<b>23</b>	<b>12</b>	<b>0.352</b>	<b>0.896</b>
<b>Ulnar Loop</b>	<b>90</b>	<b>103</b>	<b>60</b>	<b>51</b>	<b>0.011*</b>	<b>0.001*</b>
<b>Arches</b>	<b>20</b>	<b>34</b>	<b>63</b>	<b>72</b>	<b>0.003*</b>	<b>0.028*</b>

\* significant P1 comparison between right hands of the control females and RA females patients

P2 comparison between left hands of the control females and RA females patients

**Table 3: Total finger ridge count of males and females included in the study:**

	Control (n=60)		RA (n=60)		P1	P2
	Right hand	Left hand	Right hand	Left hand		
<b>Males</b>						
Range	<b>48- 96</b>		<b>50-114</b>		<b>44-72</b>	
Mean $\pm$ SD	<b>81.85<math>\pm</math>10.45</b>		<b>81.33<math>\pm</math>18.07</b>		<b>32-61</b>	
					<b>0.875</b>	<b>0.002*</b>
<b>Females</b>						
Range	<b>45-93</b>		<b>48-95</b>		<b>31-64</b>	
Mean $\pm$ SD	<b>82.87<math>\pm</math>10.64</b>		<b>85.12<math>\pm</math>9.15</b>		<b>41-65</b>	
					<b>0.001*</b>	<b>0.587</b>

\* significant P1 comparison between right hands (male & female) of control and RA patients

P2 comparison between left hands (male & female)of control and RA patients.

**Table 4: Main pattern intensity of males and females included in the study:**

	Control (n=60)		RA(n=60)		P1	P2
	Right hand	Left hand	Right hand	Left hand		
<b>Males:</b>						
Range	5-14	5-12	4-9	3-5	0.445	0.019*
Mean $\pm$ SD	7 $\pm$ 1.32	6.41 $\pm$ 0.76	6.58 $\pm$ 1.32	4.08 $\pm$ 0.425		
<b>Females:</b>						
Range	4-15	4-13	3-8	4-7	0.012*	0.263
Mean $\pm$ SD	6.56 $\pm$ 0.95	6.33 $\pm$ 0.83	5.77 $\pm$ 0.73	6.18 $\pm$ 0.91		

\* significant P1 comparison between right hands (male & female ) of the control and RA patients  
 P2 comparison between left hands (male & female) of control and RA patients.

**Table 5: a-b ridge count of males and females included in the study:**

	Control (n=60)		RA(n=60)		P1	P2
	Right hand	Left hand	Right hand	Left hand		
<b>Males</b>						
Range	30-42	30-45	29- 40	28-35	0.095	0.002*
Mean $\pm$ SD	37.66 $\pm$ 3.98	38.75 $\pm$ 4.33	34.83 $\pm$ 3.9	32 $\pm$ 2.86		
<b>Females</b>						
Range	38-50	36-47	29-36	31-40	0.001*	0.001*
Mean $\pm$ SD	44.66 $\pm$ 4.33	41.2 $\pm$ 3.38	32.77 $\pm$ 2.46	37.68 $\pm$ 2.56		

\* significant P1 comparison between right hands(male & female ) of control and RA patients  
 P2 comparison between left hands(male & female ) of control and RA patients.

**Table 6: Unusual palmar creases of males included in the study:**

Patterns of palmar creases	Control (n=12)		RA (n=12)		P1	P2
	Right hand	Left hand	Right hand	Left hand		
Partial simian	1	0	1	1	0.963	-
Complete simian	0	1	1	1	-	0.963
Sydney line	1	0	2	0	0.756	-

\* significant P1 comparison between right hands of controls and RA patients  
 P2 comparison between left hands of controls and RA patients.

**Table 7: Unusual palmar creases of females included in the study:**

Patterns of palmar creases	Control (n=48)		RA (n=48)		P1	P2
	Right hand	Left hand	Right hand	Left hand		
Partial simian	1	0	1	1	0.963	-
Complete simian	0	0	1	0	-	-
Sydney line	1	2	3	2	0.050*	0.865

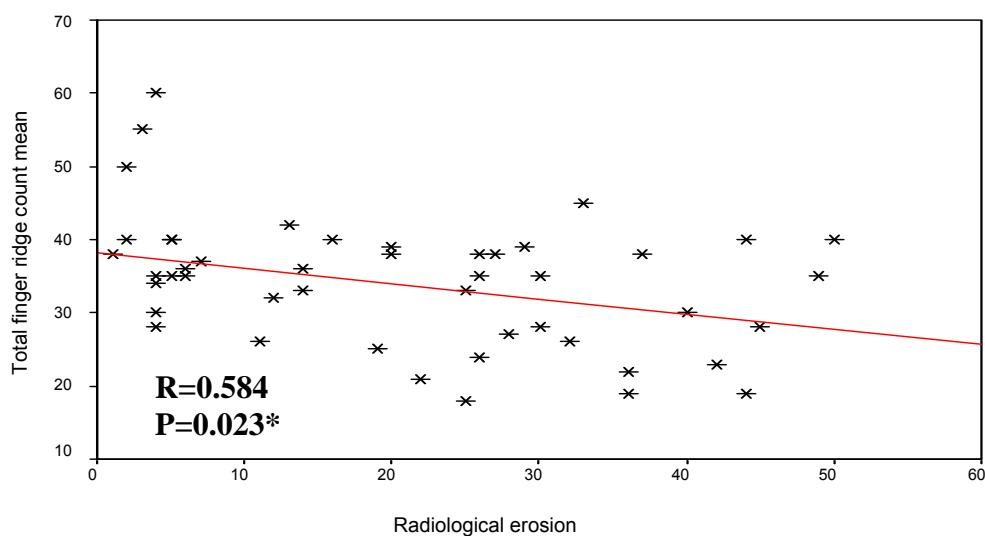
\* significant P1 comparison between right hands of the female controls and RA female patients  
 P2 comparison between left hands of the female controls and RA female patients

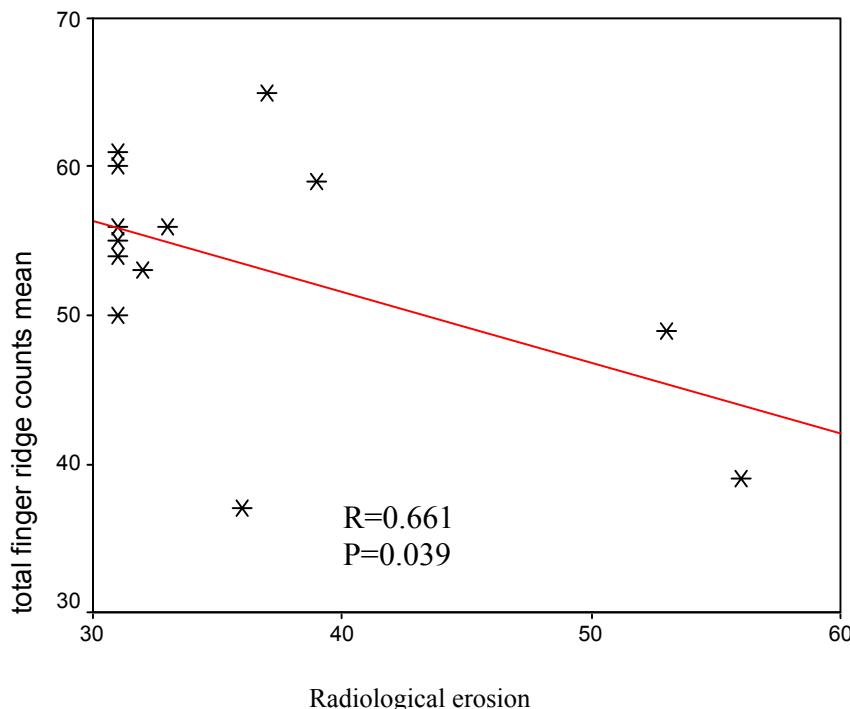
**Table 8:** Pearson's correlation coefficient between RA male patients with radiological erosion and significant dermatoglyphic variables.

Variable	Radiological erosion	
	R	p
Ulnar Loop	-0.167	0.219
Arches	-0.042	0.243
Total finger ridge count mean	-0.584	0.023*
a-b ridge count mean	-0.144	0.343
Pattern intensity mean	0.104	0.619

**Table 9:** Pearson's correlation coefficient between RA female patients with radiological erosion and significant dermatoglyphic variables.

Variable	Radiological erosion	
	R	p
Ulnar Loop	-0.132	0.668
Arches	-0.128	0.677
Total finger ridge count mean	-0.661	0.039*
a-b ridge count mean	-0.007	0.983
Pattern intensity mean	-0.125	0.684

**Fig. (7):** Correlation between RA male patients with radiological erosion and total finger ridge count mean.



**Fig. (8): Correlation between RA female patients with radiological erosion and total finger ridge count mean.**

#### Corresponding author

Mervat El-Sergany

Anatomy Departments, Faculty of Medicine, Tanta University, Tanta, Egypt.

[elahm77@hotmail.com](mailto:elahm77@hotmail.com)

#### 4. DISCUSSION

Different qualitative dermatoglyphic patterns were studied in this work. It was noticed that loops were the most common type observed in normal individuals followed by whorls, and lastly arches. Similarly, *Igbigbi and Msamati (2002)* demonstrated that ulnar loops were the most predominant digital pattern type in both sexes followed by whorls in males and arches in females. Their study documented similarities in digital ridge patterns between some African countries indicating their close historical and anthropological relationship. However, significant differences were demonstrated between them and Europeans. These results emphasize that digital patterns are specific in differentiating ethnic and population groups.

In the present work, ulnar loops were significantly decreased in both hands of both sexes of RA patients compared with controls, however, radial loops showed non significant changes. Going in line with these results, *Taneja et al., (1993)* reported a significant decrease in ulnar loops in the right hand of females and both hands of male patients with RA.

Radial loops showed also significant decrease in both hands of male patients. In another study conducted on finger print patterns on individual fingers of RA patients, loops were especially decreased in the 3rd finger of males and in the 1st and 4th fingers of females (*Ravindranath et al., 2003*).

In the present work, arches were significantly increased in both hands of male and female patients. This finding was partially coincided with *Taneja et al., (1993)* who reported an increase in arches on both hands of females and left hand only of males. Also, *Ravindranath et al., (2003)* noted that with both hands together, arches were significantly increased in male patients but female patients showed significant increase only in some individual fingers (3<sup>rd</sup> and 4<sup>th</sup> fingers). *Todd et al., (2006)* mentioned that the embryologic timing of the ridge pattern formation was associated with the type of dermatoglyphic pattern development. Early ridge formation was associated with whorls, late formation with arches, and intermediate formation with loops. They suggested that clinical syndromes which arrested embryologic development and decreased developmental maturation tend to have more arches and fewer whorls.

Regarding whorls, non significant change was noticed in the present work, not only in females but also in male patients. In a previous study,

frequency of whorls was reported to be increased on the right hand of male, and both hands of female RA patients (*Taneja et al.*, 1993). This was in contrast to the study conducted by *Ravindranath et al.*, (2003) on RA who found significant decrease in whorls in both hands of males and non significant change in females.

The main pattern intensity (number of triradii in all fingers) was also considered for correlation in the present study. It was decreased in RA patients than controls in both sexes. However, it was significant in left hand of males and right hand of females. Reviewing the available literature, the relation between pattern intensity and RA disease was not reported. The decreased number of triradii (pattern intensity) observed in this study can be explained by the decreased whorls (each whorl has two triradius) and significant increase in arches (the arch has no triradius) noticed in RA patients.

Although *Taneja et al.*, (1993) did not observe any difference between RA patients and controls regarding the total finger ridge count, the results of *Rajangam et al.*, (2008) showed a significant increase of the total ridge count only in the right hand of male patients. In the present study total finger ridge count was decreased in patients as compared to controls and this decrease was statistically significant in left hand of males and right hand of females.

The mean a-b ridge count of the palm of RA patients was also decreased in both hands of males and females when compared to the controls. This finding don't coincides neither with *Taneja et al.*, (1993) who did not observe any significant difference between patients of rheumatoid arthritis and controls for the a-b ridge count, nor with *Rajangam et al.*, (2008) who noted a significant increase in the a-b ridge count in both male and female patients.

It was suggested that the a-b ridge count is more environmentally determined and less heritable than other dermatoglyphic traits such as finger tips patterns. The a-b ridge count is sensitive to environmental stress because the area of the palm in which a-b ridge count is situated, the second interdigital region, begins to develop earlier than the fingers and its progression is more slowly. Thus the ridges in this region may develop over a longer period of time, exposing the area to potential environmental insult. On the other hand, total ridge count appears to be under relatively strong genetic control and little influenced by environmental factors (*Fearon et al.*, 2001). In the present work, the significant decrease in both a-b ridge count and total finger ridge count in RA patients confirm that RA disease is influenced by both genetic and environmental factors.

The distribution of unusual palmer flexion creases in patients and controls was also studied in this work. Sydney line, Simian and partial Simian creases were the most frequent creases observed, however, significant increase was noticed only in Sydney line of female patients right hand. In the study conducted by *Ravindranath et al.*, (2003), partial Simian crease was the only significantly increased unusual pattern in male patients, while Sydney line was absent in both males and females.

In the field of anthropology, researchers found that there are divergences of different degrees between races, nationalities, and even populations. Diagnosis on individuals of different nationalities cannot use the same standards (*Hui et al.*, 2003). Significant correlation between dermatoglyphics and geographic distances was also noticed by *Natekar et al.*, (2006). This may explain the presence of variations between different results in this field. Dermatoglyphics by themself are not enough to diagnose RA disease but the results of this work suggest that dermatoglyphics can at least serve to strengthen the diagnostic impression about RA disease especially in unidentified patients or those with early signs of joint inflammation.

Dermatoglyphic deviations observed in the present work were tested for correlation with radiologic diagnosis. The mean total finger ridge count showed significant inverse correlation with radiographic erosion. This finding indicates that this specific dermatoglyphic variable may help to predict the aggressive type of RA disease. Moreover, the significant correlation between radiographic erosion and total ridge count observed in this work together with the suggestion of *Fearon et al.*, (2001) that total ridge count appears to be under relatively strong genetic control and little influenced by environmental factors lead us to the suggestion that erosive changes in RA was more likely to be due to genetic causes. So, it is possible to estimate the individual predisposition to this aggressive type of the disease and thus to guide therapy decisions early to attenuate cumulative inflammation as soon as symptoms appear.

## 5. Conclusion:

The findings of the present work demonstrate the association between some dermatoglyphic patterns and RA disease suggesting that dermatoglyphics can represent an anatomical, non-invasive, inexpensive tool for screening high-risk population, and thus facilitate early detection and management. Also the relationship between total ridge count and the aggressive type of RA indicate that this dermatoglyphic variable might play a significant role not only for screening but also for

studying the behavior of the disease. Further studies are recommended to support our findings regarding the importance of dermatoglyphics as an anatomical marker in rheumatoid arthritis.

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8/3/2010

# High Efficiency Production of Mosquitocidal Toxin by a novel *Bacillus sphaericus* isolate from Egyptian Soils on Local Agroindustrial Byproducts

**M.S.Foda,<sup>1\*</sup> Fawkia M. El-Beih,<sup>2</sup> Maysa E. Moharam,<sup>1</sup> Nora N.A.El-Gamal<sup>1</sup>**

<sup>1\*</sup> Microbial Chemistry Department, National Research Center, Dokki, Giza, Egypt.

<sup>2</sup> Faculty of Science, Ain Shams University, Cairo, Egypt.

<sup>\*</sup>[foda302002@yahoo.com](mailto:foda302002@yahoo.com)

**Abstract:** Eighty six cultures were isolated from soil of different Egyptian Governorates including Quina, El-Menofeya, El-Gharbia, El-Sharkia, El-Behera and Kafr EL-Sheikh Governorates. Investigations on the mosquitocidal Egyptian isolates have revealed that isolate No.1 have the ability to form more toxin than the international reference strain *Bacillus sphaericus* 2362(Bs 2362).The selected isolate No.1 exhibited a lower LC<sub>50</sub> and LC<sub>90</sub>values than the International strain B.s 2362 upon bioassay against second instars' larvae of *Culex pipiens*. The Egyptian isolate No.1was identified morphologically and biochemically as *Bacillus sphaericus*. Physiological factors affecting growth and toxin formation in *B. sphaericus* No 1 in comparison to B.s 2362 were carried out. THE organism grown on modified Nutrient broth medium yielded the highest larval toxicity against the second instars' of *Culex pipiens* for both *Bacillus sphaericus* isolate No 1 and the international strain *Bacillus sphaericus*. The Optimum air : medium ratio were 9:1 and 4:1 of the flask volume for 4 and 3 days incubation periods using 2%and 3% sizes of inocula for *B. sphaericus* 2362 and the Egyptian isolate *B. sphaericus*No.1, respectively. Sodium acetate was the suitable carbon source for the isolate *B. sphaericus* No.1, while *B.s* 2362 was capable to utilize both sodium acetate and sodium succinate as carbon sources.The Egyptian isolate *B. sphaericus* No.1exhibited the highest mosquitocidal activity upon growth on kidney beans seeds and sesame meal as nutrient substrates at 3% final concentration, while *B.s* 2362 exhibited the highest mosquitocidal activity by utilizing soy beans, lentils and sesame meal as complete media for growth and mosquitocidal toxin production.

[M.S. Foda, Fawkia M. El-Beih, Maysa E. Moharam, Nora N.A.El-Gamal. High Efficiency Production of Mosquitocidal Toxin by a novel *Bacillus sphaericus* isolate from Egyptian Soils on Local Agroindustrial Byproducts. Journal of American Science 2010;6(11):467-475]. (ISSN: 1545-1003).

**Key words:** *Bacillus sphaericus*, isolation, characterization, mosquitocidal toxin, physiology, agroindustrial byproducts.

## 1. Introduction:

The mosquito acts as a vector for many of the world's most serious diseases, both parasitic e.g. malaria (*Anopheles*), lymphatic filariasis (*Anopheles*, *Aedes*, *Culex* and *Mansonia*) and viral e.g. yellow fever, dengue (both *Aedes*) and encephalitis (*Culex*). (Baumann *et al.*, 1991).

*Bacillus sphaericus* has been successfully used for the biological control of numerous of disease -transmitting mosquitoes and black fly species (Lacey and Undeen, 1986). The prime advantage of the *B. sphaericus* strain lies in their ability to persist for longer periods in the environment than *Bacillus thuringiensis* var. *israelensis*. This may be due to recycling and amplification of spores in larval cadavers under certain aquatic situations or may be simply due to the long-term persistence of sufficient and accessible toxin in the environment or a combination of both of

the above (Singer, 1990; Correa & Yousten, 1995). Major advantage of these bacterial insecticides are their safety, biodegradability, and low environmental impact (Maramorosch, 1987)

Opota *et al.* (2008) reported that the binary toxin (Bin) from *Bacillus sphaericus* exhibits a high insecticidal activity against *Culex* and *Anopheles* mosquitoes. The cytotoxicity of Bin requires an interaction with a specific receptor present on the membrane of midgut epithelial cells in larvae, a direct correlation exists between binding affinity and toxicity. The toxin binds with high affinity to its receptor in its primary target namely, *Culex pipiens* (Baumann, *et al* 1991).

The present work paper aims for isolation of new *Bacillus sphaericus* strain with mosquitocidal activity that exceeds the existing international strains e.g. *B. sphaericus*2362. In the hope to reduce Production costs of mosquitocidal toxin used for

biological control of disease-transmitting mosquitoes in the developing countries production physiology of the bacterial toxin was studied on synthetic and agroindustrial byproducts.

## 2. Materials and methods

### Microorganisms

The International strain *Bacillus sphaericus* 2362 was kindly obtained from prof. F.G. Priest, school of life sciences, Heriot watt university, UK

A new *Bacillus sphaericus* isolate namely No.1 was isolated from soils of Quina Governorate, Egypt.

### Media

Synthetic media used for cultivation of the pure organisms and their activation prior to physiological studies.

- Nutrient Broth medium: (g/l) peptone 5, beef extract 3, for solidification 25 g agar were added.
- Luria- Burtani(LB) medium: (g/l) peptone 10, yeast extract 5, sodium chloride 10.
- NYSM broth medium: nutrient broth, yeast extract 0.5 g/l

Trace elements, (g/100ml): Manganese chloride 0.09, Calcium chloride 1.03, Magnesium chloride 2.03.

1 ml of the filter sterilized trace elements solution was added to 100 ml of the medium.

2. Media used for growth, sporulation and mosquitocidal toxin production in shake cultures.

#### a- Media based on Agroindustrial by-products:

These media included offal's meal, feather meal and cotton seed meal (Ministry of Agriculture). Most of these agroindustrial by-products are currently used in animals feed and available in Egyptian market.

#### b-Media based on cheap, locally available plant proteins:

Certain legume seeds that are locally available in Egypt were examined as protein sources for growth, sporogenesis and mosquitocidal toxin production. These legumes seeds such as soy beans, kidney bean, black eyed bean, yellow split pea, and lentils were finely grinded and used in conjunction with the standard mineral salt solution ( $\text{KH}_2\text{PO}_4$ , 0.5g/L,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.25g/L,  $\text{CaCl}_2$ , 0.1g/L,  $\text{FeSO}_4 \cdot 5\text{H}_2\text{O}$ , 0.01 g/L) at appropriate concentration.

### Bioassay of bacterial toxins against Mosquitoes larvae.

Bioassay of locally isolated *Bacillus* cultures including *B. thuringiensis* and *B. sphaericus* were carried out as described by Priest and Yousten (1991). Toxicity was determined with laboratory

reared *Culex pipiens*. Serial dilutions in distilled water were tested in a preliminary toxicity screen. The range of concentration of full grown whole culture (FWC) which killed 50% and 90% of the larvae were identified. Then further toxicity tests were done in the range recorded to evaluate precisely the  $\text{LC}_{50}$  and  $\text{LC}_{90}$  values for each highly promising bacterial culture.

The corrected mortality was then plotted against culture dilution of cells/ml on log paper to determine  $\text{LC}_{50}$  and  $\text{LC}_{90}$  values for each highly promising bacterial cultures.

The bacterial dilutions were placed in small cups in duplicates along with 10 second instar larvae. Appropriate controls were run simultaneously using distilled water instead of cultures. The cups kept at room temperature at  $27 \pm 2^\circ\text{C}$ . The mortality percentage was recorded by counting the number of live larvae and corrected by using appropriate control and applying Abbott's formula (Abbott, 1925). The medium lethal concentrations  $\text{LC}_{50}$  of potent isolates was computed through probit analysis within 95% confidence limits using propan program.

Abbott's formula:

$$\text{Corrected mortality \%} = \frac{\text{Observed mortality \%} - \text{Control mortality \%}}{100 - \text{Control mortality \%}} \times 100$$

## 3. Results

### 1. Isolation, Identification and Mosquitocidal Toxin Production by *Bacilli* isolated from the Egyptian environments

Eighty six isolates were obtained from soils and mud samples of six different Egyptian Governorates including Quina, El-Menofeya, El-Gharbia, El-Sharkia, El-Behera and Kafr EL-Sheikh. Among these isolates, isolate No.1 obtained from Quina Governorate was the only isolate giving 100% mortality up to culture dilution  $10^{-5}$ . Accordingly this isolate obtained from Quina Governorate was selected for further investigation.

### 2. Determination of $\text{LC}_{50}$ and $\text{LC}_{90}$ values of the Egyptian isolate No.1 obtained from Quina Governorate soils.

$\text{LC}_{50}$  and  $\text{LC}_{90}$  of isolate No.1 and *B. sphaericus* 2362 bioassayed against second instar larvae of *Culex pipiens* revealed that the Egyptian isolate No.1 is more toxic than the reference strain 2362. (Table 1).

### 3. Identification of the Egyptian isolate No.1 isolated from Quina Governorate.

The colonies exhibited beige color with medium size colonies, the texture is smooth semi-

glistening with round margin; the appearance of colonies is shiny with little elevation and flat.

Examination of the cells with the electron microscope revealed the rod-shaped of the vegetative cells as shown in Fig (1); sporulated cells (sporangia) with subterminal spores that are round in shape giving the sporangia club shaped appearance as shown in Fig (2). Also it was observed that isolate No.1 produced a spherical spore and round crystals when examined under the electron microscope as shown in Fig (3).

Some biochemical tests were carried out for the identification of the Egyptian isolate No.1 obtained from Quina Governorate (Table 2).

#### 4. Comparative Physiological studies on factors affecting growth, sporulation, and toxin production of the Egyptian isolate *B. Sphaericus* No.1 and the International *B. sphaericus* 2362 strain under submerged fermentation conditions

##### 4.1. Effect of types of media on growth parameters, sporulation titer and mosquitocidal toxicity under submerged conditions.

Four types of media were used in this study namely Nutrient yeast salt medium, Luria –Bertani medium, Nutrient broth (NB), and modified Nutrient broth (NB+ 0.5% yeast extract) The obtained results showed that modified Nutrient broth medium yielded the highest larval toxicity against the second instars' larvae of *Culex pipiens* for both *B.s* isolate No1 and the international strain *B.s* 2362, (Tables 3, 4)

##### 4.2 Effect of aeration level on growth and toxicity of *B. sphaericus*

In this experiment the extent of aeration was altered by varying the air : medium ratio (amount of medium in the culture flask). The effect of aeration extent on growth parameters and toxicity of the mosquitocidal agent produced by the organisms under study are shown in Figure (4). It was noted that the viable count and toxicity increased with increasing the air: medium ratio. Furthermore, The sporulation and toxin production gave the highest titers when the medium volume occupied 10% and 20%, i.e. corresponding to air: medium ratio 9:1 and 4:1 of the flask volume for *B. sphaericus* 2362 and the Egyptian isolate *B. sphaericus* No.1, respectively.

##### 4.3. Effect of different carbon sources utilized by *B.sphaericus* on growth parameters and mosquitocidal toxin formation.

It is known that *B. sphaericus* can not utilize carbohydrates for growth due to the lack of many of the enzymes of Embden Meyerhof and hexose monophosphate path ways (Russel *et al.*, 1989). In this experiment different carbon sources were used

for testing the ability of the tested organisms *B. sphaericus* No.1 and *B. sphaericus* 2362 to utilize this carbon sources. The results revealed that sod. acetate was utilized by the isolate *B. sphaericus* No.1, at which the sporulation and toxin production yielded the highest titers. On the other hand, *B. sphaericus* 2362 was capable to utilize sod. acetate and sod. succinate, as shown in Figure (5).

##### 4.5. Effect of inoculum size on growth parameters and mosquitocidal toxin formation of *B. sphaericus*.

Different volumes of overnight growing culture were used as inocula for a set of 250 ml conical flasks each containing 25ml of modified nutrient liquid medium . The results of growth parameters and toxin production of tested organisms are illustrated by Figure (6).

The increase of inoculum size has led to the increase of sporulation titer and toxin production up to 3% inoculum size, and then decreased with the increasing of inoculums size in case of the Egyptian *B. sphaericus* No.1. However the sporulation and toxin production of *B. sphaericus* 2362 showed a little effect by changing the inoculum size. The highest toxicity were achieved using 3% inoculums size and 2% by isolate *B. sphaericus* No.1 and *B. sphaericus* 2362, respectively.

##### 4.6. Effect of incubation period on growth parameters and mosquitocidal toxin formation of *B. sphaericus*.

This experiment was carried out under standard conditions by using a set of 250 ml conical flasks containing 25ml of modified nutrient liquid medium, then the extent of growth, sporulation titer and toxin production were followed and determined by harvesting after 2, 3, 4 and 7 days of incubation at  $28 \pm 2^{\circ}\text{C}$  on a rotary shaker.

The results are shown in Figure (7). The mortality increased with increasing the incubation period until 3 days incubation period in case of *B. sphaericus* No.1 and 4 days for *B. sphaericus* 2362

##### 4.7. Effect of different by-products and grinded legumes seeds used as complete media on growth, sporulation and toxin production of *Bacillus sphaericus*.

Ten agro industrial byproducts that are available in Egypt were examined as a complete cost effective media for toxin production. The data in Figure(8) illustrated that the Egyptian isolate *B. sphaericus* No.1 exhibited the highest mosquitocidal activity by utilizing kidney beans and sesame meal as nutrient substrate at 3% final concentration, while *B. sphaericus*2362 exhibited the highest mosquitocidal activity by utilizing soy beans, lentils and sesame meal as growth media for growth and mosquitocidal toxin production.

**Table(1): Values of LC<sub>50</sub> and LC<sub>90</sub> for mosquitocidal toxins of the Egyptian isolate No.1 in comparison with those of the International strain of *Bacillus sphaericus* 2362 at confidence limits(95%). The bioassay was carried out against second instar larvae of *Culex pipiens*.**

Isolate	LC <sub>50</sub> by $\mu$ l (p 0.05)	LC <sub>90</sub> by $\mu$ l (p 0.05)
The Egyptian isolate <i>B. sphaericus</i> No.1 from Quina. Egypt	264.4 (155.3-365.8)	725.9 (517.3-1351.7)
The Internationalstrain <i>B. sphaericus</i> 2362	359.2 (228.5-479.3)	932.4 (674.3-1818.9)

**Table (2): Some biochemical tests for the identification of the Egyptian isolate No.1 obtained from Quina Governorate as compared with *B. sphaericus* 2362.**

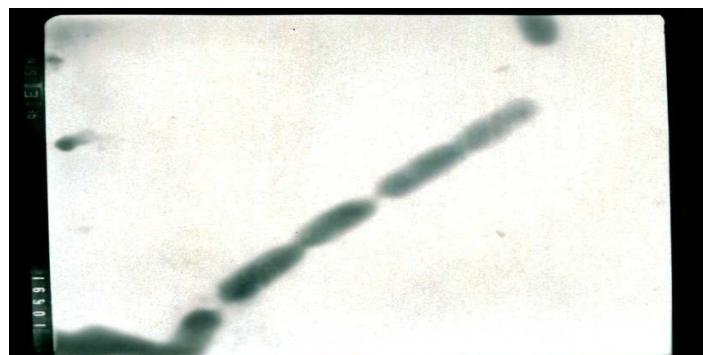
Biochemical tests	Standard strain <i>B. sphaericus</i> 2362	The Egyptian isolate No.1
Tolerance to NaCl 2%	+	+
5%	+	+
7%	-	-
10%	-	-
Degradation of adenine	+	+
Decomposition of urea	+	+
Hydrolysis of casein	+	+
Hydrolysis of Starch	+	+
Hydrolysis of gelatin	+	+
Utilization of citrate	-	-
Methyl red test	+	-
Vogesproskauer test	-	-
Catalase test	-	-
Nitrate reduction test	-	-

**Table (3): Growth parameters and sporulation titers of the Egyptian isolate *B. sphaericus* No.1 obtained from soils of Quina Governorate grown on five media for 3 days under submerged conditions.**

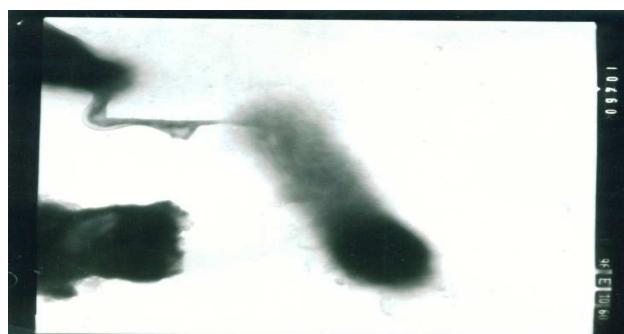
Type of growth media	Total viable counts/ml culture at ( $10^6$ )	Spore counts/ml culture at ( $10^6$ )	Mortality% after 48 hr at ( $10^5$ )	Mortality% after 48 hr at ( $5 \times 10^6$ )
<b>NYSM Medium</b>	140	83		
	140	85		
	140	85	70	50
	140	88		
<b>LB Medium</b>	47	<10		
	40	<10	40	10
	39	<10		
	45	<10		
<b>NB Medium</b>	20	<10		
	22	<10	95	90
	22	<10		
	22	<10		
<b>NB+Y.Ext Medium</b>	135	34		
	140	37		
	140	37	100	100
	144	37		
<b>Modified Spizizen Medium</b>	30	35		
	30	35		
	30	30	10	10
	29	30		

**Table (4): Growth parameters and sporulation titers of the International *B. sphaericus* 2362 grown on five media for 3 days at  $28 \pm 2^\circ\text{C}$ , under submerged conditions.**

Type of growth media	Total viable counts/ml culture at ( $10^6$ )	Spore counts/ml culture at ( $10^6$ )	Mortality% after 48 hr at ( $10^5$ )	Mortality% after 48hr at ( $5 \times 10^6$ )
<b>NYSM Medium</b>	99	50		
	90	48		
	90	47	75	40
	85	44		
<b>LB Medium</b>	47	25		
	40	17	10	0
	39	17		
	45	15		
<b>NB Medium</b>	55	23		
	56	20		
	54	20	95	80
	50	19		
<b>NB+Y.Ext Medium</b>	40	18		
	34	15		
	30	15	100	80
	30	15		
<b>Modified Spizizen Medium</b>	100	54		
	100	50	55	35
	95	50		
	95	44		



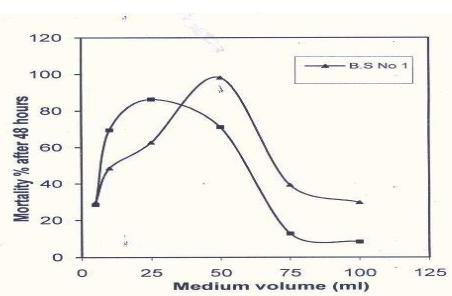
**Fig (1)** E.M. showing chain of vegetative cells of the Egyptian isolates *B. sphaericus* No.1 isolated from Quina Governorate (X 10,000).



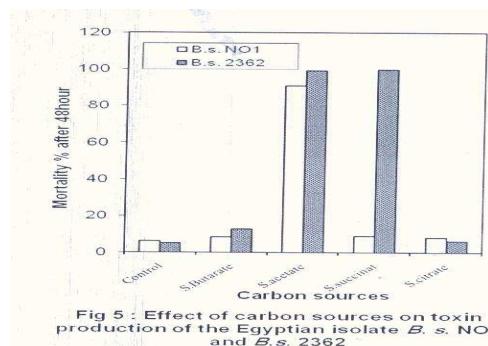
**Fig (2)** E.M. of the Egyptian isolates No.1 isolated from Quina Governorate grown on nutrient liquid medium showing the club-shaped cells (X 20,000).



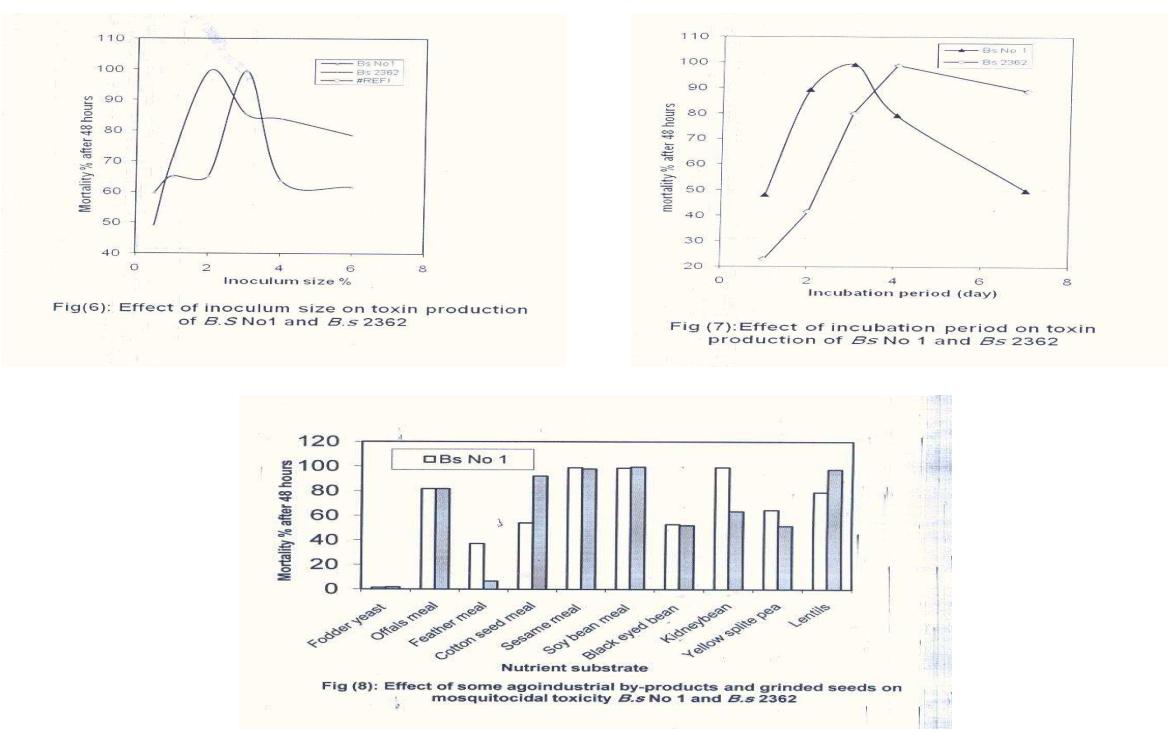
**Fig (3)** Electron Micrograph (E.M.) showing spherical spore and crystal of the Egyptian *B. sphaericus* isolates No.(1) after 3 days of incubation (X 20,000).



**Fig. 4** Effect of volume of media of toxin production by the Egyptian isolate *B. s.* No 1 and *B. s.* 2362



**Fig 5 :** Effect of carbon sources on toxin production of the Egyptian isolate *B. s.* NO1 and *B. s.* 2362



#### 4. Discussion:

The mosquito acts as a vector for many of the world's most serious diseases, both parasitic e.g. malaria (*Anopheles*), lymphatic filariasis (*Anopheles*, *Aedes*, *Culex* and *Mansonia*) and viral e.g. yellow fever, dengue (*Aedes*) and encephalitis (*Culex*). The present work aims to isolate some local isolates of *B. sphaericus* pathogenic to mosquito larvae from the Egyptian environment. It was also devoted to investigate the growth physiology and various factors that are affecting growth, sporulation and toxins formation. On the other hand, special attention was given to search for suitable media that are low-priced and locally available in Egypt for *B. sphaericus* production on a large scale. The goal stemmed from the fact that the feasibility of economic production of spores and toxin crystals of *B. sphaericus* is dependant to a large extent on production costs and availability of raw materials under the local conditions. Physiological factors affecting growth and toxin formation in *B. sphaericus* revealed that modified Nutrient broth medium yielded the highest larval toxicity against the second instars' of *Culex pipiens* for both *Bacillus sphaericus* isolate No 1 and the international strain *Bacillus sphaericus* 2362. The requirements of individual bacterial strain for nutrients may vary for different strains and also of different isolates within the same strain within the same species. Thus optimal concentration of nutrients for one isolate may not necessarily be suitable for another. Therefore, it is impossible to recommend a fermentation medium that will be best for all isolates of the same species (Foda *et al.* 2000).

It is established that *B. sphaericus* is an obligate aerobe and adequate air supply is needed for

growth, initiation of sporulation and toxin synthesis (Yousen and Wallis, 1987). In our studies, it was found that the maximum sporulation and toxicity were achieved when the medium volume to air ratio was 1:4 for the Egyptian isolate *B. sphaericus* No.1 and 1:9 for the International strain *B. sphaericus* 2362 that was used for comparative purposes. The increase in medium volume to air ratio has lead to the decrease in sporulation and toxicity. This result agrees with what reported by Yousen and Wallis (1987). They found that oxygen was required for toxin production by *B. sphaericus* strain 2362. However, they reported that increasing the level of dissolved oxygen (DO) in the medium by use of pure oxygen in the gas stream lowered toxin production, while in case of strain 1593, (another *B. sphaericus* International strain), increased (DO) produced a block in sporulation, but toxin synthesis was normal (Yousen *et al.*, 1984).

The result of growth parameters of tested organisms indicated that *B. sphaericus* isolate No.1 gave high sporulation titer and toxicity at inoculum size 3% and a decrease in toxicity was recorded by increasing the inoculum size. However the highest sporulation and toxin production levels of strain 2362 were achieved by inoculum size 2%. This result agrees with that reported by Foda *et al.* (2000), they reported that the sporulation of the Egyptian isolate No. 69 increased by decrease in the inoculum size to reach  $7.5 \times 10^6$ /ml viable count whereas the sporulation of strain *B. sphaericus* 2362 exhibited a little effect by changing the inoculum size.

*B. sphaericus* can not use carbohydrates for growth due to the lack of many of the enzymes of Embden Meyerhof and hexose monophosphate pathways (Russel *et al.*, 1989).

In the present study, it has been found that the Egyptian isolate *B. sphaericus* No.1 grew well using acetate as sole carbon source. On the other hand the International strain *B. sphaericus* 2362 grew with acetate or succinate as sole carbon source. This result agrees with what reported by (Gordon *et al.*, 1973; De BarJac *et al.*, 1980; Klein *et al.*, 1989; Widjaya *et al.*, 1992 and Ahmed *et al.*, 1993 and 1996). They reported that numerous strains of *B. sphaericus* grew with acetate, Pyruvate, lactose, glutamate, succinate, histidine and arginine, as sole major carbon and energy sources.

In the present study, ten leguminous seeds and agroindustrial by-products were used as nutrient substances at 3% concentration and the result indicated that soy beans, kidney beans and sesame seed meal could be used efficiently as nutrients sources to support growth, sporulation and toxin production of the Egyptian isolate *B. sphaericus* No.1. High levels of toxicity were obtained even at low concentration of diluted culture ( $3 \times 10^6$ ), as inocula. On the other hand, *B. sphaericus* 2362 grew well on a medium contained soy beans, lentils and sesame seed meal and the growth, sporulation and high levels of toxin production were achieved at the same culture dilution. Uses of such various by-products as well as legume seeds have shown that local production from inexpensive ingredients available in different regions is possible. Such studies may pave the way for mass production on industrial scales. Dulmage *et al.* (1970) cultured *B. sphaericus* 1593 and 2362 separately in a fermentor on peptonized milk medium with yeast extract and mineral supplements. The fermentor beer was centrifuged and then resuspended in lactose solution and precipitated with acetone. These powders were highly insecticidal to *Culex quinquefasciatus* larvae producing LC<sub>50</sub> values in the range of  $10^2 \mu\text{g}/\text{ml}$ . Obeta and Okafor (1983) formulated five media from dried cow blood, mineral salts and seeds from four species of legumes (ground nut cake, cowpea, mambara beans and soy beans) for production of *B. sphaericus* 1593. Good growth, sporulation and toxin activity of *B. sphaericus* 1593 were obtained with all tested media. Dharmsthiti *et al.* (1985) grew *B. sphaericus* on a medium containing 7% hydrolyzed liquor by-product from a monosodium glutamate factory. Klein *et al.* (1989) used hydrolyzed industrial peptones (waste product of industry) for constructing seven media for production of *B. sphaericus* larvicides. These media contained 5 g/l industrial peptone in 50 mM phosphate buffer (pH 7.0) in combination with other carbon and nitrogen sources. Industrial peptone medium supplemented with glycerol was the most efficient medium for growth and larvicides production by *B. sphaericus* 2362. The local availability of proteinaceous materials is vitally important for *B. sphaericus* fermentation. For example, one of the most useful nitrogen sources is cotton seed flour (Dulmage *et al.* 1990). They reported that several nitrogen sources are used in *Bt* fermentation, including soybean flour, cotton seed flour and fish meal. The soy

flour and cotton seed flour were both very good sources of nutrients for both *Bt* and *B. sphaericus* production. Gangurde and Shethna (1995) concluded that mustard seed meal (MSM) contains 40% protein, with glutamic acid and arginine as a major amino acids. Therefore, growth and larvicidal activity of *B. sphaericus* 2362 and 1593 produced in MSM can be attributed in part to the presence of these amino acids. Ampofo (1995) used some local raw-materials for production of *Bs* insecticides in Ghana. He tested anchovy, spent grain form breweries, bambara beans and sprout maize as media for production of *B. sphaericus* IAB 881. He reported that larvicidal activity of *Bs* IAB 881 grown in anchovy, spent grain, bambara beans and sprout maize, was similar to that obtained in synthetic medium with LC<sub>50</sub> ranging from  $0.3 \times 10^{-5}$  to  $0.68 \times 10^{-6}$  (dilution). Cell counts were in the range of  $11 \times 10^8$  –  $36 \times 10^8$  CFU/ml and spore counts were between  $29 \times 10^7$  and  $61 \times 10^7$  CFU/ml. El-Bendary (1999) used ground agroindustrial by-products and leguminous seeds at 2% final concentration as media for production of *B. sphaericus* in distilled water with or without addition of NYSM salts. The obtained results indicated that most of the tested substances supported formation of highly efficient media for *Bs* toxin production of appreciably high sporulation yield and toxicity. She also reported that the most efficient media for *B. sphaericus* toxin production were soy flour, cotton seed flour, corn steep solids and offals meal. Furthermore, it was observed that addition of NYSM salts to these substances increased the *B. sphaericus* toxicity. Moreover, the toxicity of *B. sphaericus* increased about 1.5-4.5 times when these agroindustrial by-products were partially hydrolyzed by nuclease or alkalase enzymes before using as media. El-Bendary *et al.* (2008), used whey permeate (WP) for production of mosquitocidal toxin by *B. sphaericus* 2362 and the Egyptian isolate, *B. sphaericus* 14N1 under both submerged and solid state fermentation conditions. Under submerged fermentation, high mosquitocidal activity was produced by *B. sphaericus* 2362 and *B. sphaericus* 14N1 at 50% -100% and 25% -70% whey permeate, respectively.

#### Corresponding author

M.S.Foda

Microbial Chemistry Department, National Research Center, Dokki, Giza, Egypt.

[foda302002@yahoo.com](mailto:foda302002@yahoo.com)

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# Novel Validated Chromatographic Method for Determination of Some Anti-hypertensive Drugs

**M. Farouk<sup>1</sup>, O. Abd ELAziz<sup>1</sup>, A. Hemdan<sup>\*2</sup>, M. Shehata<sup>2</sup>**

<sup>1</sup> Analytical Pharmaceutical Chemistry Department, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt.

<sup>2</sup> Pharmaceutical Chemistry Department, Faculty of Pharmacy, Ahram Canadian University, 6<sup>th</sup> October, Egypt.

[<sup>\\*</sup>hemmdan@yahoo.com](mailto:hemmdan@yahoo.com)

**Abstract:** Accurate, precise and reproducible isocratic RP-HPLC method was developed and subsequent validated for the analysis of Torasemide (I), Irbesartan (II) and Olmesartan medoxomil (III) at ambient temperature, using Atlantis 4.6 mm x 250 mm RP-C18 Column, with a flow rate of 1.5 ml.min<sup>-1</sup>, and UV. detector at 288 nm and 260 nm for (I) and (II and III), respectively. By adopting the mentioned chromatographic technique, (I) and (III) were determined in the presence of their acidic and alkaline-degradates separately as stability-indicating methods utilizing phosphate buffer pH = 3:acetonitrile (60:40, v/v), phosphate buffer pH = 3.2:acetonitrile (60:40, v/v) as a mobile phase, respectively, while (II) was determined in presence of Hydrochlorothiazide (HCTZ), using phosphate buffer pH = 4:acetonitrile (70 :30, v/v). All the proposed methods were validated according to the International Conference on Harmonization (ICH) guidelines and successfully applied to determine the mentioned studied drugs in pure form, in laboratory prepared mixtures and in pharmaceutical preparations. The obtained results were statistically compared to the reference methods of analysis [for I and "II and III", respectively] and no significant differences were found. [M. Farouk, O. Abd ELAziz, A. Hemdan, M. Shehata. Novel Validated Chromatographic Method for Determination of Some Anti-hypertensive Drugs. Journal of American Science 2010;6(11):476-486]. (ISSN: 1545-1003).

**Keywords:** Torasemide, Irbesartan, Olmesartan medoxomil, High Performance Liquid Chromatography, Stability Indicating method

## 1. Introduction:

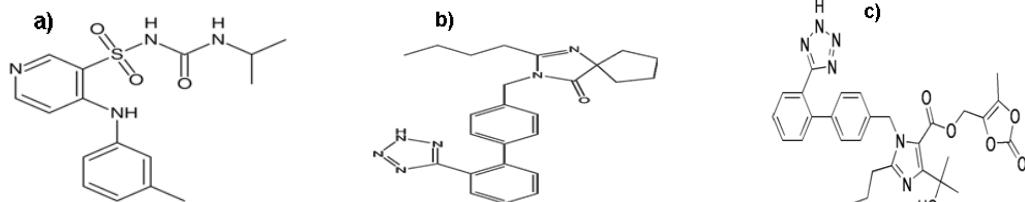


Figure (1): Chemical structure of: a) Torasemide, b) Irbesartan, c) Olmesartan medoxomil

Torasemide (I) is (1-isopropyl-3-[[4-(3-methylphenylamino) pyridine]-3-sulfonyl] urea) a loop diuretic, mainly used at low doses for the management of hypertension, where in large doses used for management of oedema associated with congestive heart failure<sup>(1)</sup>. Irbesartan (II) is 2-butyl-3-[[2-(tetrazol-5-yl)biphenyl-4-yl]-methyl]-1,3-diazaspiro[4.4]non-1-en-4-one, acts as an angiotensin-II receptor antagonist, used mainly for the treatment of hypertension<sup>(2)</sup>, while, Olmesartan medoxomil (III) is 5-methyl-2-oxo-1,3-dioxolen-4yl methyl-4-(1-hydroxy-1-methylethyl)-2-propyl-1-[4-(2-tetrazole-5-yl)phenyl] methylimidazole 5 carboxylate, used for the treatment of hypertension by the same mechanism as (II)<sup>(3)</sup>. The ICH-guidelines<sup>(4)</sup> recommends performing stress-testing of the drug substance that can help in identifying the

likely degradation-products, also can be useful in establishing the degradation-pathways and validating the stability-indicating power of the analytical procedures used<sup>(5)</sup>. Stability-indicating methods can be used for evaluating the drug in the presence of its-degradation products, excipients and additives<sup>(6)</sup>. Several methods have been reported for the determination of (I), including colorimetry<sup>(7)</sup>, differential-pulse adsorptive stripping voltammetry<sup>(8)</sup>, capillary zone electrophoresis (CZE)<sup>(9,10)</sup>, gas chromatography<sup>(11)</sup>, micellar liquid chromatography<sup>(12)</sup>, and high-performance liquid chromatography<sup>(13-22)</sup>. Alone or in combination with HCTZ, Irbesartan has been determined by derivative spectrophotometry<sup>(23-27)</sup>, kinetic Spectrophotometry<sup>(28)</sup>, spectrofluorimetry<sup>(29)</sup>, colorimetry<sup>(30)</sup>, adsorptive stripping voltammetric<sup>(31)</sup>,

A differential pulse (DP) and square wave (SW) voltammetry<sup>(32)</sup>, capillary zone electrophoresis<sup>(33-35)</sup>, micellar electrokinetic chromatography<sup>(36)</sup>, and high-performance liquid chromatography<sup>(37-43)</sup>. While for Olmesartan medoxomil (III), several methods have been reported for its determination, either alone or in combination with HCTZ, these methods were based on absorption ratio spectrophotometry<sup>(44)</sup>, ratio spectra derivative and zero-crossing difference spectrophotometry<sup>(45,46)</sup>, derivative spectrophotometry<sup>(47)</sup>, direct spectrophotometry<sup>(48,49)</sup>, capillary zone electrophoresis<sup>(50)</sup>, high performance thin layer chromatographic method<sup>(51,52)</sup>, and high-performance liquid chromatography<sup>(52-59)</sup>.

The main goal of this work is to establish accurate, precise, rapid and reproducible isocratic chromatographic methods for determination '(I), and (III) in presence of their-degradates and simultaneous determination of (II) in binary mixture with HCTZ that can be adopted as a technique for the routine quality control analysis of these drugs in raw material and pharmaceutical preparations as well as for stability studies.

## 2. Experimental:

### 2.1. Chemicals and reagents

Torasemide was kindly provided by Apex Pharma-Egypt and certified to contain 99.70%. Examide® tablets: batch number: MT1120410, manufactured by Apex Pharma-Egypt Company. Each tablet was labeled to contain 20 mg of Torasemide. Irbesartan was kindly obtained by Sanofi-Aventis Egypt and certified to contain 99.90%. Co-Approval® tablets: batch number: 1145, manufactured by Sanofi-Aventis Egypt. Each tablet was labeled to contain 300 mg of Irbesartan and 12.5 mg Hydrochlorothiazide. Hydrochlorothiazide (HCTZ) was kindly provided by Multi-Pharma Egypt and certified to contain 99.50%. Olmesartan medoxomil was kindly provided by Apex Pharma-Egypt and certified to contain 99.70%. Erastapex® tablets: batch number: MT3241009, manufactured by Apex Pharma-Egypt Company. Each tablet was labeled to contain 40 mg of Olmesartan medoxomil.

Acetonitrile, ethyl acetate, methanol and bi-distilled water (Riedel-dehaen, Sigma-Aldrich, Germany), hydrochloric acid, sodium hydroxide and sulfuric acid (BDH), each 'aqueous 0.1, '0.1 and 6.6' and 5M. 'Monobasic potassium phosphate and O-phosphoric acid (Adwic)' and triethylamine (Fluka).

All chemical and reagents used through this work are of chromatographic analytical grade. Bi-distilled water is used throughout the whole work and is indicated by the word "water".

### 2.2. Instruments

The HPLC Schimadzu LC-Lab Solution instrument comprised an isocratic pump model Schimadzu LC-20AD, connected to PC and software (LC-Solution), SIL 20A auto-sampler - Schimadzu injector and a Schimadzu SPD20A UV detector. The chromatographic separation was performed using Atlantis C18 column (5μm, 250 x 4.6 mm i.d.) at ambient temperature.

Ultrasonic vibrator, Crest Ultrasonic-Tru / Sweep; Model 575TAE, N. Y, U.S.A.

A (Jenway 3510, UK) pH-meter, equipped with combined glass electrode for pH adjustment.

### 2.3. Standard Solutions

#### 2.3.1. Standard solutions of the investigated drugs

Stock standard solutions of (I, II and III), each having concentration of (0.5 mg.ml<sup>-1</sup>), were prepared in methanol, respectively, where all the prepared solutions were used as a working standard solutions.

#### 2.3.2. Standard solution of Hydrochlorothiazide

Stock standard solution of HCTZ having concentration of (0.5 mg.ml<sup>-1</sup>), was prepared in methanol, where it was used as a working standard solution.

#### 2.3.3. Standard solution of degradates

##### 2.3.3.1. Standard solution of Torasemide degradates

Standard stock solution of acid-degrade, was prepared, by mixing 50.0 mg of (I) with 20 ml of 5.0 M sulfuric acid, refluxing for 12 hours, cooling, then neutralizing the media with 6.6 M NaOH, and making the volumes to 100 ml with methanol to obtain a concentration of 500 μg.ml<sup>-1</sup>.

##### 2.3.3.2. Standard solution of Olmesartan degradates

Standard stock solution of alkaline-degrade, was prepared, by mixing 50.0 mg of (III) with 10 ml of 0.1M NaOH, refluxing for 20 minutes, cooling, then neutralizing the media with 0.1M HCl and making the volumes to 100 ml with methanol to obtain a concentration of 500 μg.ml<sup>-1</sup>.

Complete degradation was checked by TLC using silica gel 60 F254 plates and chloroform: ethyl acetate: methanol [8 : 8 : 4 ] as a mobile phase.

### 2.4. Procedures:

Stationary phase, Atlantis C18 column (5μm, 250 x 4.6 mm i.d.), acetonitrile: phosphate buffer 'pH 3' in a ratio (40:60, v/v), acetonitrile: phosphate buffer 'pH 4' in a ratio (30:70, v/v) and acetonitrile: phosphate buffer 'pH 3.2' in a ratio (40:60, v/v) as 'degassed and filtered' mobile phases with a flow rate of 1.5 ml.min<sup>-1</sup> were the chromatographic conditions

adopted for determination of Torasemide, and 'Irbesartan and Olmesartan medoxomil' using UV detection at 288 and 260 nm, respectively. Construction the calibration curves were performed by transferring aliquots of each working standard solution separately into a series of 25 ml volumetric flasks and diluting with the mobile phase to the volume, having a concentration range of 0.2 – 25, 0.1-20, 0.5-30  $\mu\text{g mL}^{-1}$  for the investigated drugs, respectively. Under the previously mentioned chromatographic conditions, 100 $\mu\text{l}$ -volume from each solution was injected in triplicate, using HCTZ and Torasemide as an internal standards in a concentration of 50 and 4  $\mu\text{g mL}^{-1}$  for determination of I and (II and III), respectively. The obtained average peak area for each concentration of each drug was plotted versus concentration and the regression equation was then computed.

#### 2.5. Assay of the pharmaceutical formulations:

Five tablets of Examide®, Co-Approval® and Erastapex® were accurately weighed and finely powdered separately. Portion of each powder equivalent to 10 mg (I, II and III) were accurately weighed, transferred to 100 ml volumetric flask, shaked for 15-minutes with 50 ml methanol, filtered completed to the volume with methanol, to obtain a concentration of 100  $\mu\text{g mL}^{-1}$  and then the mentioned procedure under 2.4. was adopted.

### 3. Results and Discussion:

#### 3.1. Method development:

##### Torasemide and Olmesartan

Separation of I and III from their degradation-products has been performed on Atlantis C18 column (5 $\mu\text{m}$ , 250 x 4.6 mm i.d.). The proportion of the mobile phase components was optimized to reduce each of 'retention time and tailing' and to enable good resolution from its-degradates. At high acetonitrile ratio, retention time of different components decrease but with excessive tailing of its peak. High resolution was obtained by using acetonitrile: phosphate buffer 'pH 3' in a ratio (40:60, v/v) and acetonitrile: phosphate buffer 'pH 3.2' in a ratio (40:60, v/v) as a mobile phase, with a flow rate 1.5  $\text{ml min}^{-1}$ , and detection at 288 and 260 nm, respectively, where the maximum sensitivity was observed. The average retention time was 3.56 ± 0.03 and 3.85 ± 0.03 min, respectively, as shown in (Figures 2, 3, 6 and 7).

##### Irbesartan

Separation of ibesartan from HCTZ in binary-mixture has been performed on Atlantis C18 column (5 $\mu\text{m}$ , 250 x 4.6 mm i.d.). The proportion of the mobile phase components was optimized to

reduce each of 'retention time and tailing' and to enable good resolution of II from HCTZ. At high acetonitrile ratio, retention time of different components decrease but with excessive tailing of its peak. High resolution was obtained by using acetonitrile: phosphate buffer 'pH 4' in a ratio (30:70, v/v) as a mobile phase, with a flow rate 1.5  $\text{ml min}^{-1}$  and detection at 260 nm, where the maximum sensitivity was observed. The average retention time was 9.89 ± 0.03 min as shown in (Figures 4-5).

#### 3.2. Methods validation.

ICH-guidelines<sup>4)</sup> for method validation were followed. All validation parameters are shown in (Table 1).

##### 3.2.1. Linearity:

A linear correlation was obtained between peak area and concentration of (I, II and III) in a range of 0.2 – 25, 0.1-20, 0.5-30  $\mu\text{g mL}^{-1}$  with correlation coefficient [r] = 0.9998, 0.9999 and 0.9999, respectively.

##### 3.2.2. Accuracy:

Accuracy of the proposed methods was tested by analyzing freshly prepared solutions of the studied drugs in triplicate. The recovery percent and standard deviations (S.D.) revealed excellent accuracy. The results obtained by applying the proposed chromatographic methods were statistically compared with those results obtained by the reference methods<sup>(60-62)</sup>. It was concluded that with 95% confidence, there is no significant difference between them since the calculated t and F values are less than the theoretical values<sup>(63)</sup> (Tables 2a-2c).

##### 3.2.3. Repeatability and reproducibility:

The intra- and inter-day precision was evaluated by assaying freshly prepared solutions in triplicate, as shown in (Table 1).

##### 3.2.4. Specificity:

The specificity of the adopted HPLC method was illustrated by the complete separation of the studied drugs, as shown in (Figures 2-7). The Rs-values from main (acid alkaline-degradates) and from HCTZ were always above 2, which ensured complete separation. Furthermore, I and III were determined in solutions of laboratory prepared mixtures containing their acid and alkaline-degradates and III from HCTZ by the proposed method. The Recovery % and R.S.D. % proved the high specificity of these methods (Table 3).

### 3.2.5. Robustness and system suitability of the HPLC method:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected after slight but deliberate changes in the analytical conditions. Separation of the studied drugs from their different-degradates or from other drug in-combination was performed under these conditions. There was slight decrease or increase in the Rs-values of all peaks. However, the calculated Rs-values were always above 2, ensuring complete separation. The system suitability parameters of HPLC method were evaluated<sup>(64)</sup> (Tables 4a-4c).

### 3.3. Standard addition technique:

The proposed methods were applied for the determination of the studied drugs in the commercial tablets. The results shown in (Tables 5a-5c), were satisfactory and with good agreement with the labeled amount. Moreover, to check the validity of the adopted proposed methods, the standard addition method was applied by adding known amounts of the studied drugs to the previously analyzed tablets. The recoveries were calculated by comparing the concentration obtained from the spiked samples with that of the pure drug. The results of the commercial tablets analysis and the standard addition method (recovery study) (Tables 5a-5c) suggested that there is no interference from any excipients, which are normally present in tablets.

### 3.4. Identification of Torasemide acid-degrade and Olmesartan medoxomil alkaline-degrade:

#### 3.4.1. Identification of Torasemide acid-degrade

Structure elucidation of Torasemide acid-degrade exhibiting terminal amide bond cleavage, resulting in formation of hydroxyl and carbonyl groups, which was explained by utilizing FT-IR

"Fourier transform spectroscopy" and M.S., techniques. In the FT-IR technique, the acid-degrade showed a similar absorption pattern to (I) except the appearance of the acid-degrade bands at 3463.4 and 1735.7 cm<sup>-1</sup>, respectively, while in M.S., two peaks were delivered at m/z 59 and 307, respectively, (figures 8a-8c).

#### 3.4.2. Identification of Olmesartan alkaline-degrade

By the same manner, the structure elucidation of Olmesartan alkaline-degrade exhibiting ester bond cleavage, resulting in formation of hydroxyl and carbonyl groups, which was explained by utilizing FT-IR "Fourier transform spectroscopy" and M.S., techniques. In the FT-IR technique, the alkaline-degrade showed a similar absorption pattern to (III) except the disappearance of the ester carbonyl band at 1737.2 cm<sup>-1</sup> and the appearance of the corresponding Hydroxyl and carbonyl bands of the carboxylic group of the degradation product at 3423.5 and 1712.7 cm<sup>-1</sup>, respectively, on the other hand, mass spectrum of the alkaline degradation product exhibited two new peaks at m/z 130 and 446, respectively, (figures 9a-9c).

## 4- Conclusion:

The proposed HPLC methods were precise, specific, accurate and reproducible , where Torasemide, Irbesartan and Olmesartan can be determined in bulk powder and in pharmaceutical formulations without interference from excipients present, as well as in the presence of their different-degradates or other drug in-combination by the d. ICH-guidelines were followed throughout method validation and the suggested methods can be applied for routine quality control analysis and stability studies.

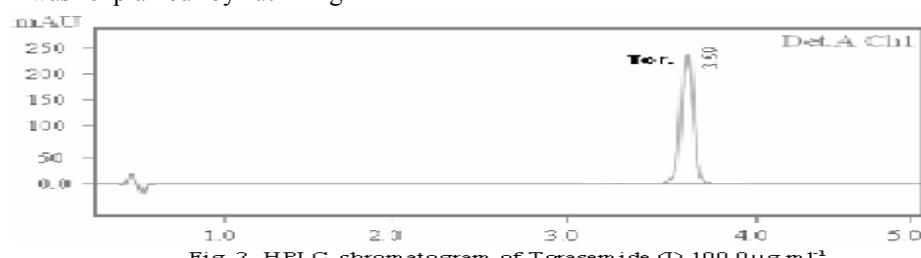


Fig. 2. HPLC chromatogram of Torasemide (1) 100.0 µg ml<sup>-1</sup>.

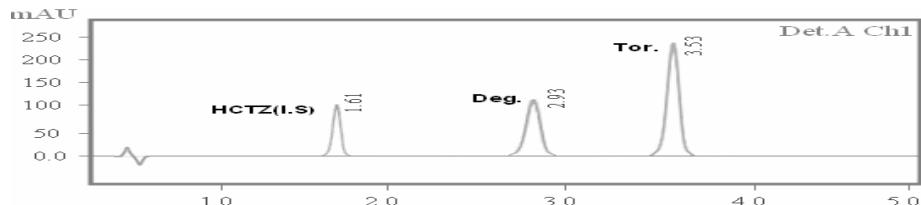


Fig. 3. HPLC chromatogram of mixture solution containing Torasemide and its acid degrate (each 100.0 µg ml<sup>-1</sup>), using HCTZ (50.0 µg ml<sup>-1</sup>) as an internal standard

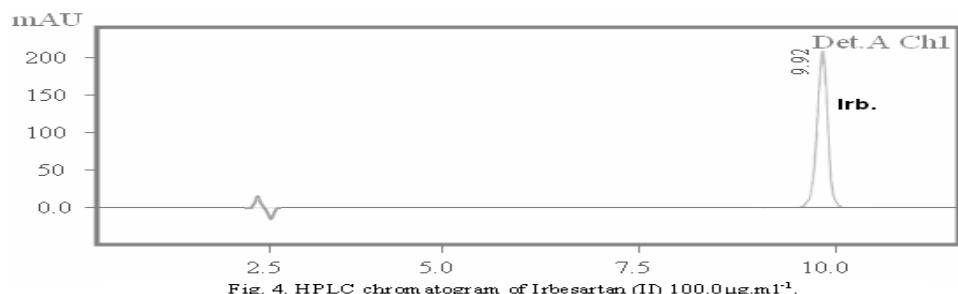
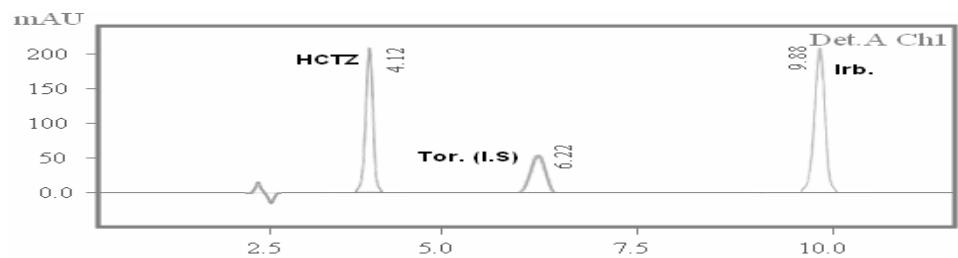
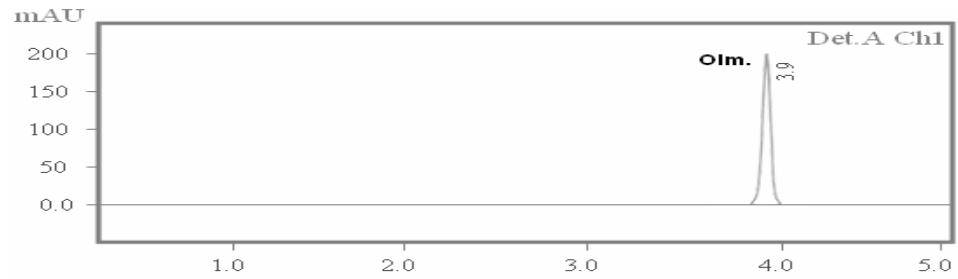
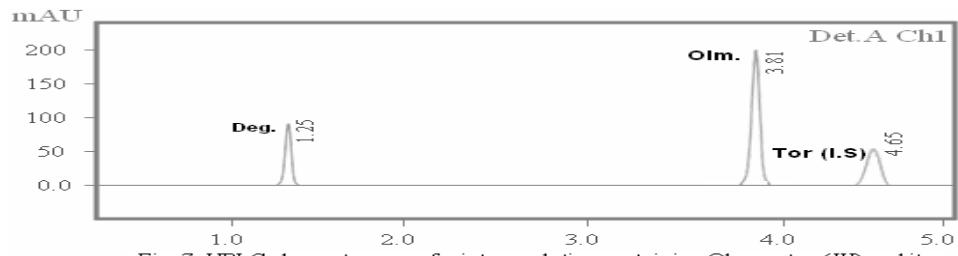
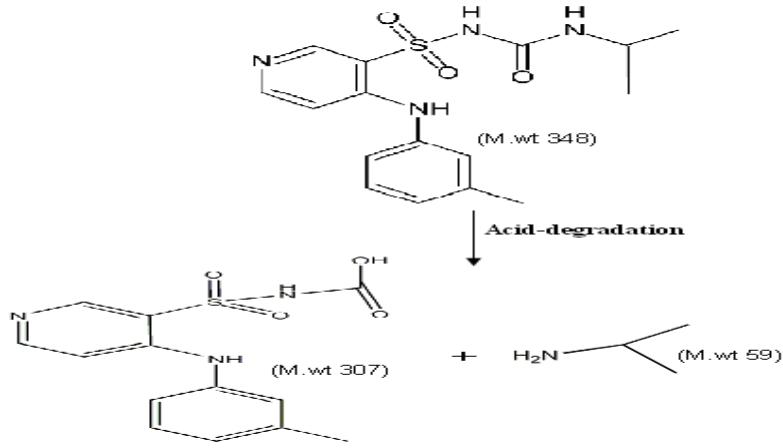
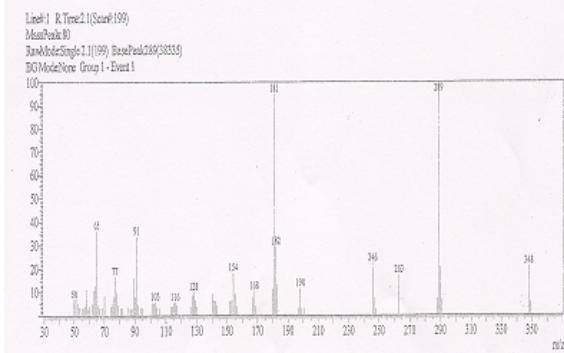
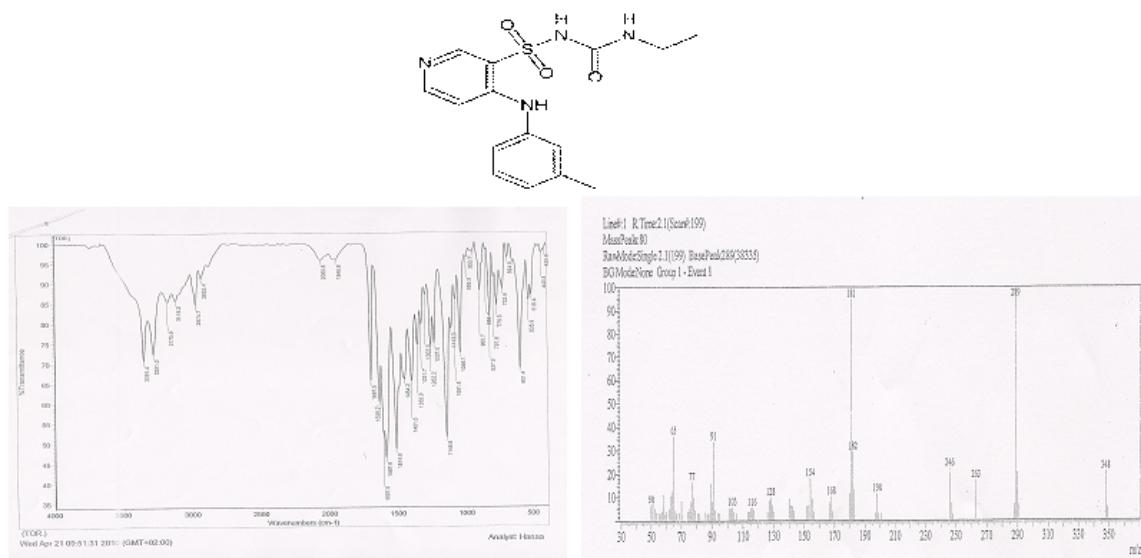
Fig. 4. HPLC chromatogram of Irbesartan (II) 100.0  $\mu\text{g.ml}^{-1}$ .Fig. 5. HPLC chromatogram of mixture solution of Irbesartan (II) in presence of Hydrochlorothiazide (each 100.00  $\mu\text{g.ml}^{-1}$ ), using Torasemide (4.00  $\mu\text{g.ml}^{-1}$ ) as an internal standard.Fig. 6. HPLC chromatogram of Olmesartan (III) 50.0  $\mu\text{g.ml}^{-1}$ .Fig. 7. HPLC chromatogram of mixture solution containing Olmesartan (III) and its alkaline degradate (each 50.0  $\mu\text{g.ml}^{-1}$ ), using Torasemide (4.0  $\mu\text{g.ml}^{-1}$ ), as an internal standard.

Fig. 8.a. Suggested degradation pathway of Torasemide

Torasemide (intact):

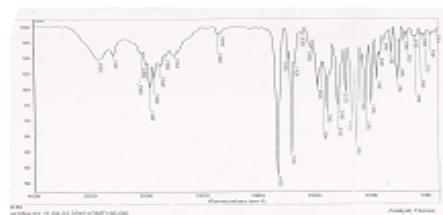
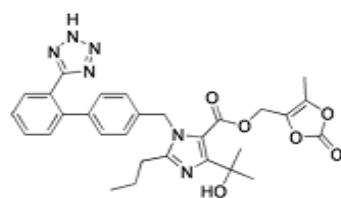
Olmesartan (intact):

Figure 9.b.1. IR spectrum of Olmesartan

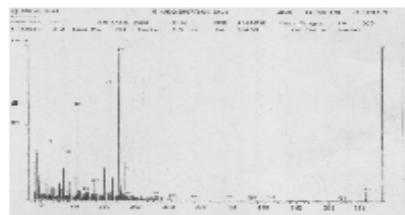


Figure 9.b.1. Mass spectrum of Olmesartan

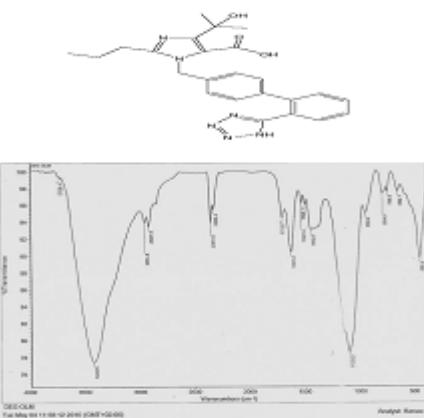
Olmesartan (alkaline-degrade):

Figure 9.c.1 IR spectrum of the Olmesartan alkaline-degrade.

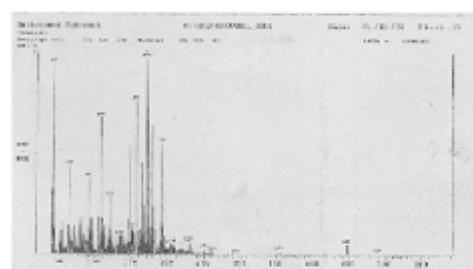
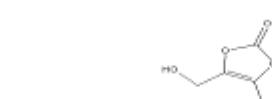


Figure 9.c.2 Mass spectrum of the Olmesartan alkaline-degrade.

**Table 1: Validation report of the proposed HPLC methods for determination of Torasemide (I), Irbesartan (II) and Olmesartan (III).**

Parameters	Torasemide	Irbesartan	Olmesartan
Linearity	0.2-25 $\mu$ g.ml <sup>-1</sup>	0.1-20 $\mu$ g.ml <sup>-1</sup>	0.5-30 $\mu$ g.ml <sup>-1</sup>
Intercept	0.0115	0.053	0.0485
Slope(b) <sup>a</sup>	0.0381	0.7189	0.6103
Correlation coefficient (r)	0.9998	0.9999	0.9999
Accuracy <sup>b</sup>	99.97±0.97	100.59±0.74	100.71±0.57
Precision:			
Repeatability <sup>b</sup>	100.40 ± 0.430	100.50 ± 0.670	99.80 ± 0.480
Intermediate precision <sup>b</sup>	100.70 ± 0.610	99.37 ± 0.750	100.80 ± 0.610

<sup>a</sup>Regression equation = “A = a + bc” for HPLC; where “A” = peak area and “c” = the concentration ( $\mu$ g.ml<sup>-1</sup>).

<sup>b</sup>Mean ± S.D.

**Table 2a: Statistical comparison between the proposed method and the reference method<sup>(60)</sup> for the determination of Torasemide (I).**

Methods	Parameters					
	Mean	S.D.	n	Variance	Student's t-test	F test
Reference method	100.2	0.48	6	0.230	-	-
HPLC	99.97	0.97	6	0.940	0.52 (2.23)	4.08 (5.19)

Values in parenthesis are the theoretical values of *t* and F at P=0.05.

**Table 2b: Statistical comparison between the proposed methods and the reference method<sup>(61)</sup> for the determination of Irbesartan(II).**

Methods	Parameters					
	Mean	S.D.	n	Variance	Student's t-test	F test
Reference method	99.8	0.65	6	0.422	-	-
HPLC	100.59	0.74	6	0.547	1.96 (2.23)	1.29 (5.19)

Values in parenthesis are the theoretical values of *t* and F at P=0.05.

**Table 2c: Statistical comparison between the proposed methods and the reference method<sup>(62)</sup> for the determination of Olmesartan medoxomil (III).**

Methods	Parameters					
	Mean	S.D.	n	Variance	Student's t-test	F test
Reference method	100.5	0.47	6	0.220	-	-
HPLC	100.71	0.57	6	0.324	0.69 (2.23)	1.47 (5.19)

Values in parenthesis are the theoretical values of *t* and F at P=0.05.

**Table 3: Determination of Torasemide (I), Irbesartan (II) and Olmesartan (III) in laboratory prepared mixtures containing their degradates by the proposed HPLC methods:**

Sample no.	% Degradates	% Recovery*		
		Torasemide	Irbesartan	Olmesartan
1	20	99.34	101.5	101.61
2	40	101.97	100.66	100.96
3	60	99.08	100.8	101.29
4	80	101.71	99.27	100.63
5	100	99.29	99.36	100.14
6	120	100.92	101.22	100.79
Mean		100.38	100.47	100.9
R.S.D.%		1.30	0.938	0.51

\*Mean of three determinations.

**Table 4a: Results from robustness testing of the proposed HPLC method for Torasemide (I).**

Conditions	R <sub>t</sub>	N	T	R <sub>s</sub>
<b>Flow rate:</b>				
1.3 ml.min <sup>-1</sup>	4.25	1470	1.21	2.4
1.5 ml.min <sup>-1</sup>	3.53	3073	1	2.6
<b>Mobile phase composition:</b>				
phosphate buffer :acetonitrile (60:40, v/v)	3.53	3073	1	2.6
phosphate buffer :acetonitrile (70:30, v/v)	5.21	2450	1.12	2.3
<b>pH:</b>				
3	3.53	3073	1	2.6
3.5	4.68	1680	1.1	2.3

**Table 4b:** Results from robustness testing of the proposed HPLC method for Irbesartan

<b>Conditions</b>	<b>R<sub>t</sub></b>	<b>N</b>	<b>T</b>	<b>R<sub>s</sub></b>
<b>Flow rate:</b>				
1.3 ml.min <sup>-1</sup>	12.6	10354	1.2	14.3
1.5 ml.min <sup>-1</sup>	9.88	12284	1	16.2
<b>Mobile phase composition:</b>				
phosphate buffer :acetonitrile (60:40, v/v)	8.12	11542	1.4	15.4
phosphate buffer :acetonitrile (70:30, v/v)	9.88	12284	1	16.2
<b>pH:</b>				
3.5	8.23	10563	1.25	13.2
4	9.88	12284	1	16.2

**Table 4c:** Results from robustness testing of the proposed HPLC method for Olmesartan

<b>Conditions</b>	<b>R<sub>t</sub></b>	<b>N</b>	<b>T</b>	<b>R<sub>s</sub></b>
<b>Flow rate:</b>				
1.3 ml.min <sup>-1</sup>	5.21	10268	1.24	13.9
1.5 ml.min <sup>-1</sup>	3.81	12588	1	15
<b>Mobile phase composition:</b>				
phosphate buffer :acetonitrile (60:40, v/v)	3.81	12588	1	15
phosphate buffer :acetonitrile (70:30, v/v)	4.8	11563	1.21	14.1
<b>pH:</b>				
2.8	3.5	11563	1.1	14.4
3.2	3.81	12588	1	15

**Table 5a:** Determination of Torasemide (I) in pharmaceutical formulation using the proposed HPLC and application of standard addition technique:

<b>Pharmaceutical Preparation</b>	<b>Found*</b>	<b>Standard Addition Technique</b>			
	%± S.D	<b>Taken</b> <b>µg ml<sup>-1</sup></b>	<b>Taken</b> <b>µg ml<sup>-1</sup></b>	<b>Taken</b> <b>µg ml<sup>-1</sup></b>	<b>Taken</b> <b>µg ml<sup>-1</sup></b>
Examide® 20 mg Batch No: MT1120410	100.60 ± 0.36	10	2	2.006	100.3
			5	5.06	101.2
			7	7.035	100.5
			10	9.98	99.8
			12	12.024	100.2
			15	15.045	100.3
Mean ± S.D					100.38 ± 0.46

\* The mean percentage recovery of 4-separate determinations for the pharmaceutical preparation

**Table 5b:** Determination of Irbesartan in pharmaceutical formulation using the proposed HPLC and application of standard addition technique:

<b>Pharmaceutical Preparation</b>	<b>Found*</b>	<b>Standard Addition Technique</b>			
	%± S.D	<b>Taken</b> <b>µg ml<sup>-1</sup></b>	<b>Taken</b> <b>µg ml<sup>-1</sup></b>	<b>Taken</b> <b>µg ml<sup>-1</sup></b>	<b>Taken</b> <b>µg ml<sup>-1</sup></b>
Co-Approvel® 300mg/12.5mg Batch No: 1145	101.5 ± 0.56	10	1	1.002	100.2
			2	2.002	100.1
			4	3.992	99.8
			6	6.018	100.3
			8	8.096	101.2
			10	9.98	99.8
Mean ± S.D					100.23±0.52

\* The mean percentage recovery of 4-separate determinations for the pharmaceutical preparation

**Table 5c: Determination of Olmesartan (III) in pharmaceutical formulation using the proposed HPLC and application of standard addition technique:**

Pharmaceutical Preparation	Found*	Standard Addition Technique			
	% $\pm$ S.D	Taken $\mu\text{g ml}^{-1}$	Taken $\mu\text{g ml}^{-1}$	Taken $\mu\text{g ml}^{-1}$	Taken $\mu\text{g ml}^{-1}$
ERASTAPEX® 40mg Batch No: MT3241009	100.7 $\pm$ 0.84	10	1	1.003	100.3
			2	1.99	99.5
			4	3.984	99.6
			6	6.048	100.8
			8	8.04	100.5
			10	9.96	99.6
Mean $\pm$ S.D				100.05 $\pm$ 0.55	

\* The mean percentage recovery of 4-separate determinations for the pharmaceutical preparation

#### Corresponding author

A. Hemdan  
Pharmaceutical Chemistry Department., Faculty of Pharmacy, Ahram Canadian University, 6<sup>th</sup> October, Egypt.  
[hemmdan@yahoo.com](mailto:hemmdan@yahoo.com)

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9/1/2010

## Diversity of *Staphylococcus aureus* Isolated from Human and Bovine Estimated by PCR - Gene Analysis

**<sup>1</sup>J.El-Jakee, <sup>2</sup>Ata S. Nagwa , <sup>1</sup> Gad El-Said, W.A., <sup>2</sup>Bakry,M.A., <sup>2</sup>Samy, A.A., <sup>2</sup>Khairy E.A., <sup>2</sup> Elgabry , E.A.**

<sup>1</sup> Department of Microbiology Faculty of Veterinary Medicine

<sup>2</sup> Department of Microbiology & Immunology National Research Center, Cairo Egypt

**Abstract:** The present investigation studied the diversity of 19 *S. aureus* isolates (9 from bovine and 10 from human sources) in comparison with the standard Cowan I strain by conventional methods and by PCR technology. The latter uses primers targeted to species-specific parts of genes encoding coagulase (*coa*), enterotoxin A (*sea*) and B (*seb*), *mec A* gene encoding methicillin resistant *S. aureus* (MRSA) and *Staphylococcus* protein A (*spa*) gene. *S. aureus* isolates (19) as well as the Cowan 1 strain were tested for antimicrobial sensitivity with 15 antibiotics by disk diffusion method and classified as susceptible, intermediate and resistant. 57.9% of isolates had a relatively high molecular weight plasmid. The *mec A* gene among the chosen MRSA *S. aureus* isolates recovered from human and bovine sources was discussed. Polymorphisms of *coa* and *spa* genes were detected among *S. aureus* isolates. The examined isolates had coagulase gene ranging from 423 bp to 658 bp and the Cowan -1 strain had amplified fragment at 642 bp. All examined *S. aureus* isolates gave an amplified *spa* gene product at approximately from 396-464 bp. The prevalence of enterotoxin genes *sea* and *seb* were determined and the diversity among the chosen isolates was recorded.

[J.El-Jakee, Ata S. Nagwa, Gad El-Said, W.A., Bakry,M.A., Samy, A.A., Khairy E.A., Elgabry, E.A. Diversity of *Staphylococcus aureus* Isolated from Human and Bovine Estimated by PCR - Gene Analysis. Journal of American Science 2010;6(11):487-498]. (ISSN: 1545-1003).

**Keywords:** *S. aureus*, antibiogram sensitivity, MRSA, Enterotoxins, coagulase gene, *spa* gene.

### 1. Introduction

*Staphylococcus aureus* is recognized as causing health care associated and community-acquired infections in every region of the world. Enterotoxigenic *S. aureus* in milk posses a potential health hazard to consumers, the identification of such strains should be used as apart of a risk analysis of milk and milk products (Zouharova and Rysanek, 2008). *S. aureus* is among the most important nosocomial pathogens because of both the diversity and the severity of the infections it causes, including superficial, deep skin and soft-tissue infections, endocarditis, and bacteremia, as well as a variety of toxin-mediated diseases such as gastroenteritis, staphylococcal scalded-skin syndrome, and toxic shock syndrome (Waldvogel, 1995 and Lowy, 1998).

Staphylococcal enterotoxins (SEs) are serologically grouped into five major classical types which are SEA, SEB, SEC, SED and SEE in addition to toxic shock syndrome toxin (TSST-1) which causes toxic shock syndrome in human, SEA and SEB are usually more common in milk and milk products (Chiang *et al.*, 2006). The resistance to antimicrobial agents among staphylococci is an increasing problem; these strains often reveal resistance to various drug classes in addition to beta-lactam resistance (Dizbay *et al.*, 2008).

Results obtained from analysis of enterotoxigenic *S. aureus* biochemically and

genotypically by using PCR for encoding genes revealed a strong correlation between each other (Lawrynowicz-Paciorek *et al.*, 2007). A reliable and rapid identification of *S. aureus* colonies from samples is a cornerstone in the control of *S. aureus* infection. Identification of bacterial pathogens still relies mainly on phenotypic criteria. Based on the above it is important to study *S. aureus* using modern differentiating diagnostic techniques like PCR and gene analysis. The goal of the present investigation is to study phenotypic and genotypic characterizes of *S. aureus* recovered from human and bovine sources.

### 2. Materials and Methods

#### Samples:

A total of 830 samples were collected from cattle, buffaloes and human for isolation of *Staphylococcus* species. They were collected from milk samples and septic wounds from bovine's dairy farms in Giza, Fayoum and Animal Health Research Institute (AHRI) Dokki. Giza. Egypt. Urine, septic wounds and nasal swabs from cases with respiratory symptoms were obtained from workers in the farms, out patient clinics of Cairo University Hospitals (CUH) and human laboratories (Cairolab, Elborg and Alfa Lab.) were also collected in nine months period from April 2007 to January 2008 as shown in Table (1).

**Table (1): Types and numbers of the samples collected.**

Source of the isolates	Type of samples	No. of The examined samples
Bovine	- Milk from mastitic cows	200
	- Milk from mastitic Buffaloes	150
	- Swabs from cow septic wounds	50
Human	- Urine from infected urinary tract	150
	- Swabs from septic wounds	150
	- Nasal swabs from cases with respiratory symptoms	130
Total		830

**Identification of staphylococci:**

The collected samples were cultured onto nutrient agar "Difco", sheep blood agar and Bacto-Mannitol salt agar "Difco". The inoculated plates were incubated for 24-48 hours at 37°C. The suspected colonies were picked up and propagated in nutrient agar slope for further examinations. Staphylococci were identified according to Quinn *et al.* (2002).

**Characterization of *S.aureus* isolates (Cruickshank *et al.*, 1975):**

The *S. aureus* isolates were identified by using the following tests: Catalase, coagulase, maltose fermentation, urease activity, mannitol fermentation, pigment production onto nutrient agar "Difco", hemolytic activity on sheep and human blood agar, DNase activity on DNase medium "Oxoid", lysozyme activity, gelatinase activity, growth on Baird-Parker Medium "Biomerieux" containing 1% potassium tullerite, lipase activity on egg yolk agar medium, protease activity of *S. aureus* on milk agar medium, fibrinolysin activity on plasma agar medium, Vogues Proskauer test for detection of acetone production, detection of SpA by agglutination using SpA agglutination kits (Wellcome Diagnostics). As well as Crystal violet agar growth type according to Rodgers *et al.* (1999). Crystal violet agar plates were prepared by adding 6 or 8 ug/ml of crystal violet to tryptose agar (Oxoid). Few colonies of the isolates were spot inoculated on plates of both concentrations, incubated at 37°C, and examined after 24 hours. The 6 ug/ml plate was examined if growth was inhibited on the 8 ug/ml plate. Growth of a cream to yellow color with or without violet margins was recorded as growth type A. Growth mainly of blue or violet color

was recorded as growth type C and white color was recorded as growth type E.

**Susceptibility of *S. aureus* isolates to antibacterial agents:**

15 antibacterial disks "Oxoid" were used and the disk diffusion technique was adapted according to Finegold and Martin (1982). After incubation, the degree of sensitivity was determined according to NCCLS (2002) Cheesbrough (2006) and Bannerman and Peacock (2007).

**Detection of plasmid.**

Plasmid DNA extraction was performed in Biotechnology Centre for Services and Research (BCSR) in Faculty of Veterinary Medicine, Cairo University. Extraction of miniprep performed according to Sambrook and Russel (2001). The extracted plasmid was evaluated as visible bands being sized by DNA molecular marker (Hind III digest), that measures molecular weight 81-23000 bp ( Gibbco).

**PCR procedure (Sambrook and Russel, 2001)**

Polymerase chain reaction was performed in Biotechnology Centre for Services and Research (BCSR) in Faculty of Veterinary Medicine, Cairo University. Qiagen extraction kit for DNA extraction from *S. aureus* isolates staphylococcal species was used as described by manufacturer manual of Qiagen, Germany. Primers were synthesized by Metabion Company, Germany as mentioned in Table (2). The presence of specific amplified DNA bands was detected by visualization with UV light at wave length 421 nm and compared with molecular size marker (Ladder) with MW 100 bp and measures MW 100-1500 bp obtained from Amersco Cleveland Ohio, USA. Cowan I strain of *S.aureus* obtained from the Namru 3 in Egypt was used as positive control.

**Table (2): Types of Primers, Primers Designs and References.**

Primer	Primer Design	Product size bp	Reference
<b>Coagulase gene F</b>	5'-ATAGAGATGCTGGTACAGG-3'		Hookey <i>et al.</i> (1998)
<b>Coagulase gene R</b>	5'-GCTTCCGATTGTTCGATGC-3'	433-638	
<b>SAEA-F</b>	5'-CCTTGGAAACGGTTAAAACG- 3'		
<b>SAEA-R</b>	5'-TCTGAACCTTCCCATCAAAAAC- 3'	127	
<b>SAEB-F</b>	5'-TCGCATCAAACGTACAAACG- 3'		Becker <i>et al.</i> (1998)
<b>SAEB-R</b>	5'-GCAGGTACTCTATAAGTGCC- 3'	477	
<b>SPA F</b>	5'-CAAAGATCAACAAAGCGCC- 3'		
<b>SPA R</b>	5'-CGAAGGATCGTCTTAAGGC- 3'	412	Annemüller and Zschock (1999)
<b>MRSA gene F</b>	5'-GGAGACGAGCACTAAAACC-3'		
<b>MRSA gene R</b>	5'-TCGGACGTTCAGTCATT-3'	182	Weller (1999)

SAEA = *Staphylococcus aureus* enterotoxin ASAEB = *Staphylococcus aureus* enterotoxin BMRSA = Methicillin (Oxacillin) resistant *Staphylococcus aureus*. SPA = *Staphylococcus* protein A.

### 3. Results and Discussion

Analyses of the genotype distributions of *S. aureus* strains of diverse origin demonstrated a certain host specificity. It seems that the occurrence of some staphylococcal lineages is restricted to animals (Sung *et al.*, 2008 and Smyth *et al.*, 2009). Livestock-associated *S. aureus* seems to be an underappreciated source of pathogenic strains (Bystron *et al.*, 2010). Several methicillin resistant *S. aureus* (MRSA) clones have disseminated worldwide (Deurenberg *et al.*, 2007). Although bacterial interaction is a well recognized phenomenon, there has been surprisingly little research with respect to MRSA and MSSA. The mechanism/s responsible for this phenomenon is not readily apparent (Al-Kulaifi *et al.*, 2009).

In this study a total of 830 samples were investigated bacteriologically to detect the occurrence of staphylococci among bovine and humans samples. The isolation rate among human samples was 33% while it was 28.3% in bovine samples. 209 *S. aureus*, 21 *S. intermedius* and 25 *S. hyicus* isolates secured from bovine and humans' origins were identified using the most important conventional biochemical tests as catalase, coagulase and acetone production as shown in Table (3).

A number of different phenotypic and genotypic techniques are available to classified strains for epidemiological investigation in the detection and tracking of outbreaks (Wildemauwe *et al.*, 2010). In veterinary microbiology, many phenotypic methods include (pigment production, hemolytic activities, DNase, etc have been applied for characterization of *S. aureus* strains. As shown in

Table (4) it is clear that all isolates were positive for coagulase test, mannitol fermentation, acetone production and show a characteristic growth on Baird parker and crystal violet media which considered being selective media for *S. aureus*. In this concern, Brown and Ngeno (2007) recorded that all positive isolates gave positive reactions in mannitol salt fermentation, in catalase and tube coagulase and latex agglutination tests also, sixteen isolates demonstrated beta hemolysis on horse blood agar while four were not beta hemolytic.

In the present investigation characterization of 19 *S. aureus* isolates (9 from bovine and 10 from human sources) in comparison with the standard Cowan I strain was performed by conventional methods and by PCR technology. Worldwide, the prevalence of multi-resistant *S. aureus* strains has been increased problematically. Increased attention has been focused on plasmid-encoded resistance to antiseptics and disinfectants in antibiotic resistant staphylococci (Bjorland *et al.*, 2003). 57.9% of isolates had a high molecular weight plasmid (more than 18000kbp) as well as Cowan 1 strain as shown in photo (1). Lindsay (2010) recorded that plasmids in *S. aureus* are predominantly of two types, small rolling circle plasmids often encode only one or two resistance genes, such as pT181 (Khan, 2005). The larger plasmids replicate by the theta mechanism and can carry a combination of resistance genes including penicillinase, heavy metals, detergents, trimethoprim and aminoglycosides, some of which are due to integrated small plasmids or transposons (Berg *et al.*,

1998). Some larger plasmids also encode the *tra* genes for conjugative transfer and many strains of *S. aureus* carry one or more plasmids (Lindsay, 2010).

Methicillin (oxacillin) -resistant *S. aureus* (MRSA) was first described in 1961 (Jevons, 1961) and since then has become a significant pathogen in nosocomial infections (Hartman and Tomasz, 1986). For clinicians, the spread of these methicillin-resistant strains has been critical as the therapeutic outcome of infections that result from MRSA is worse than those from methicillin-sensitive strains (MSSA) (Cosgrove *et al.*, 2003). This study aimed to assess the antimicrobial susceptibility patterns and prevalence of methicillin resistance among the chosen *S. aureus* isolated (19 isolates) from human and bovine sources, as well as the Cowan 1 strain as shown in Tables (5-7). High resistance was recorded to methicillin (60%) among the examined *S. aureus* isolates, followed by oxytetracycline (55%) ampicillin & sulphamethoxazole-timethoprim (45% each). Then amoxicillin (40%), ofloxacin (30%), clindamycin & erythromycin (25% each) and amoxicillin clavulanic acid, cefoperazone & cefotaxime (15% each) as shown in Table (5). Meanwhile 95% of the examined *S. aureus* isolates were sensitive to vancomycin, 85% to cefotaxime and 80% to amoxicillin clavulanic acid and cefoperazone. The human isolates were often multidrug resistant, unlike the animal isolates (Lindsay, 2010). 7 out of 10 isolates from human origin were MRSA (70%) and 5 out of 9 *S. aureus* isolates (55.6%) of bovine origin were MRSA, in addition to the Cowan 1 strain as shown in Tables (6 &7).

Interestingly, VRSA are less fit than MRSA in the presence of low concentrations of vancomycin which may be prevalent in hospitals (Foucault *et al.*, 2009). However, in the absence of vancomycin they are fit, yet, no spread of VRSA in hospitals has been reported (Lindsay, 2010). Only one isolate showed an intermediate resistance to vancomycin as shown as in Table (5). Outbreaks of VISA are not reported, and their endemic potential is probably low. Of more concern are fully VRSA strains, first reported in the USA in 2002 (Zhu *et al.*, 2008).

Methicillin-resistant staphylococci carry the *mecA* gene, which encodes a specific low-affinity penicillin-binding protein 2a (PBP<sub>2a</sub>), this protein is responsible for the methicillin resistance in staphylococci (Hackbart and Chambers, 1989). As shown in Photo (2) all methicillin-resistant *S. aureus* isolates were *mecA* gene positive by PCR among the examined isolates and the standard strain. Polymerase chain reaction and DNA hybridization detection of the *mecA* gene in staphylococci is unaffected by the level of its expression (Mo and Wang, 1997).

Comparable PCR-based systems for identification of *S. aureus* isolates under investigation have been used. The coagulase gene (*coa*) typing (Reinoso *et al.*, 2008) have been used to identify and compare *S. aureus* genotypes. As shown in Photo (3), all isolates examined had coagulase gene. Two different PCR products were detected, one in size ranging from approximately 423 bp to 484 bp and another at 608 to 658bp. The standard Cowan I strain had an amplified PCR fragment at 642bp (Photo, 3). Length and sequence polymorphisms of the coagulase gene and its use for genotypic characterization of *S. aureus* had been already shown (Stephan *et al.*, 2000 and Su *et al.*, 2000). Studies carried out by other researchers (Kalorey *et al.*, 2007; Reinoso *et al.*, 2008) showed different coagulase gene types. The reason for this polymorphism among *S. aureus* isolates is unclear, but it seems to be because of deletion or insertion mutations by which a portion of the 3' end region of the *coa* gene is deleted or several nucleotides are inserted and as a consequence change the *coa* gene size and probably antigenic properties of the coagulase enzyme (Saei *et al.*, 2009). Mobile genetic elements (MGE) are discrete pieces of DNA that encode factors allowing them to mobilise within or between genomes (Lindsay, 2008). In *S. aureus*, the major MGE are bacteriophage, pathogenicity islands (SaPI), plasmids, transposons and staphylococcal cassette chromosomes (SCC). Most MGE show evidence of frequent horizontal transfer and recombination (Lindsay, 2010). The evolution of new human and animal pathogenic strains of *S. aureus* has been due to the accumulation of mobile genetic elements (MGE) encoding methicillin resistance and virulence factors into successful lineages (Lindsay, 2010).

*S. aureus* is able to produce a number of virulence factors such as protein A or leukocidins (Kerro Dego *et al.*, 2002). Protein A is located in the cell wall and captures antibodies (Foster and McDevitt, 1994). Photo (4) showed agarose gel electrophoresis of *spa* gene amplification products. It is clear that all examined *S. aureus* isolates gave an amplified *spa* gene product at approximately from 396-464 bp. Tang *et al.* (2000) had shown that detection of genetic polymorphisms in the X region of the *spa* gene can be used as a typing method to determine the epidemiologic relatedness of MRSA isolates. Protein A is a component of the *S. aureus* cell wall and is covalently bound to the peptidoglycan. The *spa* gene is approximately 2,150 bp and contains three distinct regions: the Fc portion, the X region, and the C terminus, the polymorphic X region contains various numbers of 24 bp repeats with various sequences had been described by Frénay *et al.* (1996). With the *spa* typing, a great number of

different types were obtained (Wildemauwe *et al.*, 2010). The variability of this gene indicate that sequence analysis of the *spa* gene could be used as an alternative system for the molecular typing of *S. aureus* isolates.

*Staphylococcus aureus* is one of the most common agents in bacterial food poisoning outbreaks. Its strains produce a spectrum of protein toxins and virulence factors thought to contribute to the pathogenicity of this organism (Adwan *et al.*, 2005). The staphylococcal enterotoxins (SEs) have been classified into many different types. The most common types of these enterotoxins are SEA to SEE. Isolates carrying toxin genes *sea* to *see* are responsible for 95% of staphylococcal food poisoning outbreaks (Bergdoll, 1983). The remaining staphylococcal food-borne disease outbreaks may therefore be associated with other newly identified SEs (MacLauchlin *et al.*, 2000; Omoe *et al.*, 2002 and Rosec & Gigaud, 2002). This study was conducted to determine the prevalence of enterotoxin genes A (*sea*) & B (*seb*) among the chosen *S. aureus* isolates recovered from human and bovine sources. The results in Photo (5) show that bovine strains (33%) were positive for both *sea* and *seb* genes, while 11.1% were positive for *seb* gene only. Among human strains, 20% were positive for *sea* gene and *seb* gene each. The Cowan 1 strain was positive for both *sea* and *seb* genes. Out of the 100 *S. aureus* isolates (milk sheep origin =52: milk cows origin =

48) tested by Adwan *et al.* (2005) for SE-genes, 37 (37%) were positive and the majority of these positive toxin gene isolates, 20 (54.1 %), were *seb*-positive. This result was consistent with previous reports from Japan, Poland and Slovakia, where 64% to 85% of the enterotoxigenic *S. aureus* isolates recovered from raw poultry meat or different food samples and food manufacturers harbored the toxin gene *seb* (Holeckova *et al.*, 2002 and Kitai *et al.*, 2005). In this study 10 out of 20 strains were negative for enterotoxins genes. Also 15 *S. aureus* poultry isolates were found to be non enterotoxigenic by Bystron *et al.* (2010).

It could be concluded that antibiogram clarifying the developed resistance of *S. aureus* strains to commonly used antibiotics ensuring that the right use of antibiotic of choice is very important in line of treatment and control of the infections caused by *S. aureus* especially MRSA strains. Genotyping by PCR is highly effective in detection of *S. aureus* with high sensitivity and specificity especially with polymerization of *coa* and *spa* genes which considered the cornerstone markers for detection and study of *S. aureus*. PCR results of *mecA* gene gave sharp differentiation between many strains which help in determination of the suspected source of infection especially in nosocomial infection cases also in case of repeated infections with the same strain in case of treatment failure or insufficient disinfection.

**Table (3): Prevalence of *Staphylococcus* species from the collected samples**

Source of the isolates	No. of the examined samples	<i>Staphylococcus</i> species						Total	
		<i>S. aureus</i>		<i>S. intermedius</i>		<i>S. hyicus</i>			
		No.	%	No.	%	No.	%	No.	%
Bovine	400	86	21.5	18	4.5	9	2.3	113	28.3
Human	430	123	28.6	3	0.69	16	3.7	142	33
Total	830	209	25.2	21	2.5	25	3	255	30.7

No.: Number of Positive %: was calculated according to number of the examined samples

**Table (4) Characteristic features of the examined *S. aureus* isolates:**

Source of the Isolates	Number of examined samples	Number of <i>S. aureus</i> isolates	Colony pigment				Hemolytic activity						DNase activity		Lysozyme activity		Gelatinase activity		Lecithinase activity		Lipase activity			
			White		Creamy		Golden yellow		Sheep blood agar		Human blood agar													
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%		
Bovine	400	86	4	4.7	20	23.3	62	72.1	79	91.9	26	30.2	7	8.1	73	84.9	82	95.3	82	95.3	72	83.7	57	66.3
Human	430	123	12	9.8	22	17.9	89	72.4	112	91.1	12	9.8	11	8.9	116	94.3	121	98.4	120	97.6	111	90.2	103	83.7
Total	830	209	16	7.7	42	20.1	151	72.2	191	91.4	38	18.2	18	8.6	189	90.4	203	97.1	202	96.7	183	87.6	160	76.6
Characteristic features of the selected 20 strains for plasmid detection and PCR – gene analysis			0	0	2	10	18	90	20	100	8	40	0	0	19	95	20	100	20	100	20	100	18	90

**Table ( 4 ) : Continue**

Protease activity		Tellurite reduction		Fibrinolysin		SpA by agglutination		Crystal violet medium						Mannitol		Novobiocin (S) 30 ug "Biomerieux".		Acetone production		Coagulase test	
								Yellow (A)		Violet (C)		White (E)									
No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
69	80.2	81	94.2	61	70.9	54	62.8	26	30.2	47	54.7	13	15.1	86	100	-	-	86	100	86	100
107	87	122	99.2	119	96.7	102	88.9	81	65.9	28	22.8	14	11.4	123	100	-	-	123	100	123	100
176	84.2	203	97.1	180	86.1	156	74.6	107	51.2	75	35.8	27	12.9	209	100	-	-	209	100	209	100
18	90	20	100	19	95	20	100	13	65	6	30	1	5	20	100	20	100	20	100	20	100

No. Positive number

% was calculated according to the number of samples

S= sensitive

**Table (5): Results of chemotherapeutic sensitivity test of the examined *S. aureus* isolates**

No.	Antimicrobial agents	Disc potency µg/disc	Resistant		Intermediate		Sensitive	
			No.	%	No.	%	No.	%
1	Amoxicillin (AML)	25	8	40	7	35	5	25
2	Amoxicillin / clavulanic (AMC)	20+10	3	15	1	5	16	80
3	Ampicillin (AMP)	10	9	45	5	25	6	30
4	Azithromycin (AZ)	5	4	20	1	5	15	75
5	Cefoperazone (CB)	1	3	15	1	5	16	80
6	Cefotaxime (CX)	30	3	15	0	0	17	85
7	Ciprofloxacin (CF)	10	4	20	5	25	11	55
8	Clindamycin (CD)	2	5	25	2	10	13	65
9	Erythromycin (E)	15	5	25	1	5	14	70
10	Methicillin (Oxacillin) (OX)	5	12	60	1	5	7	35
11	Oflloxacin (ON)	20	6	30	2	10	12	60
12	Oxytetracycline (OT)	30	11	55	4	20	5	25
13	Sulphamethoxazole-Timethoprim (SXT)	23.75+1.25	9	45	5	25	6	30
14	Tobromycin (TN)	20	4	20	2	10	14	70
15	Vancomycin (VN)	3	0	0	1	5	19	95

No. Positive number

% was calculated according to the number of samples

**Table (6) Analysis of PCR products of MRSA strains from bovine origin**

Strains No.	origin	Source	coa. gene		Toxins gene		spa gene Mol. wt. 396-462 bp	mec. A gene Mol. wt. (182)	Plasmi d	Most resistant antibiotics	Most sensitive antibiotics					
			Mol. wt.		Mol. wt.											
			423	608	A (127)	B (477)										
1	Bovine strains	Bovine mastitic milk	-ve	+ve	-ve	-ve	+ve 396	+ve	+ve	OX, OT, AMC, AML	CX, AZ, CB					
2			-ve	+ve 658	-ve	+ve	+ve 418	+ve	+ve	OX, AMP, CX, ON, SXT	AMC, E, TN					
3			+ve 423	-ve	+ve	+ve	+ve 464	+ve	-ve	OX, OT, TN, E	CX, AZ, CB					
4			-ve	+ve 658	-ve	-ve	+ve 422	-ve	+ve	OX, AMP, CB, AML	AMC, AZ, E, OT					
8		Bovine wounds	+ve 432	-ve	-ve	-ve	+ve 452	+ve	-ve	OX, AMP, CF	AMC, CB, ON					

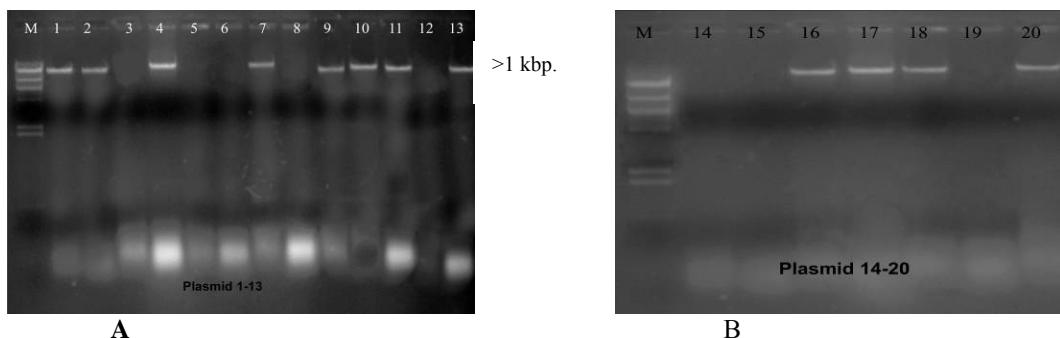
<b>20</b>	<b>Cowan-1 strain</b>	-ve	+ve 642	+ve	+ve	+ve 448	+ve	+ve	OX, OT, TN, ON, AML, E	CX, AMC, AZ, CD
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-ve= negative, +ve= positive, AML= Amoxicillin – AMC= Amoxicillin / clavulanic – AMP= Ampicillin - AZ =Azithromycin – CF= Ciprofloxacin - CX =Cefotaxime - CB =Cefoperazone – CD= Clindamycin – E= Erythromycin - OX= Methicillin (Oxacillin) – ON= Ofloxacin - OT =Oxytetracycline – SXT=Sulphamethoxazole-Timethoprim – TN= Tobromycin - VN =Vancomycin.

**Table (7) Analysis of PCR products of MRSA strains from human origin**

Strains No.	Origin	Source	<i>coa. gene</i>		Toxins gene		<i>spa</i> gene	<i>mec. A</i> gene	Plasmid	Most resistant antibiotics	Most sensitive antibiotics			
			Mol. wt.		Mol. wt.									
			423 -	608 -	A (127)	B (477)	Mol. wt. 396- 462b.p	Mol. wt. (182)bp						
1	Human strains	Respiratory infection	-ve	+ve 658	+ve	-ve	+ve 448	+ve	+ve	OX, SXT, TN, AZ	AMC, CB, E, CD, CX			
2		Septic wound	+ve 448	-	-ve	-ve	+ve 452	+ve	-ve	OX, OT, CD, ON	CX, AZ, CB, CF			
3			-ve	+ve 608	-ve	+ve	+ve 452	+ve	+ve	OX, OT, AMP, CF, CX, SXT	AMC, AZ, E, ON, TN			
4			+ve 484	-ve	-ve	-ve	+ve 418	+ve	-ve	OX, E, AML, SXT, TN	CX, AMC, CB, ON, CX			
5			+ve 484	-ve	-ve	-ve	+ve 448	-ve	-ve	OX, OT, ON, AMP	CX, AZ, E, TN, SXT			
6			-ve	+ve 658	-ve	+ve	+ve 462	-ve	+ve	OX, SXT, AZ, AML	AMC, CB, TN, VN, CF			
7		Urinary infection	-ve	+ve 642	-ve	-ve	+ve 418	+ve	+ve	OX, CD, SXT	AZ, TN, ON, CB, AMC			
8	Cowan-1 Standard strain	-ve	+ve 642	+ve	+ve	+ve 448	+ve	+ve	OX, OT, TN, AML, E, ON	CX, AMC, AZ, CD				

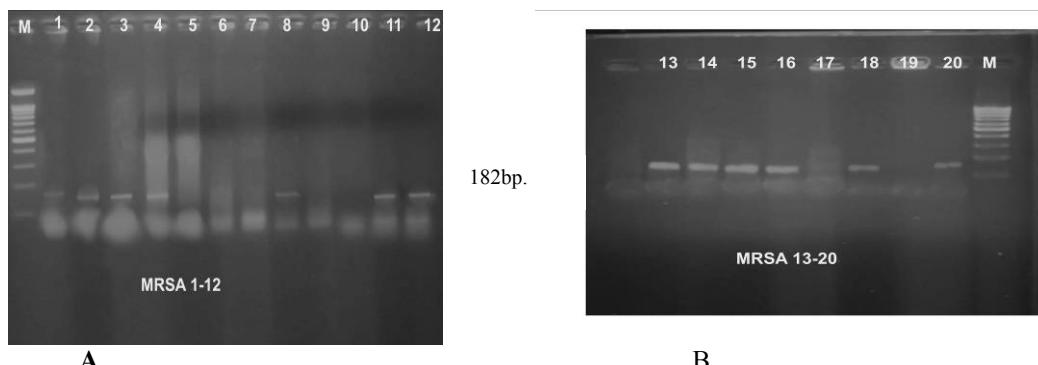
-ve= negative, +ve= positive, AML= Amoxicillin – AMC= Amoxicillin / clavulanic – AMP= Ampicillin - AZ =Azithromycin – CF= Ciprofloxacin - CX =Cefotaxime - CB =Cefoperazone – CD= Clindamycin – E= Erythromycin - OX= Methicillin (Oxacillin) – ON= Ofloxacin - OT =Oxytetracycline – SXT=Sulphamethoxazole-Timethoprim – TN= Tobromycin - VN =Vancomycin.



**Photo (1): Agarose gel electrophoresis showing plasmid profile in *S. aureus* isolated strains**

- (A) M: DNA molecular weight marker adapted by (Hind III digest). Lane 1: Cows milk (Positive for plasmid). Lane 2: Cows milk (Positive for plasmid). Lane 3: Cows milk (Negative for plasmid). Lane 4: Cows milk (Positive for plasmid). Lane 5: Cows milk (Negative for plasmid). Lane 6: Buffaloes milk (Negative for plasmid). Lane 7: Buffaloes milk (Positive for plasmid). Lane 8: Bovine septic wounds (Negative for plasmid). Lane 9: Bovine septic wounds (Positive for plasmid). Lane 10: Human respiratory infection (Positive for plasmid). Lane 11: Human respiratory infection (Positive for plasmid). Lane 12: Human septic wounds (Negative for plasmid). Lane 13: Human septic wounds (Positive for plasmid).

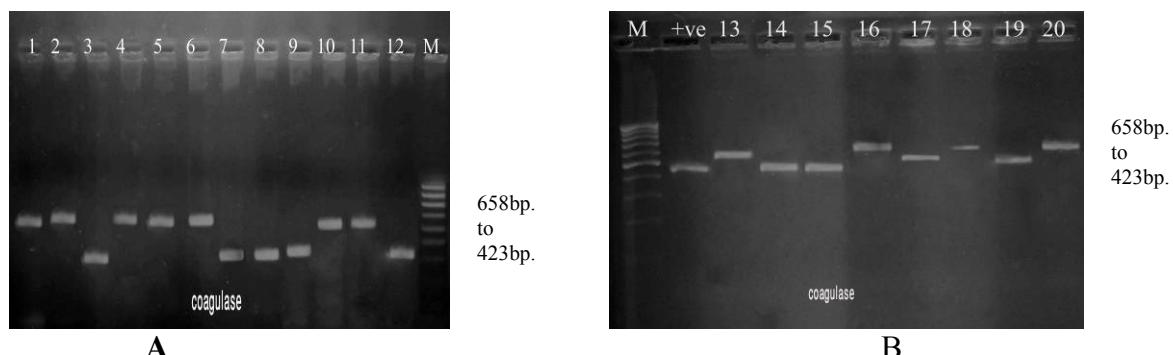
(B) M: DNA molecular weight marker adapted by (Hind III digest). Lane 14: Human septic wounds (Negative for plasmid). Lane 15: Human septic wounds (Negative for plasmid). Lane 16: Human septic wounds (Positive for plasmid). Lane 17: Human septic wounds (Positive for plasmid). Lane 18: Human infected urinary tracts (Positive for plasmid). Lane 19: Human infected urinary tracts (Negative for plasmid). Lane 20: Cowan-1 standard strain (Positive for plasmid)



**Photo (2): Agarose gel electrophoresis showing the result of amplification of *mec*. A gene (182 bp)**

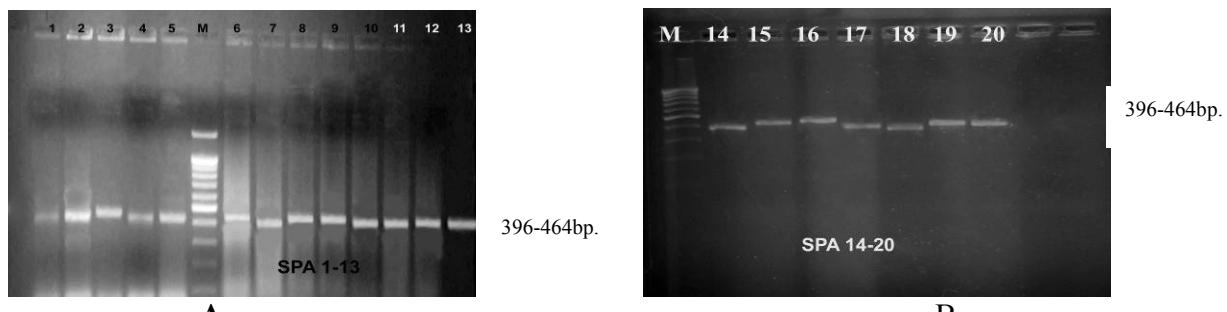
- (A) M: DNA molecular weight marker (100 bp. ladder). Lane 1: Cows milk (Positive for *mec.* A gene). Lane 2: Cows milk (Positive for *mec.* A gene). Lane 3: Cows milk (Positive for *mec.* A gene). Lane 4: Cows milk (Positive for *mec.* A gene). Lane 5: Cows milk (Negative for *mec.* A gene). Lane 6: Buffaloes milk (Negative for *mec.* A gene). Lane 7: Buffaloes milk (Negative for *mec.* A gene). Lane 8: Bovine septic wounds (Positive for *mec.* A gene). Lane 9: Bovine septic wounds (Negative for *mec.* A gene). Lane 10: Human respiratory infection (Negative for *mec.* A gene). Lane 11: Human respiratory infection (Positive for *mec.* A gene). Lane 12: Human septic wounds (Positive for *mec.* A gene)

(B) M: DNA molecular weight marker (100 b.p. ladder). Lane 13: Human septic wounds (Positive for *mec.* A gene). Lane 14: Human septic wounds (Positive for *mec.* A gene). Lane 15: Human septic wounds (Positive for *mec.* A gene). Lane 16: Human septic wounds (Positive for *mec.* A gene). Lane 17: Human septic wounds (Negative for *mec.* A gene). Lane 18: Human infected urinary tracts (Positive for *mec.* A gene). Lane 19: Human infected urinary tracts (Negative for *mec.* A gene). Lane 20: Cowan-1 standard strain (Positive for *mec.* A gene).



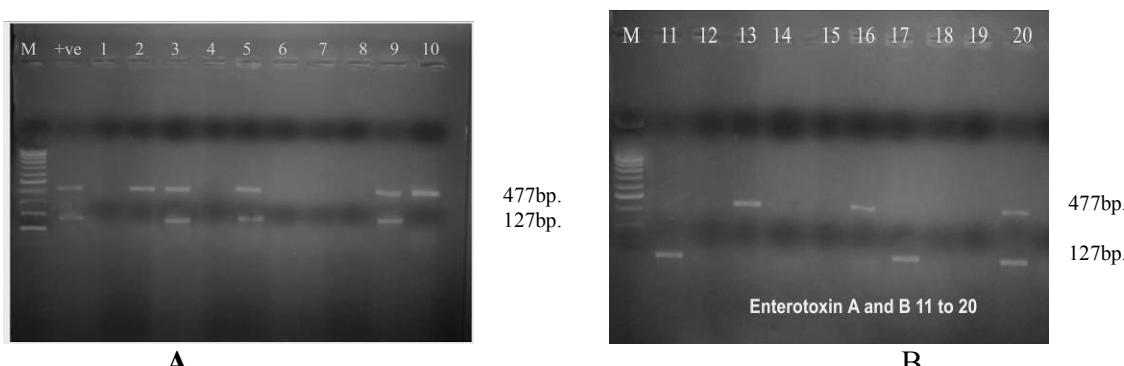
**Photo (3): Agarose gel electrophoresis showing the result of amplification of coagulase gene polymorphisms of the gene encoding staphylococcal coagulase**

- (A) M: DNA molecular weight marker (100 bp. ladder). Lane 1: Cows milk (630 bp). Lane 2: Cows milk (658 bp). Lane 3: Cows milk (423 bp). Lane 4: Cows milk (658 bp). Lane 5: Cows milk (658 bp). Lane 6: Buffaloes milk (658 bp). Lane 7: Buffaloes milk (428 bp). Lane 8: Bovine septic wounds (432 bp). Lane 9: Bovine septic wounds (456 bp). Lane 10: Human respiratory infection (658 bp). Lane 11: Human respiratory infection (658 bp). Lane 12: Human septic wounds (448 bp).
- (B) M: DNA molecular weight marker (100 bp. ladder). Lane 13: Human septic wounds (608 bp). Lane 14: Human septic wounds (484 bp). Lane 15: Human septic wounds (484 bp). Lane 16: Human septic wounds (658 bp). Lane 17: Human septic wounds (428 bp). Lane 18: Human infected urinary tracts (642 bp). Lane 19: Human infected urinary tracts (518 bp). Lane 20: Cowan-1 standard strain (642 bp). +ve: Positive control



**Photo (4): Agarose gel electrophoresis showing the result of amplification of spa gene**

- (A) M: DNA molecular weight marker (100 bp. ladder). Lane 1: Cows milk (396). Lane 2: Cows milk (418 bp). Lane 3: Cows milk (464 bp). Lane 4: Cows milk (422 bp). Lane 5: Cows milk (430 bp). Lane 6: Buffaloes milk (452 bp). Lane 7: Buffaloes milk (428 bp). Lane 8: Bovine septic wounds (452 bp). Lane 9: Bovine septic wounds (452 bp). Lane 10: Human respiratory infection (448 bp). Lane 11: Human respiratory infection (448 bp). Lane 12: Human septic wounds (452 bp). Lane 13: Human septic wounds (452 bp).
- (B) M: DNA molecular weight marker (100 bp. ladder). Lane 14: Human septic wounds (418 bp). Lane 15: Human septic wounds (448 bp). Lane 16: Human septic wounds (462 bp). Lane 17: Human septic wounds (452 bp). Lane 18: Human infected urinary tracts (418 bp). Lane 19: Human infected urinary tracts (448 bp). Lane 20: Cowan-1 standard strain (448 bp).



**Photo (5): Agarose gel electrophoresis showing the result of multiplex PCR for detection of enterotoxin genes from *S. aureus* strains**

(A) *sea*: *S. aureus* enterotoxin A (127 bp). *seb*: *S. aureus* enterotoxin B (477bp). M: DNA molecular weight marker (100 bp. ladder). Lane 1: Cows milk (negative). Lane 2: Cows milk (*seb* gene). Lane 3: Cows milk (both *sea* and *seb* genes). Lane 4: Cows milk (negative). Lane 5: Cows milk (both *sea* and *seb* genes). Lane 6: Buffaloes milk (negative). Lane 7: Buffaloes milk (negative). Lane 8: Bovine septic wounds (negative). Lane 9: Bovine septic wounds (both *sea* and *seb* genes).

*sea*: *S. aureus* enterotoxin A (127 bp). *seb*: *S. aureus* enterotoxin B (477bp). M: DNA molecular weight marker (100 b.p. ladder). Lane 11: Human respiratory infection (*sea* gene). Lane 12: Human septic wounds (negative). Lane 13: Human septic wounds (*seb* gene). Lane 14: Human septic wounds (negative). Lane 15: Human septic wounds (negative). Lane 16: Human septic wounds (*seb* gene). Lane 17: Human septic wounds (*sea* gene). Lane 18: Human infected urinary tracts (negative). Lane 19: Human infected urinary tracts (negative). Lane 20: Cowan-1 standard strain (both *sea* and *seb* genes)

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9/1/2010

# Implementation of a rapid procedure for distinguishing enterotoxigenic Clostridium perfringens

<sup>1</sup>J. El-Jakee, <sup>2</sup>Ata S. Nagwa, <sup>2</sup>Bakry, M.A., <sup>2</sup>Sohier, M. Syame, <sup>2</sup>Samy A.A., <sup>2</sup>Khairy E.A.

1Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt

2Department of Microbiology and Immunology National Research Center, Cairo, Egypt

**Abstract:** The objective of the present study is to develop an easy method for detection of toxigenic *C. perfringens* isolates .4 *C. perfringens* isolates (type A, B, C & D) were collected from chickens and reconfirmed on the basis of conventional tests and multiplex PCR. Antisera were prepared from *C. perfringens* types A, B, C & D separately in different groups of rabbits. The titres of the prepared hyperimmune sera were estimated by ELISA & staphylococci protein A (SpA) agglutination test. An attempt was carried out to detect *C. perfringens* toxins in infected fecal samples. The fecal samples were infected by 20 & 40 µg /ml *C. perfringens* toxins (A, B, C & D) and examined by double sandwich ELISA & SpA agglutination methods. In addition the sensitivity of PCR for detection of *C. perfringens* types were compared with conventional culture technique among fecal samples contaminated with *C. perfringens* types and the results were discussed.

[J. El-Jakee, Ata S. Nagwa, Bakry, M.A., Sohier, M. Syame, Samy A.A., Khairy E.A. Implementation of a rapid procedure for distinguishing enterotoxigenic Clostridium perfringens. Journal of American Science 2010;6(11):499-508]. (ISSN: 1545-1003).

**Keywords:** *C. perfringens*, ELISA, SpA agglutination, PCR.

## 1. Introduction

Clostridia are commonly found in the environment, occurring in soil, sewage, and waters, as well as in the intestines of both man and animals. Members of the genus *Clostridium* are widely recognized as enteric pathogens for man, domestic animals and wildlife (Songer, 1996). *Clostridium perfringens*, a part of normal gut flora, is commonly involved in diseases in most domestic animals and some wildlife, including horses, poultry, birds, rabbits, sheep, goats, cattle, mink, ostrich, dogs and cats (Nillo ,1993). Smyth and Martin (2010) recorded that necrotic enteritis is a serious disease of chickens and turkeys caused by *Clostridium perfringens* and demonstrated that *C. perfringens* strains from a mammalian species and from normal chickens, can cause necrotic enteritis in chickens. *Clostridium perfringens* is an important cause of both histotoxic and enteric diseases (Fernandez-Miyakawa *et al.*, 2008). *C. perfringens* type D is the etiological agent of enterotoxaemia (pulpy kidney disease) of several animal species (Blood *et al.*, 1983). According to current knowledge, the disease is caused by epsilon toxin, a major exotoxin produced by this microorganism (Uzal and Kelly, 1996).

Substantial interest and effort have been expended in establishing new methods as well as improving classical methods for the detection and isolation of *C. perfringens*. The objective of the present study is to develop an easy method for typing of *C. perfringens* isolates in the veterinary routine diagnostic laboratory.

## 2. Materials and Methods

### Diagnostic antisera:

Diagnostic *C. perfringens* antisera type A, B, C and D were obtained from Welcome, Diagnostics Dartford, England from Microbiology department, Anaerobic Unit, Abbassia. They were used as control positive among the used serological test.

### Identification of *C. perfringens* isolates:

Four isolates of *C. perfringens* type A, B, C and D were collected from chickens. Each isolate was inoculated into freshly prepared Robertson's cooked meat medium (Smith and Holdman, 1968), and incubated anaerobically at 37°C for 24 hrs. Then a loopful from this culture was streaked onto neomycin sulphate sheep blood agar (Carter and Cole, 1990). The streaked inoculated plates were incubated anaerobically at 37°C for 24-48 hrs. The catalase negative colonies were picked up and examined for their morphological, cultural and biochemical characters according to Koneman *et al.* (1992). Biotyping of *C. perfringens* isolates by dermonecrotic reaction in Albino Guinea pigs was carried out according to Stern and Batty (1975).

### Multiplex PCR:

Template DNAs for PCR were prepared according to Osek and Winiarczyk (2001). Specific oligonucleotide primers for the toxin genes (alpha ( $\alpha$ ), beta ( $\beta$ ) and epsilon ( $\epsilon$ ) of *C. perfringens* toxins

were selected on the base of published sequences (Effat *et al.*, 2007) as shown in Table (1). The PCR product using 1.5% agarose gel electrophoresis and

DNA molecular weight marker of 100 base pair ladder (Bioron GmbH) (Jena Bioscience - Germany) was analyzed according to Sambrook *et al.* (1989)

**Table (1): Oligonucleotide primers for the toxin genes  $\alpha$ ,  $\beta$  and  $\epsilon$  of *C. perfringens***

Primer Designation	5'-----3' sequence	Amplified product size (bp)
CP ALPHA toxin F	GTTGATAGCGCAGGACATGTTAAG	402 bp
CP ALPHA toxin R	CATGTAGTCATCTGTTCCAGCATC	
CP BETA toxin F	ACTATACAGACAGATCATTCAACC	236 bp
CP BETA toxin R	TTAGGAGCAGTTAGAACTACAGAC	
CP EPSILON toxin F	ACTGCAACTACTACTCATACTGTG	
CP EPSILON toxin R	CTGGTGCCTTAATAGAAAGACTCC	541 bp

#### **Preparation of hyperimmune sera against *C. perfringens* isolates:**

Sixteen healthy rabbits of New Zealand breed, each of 1.5-2 kg body weight were obtained from the farm of "Animal Health Research Institute, Dokki, Giza". They were rest under strict good hygienic conditions during the whole period of experiment and provided with a balanced diet. They proved to be free from clostridial infections. Before beginning of the experiment, 3 ml blood sample from the ear vein of each rabbit was taken and the serum was separated and kept as non-infected control negative sample and kept at -20°C. The rabbits were classified into 4 groups, each group contains 4 rabbits. The first group was injected with *C. perfringens* type A, the second group was injected with *C. perfringens* type B, the third group was injected with *C. perfringens* type C and the fourth group was injected with *C. perfringens* type D. Each animal of each group was inoculated with 18 hrs culture of *C. perfringens* grow in medium containing 2% (w/v) polypeptone (Daigo, Osaka, Japan), 1% (w/v) glucose, 0.5% NaCl (pH 7.2) and heated at 100°C for 60 min. The immunization was carried out by injection of 0.5, 1.0, 1.0, 1.0, 1.5, 2.0, 2.0, 3.0, 3.0 and 3.0 ml of the suspension, respectively into ear vein of a rabbit at 3 day intervals (Yamagishi *et al.*, 1971). The sera of inoculated rabbits were collected and kept at 20°C till use.

#### **Preparation of toxin:**

*C. perfringens* types A, B, C and D were cultivated in cooked meat broth anaerobically at 37°C for 24 hrs. Each one of *C. perfringens* type A, B, C and D was inoculated into toxin production medium (Roberts *et al.*, 1970) and incubated for 5 hrs except type D incubated for 48 hrs. Centrifugation for type

A, B, C and D and the supernatant was taken. Trypsin was added to supernatant of type D and incubated for 1 hour. Dialysis was used for each toxin to remove small molecular contaminants according to Judson *et al.* (1987). The dialyzed toxin inside the dialysis bags were covered with polyethylene glycogen and left at 4°C to avoid protein denaturation until the solutions were concentrated to 5 ml (volume) in each bag. The bags were washed with distilled water and the solutions were collected in tubes and then deposited in a lyophilizer to concentrate the volume to 0.5 ml in each sample. The concentrated toxins were stored at -20°C till use. The protein content of 4 toxins of *C. perfringens* type A, B, C and D were measured using the modified Lowry's assay according to Lowry *et al.* (1951).

#### **Antibody assay (titration):**

Titration of the prepared hyperimmune sera was estimated by ELISA and SpA agglutination test.

#### **Enzyme linked immunosorbent assay (ELISA):**

Indirect ELISA was used to detect the antibodies against toxins of *C. perfringens* type A, B, C and D according to Harlow and Lane (1988). The sera were considered to be positive when the absorbency values were as or more than the cut-off value (the cut-off = double fold of the mean negative sera) according to Timmerck (1994).

#### **SpA agglutination test (Subramanayam *et al.*, 2000):**

A Cowan 1 strain of *S. aureus* (It was obtained in freeze lyophilized dried ampoules from the Namru 3 in Egypt) was spread over on brain heart infusion agar medium (Oxoid). Incubation was carried out at 37°C for 48 hrs, bacterial growth was scooped by a bent glass rod with PBS pH 7.2 into test

tubes. The cells were centrifuged at 2000 rpm for 15 minutes at room temperature. The cells were treated overnight with formalin to make a final concentration of 2% to inactivate the cells. Then the *S. aureus* cells were treated at 80°C for 5 minutes in a water bath. The cells were cooled immediately by immersing in an ice bath. Then cells were washed five minutes with PBS, pH 7.2 and 10% of *S. aureus* suspension was prepared in PBS. 2 folds serial dilution of antitoxins were added in U-shaped microtiter plate (25 µl/well). Amount of 25 µl of the prepared 10% SpA suspension was then added to each well and incubated at 37°C for 1 hr. with periodical shaking. 25 µl of *C. perfringens* toxin prepared in PBS were added to SpA mixture then incubated at 4°C. Agglutination was read within 2 hrs.

#### Detection of *C. perfringens* in fecal samples:

The prevalence of *C. perfringens* in feces was determined by selective culture, PCR, SpA and ELISA to permit validation of the ELISA, SpA and PCR.

Artificially contaminated fecal rabbit samples of enterotoxin for *C. perfringens* type A, B, C and D separately were prepared by adding 0.1 ml of toxin at concentration of 20 µg and 40 µg separately to 1 g of feces and 9 ml of water and centrifugation for 5 min at 500 xg. The double sandwich ELISA was performed according El Idrissi and Ward (1992) in 96-well disposable flat-bottomed plates to detect *C. perfringens* toxins in contaminated feces. As well as, SpA agglutination test (Subramanayam *et al.*, 2000) used for detection of *C. perfringens* toxins in the contaminated feces.

#### Sensitivity of the multiplex PCR

The sensitivity of the multiplex PCR assay for detection of *C. perfringens* in feces was conducted according to Kanakaraj *et al.* (1998). Artificially contaminated fecal rabbit samples of *C. perfringens* A, B, C and D were prepared by adding 0.1 ml of a 12 hrs BHI broth culture to 1 g of feces and 9 ml of water then centrifuged for 5 min at 500 xg. One ml of the supernatant was serially diluted in sterile water. From 0.8 ml aliquots of each dilution DNA template was extracted (Stahl *et al.*, 1988) and counts were performed by plating 100 µl aliquots on TSC agar. The sensitivity of the PCR assay was calculated as the number of colony forming units (CFU) of *C. perfringens* in the greatest dilution which was PCR positive.

### 3. Results and Discussion

*C. perfringens* type A ( $\alpha$ -toxin producer) is common in the intestinal tract of chicks, soil, dust-contaminated feed and litre (Kalender and Ertafi, 2005). The  $\alpha$ -toxin ( $\alpha$ ) the principal lethal toxin of *C.*

*perfringens* is a multifunctional phospholipase produced by nearly all isolates. The toxin is haemolytic, necrotizing and potently lethal. Detection of *C. perfringens* toxin types is critical for a better understanding of the epidemiology of *C. perfringens* infections and may be helpful in the development of effective preventive measures.

In the present study, 4 *C. perfringens* isolates (type A, B, C & D) were collected from chickens and reconfirmed on the basis of morphological, cultural and biochemical characteristics. All isolates were Gram-positive spore-forming bacilli as shown in Photo (1). Colonies of *C. perfringens* are up to 5 mm in diameter, circular, flat, greyish and surrounded by zone of double haemolysis after cultured anaerobically on blood agar at 37 °C for 48 hrs (Photo.2). The biochemical behaviour of the isolates complies with these of *Clostridium* typical reactions as described by (Quinn *et al.*, 2002). Toxins of *C. perfringens* isolates were detected using inoculation of guinea pig to detect necrosis as shown in photo. (3)

The collected strains were investigated for production of toxins by PCR. This test was established to replace animal testing and to reduce cost and time. Rapid detection of enterotoxigenic *Clostridium perfringens* in meat samples was accomplished by Yang *et al.* (2010) with an immunomagnetic separation polymerase chain reaction (IMS-PCR). Photo (4) shows amplification of alpha toxin gene (402 bp) from all isolates & amplification of beta toxin gene (236 bp) from *C. perfringens* type B & C, while epsilon toxin gene (541 bp) was recorded in *C. perfringens* type D. The  $\alpha$ -toxin is present in all types of *C. perfringens* and lies within the chromosome of the bacterial DNA (Albini *et al.*, 2008). Molecular typing allowed for an easier in vitro test (Effat *et al.*, 2007).

The development of a useful serological test is dependent upon the identification of defined antigens and the examination of the antibody responses to these antigens. Therefore, antisera were prepared from *C. perfringens* types A, B, C & D separately. Serum samples were collected from rabbits after inoculations with the isolates and the antibody titre against homologous isolate was estimated by ELISA & SpA agglutination test as shown in Tables (3&4). Specificity and avidity of antibody binding to target antigen is critical for the success of antibody-based pathogen detection methods (Bhunia, 1997).

The optical density (OD) of ELISA among the prepared hyperimmune sera was estimated as shown in Table (2) after application of checkerboard titration. It is clear that, positive ELISA OD 0.522, 0.687, 0.504 and 0.534 were recorded among the

prepared hyperimmune sera of *C. perfringens* type A, B, C, and D diluted to 1:800, 1:1600, 1:800 and 1:1600. Using an enzyme-linked immunosorbent assay (ELISA) for measuring levels of specific antibodies against alpha-toxin Heier *et al.* (2001) found a variation in level of maternal antibodies against  $\alpha$ -toxin in broilers. The variation in antibody levels between broiler flocks of different origin indicated that some parent flocks has raised an antibody response to naturally-occurring alpha-toxin. If broiler chickens are able to mount a similar response, this response may have value for protection against the disease, as well as diagnostic value to reveal subclinical disease in broiler flocks (Lovland *et al.*, 2003).

In the present study, SpA agglutination test was used for estimation of *C. perfringens* antibodies among the prepared hyperimmune sera. Coagglutination is similar to latex agglutination technique for detecting antigen protein A, a uniformly distributed cell wall component of *S. aureus* is able to bind to the Fc region of most IgG isotype antibodies leaving Fab region free to interact with antigens present in the applied specimens. The visible agglutination of *S. aureus* particles indicates the antigen-antibody reaction. Data present in Table (3) illustrated that positive coagglutination antibody titers could be detected at 1/4 up to 1/64. 1/16 was used as a CoA reagent for various tests in 12 studies and the results obtained are in agreement with Rahman *et al.* (1989) in *Salmonella* enterotoxin system and with Batra *et al.* (1989) in *Brucella* antigens.

Cross reactivity in vitro was tested to figure out the possible diagnosis of *C. perfringens* using concentrations of 20  $\mu\text{g}/\text{ml}$  toxins ( types A, B, C and D). In this study cross reactivity between *C. perfringens* types was tested as shown in Figures (1-5) using ELISA. These cross reactivity may be due to use of crude toxins which contain  $\alpha$ -toxins among all types.  $\alpha$ -toxin, a necrotizing toxin commonly produced by all five types of *C. perfringens*, is believed to be a major factor responsible for the organism tissue pathology and has been suggested to be a key virulence determinant and predominant product of *C. perfringens* type A (Scott *et al.*, 2004). Also Effat *et al.* (2007) revealed that  $\alpha$ -toxin gene is found in all types of *Clostridium perfringens*.

An attempt was carried out to detect *C. perfringens* in infected feces using ELISA, coagglutination test & PCR assay. Fecal samples were infected by 20 & 40  $\mu\text{g}/\text{ml}$  toxins (A, B, C & D). The infected samples were examined by double sandwich ELISA methods as shown in Tables (4-7). It is clear that 20  $\mu\text{g}/\text{ml}$  is a concentration of choice. The ELISA method of Vaikosen and Ikgatua (2005)

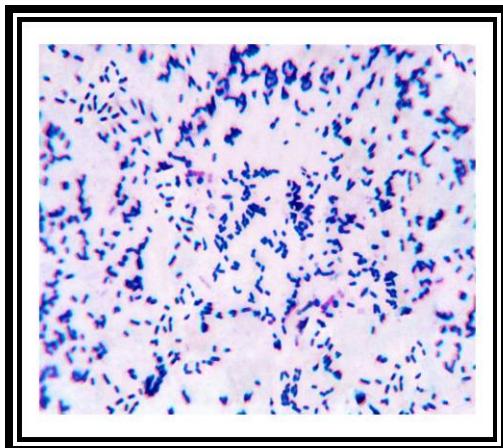
was able to detect as low as 0.1  $\mu\text{g}/\text{ml}$  of enterotoxin corrected optical density (OD) at 405nm, the value of 0.4 OD units was used to estimate the enterotoxin levels of the isolates, and mean value of 0.66 OD units for *C. perfringens* type D, while the mean value of 0.94 OD units for *C. perfringens* type C were obtained. The detection of *C. perfringens* in feces of horses by ELISA could be diagnostically beneficial in a clinical setting (Waggett *et al.*, 2010). Using specific antibodies for beta and epsilon toxins of *C. perfringens*, two double sandwich ELISAs were developed for detection of these toxins in buffers, culture supernatants and intestinal contents by El Idrissi and Ward (1992). In both assays absorbance readings were directly related to the  $\text{Log}_{10}$  of the toxin concentration over 3-4 points between 31 and 250  $\text{ng}/\text{ml}$  for beta toxin and between 8 and 125  $\text{ng}/\text{ml}$  for epsilon toxin. The reason for this difference may be due to we used crude toxins as antigens while El Idrissi & Ward (1992) used a purified activated epsilon toxin, which may have increased both the specificity and sensitivity of the test.

As shown in Tables (8-11) the infected fecal samples were analyzed by SpA agglutination test. The epsilon toxin was detected in five samples by coagglutination and mouse neutralization tests by Subramanyam *et al.* (2000), these samples were made by serial two-fold dilutions from 1/2 to 1/32. Previously Dobosch (1983) describe staphylococcal coagglutination procedure for assaying *C. perfringens* enterotoxin; its sensitivity and specificity were studied. *C. difficile* A and B toxins and *C. perfringens* type A enterotoxin was studied by Giulazian *et al.* (2008) employing the immunological test systems in the coagglutination test using the plates.

The sensitivity of PCR for detection of *C. perfringens* types were compared with conventional culture technique. As shown in Table (12) and photos.(5-8) *C. perfringens* could be isolated onto medium only from  $10^3$  dilution, meanwhile *C. perfringens* toxins could be detected from all dilutions of different *C. perfringens* types by PCR assay. PCR provides a simple and rapid assay for detection of *C. perfringens* under condition where the current levels of sensitivity and specificity are acceptable; it should be immediately useful in epidemiologic and diagnostic studies.

In conclusion, the *C. perfringens* toxin can be detected instantaneously (within 4 minutes) on a slide by SpA agglutination test. Moreover, this test requires minimum number and amounts of reagents which could be presented and used in the form of portable diagnostic kit for use in the farm premises. The results demonstrate the suitability and reliability of multiplex PCR for the routine diagnostic

laboratory. The procedure will improve the diagnosis of food-borne intoxications and will help to discover further epidemiological and aetiological aspects of these diseases.



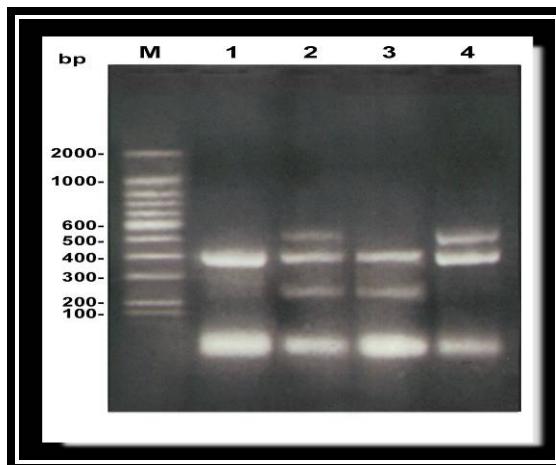
**Photo (1):** *C. perfringens* showed Gram-positive bacilli



**Photo (2):** Double zone of hemolysis showed by *C. perfringens* grown on sheep blood agar.



**Photo (3):** The dermonecrotic reaction of *C. perfringens* toxin type A



**Photo (4):** Amplification of *cpa* (402 bp), *cpβ* (236 bp) & *etx* (541 bp) genes from *C. perfringens* isolates using Multiplex PCR. Lane M:100 bp adder, Lane 1: *C. perfringens* type A, Lane 2: *C. perfringens* type B, Lane 3: *C. perfringens* type C, Lane 4: *C. perfringens* type D.

**Table (2): Results of ELISA absorbance values among the prepared hyperimmune sera using *C. perfringens* toxin types A, B, C and D (20ug/ml).**

Dilution of antisera	<i>C. perfringens</i> toxin types			
	A	B	C	D
1/50	0.851	1.521	0.980	1.022
1/100	0.811	1.103	0.850	0.972
1/200	0.742	0.950	0.801	0.842
1/400	0.651	0.870	0.622	0.685
1/800	0.522	0.742	0.504	0.601
1/1600	0.434	0.687	0.475	0.534

+ve OD≥ 0.5

**Table (3) Results of SpA among the prepared hyperimmune sera using *C. perfringens* toxin types A, B, C and D (20 ug/ml).**

Dilution of antisera	<i>C. perfringens</i> toxin types			
	A	B	C	D
1/4	+	+	+	+
1/8	+	+	+	+
1/16	+	+	+	+
1/32	+	+	+	+
1/64	+	+	+	+

**Table (4): Results of ELISA among fecal sample infected with 40μg/ml and 20 μg/ml of *C. perfringens* toxin type A.**

<i>C. perfringens</i> toxin type A								
Dilution of antisera	A		B		C		D	
	40 μg/ml	20 μg/ml						
1/800	0.792	0.530	0.623	0.369	0.560	0.301	0.523	0.286
1/1600	0.498	0.430	0.422	0.275	0.356	0.199	0.301	0.136

**Table (5): Results of ELISA among fecal sample infected with 40 μg/ml and 20 μg/ml of *C. perfringens* toxin type B.**

<i>C. perfringens</i> toxin type B								
Dilution of antisera	A		B		C		D	
	40 μg/ml	20 μg/ml						
1/800	0.560	0.329	0.880	0.721	0.602	0.301	0.591	0.276
1/1600	0.305	0.225	0.476	0.435	0.376	0.170	0.322	0.122

**Table (6): Results of ELISA among fecal sample infected with 40 μg/ml and 20 μg/ml of *C. perfringens* toxin type C.**

<i>C. perfringens</i> toxin type C								
Dilution of antisera	A		B		C		D	
	40 μg/ml	20 μg/ml						
1/800	0.512	0.310	0.690	0.331	0.700	0.495	0.430	0.272
1/1600	0.318	0.154	0.350	0.207	0.465	0.309	0.301	0.120

**Table (7): Results of ELISA among fecal sample infected with 40 $\mu$ g/ml and 20  $\mu$ g/ml of *C. perfringens* toxin type D.**

Dilution of antisera	<i>C. perfringens</i> toxin type D							
	A		B		C		D	
	40 $\mu$ g/ml	20 $\mu$ g/ml	40 $\mu$ g/ml	20 $\mu$ g/ml	40 $\mu$ g/ml	20 $\mu$ g/ml	40 $\mu$ g/ml	20 $\mu$ g/ml
1/800	0.523	0.312	0.602	0.414	0.530	0.295	0.834	0.552
1/1600	0.307	0.187	0.381	0.255	0.337	0.115	0.442	0.399

**Table (8): Results of SpA test among fecal sample infected with *C. perfringens* toxin type A.**

Dilution of antisera	<i>C. perfringens</i> toxin type A							
	A		B		C		D	
	40 $\mu$ g/ml	20 $\mu$ g/ml	40 $\mu$ g/ml	20 $\mu$ g/ml	40 $\mu$ g/ml	20 $\mu$ g/ml	40 $\mu$ g/ml	20 $\mu$ g/ml
1/32	+	+	+	-	-	-	-	-
1/64	+	-	+	-	-	-	-	-

**Table (9): Results of SpA test among fecal sample infected with *C. perfringens* toxin type B.**

Dilution of antisera	<i>C. perfringens</i> toxin type B							
	A		B		C		D	
	40 $\mu$ g/ml	20 $\mu$ g/ml	40 $\mu$ g/ml	20 $\mu$ g/ml	40 $\mu$ g/ml	20 $\mu$ g/ml	40 $\mu$ g/ml	20 $\mu$ g/ml
1/32	-	-	+	+	+	-	-	-
1/64	-	-	+	-	-	-	-	-

**Table (10): Results of SpA test among fecal sample infected with *C. perfringens* toxin type C.**

Dilution of antisera	<i>C. perfringens</i> Toxin type C							
	A		B		C		D	
	40 $\mu$ g/ml	20 $\mu$ g/ml	40 $\mu$ g/ml	20 $\mu$ g/ml	40 $\mu$ g/ml	20 $\mu$ g/ml	40 $\mu$ g/ml	20 $\mu$ g/ml
1/32	-	-	+	+	+	+	-	-
1/64	-	-	-	-	-	-	-	-

**Table (11): Results of SpA test among fecal sample infected with *C. perfringens* toxin type D.**

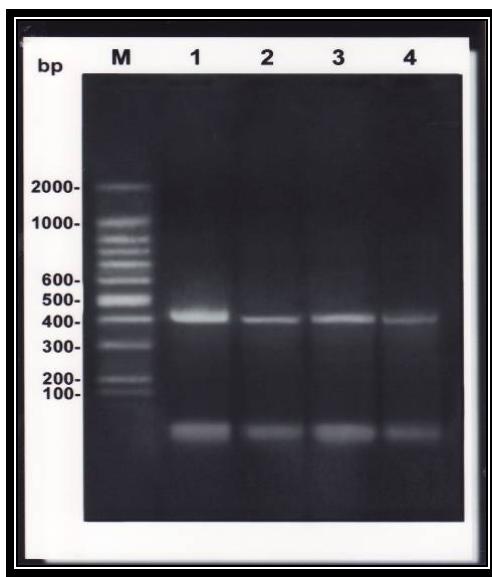
Dilution of antisera	<i>C. perfringens</i> Toxin type D							
	A		B		C		D	
	40 $\mu$ g/ml	20 $\mu$ g/ml	40 $\mu$ g/ml	20 $\mu$ g/ml	40 $\mu$ g/ml	20 $\mu$ g/ml	40 $\mu$ g/ml	20 $\mu$ g/ml
1/32	-	-	+	-	-	-	+	+
1/64	-	-	-	-	-	-	-	-

**Table (12): Sensitivity of the PCR assay for detection of *C. perfringens* toxins\* in feces**

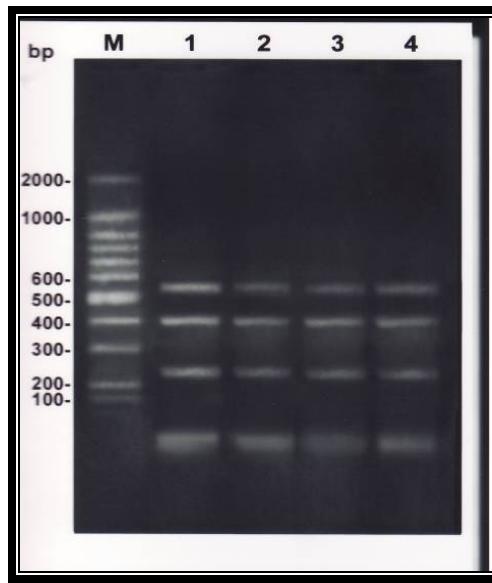
different dilution of Feces infected with <i>C. perfringens</i> type A	Colony count on TSC agar	PCR
$10^3$	1-2 colonies	+ve
$10^4$	-	+ve
$10^5$	-	+ve
$10^6$	-	+ve

\* *C. perfringens* toxin types A, B, C or D

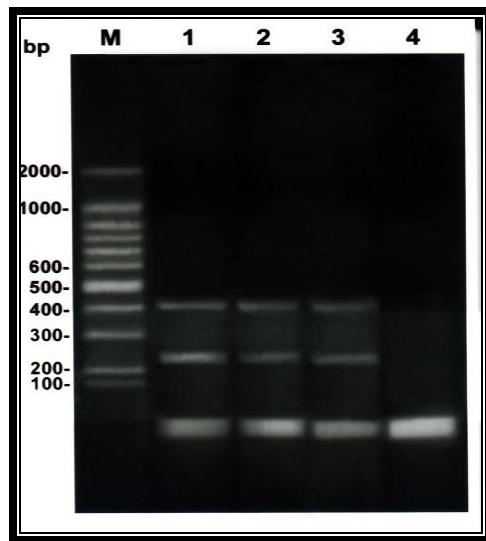
TSC agar: trypticase soy agar.



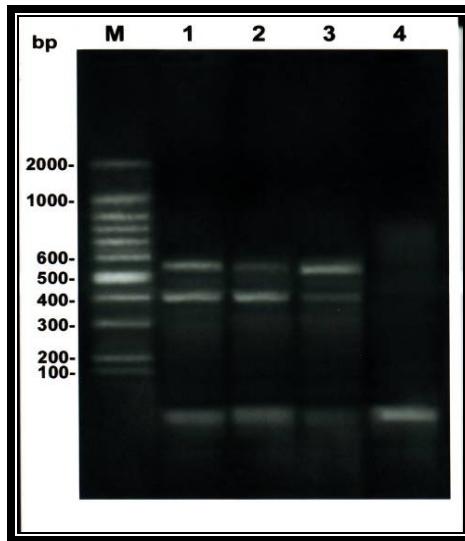
**Photo (5):** Amplification of *Cpa* (402 bp) from feces infected with *C. perfringens* type A by multiplex PCR. Lane M: 100 bp ladder, Lanes1, 2, 3&4 *C. perfringens* type A ( $10^3$ ,  $10^4$ ,  $10^5$  &  $10^6$  dilutions respectively).



**Photo (6):** Amplification of *Cpa* (402 bp), *cpβ* (236 bp) and *etx* (541 bp) genes from feces infected with *C. perfringens* type B by multiplex PCR. Lane M: 100 bp ladder, Lanes1, 2, 3&4 *C. perfringens* type B ( $10^3$ ,  $10^4$ ,  $10^5$  &  $10^6$  dilutions respectively)



**Photo (7):** Amplification of *cpa* (402 bp) and *cpβ* (236 bp) genes from feces infected with *C. perfringens* type C by multiplex PCR. Lane M: 100 bp ladder, Lanes1, 2 and 3 *C. perfringens* type C ( $10^3$ ,  $10^4$  &  $10^5$  dilutions respectively).



**Photo (8):** Amplification of *cpa* (402 bp) and *etx* (541 bp) genes from feces infected with *C. perfringens* type D by multiplex PCR. Lane M: 100 bp ladder, Lanes 1, 2 and 3 *C. perfringens* type D ( $10^3$ ,  $10^4$  &  $10^5$  dilutions respectively).

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9/9/2010

# Comparative Study of Software Engineering Processes in Egyptian Cmmi Companies

Alaa El-Din Hamouda and Mohammad Abdрабو إلوهش

Computers & Systems Engineering Dept., Al-Azhar University Cairo, Egypt.  
[Alaa\\_ham@giga.net](mailto:Alaa_ham@giga.net), [eng.md.elwahsh@gmail.com](mailto:eng.md.elwahsh@gmail.com); [www.elwahsh.com](http://www.elwahsh.com)

**Abstract:** The Egyptian government has paid special attention to the software industry as Egypt to provide it with a competitive advantage that makes this emerging industry promising. Thus, the State has supported the Egyptian companies to make use of the Capability Maturity Model Integration (CMMI). Since 2009, more than thirty companies obtained the CMMI at different levels. However, these companies suffer from lack of a mechanism to exchange experience and information among themselves although they could be similar in the culture of their engineers and perhaps in the nature and size of their software projects. So, we provide in this research a survey to gauge the quality of methods, tools and processes used in these Egyptian companies winning the CMMI. Then we analyzed the results to reach the recommendations aimed at enriching the software industry in Egypt.

[Alaa El-Din Hamouda and Mohammad Abdрабو إلوهش. Comparative Study of Software Engineering Processes in Egyptian Cmmi Companies. Journal of American Science 2010;6(11):509-514]. (ISSN: 1545-1003).

**Keywords:** CMMI in Egypt, software engineering processes, survey.

## 1. Introduction

In 1993, the Software Engineering Institute (SEI) released the Capability Maturity Model Integration (CMMI) with five staged maturity levels as a means to both appraise maturity level and guide process improvement efforts for software organizations. This model has since been widely accepted around the world, especially in Egypt where the CMMI has helped many software companies [1, 2, 3, 4, and 5].

CMMI combines software engineering, systems engineering, integrated products and procurement to design and improve all types of processes. CMMI has become an international standard for devising software development processes and is credited with helping Egypt rise rapidly to become among the world's software exporters. CMMI provides guidance to improve organizations processes and ability to manage the development, acquisition, and maintenance of products or services. The process areas are grouped into four categories: Process Management, Project Management, Engineering, and Support [6, 7, 8, 9, and 10].

Around the world, there are fast growing CMMI companies. Many countries use the CMMI model extensively e.g. India, China, Japan, Australia, Russia, USA, S.Korea, France, Germany, Brazil, Argentine, Canada and Taiwan. For example in Taiwan seven companies hold Level 2 accreditation, two have Level 3 accreditation and one(IBM Taiwan) has already achieved Level 5 accreditation. In 2009

more than 500 companies in the U.S. were certified to CMMI standard [11, 12, and 14].

Today, use of CMMI in Software industry in Egypt has been increasing to improve software processes. By June 2009, thirty-one software companies achieved CMMI accreditation levels, from Level 2 to Level 5. One of the problems that face CMMI companies in Egypt is lack of conferences that enable specialists to meet to share their experience about software engineering processes. Also, there is lack of researches that reflect the experiences and provides comparative studies. So, we made the CMMI Survey in 2009 to help organizations identify the best practices and enhance the maturity of their processes. By investigating most of the organizations that have been appraised, processes automation, success factors, keep performance indicator (KPI), benefits of CMMI implementation are identified [13].

## 2. Survey Design

We designed a Survey for CMMI companies in Egypt. The target of the survey is to make a comparative study of the process implementation, best practices, tools, and techniques used in these companies. The target of these questions is to give informative details about the CMMI companies to get clear and transparent information about the software industry in Egypt [13]. These survey requirements were divided into twelve sections, covering most areas of operations in the maturity model with the aim of measuring the capacity of the second and third division levels.

The results were as follows: The first section was to get general information about the company characteristics in terms of size and structure, other sections of the survey focus on processes implementation and tools used for different process areas of levels two and three. Level two includes Project Management, Requirement Management, Measurements and Analysis, Quality Assurance and Configuration Management. Level three includes Technical Solutions, Product Integration, Risks Management, Testing, Decision Analysis and Resolutions , Process Improvement, and CMMI satisfaction[3,9].

The survey was launched in 2009. Through Software Engineering Competence Center (SECC) in the Egyptian Ministry of Communications, the thirty-one Egyptian CMMI companies were requested to fill the survey to answer 65 questions addressing different sections of programming activities in Egypt. Then four experts from software engineering processes and CMMI section were contacted to define the key points which Egyptian companies need to relay their experience. Based on these needs we divided the survey as follows:

- 1) General Information
- 2) Individual Evaluation System
- 3) Engineering Processes Group
- 4) Project Management
- 5) Requirement Management
- 6) Measurements and Analysis
- 7) Quality Assurance
- 8) Technical Solution and Product Integration
- 9) Testing
- 10) Risks
- 11) Process Improvement
- 12) Satisfaction with CMMI based processes

### **3. Survey Implementation**

This survey was sent to 31 companies in Egypt that obtained the Capability Maturity Model Integration (CMMI) at different levels: eighteen companies were granted the second level (58.2%), ten received third level (32.3%), one got level four (3.2%), and two obtained the fifth level (5.6%).

From the results of the survey, we find that 14 companies participated as follows: 8 companies got the second level (57.1%) got the third level (28, 6%), and two got the fifth level (14.3%). most companies responded to all questions included in the survey. However, some vague points were not answered by few companies.

### **4. Results and Evaluations**

#### **A. General Information**

As a result of evaluation, the following rates were obtained from responding companies:

- ❖ A percent of 58.3 % have projectized structures, while 41.7 % are matrix organizations. This is striking if we bear in mind that most companies and projects are individual and small-scale projects. The importance of the adoption of the Matrix Organization is demonstrated when the companies are large, including a multiplicity of departments and skills. But in a small institution, it is usually advisable to adopt a system of Projectized Organization, where the responsibilities and tasks are more specific and there is speed in decision-making, flexibility in management, and easy follow-up on the other hand. Please note that in medium-sized companies (where the number of employees is less than fifty) 50% of the employees use the matrix model. So we recommend that these medium-sized companies adopt a projectized system.
- ❖ 36% of the companies got the Capability Maturity Model Integration (CMMI) before 2006, 36.4% before 2007, and 27.6% by the year 2008.
- ❖ All companies on the second level have valid plans to get the third level in a year or two, which indicates:
  - a. Growing awareness of the importance of quality systems and the positive repercussions on companies.
  - b. Companies are satisfied with returns resulting from the application of quality systems (Return on Investment).
- ❖ All the companies that got the third level in re-evaluation (reappraisal) after expiry of the first evaluation aim at higher level of the CMMI Levels 4 and 5 but this is impaired by the financial constraints, as the Egyptian Government supports only the companies that plan to get the second and third levels.
- ❖ The ISO 9001 certification was a good start for Egyptian companies, as 43% of the participants, received the ISO certification before they got the CMMI. This can be explained as the required specifications in ISO 9001 focus on the administrative side, at the same time the CMMI standards focus on the specifications of the technical operations, especially in the third level and above. This is why we recommend the companies that got the ISO certification or wishing to start a quality journey to follow the experience gained and the methodology proposed by [Chanwoo, 2006].

#### **B. Individual Evaluation System**

Individual Evaluation is a key success factor for organization. Having good measures and processes for performance evaluation affects the employee's satisfaction and turn over rate. In 55% of the companies, where the individual survey system was applied the direct manager alone filled the survey. This reflects two facts:

- a. Companies need performance indicators which truly reflect the real level of performance of engineers.
- b. Companies need to design questionnaires on specific scientific basis which reflect the level of performance of software engineers from different points of view. For example, designing a system of individual performance appraisal method based on the Three-Hundred and Sixty Degrees method can be a good choice. Hence it is recommended that researchers in the field of software engineering would address this need and give it priority in their research.

#### C. Engineering Process Group (EPG)

At the beginning of the processes improvement initiative, there are usually some important questions, e.g. How many people are needed for the EPG? Should they be dedicated process engineers or normal software engineers who spend some time in processes? What are the criteria for EPG member selection? The paragraphs below help the decision maker through highlighting the actual performance.

- ❖ 83% of the companies that dealt with the survey are of small and medium SME.
- ❖ 53.8% of the companies prefer to have the engineering team dedicated to this work size (Static EPG), while in 46.2% of the companies, EPG members are originally working in software projects (such as a systems analyst, developer, tester) and those who deal with process improvement tasks would, take over these functions to complement their own tasks (Virtual EPG).

The average overall efforts to improve operations in companies have a monthly rate of 1.25 employees. This will be useful later to show the amount of spending on improving processes, calculate the resulting returns and consequently access the return on expenditure (Return on Investment, ROI).

#### D. Project Management (PM)

Project managers face challenges of selecting the suitable quick and detailed estimation techniques and the adopted software life cycle. They are also required to select the project management tools and decide about the meetings frequency. Challenge facing the project manager to select the appropriate method to estimate the size of the product backend

forums and then estimate the cost and time, has emerged from the questionnaire which also revealed that:

- ❖ 42.9% of the companies use the Microsoft Professional project management (Microsoft Project Professional).
- ❖ 36% use the Microsoft Advanced Project Management (Microsoft Enterprise Project).
- ❖ 21.1% are using a spreadsheet (Excel sheet). These rates are consistent with the nature of projects which are based on dealing with the user.
- ❖ 53. 8% Use Case Points (Use Case Point) as a tool to estimate the size of projects.
- ❖ 38.2% use point of the task (Function Point), and 8% use COSMIC tool.
- 78.6% have a preliminary technical assessment (Initial Estimation Technique), and 21.4% do not.

#### E. Requirement Management

A percent of 46.2% of the companies use spreadsheet software for management of requirements. However, complex software systems steadily increase the list of requirements which makes it difficult to manage and follow-up. It is also difficult to link design and test programs and schemes corresponding to each requirement (Traceability Matrix). Accordingly, we recommend, in such case, use of special programs to manage the requirements of software systems to enhance the efficiency of the management process requirements.

#### F. Measurements and Analysis (M&A)

The number of key performance indicators (KPI) in projects and institutions in general was great compared to company sizes. So as, each KPI has a cost for managing it (e.g. collecting KPI values, verifying their validity, and analyzing them), we recommend training specialists to calculate the cost of these indicators (cost/benefit analysis) in order to be able to take the right decision for selection of numbers and quality.

In 50% of the companies, key performance indicators for the (KPIs) are less than 4 and more than 7, and 33% less than 4, in 17% it is more than 4, this is compared to the number of key performance indicators where in 64.2% of companies the number of KPI is between 4 and 7, and in 30.8% the number of is less than 4, and in 23% is more than 7. We find that 85.7% of the companies take advantage of the actual analysis of performance indicators to improve operations.

#### G. Quality Assurance (QA)

How many process QA engineers are needed? Is it useful to get QA approval before a project is closure?

When should QA issues be escalated? The results below express the real situation in Egyptian CMMI Companies. The senior management support to a quality assurance is an essential element in the commitment of staff operations that are in line with quality systems. It has been found that 57.1% of the companies need to get the approval of the project manager of quality assurance team (QA approval) at the end of any project, and in 71.4% of the companies, the number of quality assurance engineers was more than three. These are good promising an indicator of the manager's concern to stress the value of the quality of operations and give it direct support.

#### H. Technical Solution and Product Integration

For developers, it is important to determine how unit testing is performed and to define dedicated positions for architecture, analysis, and design are used. It is equally important to define who is responsible for support documentation. The results of that survey are given below.

- ❖ 78.6% of the companies perform automatic unit testing, and 57.1% perform the testing manually. This reduces efficiency as much as it lowers the productivity of the developer/tester. These companies are recommended to adopt unit testing and general testing. We also recommend training developers on tools unit testing automatically, and training testers to use automated testing tools.
- ❖ 27.3% of the companies use dot-net (Dot Net) as a tool for program development. 27.3% use the Java language (Java), 9.1% use the Oracle (Oracle) language, while 36% use other languages such as Delphi (Delphi).
- ❖ There is no specialization in technical writing where developers and testers take the responsibility of preparing documents associated with the product code. This is, in most cases, not accomplished professionally which reduces companies' efficiency. So, we recommend that company's employee technical writer who would be responsible for documentation especially since the cost incurred may be less than the cost of employing a developer or tester.

#### I. Testing

- ❖ It is noted that the checking bugs (Bugs), and problem issues (Issues) are checked manually in 57.1% of the companies. Perhaps this makes it difficult to follow-up and may affect the quality of products and processes. Hence, we propose that companies automate this process, either through

their own software or through ready-made software.

- ❖ Manuscript rapid testing (rapid testing script) is very important to examine applications in a limited time. The importance of this testing is highlighted in the maintenance phase of software systems. However, we find that 71.4% of the companies do not have this facility. Therefore, we recommend its provision for testers and training them to use it.
- ❖ The rate of developers to testers in the companies was at an average of one laboratory for each 3.7 developers. This reflects the distribution of effort in software projects, where this ratio is severely limited if compared to the ratio of 1: 2 approximately [17] in COCOM I and COCOM II. This indicates a weak interest in testing the products adequately. So, it is recommended to invest more effort in testing products which would improve the quality and increase competitiveness of Egyptian software.
- ❖ 78% of the companies perform unit tests manually, and 57.1% do screening tests manually. This reduces efficiency as much as it reduces the productivity of the developer/tester. Thus, we recommend companies to adopt mechanical unit testing. We also recommend training developers to perform the Tools unit test automatically, and training examiners to use automated testing tools.

#### J. Risks

54.5% of the companies use spreadsheets to manage risks, 18.2% use radar (Radar), and 27.3% use other programs such as (Microsoft Project Server) for the management of risks.

#### K. Overall Satisfaction with CMMI

- ❖ Generally, the companies applying the CMMI are satisfied which is a good sign. However, there are complaints from the complexity of operations there is an urgent need to review the operations approved by each company in the system to alleviate any burden carried by these processes (Process overhead). To accomplish this, we recommend that companies work on some ideas inspired by the Agile Models and trying to integrate them in their quality system which is compatible with the CMMI, especially as most companies are small and thus need flexible and simple processes.
- ❖ 21% of the projects in the companies do not follow the processes set forth by the CMMI quality system. When these companies were asked, the answer was that pressure from

- customers to get the product forces them not to follow the internal CMMI quality systems. This in fact represents a threat to the quality of processes and then the quality of products in these projects. So, it is proposed that companies apply another Simple Process to speed the completion of work such as the Agile Model, and thus Subject all company projects whether complex processes or simple operations, to the internal quality system according to the standard set by the companies to follow the appropriate processes.
- ❖ The percentage of delay in software projects delivery date was 58% and the average percentage of projects costing more than the approved budget is estimated by 45.5%. This is consistent with global figures estimated by 75% and 50% respectively. It is observed that most international projects that suffer from delays and cost increase are large-scale projects, while the projects in the Egyptian companies are not huge. According to [18], the most important reasons at the global level are:
    - a. poor planning and management
    - b. changing objectives of projects during their implementation
    - c. Non-participation of senior management in the follow-up projects and consequently not giving enough support.

We find that the second and third reasons may not apply directly to the Egyptian companies which are relatively small in size. Also, the number of projects is limited, which reduces the likelihood of changing objectives and lack of support from senior management. Thus, the primary cause is the most influential and therefore it is recommended to raise the skills of project managers through training to use the specific and effective methods of project management software.

## 5. Conclusion

Through this paper we are providing the results of a questionnaire to investigate the processes and Tools used in CMMI software companies in Egypt. By analyzing the results and conclusion obtained, we recommend that:

1. Small and medium companies adopt a structural Projectized Organization process, where the responsibilities and tasks are more specific and there is speed in decision-making and flexibility in management and follow-up.
2. Training the responsible personal to estimate the cost of key performance indicators (analysis of the relationship between expenses and profit) in order to be able to reach a wise decision for selection of

performance indicators with respect to quality and quantity.

3. Carrying performance unit tests and general tests automatically. Also, training developers to perform the Tools unit tests automatically, and testers to automated testing tools.
4. Automation of the follow-up bugs (Bugs), and issues (Issues) either through their own software or through ready-made software's.
5. Training testers to prepare and implement rapid testing (rapid testing script) to support the maintenance of software products.
6. With the steady complexity of the code systems and growth of the list of requirements which is difficult to manage and follow-up, it becomes difficult to link it to design, test programs and plans corresponding to each requirement (Traceability Matrix). Preparation and use of special programs to manage the requirements of software systems, to enhance the efficiency of the management process requirements.
7. Exerting more effort in the work of a good testing of products in order to increase the quality and competitiveness of Egyptian software.
8. Companies employ a technical writer, especially since the cost incurred is less than that of employee a programmer or tester.
9. Enhancing skills of project managers through training on the unique and effective methods of project management software.
10. Companies prepare new classifications for simple operations (Simple Process) in order to be able to speed the completion of work by following the Agile model and thus subject all projects to their special quality system (both for complex or simple operations) according to the standard they set.
11. Companies holding ISO 9001 or wishing to start a trip to ISO quality should follow the experience gained and the methodology proposed in [19]. The ISO 9001 certification was a good start for many Egyptian companies. Forty-three percent of the participating companies received a certificate of the ISO before they got the Capability Maturity Model. This can be explained by the required specifications in both, where the ISO 9001 focuses on the administrative side, at the same time the Capability Maturity Model identifies standards of technical operations, notably in the third level and above.
12. There is an urgent need to review the operations approved by each company in its system, and for this process said companies try to make use of Agile models and integrate them in their quality system which is compatible with the CMMI to decrease the Process overhead,

especially as most companies are small and need to be flexible, and simple.

13. Researchers in the field of software engineering should study to find indications of a genuine performance which truly reflects the performance of engineers to design by questionnaires on the basis of special scientific programmed software which describe the performance of engineers from different points of view, for example, designing a system to assess individual performance depending on the method of Three Hundred and Sixty Degrees.

### **Future Work**

After publication of this paper we shall supply the participating companies with the results through the Egyptian Ministry of Communications represented by SECC. Through participation in Arab conferences we hope that government's private institutions Adopt these recommendations and spread them in their countries.

### **Acknowledgments**

We thank the Software Engineering Competence Center (SECC) especially Dr. Gamal Aly and Abeer Khedr for their support, and Dr. Mohammad Zaki for his valuable comments; we also thank all individuals who took the time to assist us in this survey. Finally, we thank Horizon Software Company as a sponsor of this work.

### **Corresponding author**

Alaa El-Din Hamouda  
 Mohammad Abdрабو Elwahsh  
 Computers & Systems Engineering Dept., Al-Azhar University Cairo, Egypt.  
[Alaa\\_ham@giga.net](mailto:Alaa_ham@giga.net), [eng.md.elwahsh@gmail.com](mailto:eng.md.elwahsh@gmail.com)

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9/10/2010

## Prevalence of *S. aureus* and *S. epidermidis* among patients with indwelling catheters and their antibiogram using some commonly used antibiotics.

\*De, N. and Godlove, M.

Department of Microbiology, Federal University of Technology, Yola, Adamawa State, Nigeria

e-mail: nanditamicrobio@yahoo.com

\*corresponding author

**Abstract:** The objectives of this study were to find the prevalence of *S. aureus* and *S. epidermidis* in urine samples of patients placed on catheter in Federal Medical Centre, Yola (FMCY) and State Specialist hospital, Yola (SSHY) and the efficacy of some commonly used antibiotics against the isolates of *S. aureus* and *S. epidermidis*. A total of one hundred and five samples (60 from SSHY and 45 from FMCY) were collected and inoculated into Cystine lactose electrolyte deficient (CLED) agar for isolation of Staphylococcal species. A total of seventy six presumptive Staphylococcal isolates were obtained on CLED agar and these isolates were identified using gram staining, morphological characteristics and standard biochemical tests. Serological studies revealed that out of 76 isolates, 56 were *S. epidermidis* (coagulase negative) and 20 were *S. aureus* (coagulase positive). Fifty one point eight percent (51.8%) of the isolates of *S. epidermidis* were sensitive to ceftazidime followed by ciprofloxacin (46.4 %) whereas 45% of the isolates of *S. aureus* were sensitive to ceftriaxone followed by cefotaxime and ciprofloxacin (40%). [De, N. and Godlove, M. Prevalence of *S. aureus* and *S. epidermidis* among patients with indwelling catheters and their antibiogram using some commonly used antibiotics. Journal of American Science 2010;6(11):515-520]. (ISSN: 1545-1003).

**Keywords:** ciprofloxacin; coagulase; CLED agar, catheters, *S. aureus*, *S. epidermidis*

### Introduction

Urinary tract infections (UTIs) are the most common infections acquired in hospitals and long term care facilities. Several studies have estimated the incidence of health care associated UTIs at around 2-3 patients per 100 admissions (Kreiger *et al.*, 1998). Catheter associated urinary tract infections (CAUTIs) perhaps the largest institutional reservoir of nosocomial antibiotic resistant pathogens. This could lead to complications such as pyelonephritis and bacteremia (Nwankwo *et al.*, 2007). Glynn *et al.*, 2007 have indicated that between 75 and 80 % of all health care associated UTIs follow the insertion of a urinary catheter, and a study investigating 40 English hospitals estimated that around 26% of all hospitalized patients have a urinary catheter inserted during their stay in hospital. Use of catheters is common in long term care facilities and many patients are catheterized for long period, thus increasing their risk of acquiring a CAUTI (catheter associated urinary tract infection). Ouslander *et al.*, 1994 carried out a study on patients in nursing home and illustrated the problem of CAUTI in long term care of the

elderly. During the one year study period, 80% of the patients had atleast one CAUTI and 48% of the patients had two or more CAUTIs. Nwankwo *et al.*, 2007 carried out an investigation on catheter associated urinary tract infection in a tertiary health institution in Kano, Nigeria. The results show that out of 210 patients studied, 180 patients showed bacterial growth from the aspirated urine sample. The prevalence rates of the isolates for catheter tip and aspirated urine culture were *E. coli* (38.3%), 41.2%, *P. aeruginosa* 20%, 18.8%, *Proteus* sp. 12.7%, 11.8%, *S. aureus* 8.8%, 4.7%, *Streptococcus* sp. 1.1%, 7.1%, *C. freundii* 2.7%, 0%, *Candida* sp. 2.7%, 0% and *S. epidermidis* 1.1%, 0 % respectively.

*Staphylococcus aureus* causes a variety of suppurative infections and toxioses in humans. It causes superficial skin lesions such as boils, styes and furunculosis, more serious infections such as pneumonia, mastitis, phlebitis, meningitis and urinary tract infections and deep seated infections such as osteomyelitis and endocarditis. *S. aureus* is a major cause of hospital acquired infection of surgical wounds and infections associated with indwelling

medical devices (Todar, 2005). *S. epidermidis* is known to cause infection of native heart valves, intravenous catheters, and artificial heart valves. It is a common skin resident that is sometimes responsible for endocarditis and infections of patients with lowered resistance (e.g. wound infections, surgical infections, urinary tract infections). It can cause peritonitis receiving peritoneal dialysis usually introduced via a break in the patient's skin and also causes infections in prosthetic joints ((Baron *et al.*, 1994).

CoNS are the (coagulase negative *Staphylococcus*) quite essential pathogens of medical devices. The array of virulence factors produced by CoNS is meager compared with that of the virulence factors produced by *S. aureus*, but among these few factors are substances that promote bacterial adherence to and persist on foreign bodies. CoNS infection of intravenous catheter may or may not be accompanied by signs of inflammation at the site of catheter insertion, and the degree of systemic toxicity (including fever) ranges from minimal to moderately severe (Rupp and Archer, 1994).

Multidrug resistant *Staphylococcus* isolates have been recognized as one of the major challenges in control of hospital acquired infections and community associated infections. Bacteremia caused by *S. aureus* continues to be a common problem world wide and also the coagulase negative *Staphylococcus* and antibiotic sensitivity patterns are regarded with all seriousness in clinical practice and hospital acquired and community acquired infections. Martha *et al.* (2009) isolated MRSA from AIDS patients attending some public hospitals in Yola, Adamawa State, Nigeria. Shoba *et al.*, 2005 conducted a survey work on prevalence of *Staphylococcus* sp. among hospital personnel, environment and their antibiogram with special emphasis on methicillin resistance. They reported that resistance to oxacillin was 13.84% among the 65 staphylococcal isolates. Yenda *et al.*, 2010 isolated 60 isolates of *S. aureus* from patients attending State Specialist hospital and out of these 60 isolates, 85% of the isolates were resistant to ofloxacin and 80% of the isolates were resistant to ciprofloxacin.

FMCY and SSHY are the two largest hospitals in Adamawa State. Patients that need catheter devices or any prosthetic devices, generally attend these two hospitals. This present work was aimed at determination of

prevalence of *S. aureus* and *S. epidermidis* isolated from patients with indwelling catheters and their antibiogram in these two hospitals.

## Materials and Methods

### Study area:

The study area selected for this study was Jimeta-Yola, Adamawa State. The selected hospitals were Federal Medical Centre, Yola (FMCY) and State Specialist hospital, Yola (SSHY).

### Collection of samples:

According to Procter and Peters (1998), catheter urine samples should be collected not from the catheter bag but from the tube at upper connection. For collection of sample, the catheter tube was disconnected from the catheter bag and urine was collected into a wide mouth screw capped bottle and was covered after collection. Patient's age and gender were recorded for each of the sample. All the samples were analyzed within four hours of collection.

### Isolation of *Staphylococcus* sp.:

A loopful of urine sample was inoculated on CLED agar and was incubated at 37°C for 24 hours. The round cream and white colonies of 1-2 mm in diameter were collected and labeled. These were subcultured on blood agar and kept in refrigerator for identification purpose.

### Identification of Staphylococcal isolates:

The appearance and color of the colonies on CLED agar and Blood agar and the diameter of the colonies were noted. Gram staining was done following the procedure as described in Benson, 2002. The isolates were also identified using different biochemical tests like catalase test, coagulase test, sugar utilization tests using sucrose, mannitol, trehalose and also novobiocin test using novobiocin at 5 µg/ml (Benson, 2002).

Isolates were then tested for coagulase production using Staphytect Plus test method. The reagents were purchased from Sanofi Diagnostic Pasteur, France and the method was followed as instructed by manufacturer. One drop of test latex reagent was dispensed onto one of the circles on the reaction card and one drop of control latex was dispensed onto another circle.

A loop was used to pick up 5 average-size of suspected staphylococcal colonies onto a culture media plate and mix this in the control latex reagent. The colonies were smeared in order to cover the circle. A separate loop was then used to proceed in the same way with the test latex.

The card was rocked for 20 seconds and agglutination was observed under normal lighting conditions.

A result was reported positive if agglutination of the blue latex particles occurred within 20 sec. A result was reported negative if no agglutination occurred and a smooth blue suspension remained after 20 sec in the test circle.

#### **Antimicrobial susceptibility testing:**

All 76 isolates were used for this test using streak plate method. Sensitivity disks containing conventional antibiotics like augmentin (5 µg/ml), ofloxacin (5 µg/ml), ceftriaxone ((30 µg/ml), ceftazidime (5 µg/ml), cefotaxime (10 µg/ml), sparfloxacin (10 µg/ml) and ciprofloxacin (5 µg/ml) manufactured by BIOTECH LABS., England were used for sensitivity test. A loopful of growth of each isolate on blood agar was suspended in sterile water and then was diluted in steps of 1:10 to give turbidity equivalent to the 0.5 McFarland standard (a density of  $1 \times 10^8$  cells/ml) before inoculation (NCCLS, 2002). Diagnostic sensitivity test (DST) agar plates were inoculated with 0.5 ml of cell suspension of each isolate adjusted to  $1 \times 10^8$  using a sterile spreader. Sensitivity discs containing antibiotics were placed on the surface of each DST agar plate evenly seeded with the test organism and

was incubated for 24 hrs. at 37 °C. The zone size of 20 mm or less for ciprofloxacin and 15 mm or less for ofloxacin, augmentin and sparfloxacin was considered as resistant and for other antibiotics (cephalosporin drugs) the zone size of 24 mm or less was considered as resistant for *S. epidermidis* and *S. aureus*.

#### **Results**

##### **Description of samples:**

A total number of one hundred and five samples were collected from FMCY and SSHY from patients placed on catheter. Out of 105 samples, 45 samples were from FMCY while 60 from SSHY. Out of the 45 samples from FMCY, 29 were from female patients while 16 from male patients. Out of 60 samples from SSHY, 38 were from female while 22 from male patients.

##### **Isolation and identification of isolates:**

Seventy six discrete colonies were isolated from CLED agar plates. The isolates produced cream, yellow or white colonies on CLED and blood agar plates. All the 76 isolates were gram positive cocci. The results of biochemical tests are listed in Table 1.

For isolates A1-A20, the agglutination occurred within 20 seconds signifying the fact that the isolates were *S. aureus*. For B1-B56, there was no agglutination verifying that B12-B56 were not isolates of *S. aureus*.

##### **Results of antibiotic sensitivity test:**

The results are shown in Table 2 and Table 3. The percent efficacy of different antibiotics is listed in Table 4.

**Table 1:** Biochemical characteristics of isolates

IN	S	Ca	Co	M	N	T	Organism
A1-A20	+	+	+	+	S	+	<i>S. aureus</i>
B1- B56	-	+	-	-	S	-	<i>S. epidermidis</i>

IN- isolate number, S- Sucrose, Ca- Catalase, Co- Coagulase, M- Mannitol, N- novobiocin at 5µg/ml.; T- Trehalose

**Table 2:** Zones of inhibition produced by antibiotics against isolates of *S. aureus*

IN	A	O	Ce	C	Cf	S	Ci	IN	A	O	Ce	C	Cf	S	Ci
A1	r	r	r	s	r	r	r	A11	s	s	r	r	r	r	s
A2	s	r	r	s	r	r	s	A12	r	s	s	r	s	r	s
A3	r	r	s	r	r	r	r	A13	s	r	r	r	r	s	r
A4	r	s	r	s	r	s	r	A14	r	s	s	r	r	s	r
A5	r	r	s	r	s	r	r	A15	r	s	r	s	r	s	s
A6	r	s	r	r	s	r	r	A16	r	r	r	s	r	r	s
A7	r	r	s	r	s	r	r	A17	s	r	r	s	r	s	r
A8	s	r	r	r	s	r	r	A18	r	r	s	r	s	r	s
A9	s	r	s	r	r	r	r	A19	r	r	s	r	r	s	r
A10	r	r	r	r	s	r	s	A20	s	r	s	s	s	r	s

IN=Isolate Number; A1-A20 isolates of *S. aureus*; A= augmentin; O= ofloxacin; Ce= ceftriaxone; C= ceftazidime; Cf= cefotaxime; S= sparfloxacin; Ci = ciprofloxacin; r = resistant; s = sensitive

**Table 3:** Zones of inhibition produced by antibiotics against isolates of *S. epidermidis*

IN	A	O	Ce	C	Cf	S	Ci	IN	A	O	Ce	C	Cf	S	Ci
B1	s	r	r	s	s	s	r	B29	r	s	r	s	s	r	r
B2	s	s	r	r	s	r	r	B30	s	r	s	s	s	r	r
B3	s	r	r	s	s	s	r	B31	s	s	r	s	s	r	r
B4	s	r	s	r	s	r	r	B32	r	r	s	s	r	r	s
B5	r	r	r	s	s	r	r	B33	r	r	s	s	r	r	s
B6	s	r	r	s	r	r	s	B34	r	r	s	r	s	s	r
B7	r	s	r	s	r	r	s	B35	s	s	s	r	r	r	s
B8	r	r	r	r	r	s	r	B36	s	s	s	r	r	r	s
B9	s	r	s	r	r	r	s	B37	r	r	r	s	r	r	s
B10	s	r	s	r	r	r	r	B38	r	r	s	r	r	r	s
B11	s	r	r	r	r	r	s	B39	s	r	r	s	s	r	r
B12	s	r	s	r	s	r	r	B40	r	r	r	r	s	r	r
B13	s	r	r	s	r	r	r	B41	r	r	s	s	r	r	s
B14	s	r	s	r	s	r	r	B42	r	r	s	r	s	r	r
B15	r	r	s	s	s	r	s	B43	r	r	r	s	r	r	s
B16	s	r	s	s	s	r	r	B44	r	s	r	s	s	r	r
B17	s	s	r	r	r	r	s	B45	r	r	r	r	r	r	r
B18	r	r	s	r	s	r	s	B46	s	s	r	r	r	r	s
B19	r	r	r	s	r	r	s	B47	s	r	r	s	r	r	r
B20	r	r	r	r	r	r	s	B48	s	r	r	s	r	r	s
B21	r	r	r	s	r	s	r	B49	r	r	r	s	r	s	r
B22	r	s	r	s	s	s	s	B50	r	r	r	r	s	r	r
B23	r	s	s	s	r	r	s	B51	r	r	r	r	r	r	s
B24	r	r	r	r	r	r	r	B52	r	r	r	s	s	s	s
B25	r	r	s	r	r	s	r	B53	r	s	s	s	r	r	s
B26	r	s	r	r	r	r	s	B54	r	r	s	r	r	r	s
B27	r	r	r	s	r	r	r	B55	r	s	s	r	r	r	s
B28	s	s	r	s	r	r	r	B56	r	s	s	r	s	r	r

IN=Isolate Number; B1-B56- isolates of *S.epidermidis*; A= augmentin; O= ofloxacin; Ce= ceftriaxone; C= ceftazidime; Cf= ceftriaxone; S= sparfloxacin; Ci = ciprofloxacin; r- resistant; s-sensitive

**Table 4:** Percentage efficacy of different antibiotics against isolates of *S. aureus* and *S. epidermidis*

Antibiotics	<i>S. aureus</i> No. of sensitive isolates	percentage efficacy	<i>S. epidermidis</i> No. of sensitive isolates	percentage efficacy
Augmentin	7	35.0	22	39.3
Oflloxacin	6	30.0	16	28.6
Ceftriaxone	9	45.0	23	41.0
Ceftazidime	7	35.0	29	51.8
Cefotaxime	8	40.0	22	39.2
Sparfloxacin	6	30.0	10	17.8
Ciprofloxacin	8	40.0	26	46.4

## Discussion

The results from this study show that *S. aureus* and *S. epidermidis* were isolated from the urine samples of patients placed on catheter devices. Jernigan and Farr, 1993 reported that the catheter related infections are mainly caused by *C. albicans*, *S. aureus* and *S. epidermidis*. Some studies showed that *S. epidermidis* is a significant nosocomial pathogen, preferentially affecting immunocompromised patients and the cause of septicemia has been frequently associated with wounds and catheters or others indwelling devices (Hunton *et al.*, 1985). CoNs are the most common pathogens complicating the use of intravenous catheters, hemodialysis shunt and grafts, cerebrospinal fluid shunts, peritoneal dialysis catheters, pacemakers wires and electrodes, prosthetic joints, vascular grafts and prosthetic valves (Rupp and Archer, 1994). The results obtained from this study also show that the prevalence of *S. epidermidis* (74%) is higher than that of *S. aureus* (26%) among patients placed on urinary catheter. This agrees with the findings of Buchman *et al.*, 2007 who reported that coagulase negative staphylococcus infections are associated with medical devices and removal of such devices is often required for cure. Fidalgo *et al.*, 1990 showed that *S. epidermidis* is the casual agent of true bacteremia on the basis of microbiologic, epidemiologic and prognostic data on 60 episodes of *S. epidermidis* bacteremia recorded in the hospital Covadonga of Oviedo, Spain during 1982-1986. Fifty one of SEB (*Staphylococcus epidermidis* bacteremia) were associated with indwelling devices, which in 44 cases were intravascular catheters; these included 23 central venous catheters and 21 peripheral intravascular catheters. The duration of catheterization ranged from 3 to 21 days for

patients with central venous catheters and from 5 to 27 days for patients with peripheral intravascular catheters. For catheter insertion, rigorous attention should be given to aseptic techniques which involve washing of hands, application of 2% chlorhexidine to the site before catheter insertion, using of normal saline to flush the devices e.g. arterial catheters. Failure to follow these steps during catheter insertion might be responsible for the prevalence of *S. epidermidis* and *S. aureus* in urine samples of catheter patients in FMC, Yola and SSHY, Yola.

In regard to percentage efficacy of different antibiotics, two isolates of *S. epidermidis* (B24 and B45) were resistant to all the antibiotics tested and less than 50% of the isolates of *S. aureus* were susceptible to all the six antibiotics tested. A feature of *S. epidermidis* is the high rate of multiresistant strains. In this study we found that 60% of the isolates of *S. epidermidis* were resistant to augmentin; 81% were resistant to ofloxacin, 57% to ceftriaxone, 48% to ceftazidime, 54% to cefotaxime, 82% to sparfloxacin, 53% to ciprofloxacin. It has been shown that between 75 and 85% of all health care associated UTIs follow the insertion of a urinary catheter and a study investigating 40 English hospitals estimated that around 26% of all hospitalized patients have a urinary catheter inserted during their stay in hospital (Glynn *et al.*, 2007). Ceftriaxone showed highest antibacterial activity against *S. aureus* isolates with percentage efficacy of 45% followed by ciprofloxacin, cefotaxime (40%) and ofloxacin and others (30%). This resistance might be attributed to the production of chemical substances and endotoxins by the isolates. Yenda *et al.*, 2010 isolated 60 isolates of *S. aureus* from patients attending State Specialist

hospital and out of these 60 isolates, 85% of the isolates were resistant to ofloxacin and 80% of the isolates were resistant to ciprofloxacin. Fidalgo *et al.*, 1990 reported among all isolates of SEB, 50% exhibited resistance to five antibiotics namely Penicillin G, ampicillin, oxacillin, erythromycin and clindamycin. This study shows that less than 50% of isolates of *S. aureus* and *S. epidermidis* were susceptible to quinolone antibiotics (Ofloxacin and sparfloxacin) and to some 3<sup>rd</sup> generation cephalosporins like cefotaxime and ceftriaxone.

#### **Corresponding author:**

Nandita De  
Dept. of Microbiology  
Federal university of Technology, Yola  
Adamawa state, Nigeria  
e-mail:nanditamicrobio@yahoo.com

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## Biochemical and Molecular Genetic Studies on Rice Tolerance to Salinity

\*El-Mouhamady, A.A.; I.S.El-Demandash and K.A.Aboud

Department of Genetics and Cytology, National Research Center, Cairo, Egypt.

\*elmouhamady@yahoo.com    lola\_El-Demandash@yahoo.com    Kamal\_Aboud@yahoo.com

**Abstract:** The present investigation was carried under green house conditions from (october2009) to (march 2010) seasons included two conditions (normal irrigation and salinity) using model of half diallel analysis by five cultivars of rice "Gz1368-S-5-4, Hybrid1, Sakha102, Giza 181 and IET444". Five parents and ten crosses were grown under two conditions and the results showed that:

1. The most desirable mean value were positive and highly significant for heterosis, general and specific combining ability effects for all genotypes under normal and salinity conditions were observed from the genotypes.; Gz1368-S-5-4, hybrid1, IET1444, Gz1368-S-5-4 x hybrid 1, Gz1368-S-5-4 x IET1444, Hybrid 1 x IET1444, Sakha 102 x IET1444 and Giza 181 x IET 1444 under normal and salinity conditions.

2. From the foreign discussion, it could be concluded that the crosses Gz1368-S-5-4 x Hybrid 1, Gz1368-S-5-4 x IET1444, Hybrid 1 x IET1444 and Giza 181 x IET1444 were contained of 1, 5, 1 and 5 bands using PM15 primer, 6, 3, 6 and 6 bands using AY334988 primer and 6, 2, 4 and 5 bands using HL-17 primer, which indicated that these bands were found to be index and marker for salinity tolerance in rice by increasing  $K^+$  content and decreasing of  $Na^+$  content. Journal of American Science 2010;6(11):521-535]. (ISSN: 1545-1003).

**Key words:** Rice, salinity, yield components, some traits related to salinity

### 1. Introduction:

Rice is moderately susceptible to salinity. The degree of injury, however, depends on the nature and concentration of salts, soil pH, water regime, method of planting, seedling age, growth stage of the plant, duration of exposure to salt and temperature.

Most rice cultivars are severely injured in submerged soil cultured on EC of 8-10 dSm<sup>-1</sup> at 25 °C, sensitive ones are damaged even at 2 dSm<sup>-1</sup> (Mass and Hoffman, 1997). Rice that is tolerant to salinity during germination becomes very sensitive during the early seedling stage; gains tolerance vegetative growth again becomes sensitive during pollination and fertilization and then becomes increasingly more tolerant at maturity. Salinity during reproductive stage decreases grain yield much more than salinity during vegetative stage (Akbar and Ponnamperuma, 1982).

Symptoms of salt injury in rice are stunted growth, rolling of leaves, white leaf tips, white blotches in the laminae, drying of older leaves and poor root growth. The percentage of dead leaves is a good measure of salt injury (RRTC, 2002).

### 2. Materials and methods

The present study was carried out under green house conditions during October 2009 to march 2010. Five rice varieties utilized in this study namely, Gz1368-S-5-4, hybrid 1, Sakha 102, Giza 181 and IET1444 were grown on three successive dates of planting at fifteen days intervals in order to overcome the differences in flowering time for each parent. The pedigree of the parental varieties is shown in Table (1). Each parent was grown in five rows, each row was 5 meter long and contained 25 hills. At flowers, the six parents were diallel crossed, i.e., in all possible

Table (1):Origin and main characters of the five rice varieties used as parents in the studied diallel cross

Varieties	Origin	Type	Duration (days)	Grain type	Reaction to salinity
GZ1368-S-5-4	(IR1615-31/BG94-2) Egypt	Indica	140	Short	Tolerant
Hybrid 1	(IRmolesterility/G.178) Egypt	Japonica	135	Short	Moderate
Sakha 102	(Gz4098-7-1/Giz 177) Egypt	Japonica	125	Short	Susceptible
Giza 181	(IR1626-203/IR28//IR22) Egypt	Indica	145	Long	Susceptible
IET1444	(TN1/CO29) Egypt	Indica	135	Long	Tolerant

a,h, Japonica, I, Indica

combinations (excluding reciprocal) to produce following the method proposed by Jodon (1938) and modified by Butany (1961).

In 2010 season, the parents and their F<sub>1</sub> hybrid were growing in a randomized complete block design with three replications in two locations (normal and saline). These cultivars were taken from RRTC (Rice Research and Training Center, Sakha, Kafrel-sheikh).

Fertilizers were added as recommended rate and time of application (15 days after transplanting at the rate of 40 kg N/fed., and 15 kg P<sub>2</sub>O<sub>5</sub>/feddan) and hand weeding was done when it was needed and were chemical controlled by adding 2 liter Saturn/feddan, four days after transplanting. All the data were recorded on the parents and their crosses under two locations. All recommended agriculture practices were applied. At ripening each plant was harvested individually

### Soil analysis:

Before conducting the experiments, soil samples were taken from different sites of the experimental area. Each sample was taken from a depth of 0-30 cm, both soil samples normal and saline treatment. the chemical analysis was carried out for each soil extract 1:5 to estimate the soluble anions, cations and total dissolved salts (TDS). The electrical conductivity (EC) was estimated in the extract of the soil saturate paste. The procedure for preparation and measurements of the soil extract was taken according to the method of Black *et al.* (1965). The methods of Chapman and Parker (1961) of soil chemical analysis were followed. The description of both normal and saline types of soil used in the investigation are shown in Table (2).

Table(2): Some chemical characteristics of experimental soil at normal and saline soil (lyzimeter).

Characteristics	Normal soil (Tap water)	Saline water (Lyziemter)
EC (dS/m)	1.84	7.6-7.8
pH (1:2.5)	7.0	8.5
TDS mg/litre (ppm)	718.50	3216.7-4214.8
Ca <sup>++</sup>	3.90	17.80-19.250
Mg <sup>++</sup>	2.70	14.85
Na <sup>+</sup>	11.34	50.0-54.0
K <sup>+</sup>	0.43	0.14
CO <sub>3</sub> <sup>2-</sup>	0.03	0.07
HCO <sub>3</sub> <sup>-</sup>	4.50	1.28
Cl <sup>-</sup>	16.80	47.30
SO <sub>4</sub> <sup>2-</sup>	1.70	13.70
Texture	Clay	Clay

EC = Electrical conductivity

TDS = Total dissolved salts

\* Measure of soil saturation

\*\* Measure of soil water extract 1:5

For raising seedlings, wooden 60 x nurseries were used which irrigated by tap water. After thirty days from sowing, seedlings of each parent and their crosses were individually transplanted in 1 row, 1 m length for each variety, with a spacing of 15 x 15 cm between rows and recommended culture practices were followed. The plots were salinized 15 days after transplanting and salinization was fixed till harvesting. Plants were irrigated every day by auto-pumping the salt solution from the tanks. Drainage was practiced every 48 hours through bottom out lets and water electrical conductivity (EC) were measured through the crop season.

Studied characters:

A. Yield and its components characters:

1. Heading date (days)

It was determined as the number of days from date of sowing to the date of the first panicle exertion.

2. Plant height (cm):

Length of the main culm was measured from the soil surface to the tip of the main panicle at maturity

3. Number of filled grains per panicle:

Filled grains of the main panicle was separated and counted.

4. 1000-grain weight (g):

Was recorded as the weight of 1000 random filled grains per plant.

5. Grain yield per plant (g):

Was recorded as the weight of grain yield of each individual plant and adjusted to 14% moisture content.

B. Some characters related to salinity:

Were measured to determine the chemical characters Na<sup>+</sup> uptake, K<sup>+</sup> uptake Na/K ratio and salinity index for grain yield/plant.

Determination of Na<sup>+</sup> uptake, K<sup>+</sup> uptake and Na/K ratio:

Shoot sampling was determined 25 days from salinization by using different salinity levels when the sensitive parents were severely affected. the shoot samples were weighed and dried for three days at 70°C. samples were finally grounded and 1 gram dried powder from each sample was taken for Na<sup>+</sup> and K<sup>+</sup> determination by flame photometer.

Salinity index (SI):

The salinity index (SI) for each character was calculated by using the formula of Dwivedi *et al.* (1991).

$$SI = \frac{\text{Value of each character under saline situation}}{\text{value of each character under normal situation}} \times 100$$

Statistical analysis:

At first, the data were analyzed by using the ordinary analysis of variance to test the significance of differences among the genotypes studied (six parents and their crosses). If the genotypes mean squares were found to be significant, there was a need to proceed for further analysis; i.e., Griffing(1956) mode 1, method 2.

Estimation of heterosis effects:

The heterosis of an individual cross was determined for each trait as the increase of the F<sub>1</sub> hybrid mean over its better parent, (i.e. heterobeltiosis), as follows:

$$\frac{\bar{F}_1 - \bar{B.P.}}{\bar{B.P.}}$$

Heterosis over the better parent % =  $\frac{\bar{F}_1 - \bar{B.P.}}{\bar{B.P.}} \times 100$

Where:

$\bar{F}_1$  = Mean value of the first generation.

$\bar{B.P.}$  = Mean value of the better parent.

L.S.D. values were calculated to test the significance of the heterosis effects, according to the following formula suggested by Wyanne *et al.* (1970).

L.S.D. for heterosis over better parent

$$= t \sqrt{\frac{2MSe}{r}}$$

Where:

t = Tabulated value at the specified level of probability for the experimental error.

MSe = The experimental error mean squares

r = Number of replications.

r = Number of replications.

MSeGCA-Mse TERM/B+2

$$GCA/SCA = \frac{MSeGCA-Mse TERM}{MSeSCA-Mse TERM}$$

B: Number of parents

Mse TERM=mean square of error from ANOVA.

According to Griffing (1956).

Estimation of combining ability:

Griffing (1956) stated that the mathematical model in this case was as follows:

$$X_{ij} = U + g_i + g_j + s_{ij} + e_{ijk}$$

Where:

X<sub>ij</sub>= The value of a cross between parent (i) and parent (j)

U= The population mean

g<sub>i</sub>= The general combining ability (gca) effect of the i<sup>th</sup> parental variety.

g<sub>j</sub>=The general combining ability (gca) effect in j<sup>th</sup> parental variety.

s<sub>ij</sub>=Specific combining ability effect (sca) for the cross.

e<sub>ijk</sub> = The mean error effect; (i.e. the environmental effect associated with the individual observations)

Genotypes sum of squares was partitioned into GCAs and SCAs as follows:

SS due to GCA =

$$\frac{1}{P+2} \left( \sum i(x_i + x_{ii})^2 - \frac{4}{P} x^2 \right)$$

SS due to SCA =

$$\sum i < \sum jx_{ij}^2 - \frac{1}{P+2} \sum i(x_i + x_{ii})^2 + \frac{2}{(P+1)(P+2)} x^2$$

The estimates of general combining ability effects ( $\hat{g}_i$ ) and specific combining ability effects ( $\hat{s}_{ij}$ ) were computed as follows:

$$\hat{g}_i = \frac{1}{P+2} (x_i + x_{ii} - \frac{2}{P} X) ..$$

$$\hat{s}_{ij} = x_{ij} - \frac{1}{P+2} (x_i + x_{ii} + x_j + x_{jj}) + \frac{2}{(P+1)(P+2)} x ..$$

The variances of both effects and differences between effects were estimated as follows:

$$\text{var}(\hat{g}_i) = \frac{P-1}{P(P+2)} \sigma^2 e$$

$$\text{var}(\hat{s}_{ij}) = \frac{2P+P+2}{(P+1)(P+2)} \sigma^2 e(i \neq j)$$

$$\text{var}(\hat{s}_{ij} - \hat{s}_{ik}) = \frac{2(P+1)}{(P+2)} \sigma^2 e \quad (i \neq j, k, 1; j \neq K1 \text{ and } K \neq 1)$$

## 2. Biochemical and molecular genetic analysis

### 2.1. PCR-based DNA analysis:

DNA was extracted from the leaves of the selected plants of all genotypes studied which different reaction of salinity "tolerant, moderate and sensitive". The samples were single leaves for parents and first generation according to the method of Graham and Henry (1997).

### 2.2. Gel electrophoresis buffers

TBE buffer	10x
Tris	10.89
Boric acid	5.50 g
EDTA	0.74 g
H <sub>2</sub> O (dd) up to 100 mL	

### 2.3. Loading buffer:

Tris	10.89
Boric acid	5.50 g
EDTA	0.74 g
H <sub>2</sub> O (dd) up to 100 mL	

**Table (3):The primer names and sequences used in PCR analysis**

Primer names	Sequences
(PM15)	5'-CGGTTATGCCAACCGGCAT-3'
(AY334988)	5'-CGTTACCCCTTAAATTCTGA-3'
(HL-17)	5'-AATTCCCTCAGGTTCCCTAAC-3'

### 2.4. Agarose gel electrophoresis:

PCR amplification products were analyzed using 1.5% agarose gel electrophoresis in 1 x TBE buffer and stained with ethidium bromide. the run was performed at 100 V in Bio Rad submarine the bands of amplified DNA were visualized under UV light and the sizes of the fragments were estimated based on a DNA ladder of a 10 to 200 base pairs, and photographed with gel documentation system.

### 2.5. Gel analysis:

Gels were photographed under UV light with Polaroid film 667 and scanned with bio-rad video densitometer model 620, at a wave length of 557 software data analysis for Bio-Rad model 620 USA densitometer and computer were used.

## 3. Results and Discussion

Salinity is a major obstacle to increase production in rice growing areas. In Egypt rice is grown in the northern delta, whereas according to soil survey reports, it had been found that about 1.6 million feddan in the part of this area are damaged by excess soluble salts, exchangeable sodium accumulation and water logging conditions to an extent that causes crop yield reduction.

Rice is considered as moderately salt sensitive crop for the newly reclaimed saline areas. Therefore, developing salinity tolerance rice varieties is a very important approach not only for increasing yields, but also for conquering saline soils.

To develop and sustain high yielding rice varieties combined with salinity tolerance, it is needed to know adequate genetic information about the type and magnitude of the genetic and environmental variations within the genotypes.

### 1. Variation and interaction:

Mean squares of the ordinary analysis and combining ability analysis for all characters under normal and saline soils are presented in Table (4 and 5).

Mean squares of genotypes (parents and their crosses) were found to be highly significant for all characters studied at the normal and saline soil, indicating overall differences among these populations.

Both general and specific combining ability variances were found to be highly significant for all characters studied at two locations except plant height and Na/K ratio under normal conditions. These results would indicate the importance of both additive and non-additive genetic variances in determining the performance of these agronomic characters.

GCA/SCA ratio was used to clarify the nature of the gene action involved. GCA/SCA ratio were found to be greater than unity for grain yield/plant under all conditions, K<sup>+</sup> content, Na/K ratio and salinity index for grain yield/plant under normal and salinity conditions indicates that additive and additive x additive types of gene action were greater importance in the inheritance of these characters. It is therefore, could be concluded that selection procedures based on the accumulation of additive effects, would be successful in improving these characters. These findings were in agreement with those reported by Borgohain and Sharma (1998), El-Refaee (2002) and El-Mouhamady (2009).

### 2. Mean performance

The genotypes mean values for all studied character under normal and salinity conditions are presented in Tables (6 & 7).

For heading date. The earlier plants were obtained from the genotypes, GZ1368-S-5-4, hybrid 1, IET1444, GZ1368-S-5-4 x IET1444, Hybrid 1 x IET1444 and Giza 181 x IET1444 under both conditions. The mean values were ranged from 83.4 to 129.33 day for normal and salinity condition, respectively. These findings were in agreement with those reported by El-Said (2007) and El-Mouhamady (2009).

Regarding plant height, the parents; GZ1368-S-5-4, Hybrid 1 and IET1444 and the crosses; GZ1368-S-5-4 x

Hybrid 1, GZ1368-S-5-4 x IET1444 and Giza 181 x IET 1444 recorded the lowest values of plant height under all conditions. These results were in agreement with those obtained by Weerakoon (2008).

Concerning 1000-grain weight, the most desirable mean values were obtained from the genotypes; Hybrid 1, Sakha 102, IET1444, GZ1368-S-5-4 x Hybrid 1, GZ1368-S-5-4 x IET1444 and Giza 181 x IET 1444 under normal and salinity conditions. These findings were in harmony with those reported by Zhang *et al.* (2007), Weerakoon (2008) and El-Mouhamady (2009).

Regarding number of filled grains/panicle, the highest mean values were obtained from the genotypes; GZ1368-S-5-4, Hybrid 1, IET1444, GZ1368-S-5-4 x IET 1444, Hybrid 1 x IET 1444, Sakha 102 x IET 1444 and Giza 181 x IET 1444 under normal and salinity conditions.

With respect to grain yield/plant, the highest mean performance were showed from the genotypes; Hybrid 1, Sakha 102, IET 1444, GZ1368-S-5-4 x Hybrid 1, GZ1368-S-5-4 x IET1444, Hybrid 1 x IET 1444, Sakha 102 x IET 1444 and Giza 181 x IET 1444 under normal and salinity conditions. The values were ranged from 28.48 to 74.82 (g) for normal and salinity conditions. These results are in conformity with those reported by El-Said (2007) and El-Mouhamady (2009).

For Na<sup>+</sup> content, the most desirable mean values were obtained from the genotypes; Hybrid 1, IET1444, GZ1368-S-5-4 x IET1444, Hybrid 1 x IET1444 and Giza 181 x IET 1444 under normal and salinity conditions because these genotypes recorded the lowest level of Na<sup>+</sup> content from the soil as index for salinity tolerance in rice. These findings were in conformity with that reported by Won *et al.* (1992), Gonzalez *et al.* (1999), and Weerakoon *et al* (2008).

With respect to K<sup>+</sup> content, the genotypes; GZ1368-S-5-4, Hybrid 1, IET1444, GZ1368-S-5-4 x Hybrid 1, GZ1368-S-5-4 x IET1444, Hybrid 1 x IET 1444 and Giza 181 x IET1444 showed the highest mean values for this trait under both conditions. Similar results were obtained by Gonzalez *et al.* (1999) and Weerakoon *et al.* (2008).

Concerning Na/K ratio, the genotypes; Sakha 102, Giza 181, GZ1368-S-5-4 x Giza 181, Sakha 102 x Giza 181 and Sakha 102 x IET1444 gave the highest mean values under both normal and salinity conditions. These results were in agreement with those reported by Alam (1990), Gonzalez *et al.* (1999) and Weerakoon *et al.* (2008).

For salinity index for grain yield/plant, the parents; GZ1368-S-5-4, Hybrid 1, Sakha 102 and IET1444 and the crosses GZ1368-S-5-4 x ET1444, Hybrid 1 x Sakha 102, Hybrid 1 x IET1444, Sakha 102 x IET1444 and Giza 181 x IET1444 were recorded the highest mean values for this trait. Similar results were showed by El-Said (2007) and et al Weerakoon (2008).

### 3. Heterosis:

From Tables (8 and 9) observed the percentages of heterosis over better parents for all the characters studied under normal and salinity conditions.

With respect to heading date and plant height, heterosis percentages were highly significant and negative in the crosses, GZ1368-S-5-4xHybrid 1 and GZ1368-S-5-4 x IET1444 for heading date and the first cross in addition to Hybrid 1 x Sakha 102 and Sakha 102 x Giza 181 for plant height under all conditions, respectively which indicated, that additive gene action played an importance role to control this trait under salinity conditions.. These findings were inconformity with that reported by Sedeek (2006), weerakoon et al (2008) and El-Mouhamady (2009).

Highly significant and positive of heterosis over better parent were showed in the cross; GZ1368-S-5-4 x Giza 181 under all conditions only for 1000-grain weight and the values were 8.47 and 9.72% for normal and salinity conditions. it was found to be useful for specific combining ability which indicated, that additive gene action played an importance role to control this trait under salinity conditions. These results were obtained by El-Said (2007) and El-Mouhamady (2009).

Concerning number of grains/panicle heterosis percentages as deviation from better parents was highly significant and positive in the crosses, GZ1368-S-5-4 x Giza 181 , hybrid 1 x Sakha 102, Hybrid 1 x IET 1444, Sakha 102 x Giza 181, sakha 102 x IET1444 and Giza 181 x IET1444 under normal and salinity conditions and GZ1368-S-5-4 x sakha 102 under salinity conditions only which indicated, that additive gene action played an importance role to control this trait. Similar results were reported by El-Said (2007).

For grain yield/plant, the crosses, GZ1368-S-5-4 x Hybrid 1 under normal conditions, GZ1368-S-5-4 x IET1444 under salinity conditions and Giza 181 x IET1444 under normal and salinity conditions, respectively showed highly significant and positive of heterosis over better parent and the values were ranged from 11.22 to 11.40% under normal condition and from 4.59 to 10.78% under salinity conditions ,respectively. which indicated that additive gene action played an important role in the inheritance of grain yield/plant under salinity conditions. These results were reported by Zhang *et al.* (2007) and El-Mouhamady. (2009).

For Na<sup>+</sup> content, the crosses; GZ1368-S-5-4 x IET1444 , Hybrid 1 x IET1444 and Giza 181 x IET1444 under all conditions showed highly significant and negative of heterosis over better parent which indicated that these parents considered a good combiners for hybridization to make a programme for salinity tolerance . Similar results were obtained by El-Said (2007), Weerakoon *et al.* (2008) and El-Mouhamady (2009).

The genotypes, GZ1368-S-5-4 x Hybrid 1 , hybrid 1 x IET1444 and Sakha 102xGiza 181were showed highly significant and positive of heterosis over better-parent for k<sup>+</sup> content all conditions and the values were ranged from 2.98 to 133.3% and from 2.96 to 139.20% under normal and salinity conditions respectively. which indicated that , additive gene action played an important role in the inheritance of this trait.These findings were in conformity with those reported by Sedeek (2006), El-Said (2007), Zhang *et al.* (2007), Weerakorn *et al.* (2008) and El-Mouhamady (2009).

**Table (4): Mean squares estimates of ordinary analysis and combining ability analysis for yield and its components under normal and salinity conditions**

S.O.V	D.F	M.S									
		Heading date(day)		plant height (cm)		1000-grain weight (g)		No.of filled grains/panicle		Grain yield/ plant	
		N	S	N	S	N	S	N	S	N	S
Genotypes	14	684.76**	766.82**	701.46**	588.59**	88.01**	93.87**	8260.66**	7437.18**	825.09**	720.16**
Parents	4	312.95**	403.17**	515.86**	380.47**	103.63**	91.78**	98.9306**	8647.10**	799.03**	636.12
Crosses	9	866.01**	1000.29**	855.55**	744.50**	90.21**	102.53**	6649.71**	5879.86**	924.40**	835.19**
P V S crosses	1	540.91**	114.46**	57.08**	17.86**	5.67*	24.26**	1622.61**	16571.41**	35.45**	21.02**
Error	28	0.69	0.99	1.27	0.79	0.91	0.82	0.28	1.12	0.75	0.62
GCA	4	408.91**	499.87**	292.00**	220.99**	65.26**	59.59**	6346.04**	5670.5**	713.85**	633.45**
SCA	10	155.99**	157.90**	210.55	186.28**	14.96**	19.97**	1316.56**	1201.07**	99.50**	82.69**
GCA/ SCA		0.37	0.45	0.19	0.17	0.62	0.42	0.68	0.67	1.02	1.09
Error	28	0.23	0.33	0.42	0.26	0.30	0.27	0.09	0.37	0.25	0.21

N: normal

S: salinity

\*: Significant at 5%    \*\* Significant at 1%

**Table (5): Mean squares estimates of ordinary analysis and combining ability analysis for some characters related to salinity under normal and salinity conditions.**

S.O.V	D.F	M.S							
		Na <sup>+</sup> content		K <sup>+</sup> content		Na/ K ratio		Salinity index for grain yield plant	
		N	S	N	S	N	S	N	
Genotypes	14	0.113**	0.199**	1.927**	2.120**	0.093*	0.141**	326.17**	
Parents	4	0.103**	0.261**	2.453**	3.551**	0.150*	0.350**	265.27**	
Crosses	9	0.129**	0.191**	1.463**	1.163**	0.056	0.029**	370.46**	
P.V S. crosses	1	0.007**	0.027**	4.00**	5.00**	0.200*	0.317**	171.20**	
Error	28	0.00003	0.00001	0.0003	0.001	0.042	0.0002	18.27	
GCA	4	0.09**	0.16**	1.46**	1.41**	0.06**	0.11**	217.03**	
SCA	10	0.02**	0.03*	0.31**	0.42**	0.02	0.02**	65.40**	
GCA/ SCA		0.50	0.76	0.67	0.47	0.47	0.78	0.48	
Error	28	0.00	0.00	0.0001	0.00	0.01	0.00	6.09	

N: normal

S: salinity

\*: Significant at 5%    \*\* Significant at 1%

**Table (6): The genotypes mean performance for yield and components characters studied under normal and salinity, and interactions**

Genotypes	Heading date (day)		plant height (cm)		1000- grain weight (g)		No.of filled grains/ panicle		Grain yield/ plant (g)	
	N	S	N	S	N	S	N	S	N	S
GZ 1368-S-5-4	92.0	105.0	92.63	90.30	22.43	19.43	140.23	105.13	41.57	32.40
Hybrid I	90.73	96.10	110.50	103.37	31.50	28.37	190.38	171.30	64.43	50.60
Sakha 102	113.17	122.67	117.0	112.30	27.37	25.23	115.57	105.57	44.40	40.43
Giza 181	97.83	104.57	115.23	109.33	19.33	16.67	133.23	121.22	28.48	21.03
IET 1444	87.30	92.83	89.0	87.17	33.20	29.23	257.23	229.37	67.16	57.87
GZ1368-S-5-4 x Hybrid I	87.17	87.37	84.33	82.03	29.40	26.67	177.34	151.44	71.66	44.10
GZ1368-S-5-4 x Sakha 102	118.33	122.33	134.43	128.33	20.50	17.23	133.53	126.80	38.57	22.71
GZ1368-S-5-4 x Giza 181	116.33	122.23	125.43	120.07	24.33	21.32	150.37	120.36	27.47	19.37
GZ1368-S-5-4 x IET 1444	83.4	85.17	95.33	93.03	34.00	30.33	232.37	221.22	69.47	60.53
Hybrid I x Sakha 102	110.00	117.27	97.67	95.77	21.17	15.00	201.09	192.86	42.57	32.20
Hybrid I x Giza 181	129.33	133.33	122.37	114.40	20.93	17.00	171.30	156.29	38.40	26.57
Hybrid I x IET 1444	94.43	96.30	98.67	89.33	29.83	26.67	261.07	234.46	65.44	52.17
Sakha 102x Giza 181	120.23	126.67	108.03	95.33	19.33	15.40	241.43	221.23	28.85	19.59
Sakha 102x IET 1444	89.07	93.67	117.36	112.86	27.43	23.43	250.40	217.70	61.33	55.07
Giza 181 x IET 1444	87.38	92.89	89.09	87.37	33.80	29.83	257.83	229.39	74.82	64.11
LSD at 5%	1.39	1.67	1.88	1.48	1.59	1.51	0.88	1.77	1.45	1.32
LSD at 1%	1.88	2.25	2.55	2.01	2.16	2.04	1.19	2.39	1.96	1.78

N: normal S: salinity \*: Significant at 5% \*\* Significant at 1%

**Table (7): The genotypes mean performance for some traits related to salinity under normal and salinity conditions**

Genotypes	Na <sup>+</sup> content		K <sup>+</sup> content		Na / K ratio		Salinity index for grain yield/plant
	N	S	N	S	N	S	
GZ1368-S-5-4	0.29	0.31	2.12	2.70	0.13	0.11	77.94
Hybrid I	0.12	0.14	2.77	2.87	0.04	0.05	78.53
Sakha 102	0.63	0.91	1.09	1.12	0.57	0.81	91.05
Giza 181	0.41	0.54	1.11	1.03	0.37	0.52	73.84
IET 1444	0.32	0.34	3.02	3.43	0.10	0.09	86.16
GZ1368-S-5-4 x Hybrid I	0.31	0.41	2.91	3.20	0.10	0.13	61.54
GZ1368-S-5-4 x Sakha 102	0.48	0.61	2.03	2.78	0.23	0.22	58.87
GZ1368-S-5-4 x Giza 181	0.54	0.72	1.82	2.90	0.29	0.25	70.51
GZ1368-S-5-4 x IET 1444	0.04	0.10	3.11	2.84	0.01	0.03	87.13
Hybrid I x Sakha 102	0.22	0.22	2.52	2.64	0.08	0.08	75.64
Hybrid I x Giza 181	0.21	0.22	1.72	1.82	0.12	0.12	69.19
Hybrid I x IET 1444	0.11	0.13	4.06	4.24	0.02	0.03	79.72
Sakha 102x Giza 181	0.73	0.83	2.59	2.68	0.28	0.31	67.90
Sakha 102x IET 1444	0.38	0.33	2.78	2.91	0.11	0.11	89.79
Giza 181 x IET 1444	0.16	0.20	3.75	3.18	0.04	0.06	85.68
LSD at 5%	0.009	0.005	0.028	0.052	0.344	0.026	7.149
LSD at 1%	0.01	0.01	0.04	0.07	0.46	0.03	9.65

N: normal

S: salinity

\*: Significant at 5%

\*\* Significant at 1%

Table (8) percentages of heterosis over better parents (B.p) for grain grain yield and its components under normal and salinity conditions.

Genotypes	Heading date (day)		plant height (cm)		1000- grain weight (g)		No.of filled grains/ panicle		Grain yield/ plant (g)	
	N	S	N	S	N		N	S	N	S
GZ1368-S-5-4 x Hybrid I	-3.92**	-9.08**	-8.96**	-9.15**	-6.66*	-5.99*	-6.84	-11.59**	11.22**	-12.84
GZ1368-S-5-4 x Sakha 102	28.62**	16.50**	45.12**	42.11	-25.10**	-31.70**	-4.77**	20.41**	-13.13**	-43.89**
GZ1368-S-5-4 x Giza 181	26.44**	16.88	35.40**	32.96**	8.47*	9.72*	7.23**	-0.71	-33.91**	-40.21**
GZ1368-S-5-4 x IET 1444	-4.46**	-8.25**	7.11**	6.75**	2.40	3.76	-9.66	-3.55**	-3.43**	4.59**
Hybrid I x Sakha 102	21.23**	22.03**	-11.61**	-7.35**	-32.79**	-47.12**	5.62**	12.58**	-33.92**	-36.36**
Hybrid I x Giza 181	42.54**	38.74**	10.74**	10.67**	-33.55**	-40.07**	-10.02**	-8.76**	-40.40**	-47.49**
Hybrid I x IET 1444	8.16**	3.73**	10.86**	2.47**	-10.15**	-8.75**	1.49**	2.22**	-2.56*	-9.84**
Sakha 102 x Giza 181	22.89**	21.13**	-6.24**	-12.80**	-29.37**	-38.96**	81.21**	82.50**	-35.02**	-51.54**
Sakha 102 x IET 1444	2.02*	09.0	31.86**	29.47**	-17.37**	-19.84**	-2.65**	-5.08**	-8.68**	-4.83**
Giza 181xIET 1444	0.09	0.06	0.10	0.22	1.80	2.05	0.23	0.008	11.40	10.78**
LSD at 5%	1.39	1.67	1.88	1.48	1.59	1.51	0.88	1.77	1.45	1.32
LSD at 1%	1.88	2.25	2.55	2.01	2.16	2.04	1.19	2.39	1.96	1.78

N: normal

S: salinity

\*: Significant at 5%

\*\*: Significant at 1%

Table (9): Percentages of heterosis over better Parents (B.P) for some characters related to salinity under normal and salinity conditions.

Genotypes	Na <sup>+</sup> content		K <sup>+</sup> content		Na / K ratio		Salimty index for grain yield/plant	
	N	S	N	S	N	S		
GZ1368-S-5-4 x Hybrid I	158.33**	192.85**	5.05**		11.49**	-23.07	18.18	-21.63**
GZ1368-S-5-4 x Sakha 102	65.51**	96.77**	-4.24**		2.96**	-59.64*	-72.83**	-35.34**
GZ1368-S-5-4 x Giza 181	86.20**	132.25**	-14.15**		7.40**	-21.62	-51.92**	-9.53*
GZ1368-S-5-4 x IET 1444	-86.20**	-67.74**	2.98**		-17.20**	-92.30	-72.72**	1.12
Hybrid I x Skha 102	83.33**	57.14**	-9.02**		-8.01**	-85.96**	-90.12**	-16.92**
Hybrid I x Giza 181	75.0*	57.14*	-37.90**		-36.85**	-67.56	-76.92**	-11.89
Hybrid I x IET 1444	-8.33**	-7.14**	34.43**		23.61**	-80.00	-66.66**	-7.47
Sakha 102 x Giza 181	78.04**	53.70**	133.33**		139.28**	50.87	-61.73**	-25.42**
Sakha 102 x IET 1444	18.75*	-2.94	-7.94**		-15.16**	-80.70	-86.42**	-1.38
Giza 181xIET 1444	-50.0**	-41.17**	24.17**		-7.28**	-89.18	-88.46**	-0.55
LSD at 5%	0.009	0.005	0.028		0.052	0.344	0.026	7.149
LSD at 1%	0.01	0.01	0.04		0.07	0.460	0.03	9.650

N: normal

S: salinity

\*: Significant at 5%

\*\*: Significant at 1%

For Na/K ratio, all crosses showed highly significant and negative of heterosis over better parent for this trait under normal and salinity conditions. Similar results were obtained by Sedeek (2006) and Weerakoon et al (2008).

With respect to salinity index for grain yield/plant, six crosses were showed highly significant and negative of heterosis over better parent for example, GZ1368-S-5-4 x Hybrid 1, GZ1368-S-5-4 x Sakha 102, Hybrid 1 x Sakha 102 and Sakha 102x IET1444. It could be concluded that the most desirable crosses for all traits studied under both normal and salinity conditions were GZ1368-S-5-4 x Hybrid 1, GZ1368-S-5-4 x IET1444, hybrid 1 x IET1444 and Giza 181 x IET1444.

#### 4. Combining ability

##### 4.1. General combining ability

Estimates of the GCA effects of individual parental lines for all characters studied under normal and salinity conditions are present in Tables (10 and 11).

The varieties GZ1368-S-5-4, Hybrid 1 and IET1444 showed highly significant and negative of general combining ability effects under two conditions for heading date and plant height characters, indicating that these varieties can be considered as a good combiners for these traits under normal and salinity conditions, and found that earliness was controlled by over-dominance. These results were in agreement with the data obtained by Sedeek (2006), El-Said (007) and Weerakoon *et al.* (2008).

Highly significantly and positively of GCA effects was showed in the genotypes; Hybrid 1 and IET1444 for 1000-grain weight and grain yield/plant under the two conditions , while number of filled grains/panicle was under normal conditions only, it is clear that these varieties were found to be the best combiners for these traits and indicating that additive x additive types of gene action were of greater importance in the inheritance of this traits under both conditions. These results were in agreement with those reported by El-Mouhamady (2009).

The parents; Hybrid 1 and IET144 under both conditions and GZ1368-S-5-4 under normal conditions only showed significant and highly significant negative GCA effects for Na<sup>+</sup> content, While, hybrid 1 and IET1444 under two conditions and GZ1368-S-5-4 under normal conditions showed highly significant and positively of GCA effects for K<sup>+</sup> content.

On the other hand, the genotypes, sakha 102 under all conditions,Giza 181 under salinity conditions for Na/k ratio and the same cultivar in addition to IET1444 for salinity index for grain

yield/plant observed significant and highly significant and positive of GCA effects, which proving to be good combiners for these traits, proving to be good combiners for this traits, which indicating the importance of both additive and non-additive variances in the expression of this traits in rice. Similar results were obtained by Singh and Kumar (2005) and Weerakoon et al. (2008).

Since significant negative values of GCA effects would be of interest for earliness and short stature rice cultivars in Tables (10 and 11) for heading date, plant height and Na<sup>+</sup> content in the genotypes ; Hybrid 1 and IET1444 provide to be good combiners under both conditions, could be useful for rice breeders who breed for earliness or short stature rice cultivars and low level of Na<sup>+</sup> content considering the GCA effects for yield and its components, K<sup>+</sup> content, Na/K ratio and salinity index for grain yield/plant, it was suggested that population involving the parents GZ1368-S-5-4, Hybrid 1 and IET 1444 could be considered in making multiple crossing because they might possess desirable genes for earliness, short stature as well as high grain yielding ability under normal and salinity conditions. Accordingly, these parents would be the best choice as base populations.

From the foregoing discussion, it could be concluded that, the most desirable genotypes for grain yield and its components and some characters related to salinity under normal and salinity conditions were GZ1368-S-5-4, Hybrid 1, IET1444, GZ1368-S-5-4 x IET1444, Hybrid 1 x IET1444, Sakha 102 x IET1444 and Giza 181 x IET1444, which indicated the importance for the earlier selection for these traits under salinity conditions.

##### 4.2. Specific combining ability effects:

SCA effects for the parental combinations under the two conditions (normal and salinity) are shown in Tables (12 & 13).

For heading date, four out of ten hybrid combinations had negative and highly significant desirable SCA effects under normal and salinity conditions. The best crosses were GZ1368-S-5-4 x Hybrid 1, GZ1368-S-5-4 x IET1444, Sakha 102 x IET1444 and Giza 181 x IET1444 for this trait under two conditions. These results were in agreement with those reported by Aidy *et al* (2006) and El-Said (2007).

Concerning plant height, four out of ten hybrid studied showed highly significant and negative of SCA effects under both conditions. These results obtained in the crosses; GZ1368-S-5-4 x Hybrid 1, Hybrid 1 x Sakha 102, Sakha 102x Giza 181 and Giza 181 and x IET1444, indicated that additive and additive x additive types of gene action were of greater importance in the inheritance of plant

Table (10): Estimates of general combining ability effect for the poronet varieties evaluated for yield and its components characters studied under normal and salinity conditions.

Genotypes	Heading date (day)		plant height (cm)		1000- grain weight (g)		No.of filled grains/ panicle		Grain yield/ plant (g)	
	N	S	N	S	N	S	N	S	N	S
GZ1368-S-5-4	-2.49**	-1.63**	-2.00**	-0.61**	-0.64**	-0.30	-27.29**	-30.22**	-1.78**	-3.64**
Hybrid I	-0.61**	-1.87**	-2.11**	-2.86	0.96**	0.80**	3.78**	5.15**	6.31**	2.75**
Sakha 102	8.19**	9.39**	7.53**	6.94**	-2.06**	-2.14**	-15.36**	-10.35**	-6.10**	-3.80**
Giza 181	6.03**	6.38**	5.21**	3.90**	-3.02**	-2.89**	-11.19**	-10.27**	-11.99**	-10.22**
IET 1444	-11.12**	-12.27**	-8.63**	-7.37**	4.75**	4.52**	50.06**	45.69	13.56**	14.91**
LSD at 5%g	0.33	04.	0.45	0.36	0.38	0.36	0.21	0.42	0.35	0.32
LSD at 1%g	0.45	0.54	0.61	0.48	0.52	0.49	0.29	0.57	0.47	0.43
LSD at 5%gi	0.53	0.63	0.71	0.56	0.60	0.57	0.33	0.67	0.55	0.50
LSD at 1 %gi	0.71	0.85	0.96	0.76	0.82	0.77	0.45	0.90	0.74	0.67

N: normal

S: salinity

\*: Significant at 5%

\*\*: Significant at 1%

Table (11):Estimates of general combining ability effect for the parental varieties evaluated for some characters related to salinity under normal and salinity conditions.

Genotypes	Na <sup>+</sup> content		K <sup>+</sup> content		Na / K ratio		Salimty index for grain yield/plant
	N	S	N	S	N	S	
GZ1368-S-5-4	-0.01**	0.00	-0.08**	0.12**	-0.06	-0.04**	-3.65**
Hybrid I	-0.13**	-0.17**	0.30**	0.20**	-0.13**	-0.10**	-4.58**
Sakha 102	0.14**	0.19**	-0.37**	-0.43**	0.08*	0.17**	2.83**
Giza 181	0.09**	0.10**	-0.47**	0.47**	0.08	0.10**	-3.09**
IET 1444	-0.08**	-0.13**	0.62**	0.58**	0.02	-0.11*	8.49**
LSD at 5%g	0.00	0.00	0.01	0.01	0.08	0.01	1.71
LSD at 1%g	0.00	0.00	0.01	0.02	0.11	0.01	2.31
LSD at 5%gi	0.00	0.00	0.01	0.02	0.13	0.01	2.70
LSD at 1 %gi	0.00	0.00	0.01	0.03	0.18	0.01	3.65

N: normal

S: salinity

\*: Significant at 5%

\*\*: Significant at 1%

height and These crosses were found to be the useful crosses for heterosis. which indicated that these crosses were by Bindu and shashidhear (2006) and El-Mouhamady (2009).

For 1000-grain weight, the crosses; GZ1368-S-5-4 x Hybrid 1, GZ1368-S-5-4 x IET1444 , GZ1368-S-5-4 x Giza 181 and Giza 181x IET1444 showed highly significant and positive SCA effects under normal and salinity conditions. These crosses were found to be very important for salinity tolerance in rice by the interaction between additive and additive x additive gene actions These findings were in agreement with those reported by Weerakoon *et al.* (2008).

With respect to number of filled grains/panicle, six out of ten crosses studied had positive and highly significant of SCA effects under normal and salinity conditions. These crosses were GZ1368-S-5-4 x Hybrid 1, GZ1368-S-5-4 x IET 1444, Hybrid1 x Sakha102, Hybrid 1 x IET1444,Sakha 102xGiza 181 and Giza 181 x IET 1444. The same results were obtained by Bindu and shashidhear (2006) and El-Said (2007).

For grain yield/plant, four out of ten crosses showed highly significant and positive of SCA effects. The best of these crosses were GZ1368-S-5-4 x Hybrid 1 under salinity conditions, GZ1368-S-5-4 x IET1444,Sakha 102 X IET 1444 and Giza 181 x IET1444 under all conditions, which were found to show useful heterosis for this trait under normal and salinity conditions, which indicated that these crosses were found to be useful for salinity tolerance in rice and indicating the importance of both additive and non-additive variances in the expression of these trait in rice.. Similar results were showed by Aidy (2006), Bindu and shashidhear (2006), Abd El-Lateef *et al.* (2006) and Weerakoon *et al.* (2008).

Regarding of  $\text{Na}^+$  content, the crosses, GZ1368-S-5-4 x IET1444, Hybrid 1 x Sakha 102, Hybrid 1 x Giza 181,Sakha 102 x IET1444 and Giza 181 x IET1444 showed highly significant and negative of SCA effects under normal and salinity conditions, which indicated that these crosses were very important for development salinity tolerance in rice. Similar results were obtained by El-Mowafy (1954), Bindu and shashidhear (2006) and Weerakoon *et al* (2008).

The crosses; GZ1368-S-5-4 x Hybrid 1, GZ1368-S-5-4 x Sakha 102, Hybrid 1 x IET1444, Hybrid 1 x Sakha 102, Sakha 102xGiza 181, Sakha 102 x IET1444 and Giza 181 x IET1444 were

showed highly significantly and positively of SCA effects under all conditions for  $\text{K}^+$  content. These crosses were found to be useful for heterosis and important for salinity tolerance of rice. These results were in agreement with those reported by Bindu and shashidhear (2006) and Weerakoon *et al* (2008).

Highly significant and positive of SCA effects for Na/K ratio were found in the crosses; GZ1368-S-5-4 x Hybrid 1 and Hybrid 1 x IET1444 under salinity conditions only, proving to be the best crosses for this trait and indicating the importance of additive and non-additive genetic variance in the inheritance of these characters and that selection for these traits would be effective in early segregating generations. These results were in agreement with those reported by El-Mowafy (1994), Cheong *et al.* (1995) and El-Said (2007).

For salinity index for grain yield/plant, the crosses GZ1368-S-5-4 x IET 1444 and Giza 181 x IET 1444 showed significant and highly significant and positive of SCA effects for this trait. Similar results were showed by Cheong *et al.* (1995), Zhang *et al.* (2007) and El-Mouhamady (2009).

It was obvious in Tables (12 and 13) that most of the crosses showing high SCA effects involved diverse parents. The superior F's having SCA effects are expected to produce desirable transgressive segregates, provided that the desirable complementary genes and epistatic effects are coupled in the same direction to maximize these traits in view.

Also, desirable SCA effects in the crosses, GZ1368-S-5-4 x Hybrid 1, GZ1368-S-5-4 x IET1444 and Giza 181 x IET1444 might be recommended to be utilized in hybrid rice breeding. The population would posses desirable genetic for yield components and salinity traits, which indicated that this different origin of these parent would widen the genetic base for selection.

## 5.Biochemical genetic markers:

The importance and need of rice genotypes at global level requires evaluation of germplasm to assist the future of Salinity tolerance programs. Hence, it is essential top characterize rice genotypes using PCR-based markers such as PM15 primer in Table (14), Figure (1).

Table (12): Estimates of specific combining ability effects for the crosses evaluated for yield and its components characters studied under normal and salinity conditions.

Crosses	Heading date (day)		plant height (cm)		1000- grain weight (g)		No.of filled grains/ panicle		Grain yield/ plant (g)	
	N	S	N	S	N	S	N	S	N	S
GZ1368-S-5-4 x Hybrid I	-10.85**	-15.72**	-18.02	-15.88**	2.81**	3.42**	6.67**	2.91**	-16.67	5.49**
GZ1368-S-5-4 x Sakha 102	11.53**	7.99**	22.44**	20.62**	-3.06**	-3.08	-18.00**	-6.24**	-4.02	-9.35**
GZ1368-S-5-4 x Giza 181	11.68**	10.90**	15.75**	15.38**	1.73**	1.76**	-5.34**	-12.75**	-9.22**	-6.27**
GZ1368-S-5-4 x IET 1444	-4.10**	-7.52**	-0.50	-0.37	3.62**	3.36**	15.41**	32.14**	7.22**	9.76**
Hybrid I x Sakha 102	1.31**	3.15**	-14.22**	-9.69**	-4.00**	-6.41**	18.49**	24.45**	-8.11**	-6.28**
Hybrid I x Giza 181	22.80**	22.23**	12.79**	11.97**	-3.28**	-3.66**	-15.48**	-12.18**	-6.38**	-5.46**
Hybrid I x IET 1444	5.05	3.85**	2.94**	-1.82**	-2.15**	-1.40**	13.04**	10.02**	-4.89**	-4.99**
Sakha 102 x Giza 181	4.91**	4.31**	-11.18**	-16.89**	-1.85**	-2.33**	73.80**	68.25**	-3.53**	-5.90**
Sakha 102 x IET 1444	-9.11**	-10.04**	12.00**	11.88**	-1.53**	-1.70**	21.51**	8.75	3.40**	4.45**
Giza 181 x IET 1444	-8.72**	-7.87**	-14.05**	-10.75**	5.20**	4.85**	24.18**	20.34**	15.13**	13.68**
LSD at 5% Sij	0.86	1.03	1.17	0.92	0.99	0.93	0.55	1.09	0.90	0.82
LSD at 1% Sij	1.16	1.39	1.57	1.24	1.33	1.26	0.74	1.48	1.21	1.10
LSD at 5% Sij- sik	1.29	1.55	1.75	1.38	1.48	1.40	0.82	1.64	1.35	1.22
LSD at 1% Sij- sik	1.74	2.09	2.36	1.86	2.00	1.89	1.10	2.21	1.82	1.65
LSD at 5% Sij- sk	1.18	1.41	1.60	1.26	1.35	1.28	0.75	1.50	1.23	1.12
LSD at 1% Sij- sk	1.59	1.90	2.15	1.80	1.82	1.73	1.05	2.02	1.66	1.51

N: normal

S: salinity

\*: Significant at 5%

\*\*: Significant at 1%

Table (13): Estimates of speafic combining ability effect for the crosses evaluated for some characters related to salinity under normal and salinity conditions.

Genotypes	Na <sup>+</sup> content		K <sup>+</sup> content		Na / K ratio		Salimty index for grain yield/plant
	N	S	N	S	N	S	
GZ1368-S-5-4 x Hybrid I	0.11**	0.18**	0.25**	0.18**	0.07	0.08**	-7.10**
GZ1368-S-5-4 x Sakha 102	0.02**	0.01**	0.03**	0.31**	0.00	-0.08**	-16.16
GZ1368-S-5-4 x Giza 181	0.12**	0.20**	-0.08**	0.55**	0.05	0.01	0.12
GZ1368-S-5-4 x IET 1444	-0.21**	-0.18**	0.13**	-0.56**	-0.17	-0.01	6.19**
Hybrid I x Sakha 102	-0.13**	-0.12**	-0.56**	-0.61**	-0.04	-0.06**	0.34
Hybrid I x Giza 181	-0.07**	-0.12**	-0.56**	-0.61**	-0.04	-0.06**	0.34
Hybrid I x IET 1444	-0.01**	0.01**	0.69**	0.76**	-0.09	0.04**	-0.68
Sakha 102 x Giza 181	0.16**	0.13**	0.99**	0.89**	-0.10	-0.15**	-7.75**
Sakha 102 x IET 1444	-0.07**	-0.14**	0.08**	0.06**	-0.21	-0.15**	2.96
Giza 181 xIET 1444	-0.02**	-0.05**	0.43**	0.62**	0.12	-0.10**	4.90*
LSD at 5% Sij	0.01	0.00	0.02	0.03	NS	0.02	4.41
LSD at 1% Sij	0.01	0.00	0.02	0.04	NS	0.02	5.95
LSD at 5% Sij- sik	0.01	0.00	0.03	0.05	NS	0.02	6.62
LSD at 1% Sij- sik	0.01	0.01	0.04	0.06	NS	0.03	8.93
LSD at 5% Sij- sk	0.01	0.00	0.02	0.04	NS	0.02	6.04
LSD at 5% Sij- sk	0.01	0.01	0.03	0.06	NS	0.03	8.15

N: normal

S: salinity

\*: Significant at 5%

\*\*: Significant at 1%

NS: not significant

The results indicated that the bands number 1, 2, 3, 9 and 10 were found in the parents; GZ1368-S-5-4, Hybrid 1, Sakha 2 and the crosses; GZ1368-S-5-4 x IET1444, Hybrid 1 x Sakha 102, Hybrid 1 x Giza 181 and Giza 181 x IET 1444, which indicated that these bands were common bands in these genotypes and were index for salinity tolerance in rice. This high degree of polymorphism for these DNA markers could be a very powerful tool for studying the phylogenetic relationships among rice genotypes.

These results were found by Champoux *et al.* (1995), Roy *et al.* (1996), Yadav *et al.* (1997), Price and Tomas (1997), Thank *et al.* (1999), Tripathy *et al.* (2000), Zhang *et al.* (2001), Courtois *et al.* (2003), Lanceras *et al.* (2004), Weerakoon *et al.* (2008) and El-Mouhamady. (2009).

The bands number 1, 2, 3, 8, 9 and 10 were appeared in the genotypes; GZ1368-S-5-4, Hybrid 1, IET1444, GZ1368-S-5-4x Hybrid 1, GZ1368-S-5-4 x Sakha

Table (14) The densitometric analysis of Rapd- PCR products for all genotypes studied of rice using PM15 primer

Band No.	Base pairs	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	2000	+	+	+	-	-	-	-	-	+	+	+	-	-	-	+
2	1500	+	+	+	-	-	-	-	-	+	+	+	-	-	-	+
3	1000	+	+	+	-	-	-	-	-	+	+	+	-	-	-	+
4	950	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	800	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	750	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	500	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
8	450	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
9	350	+	+	-	-	+	-	-	-	+	-	+	-	-	-	+
10	300	+	+	-	-	+	+	-	-	+	-	+	-	-	-	+

1- 1368-S-5-4    2- Hydrid I    3- SaKHa102    4- Giza 181    5- IET 1444    6- Gz1368-S-5-4 x Hybrid I

7- GZ1368-S-5-4 x Sakha 102    8- GZ1368-S-5-4 x Giza 181    9- GZ1368-S-5-4 x IET 1444    10- Hybrid I x Skha 102

11- Hybrid I x Giza 181    12- Hybrid I x IET 1444    13- Sakha 102 x Giza 181    14- Sakha 102 x IET 1444

15- Giza 181 xIET 1444

Table (15) The densitometric analysis of Rapd- PCR products for all genotypes studied of rice using Ay334988 primer

Band No.	Base pairs	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	2000	+	+	-	-	+	+	+	-	-	-	-	+	+	+	+
2	1500	+	+	-	-	+	+	+	-	-	-	-	+	+	+	+
3	1000	+	+	-	-	+	+	+	-	-	-	-	+	+	+	+
4	950	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	800	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
6	750	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
7	500	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
8	450	+	+	-	-	+	+	+	-	-	-	-	+	+	+	+
9	350	+	+	-	-	+	+	+	-	+	-	-	+	+	+	+
10	300	+	+	-	-	+	+	+	-	+	-	-	+	+	+	+

1- 1368-S-5-4    2- Hydrid I    3- SaKHa102    4- Giza 181    5- IET 1444    6- Gz1368-S-5-4 x Hybrid I

7- GZ1368-S-5-4 x Sakha 102    8- GZ1368-S-5-4 x Giza 181    9- GZ1368-S-5-4 x IET 1444    10- Hybrid I x Skha 102

11- Hybrid I x Giza 181    12- Hybrid I x IET 1444    13- Sakha 102 x Giza 181    14- Sakha 102 x IET 1444

15- Giza 181 xIET 1444

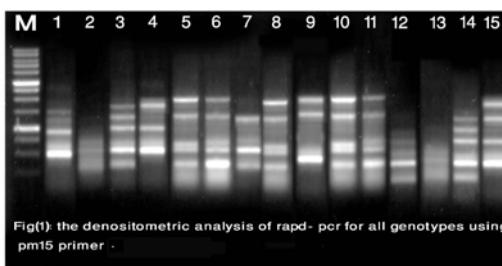
102, Hybrid 1 x IET 1444, Sakha 102 x Giza 181, Sakha 102, IET 1444 and Giza 181 x IET1444 and not appeared in the other genotypes, which means that these bands were found to be marker for salinity tolerance in these genotypes using AY334988 primer in Table (15) and Figure (2).

In Figure (16), the results indicated that the bands number 4, 5, 6, 7 and 10 were appeared in the gene types; GZ1368-S-5-4, Hybrid 1, IET1444, GZ1368-S-5-4 x Hybrid 1, GZ1368-S-5-4, hybrid 1, IET 1444, GZ1368-S-5-4 x Hybrid 1, GZ1368-S-5-4 x Sakha 102, Sakha 102 x Giza 181, Sakha 102 x IET 1444 and Giza 181 x IET 1444 and weren't appeared in the other genotypes, which indicated that, these bands were common bands and index for salinity tolerance in these crosses using HL-17 primer in Figure (3). Similar results were obtained by El-Mouhamady (2009).

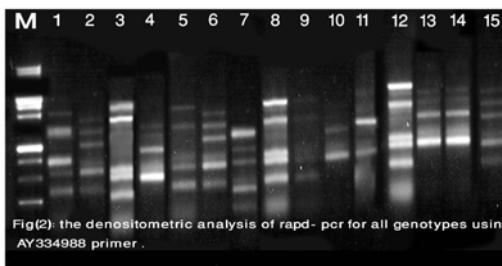
Table (16) The densitometric analysis of Rapd- PCR products for all genotypes studied of rice using HL-17 primer

Band No.	Base pairs	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	2700	-	-	+	+	-	-	-	+	-	-	+	+	+	+	+
2	2000	-	-	+	+	-	-	-	+	-	+	+	+	-	-	+
3	1500	-	-	-	+	-	-	-	+	-	+	+	+	+	+	+
4	1350	+	+	-	-	+	+	+	-	+	-	-	+	-	-	-
5	1300	+	+	+	-	+	+	+	-	+	+	-	-	+	+	-
6	1150	+	+	+	-	+	+	+	+	-	-	-	-	-	-	-
7	1000	+	+	+	+	+	+	+	-	+	-	-	+	+	+	-
8	750	-	-	-	+	-	-	+	-	+	+	+	+	+	+	+
9	500	-	+	+	+	+	+	+	+	+	+	+	+	-	-	+
10	350	+	+	-	+	+	+	+	+	-	+	+	-	+	+	+

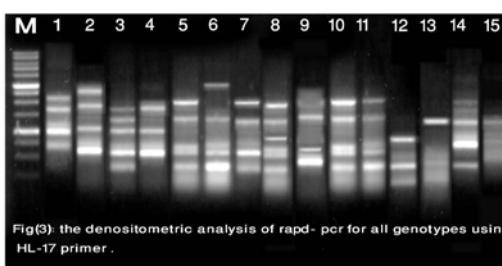
1- 1368-S-5-4      2- Hydrid I      3- SaKHa102      4- Giza 181      5- IET 1444      6- Gz1368-S-5-4 x Hybrid I  
 7- GZ1368-S-5-4 x Sakha 102      8- GZ1368-S-5-4 x Giza 181      9- GZ1368-S-5-4 x IET 1444      10- Hybrid I x Skha 102  
 11- Hybrid I x Giza 181      12- Hybrid I x IET 1444      13- Sakha 102 x Giza 181      14- Sakha 102 x IET 1444  
 15- Giza 181 xIET 1444



Fig(1): the denositometric analysis of rapd- pcr for all genotypes using pm15 primer .



Fig(2): the denositometric analysis of rapd- pcr for all genotypes using AY334988 primer .



Fig(3): the denositometric analysis of rapd- pcr for all genotypes using HL-17 primer .

#### 4. Conclusion

1.The most desirable mean value , positive and highly significant for heterosis, general and specific combining ability effects for all genotypes under normal and salinity conditions were obtained from the genotypes.: GZ1368-S-5-4, IET1444, GZ1368-S-5-4 x hybrid 1, Gz1368-S-5-4 x IET1444 and Giza 181 x IET 1444 for all traits studied.  
 2.From the foreign discussion, it could be concluded that the crosses GZ1368-S-5-4 x Hybrid 1, G21368-S-5-4 x IET1444, Hybrid 1 x IET1444 and Giza 181 x IET1444 were contained of 1, 5, 1 and 5bands using PM15 primer, 6,

3, 6 and 6 bands using AY334988 and 6, 2, 4 and 5 bands using HL-17 primer, which indicated that these bands were found to be index and marker for salinity tolerance in rice by increasing K<sup>+</sup> content and decreasing of Na<sup>+</sup> content.  
 3.The crosses GZ1368-S-5-4 x hybrid 1and Giza 181 x IET 1444 were the best crosses for salinity tolerance in rice and were important for breeder to selection the cultivars for the earlier and short stature would be the best choice as base populations.

**Corresponding author****\*El-Mouhamady, A.A**Dept., of Genetics and Cytology, National Research Center,  
Cairo, Egypt.

El-Mouhamady@yahoo.com

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9/2/2010

## Effect of Adding Urea or Ammonium Sulphate on some Herbicides Efficiency in Controlling Weeds in Onion Plants

<sup>1</sup>El-Metwally, I. M.; <sup>\*1</sup>Kowthar G. El-Rokiek; <sup>1</sup>Salah A. Ahmed; <sup>1</sup>Ebrahim R. El-Desoki and  
<sup>2</sup>Emad E. H. Abd-Elsamad

<sup>1</sup>Botany Dept., National Research Centre, Dokki, Cairo, Egypt.

<sup>2</sup>Vegetable Crops Research Dept., National Research Centre, Dokki, Cairo, Egypt.

[\\*ahmed\\_ezat2000@yahoo.com](mailto:ahmed_ezat2000@yahoo.com)

**Abstract:** Two field experiments were conducted during two successive seasons of 2008/2009 and 2009/2010 at the Agricultural Experiments Station of the National Research Centre at Nobariya, Behaira Governorate, Egypt, to study the effect of adding urea or ammonium sulphate at 2% to herbicide solution on weed control efficiency in onion fields. Weed control treatments were as follows: Metosulam at 20 ml/fed or Clodinafop-propargyl at 70g/fed with or without addition of urea or ammonium sulphate (AMS) at 2% of herbicide solution in comparison to Metosulam at 40 ml/fed, Clodinafop- propargyl at 140g/ fed, Metosulam at 20 ml + Clodinafop- propargyl at 70 g / fed, two hand hoeing and unweeded check. All weed control treatments significantly depressed weed growth when compared to the unweeded one. Two hand hoeing showed the best control of broadleaved weeds in both seasons, followed by that of Metosulam at 40 ml, Metosulam + urea and Metosulam + AMS treatments, respectively. Clodinafop – propargyl at 140 g, Clodinafop – propargyl at 70 g, Clodinafop – propargyl + urea, Clodinafop – propargyl + AMS and Metosulam + Clodinafop – propargyl were very effective in controlling most grass weeds. Meanwhile, hand hoeing, Metosulam + Clodinafop – propargyl, Metosulam at 40 ml and Clodinafop – propargyl at 140 g /fed were the most effective in controlling onion weeds. All herbicidal treatments as well as hand hoeing markedly increased onion yield in both seasons. Maximum values of bulb length, diameter, weight and bulb yield (t/fed) were recorded from Metosulam + Clodinafop – propargyl, Metosulam at 20 ml and hand hoeing twice.

[El-Metwally, I. M.; Kowthar G. El-Rokiek; Salah A. Ahmed; Ebrahim R. El-Desoki and Emad E. H. Abd-Elsamad. Effect of Adding Urea or Ammonium Sulphate on some Herbicides Efficiency in Controlling Weeds in Onion Plants. Journal of American Science 2010; 6(11):536-543]. (ISSN: 1545-1003).

**Keywords:** Onion, Urea, Ammonium sulphate, Metosulam, Clodinafop-propargyl, weeds.

### 1. Introduction:

Onion (*Allium cepa* L.) is one of the most important field and vegetable crops for both local or export market in Egypt. Weeds in transplanted onion fields not only compete with onion seedlings for growth factors but also act as hosts of insects and fungal diseases such as downy mildew that in turn infest onion plants (Ghalwash *et al.*, 2008). Unlike most crops, onion plants grow slowly and do not form a leaf canopy because of their upright growth habit. This character of onion makes competition with weeds very poor. Thus, onion is the least competitive crop against weeds (Karim *et al.*, 1998). Weed growth reduce the yield of transplanted onion by 26 – 48 % (Babiker and Ahmed, 1986).

Weed control in onion fields must be carried out, especially at the early developmental stages. Due to the severe shortage of hand labour with highly paid wages, hand weeding has become uneconomical processes. Consequently, chemical weed control would be a highly demanded alternative to decrease the cost and increase the economic return due to the increase in onion yield.

Effective weed control and high yield of onion were achieved by application of hand hoeing (Radwan and Hussein; 2001, El-Sayed *et al.*, 2002 and Ghalwash *et al.*, 2008), Clodinafop – propargyl (Khan *et al.*, 2005 and Ghalwash *et al.*, 2008) and Metosulam (El-Metwally, 2002; Sharara *et al.*, 2006; Ghalwash *et al.*, 2008 and El-Metwally and Saudy, 2009). However, the recommended dose of herbicide is relatively high and hence its cost is high and too expensive under the Egyptian conditions. Recently, some evidences have been gathered that adding some additives, especially the nitrogenous fertilizers to herbicide solution could increase its activity, consequently the dose could be lowered and its cost price could be decreased. Moreover, lowering the dose of any herbicide is much appreciated from the point of view of minimizing pollution. In addition, Metwally and Hassan (2001) and El-Metwally (2002) recorded that using some herbicides with urea or ammonium sulphate had higher efficiency in controlling annual weeds and increased yield and its components of wheat or maize as compared with other treatments used.

Therefore, the objective of this work was to study the effect of adding urea or ammonium sulphate to herbicide solutions on weed control efficiency in onion crop.

## 2. Materials and methods

Two field experiments were carried out during the two successive seasons of 2008/2009 and 2009/2010 at the Experimental Station of the National Research Centre at Nobariya, Behaira Governorate, Egypt, to study the influence of adding urea or ammonium sulphate at 2% (equall 4kg/fed) of herbicide solutions on weed control efficiency in onion crops. The soil of the experiments was sandy, the mechanical analysis (Piper, 1950) and chemical analysis (Jackson, 1960) of the soil were carried out before sowing and presented in Table (1).

**Table (1): Mechanical and chemical analysis of Nobariya soil before executing experiment.**

Components		Value
Mechanical analysis	Sand %	75.6
	Silt %	17.4
	Clay %	5.5
	Texture class	Sandy
Chemical analysis	PH	7.9
	E.C.	0.11 mmhos/cm
	CO <sub>3</sub>	—
	HCO <sub>3</sub>	2.5 meq / 100 g soil
	Cl <sup>-</sup>	1.0 meq / 100 g soil
	Ca <sup>+2</sup>	2.5 meq / 100 g soil
	Mg <sup>+2</sup>	1.0 meq / 100 g soil
	Na	1.3 meq / 100 g soil
	K <sup>+</sup>	0.05

m. equivalent / 100 g soil

A complete randomized blocks design with three replications was used in the two seasons. Weed control treatments were as follows:

- 1- Metosulam (N- 2,6 – dichloro – 3 –methyl phenyl – 5.7 – dimethoxy – (1,2,4) Triazolo (1,5a) pyrimidine – 2- sulphona mide), known commercially as Sinal 10 Sc sprayed after 30 days from transplanting at the rate of 40 ml/ fed.
- 2- Metosulam at 20 ml/fed.
- 3- Metosulam at 20 ml + urea at 2%.

- 4- Metosulam at 20 ml +ammonium sulphate (AMS) at 2%.
- 5- Clodinafop – propargyl (Prop – 2 – ynyl – (R) – 2 – (4-(5-chloro- 3- fluoro pyridine – 2- yloxy) phenoxy) = propionate , known commercially as Topik 15 WP sprayed after 50 days from transplanting at the rate of 140g/fed.
- 6 - Clodinafop – propargyl at 70 g / fed.
- 7- Clodinafop – propargyl at 70 g + urea at 2%.
- 8- Clodinafop – propargyl at 70 g + AMS at 2%.
- 9- Metosulam at 20 ml + Clodinafop – propargyl at 70 g / fed.
- 10- Hand hoeing after 30 and 50 days from transplanting (DFT).
- 11- Unweeded check (control) without hoeing or herbicide.

The herbicides were applied with knapsack sprayer equipped with one nozzle boom and water volume was 200 L/ fed (fed=4200m<sup>2</sup>). The drip irrigated was the irrigation system. Each treatment plot consisted of 3 lateral lines, each was 10 m long, 70 cm distances between drip lateral lines. The treatments plot area was 21 m<sup>2</sup>. Onion plants were transplanted in two sides of drip lateral lines, 20 cm apart between the plants. Seedlings of onion cultivar (Giza 6) were transplanted at the last week of December in the two seasons. The previous summer crop in both seasons was peanut (*Arachis hypogaea* L.). All agronomic practices for growing onion were done as recommended.

Data recorded were:

### A -Weeds:

Weeds were hand pulled randomly from one square meter from each plot after 75 and 110 days after transplanting and then were identified and classified to broadleaved, grasses and total weeds. Number and dry weight of each category was estimated.

### B- Bulb characters and onion yield:

At harvest time, ten bulbs were chosen at random from each plot and the following data were recorded:

- 1- Bulb length    2 – Bulb diameter
- 3- Bulb weight    4- Bulb yield (t/fed)

### C- Some chemical constituents of onion bulbs:

- a- Nitrogen, phosphorus and potassium contents (NPK)

Nitrogen, phosphorus and potassium contents were determined in dried tissues of onion bulbs according to the official and modified methods of analysis (A.O.A.C., 1984).

### b- Total carbohydrate contents

Total carbohydrates in onion bulbs were extracted according to Herbert *et al.* (1971) and estimated colourimetrically by the phenol-sulphuric acid method as described by Montogomery (1961).

### Statistical analysis:

All data were statistically analyzed according to the technique of analysis of variance (ANOVA) of a randomized complete blocks design. Since the obtained results of the two seasons of experiment were with the same trend, combined analysis was followed for the two seasons (Little and Hills, 1978). Least significant difference (LSD) method was used to test the differences between treatment means at 5% level probability (Gomez and Gomez, 1984).

## 3. Results and Discussion:

### Effect of different weed control treatments on:

#### A- Onion weeds:

The common weeds in both growing seasons of onion crop were:

*Chenopodium album* L.; *Ammi majus* L.; *Coronopus squamatus*, L. *Melilotus indicus* L. and *Centaurea calcitrapa* as broadleaf weeds, while the grassy weeds were *Avena fatua* L.; *Lolium multiflorum* L. and L. The effect of different weed control treatments on number and dry weight of onion weeds after 75 and 110 days from transplanting are presented in Tables 2, 3 and 4.

#### 1- Broadleaved weeds:

The results in Table 2 showed significant effects on number and dry weight of broadleaved weeds after 75 and 110 days from transplanting in both seasons. Hand hoeing exerted the highest reduction in number and dry weight of broadleaved weeds, followed by Metosulam at 40 ml, Metosulam + urea and Metosulam + ammonium sulphate treatments, respectively. These treatments decreased dry weight of broadleaved weeds than unweeded treatment by about 84.2, 60.9, 59.6 and 59.1 % at 75 days and by 86.3, 66.7, 64.2 and 63.8 %, at 110 days from transplanting, respectively.

#### 2- Grass weeds:

Number and dry weight of grass weeds were significantly decreased by different weed control treatments (Table 3). Clodinafop - propargyl at 140 and 70 g with or without urea, ammonium sulphate or Metosulam were very effective in controlling most grass weeds at 75 and 110 days from transplanting. These treatments decreased dry weight of grass

weeds by 93.3, 91.1, 89.4, 89.2 and 86.6 % at 75 days and by 94.4, 91.8, 90.6, 89.7 and 89.6 %, at 110 days from transplanting.

#### 3- Total weeds:

It is obvious from the results in Table (4) that weed control treatments revealed significant decrease on number and dry weight of total weeds. Hand hoeing twice, Metosulam + Clodinafop – propargyl, Metosulam at 40ml and Clodinafop – propargyl at 140 g /fed recorded the highest efficiency in decreasing total number of weeds at 75 and 110 days from transplanting. These treatments reduced number of total weeds than unweeded check by 84.3, 68.1, 59.1 and 53.8 %, at 75 days and by 83.3, 69.7, 60.8 and 55.6%, at 110 days from transplanting. Two hand hoeing, Metosulam + Clodinafop – propargyl, Clodinafop – propargyl at 140 and 70 g /fed treatments were very effective in controlling onion weeds when compared with other weed control treatments at 75 days from transplanting. These treatments reduced the total dry weight of weeds by 85.0, 67.5, 59.2 and 57.4 %, respectively, as compared to unweeded check. With regard to dry weight of total weeds at 110 days from transplanting, results in Table (4) cleared that the highest efficiency in decreasing dry weight of total weeds was obtained from plots treated with hand hoeing, Metosulam + Clodinafop – propargyl, Clodinafop – propargyl at 140 g, Metosulam at 40 ml and Clodinafop – propargyl at 70 g /fed + urea. These treatments decreased dry weight of total weeds than unweeded treatment by 84.2, 72.8, 62.00, 59.6 and 54.9 %, respectively at 110 days from transplanting.

Generally, results in Tables 2, 3 and 4 revealed that all herbicidal treatments used alone or mixed with urea or ammonium sulphate and hand hoeing decreased statistically the number and dry weight of broad leaved, grasses and total weeds grown with onion crop as compared with unweeded treatment. These results may be due to the inhibitory effect of herbicidal treatments on weeds growth. Two hand hoeing, Metosulam + Clodinafop – propargyl, Metosulam at 40 ml, Clodinafop – propargyl at 140 g and Metosulam + urea were the most effective for controlling the weeds. Also, Clodinafop – propargyl at 70 g, Clodinafop – propargyl + urea, Metosulam + ammonium sulphate, Metosulam + urea and Clodinafop – propargyl + ammonium sulphate treatments produced a promising effect against weed prevailing in onion fields compared with unweeded treatment. Such results may be due to that urea or ammonium sulphate had capacity to give synergistic effects with herbicides used that reflected by the higher reduction in weed growth. Similar results on the synergistic effect of herbicide and ammonium

sulphate on broad leaved weeds were obtained with Abouziena *et al.* (2009-a). In this connection, it is worthy to mention that Suwnnamek and Parker (1975) found that the synergistic mechanism of urea or ammonium sulphate when mixed with Glyphosate could be attributed to some degree of activation inside the weed plants. Abouziena *et al.* (2009-b) reported that adding AMS to the glyphosate solution increased absorption and translocation of glyphosate to 90 and 67%, respectively. Similar results were

recorded by many investigators, who showed that effective control of weeds could be obtained with Metosulam(Sharara *et al.*,2006; Ghalwash *et al.*, 2008 and El-Metwally and Saudy, 2009 ), Clodinafop – propargyl (Saini and Angiras, 2005; El-Metwally and El- Rokiek,2007 and Ghalwash *et al.*, 2008 ) as well as hand hoeing twice (Ishwar *et al.*, 2000; Ved-Prakash *et al.*,2000; Kolhe, 2001 and Ghalwash *et al.*, 2008).

**Table (2): Effect of herbicide treatments alone or mixed with urea or ammonium sulphate (AMS) on number and dry weight of broadleaved weeds after 75 and 110 days from transplanting (Combined analysis for 2008 / 2009 and 2009/2 010 seasons).**

Treatments	At 75days from transplanting				At 110 days from transplanting			
	Number	% of reduction	Dry weight (g/ m <sup>2</sup> )	% of reduction	Number	% of reduction	Dry weight (g/ m <sup>2</sup> )	% of reduction
Metosulam at 40 ml / fed	39.0	62.9	102.0	60.9	50.4	64.9	150.6	66.7
Metosulam at 20 ml / fed	46.5	55.7	125.2	52.0	58.6	59.2	174.5	61.4
Metosulam at 20 ml / fed + urea at 2%.	42.0	60.0	105.4	59.6	53.4	62.8	161.8	64.2
Metosulam at 20 ml / fed + AMS at 2%.	44.3	57.8	106.7	59.1	53.8	62.6	163.8	63.8
Clodinafop – propargyl at 140 g / fed	71.2	32.2	173.1	33.6	92.7	35.5	266.2	41.2
Clodinafop – propargyl at 70 g / fed	73.8	29.7	176.8	32.2	102.2	28.9	311.2	31.2
Clodinafop – propargyl at 70 g / fed + urea at 2%	75.3	28.3	182.6	30.0	106.4	26.0	322.2	28.8
Clodinafop – propargyl at 70 g / fed + AMS at 2%	76.4	27.2	189.7	27.3	112.5	21.7	330.1	27.1
Metosulam at 20 ml / fed + Clodinafop – propargyl at 70 g / fed	45.4	56.8	122.0	53.2	56.8	60.5	171.8	62.0
Two hand hoeing	18.3	82.6	41.2	84.2	22.9	84.1	62.0	86.3
Unweeded check	105.0	—	260.8	—	143.7	—	452.5	—
LSD at 0.05	3.97	—	5.5	—	3.04	—	4.39	—

**Table (3): Effect of herbicide treatments alone or mixed with urea or ammonium sulphate (AMS) on number and dry weight of grass after 75 and 110 days from transplanting. (Combined analysis for 2008 / 2009 and 2009 / 2010 seasons).**

Treatments	At 75days from transplanting				At 110 days from transplanting			
	Number	% of reduction	Dry weight (g/ m <sup>2</sup> )	% of reduction	Number	% of reduction	Dry weight (g/ m <sup>2</sup> )	% of reduction
Metosulam at 40 ml / fed	27.4	52.1	115.2	40.9	37.1	53.5	149.5	48.5
Metosulam at 20 ml / fed	33.1	42.1	130.2	33.2	44.7	43.9	166.5	42.7
Metosulam at 20 ml / fed + urea at 2%	38.6	32.5	148.4	23.9	49.5	37.9	185.6	36.1
Metosulam at 20 ml / fed + AMS at 2%	39.0	31.8	151.7	22.2	56.5	29.1	198.5	31.6
Clodinafop – propargyl at 140 g / fed	3.7	93.5	13.0	93.3	6.6	91.7	16.4	94.4
Clodinafop – propargyl at 70 g / fed	4.9	91.4	17.4	91.1	7.4	90.7	23.7	91.8
Clodinafop – propargyl at 70 g / fed + urea at 2%	5.5	90.4	20.7	89.4	8.2	89.7	27.3	90.6
Clodinafop – propargyl at 70 g / fed + AMS at 2%	5.9	89.7	21.1	89.2	10.3	87.1	30.0	89.7
Metosulam at 20 ml / fed + Clodinafop – propargyl at 70 g / fed	6.3	89.0	26.2	86.6	11.0	86.2	30.2	89.6
Two hand hoeing	7.1	87.6	27.3	86.0	14.4	81.9	55.4	80.96
Unweeded (Control)	57.2	—	195.0	—	79.7	—	290.3	—
LSD at 0.05	1.90	—	4.47	—	3.66	—	2.12	—

**Table (4): Effect of herbicide treatments alone or mixed with urea or ammonium sulphate on number and dry weight of total weeds after 75 and 110 days from transplanting. (Combined analysis for 2008 / 2009 and 2009 / 2010 seasons).**

Treatments	At 75days from transplanting				At 110 days from transplanting			
	Number	% of reduction	Dry weight (g/ m <sup>2</sup> )	% of reduction	Number	% of reduction	Dry weight (g/ m <sup>2</sup> )	% of reduction
Metosulam at 40 ml / fed	66.4	59.1	217.2	52.4	87.5	60.8	300.1	59.6
Metosulam at 20 ml / fed	79.6	50.9	255.4	44.0	103.3	53.8	341.0	54.1
Metosulam at 20 ml / fed + urea at 2%	80.6	50.3	253.8	44.3	102.9	53.9	347.4	53.2
Metosulam at 20 ml / fed + AMS at 2%	83.3	48.6	258.4	43.3	110.3	50.6	362.3	51.2
Clodinafop – propargyl at 140 g / fed	74.9	53.8	186.1	59.2	99.3	55.6	282.6	62.0
Clodinafop – propargyl at 70 g / fed	78.7	51.5	194.2	57.4	109.6	50.9	334.9	54.9
Clodinafop – propargyl at 70 g / fed + urea at 2%	80.8	50.2	203.3	55.4	114.6	48.7	349.5	53.0
Clodinafop – propargyl at 70 g / fed + AMS at 2%	82.3	49.3	210.8	53.8	122.8	45.0	360.1	51.5
Metosulam at 20 ml / fed + Clodinafop – propargyl at 70 g / fed	51.7	68.1	148.2	67.5	67.8	69.7	202.0	72.8
Two hand hoeing	25.4	84.3	68.5	85.0	37.3	83.3	117.4	84.2
Unweeded (Control)	162.2	—	455.8	—	223.4	—	742.8	—
LSD at 0.05	3.87	—	3.34	—	3.29	—	4.09	—

**B – Bulb criteria and onion:****1 – Bulb length:**

Bulb length significantly influenced by the different weed control treatments in both seasons (Table 5). The highest values of bulb length were recorded with Metosulam + Clodinafop – propargyl, Metosulam at 20 ml, Metosulam + urea, hand hoeing and Clodinafop – propargyl at 140 g, respectively. On the other side, unweeded plots resulted in the lowest values of bulb length. Similar results were recorded by Rizk *et al.* (1995).

**2 – Bulb diameter:**

Results in Table (5) indicated that maximum bulb diameter was obtained from the application of Metosulam + Clodinafop – propargyl followed by Metosulam at 20 ml, hand hoeing twice, Metosulam + ammonium sulphate and Metosulam + urea. These treatments increased the bulb diameter by 43.3, 36.2, 31.2, 26.1 and 24.1 %, over unweeded check. These results are coincided with those reported by Ghosheh (2004) and Ghalwash *et al.* (2008).

**3 – Bulb weight:**

Controlling onion weeds with Metosulam + Clodinafop – propargyl recorded the highest values of bulb weight followed by Metosulam at 20 ml, hand hoeing twice, Metosulam + ammonium sulphate and Metosulam + urea, respectively (Table 5). Formentioned superior treatments increased bulb weight than unweeded treatment by 125.6, 100.0, 86.1, 77.0 and 70.3 %, respectively. Chemical and mechanical weed control treatments reduced weed competition and thus afforded more efficient

utilization of available resources to onion plants to produce plants having more bulb diameter, length and weight than weedy check plants. The same conclusion was mentioned by Radwan and Hussein (2001); El-Sayed *et al.* (2002); Ghosheh (2004) and Ghalwash *et al.* (2008).

**4 – Yield of bulbs/ fed:**

The results in Table (5) indicate that Metosulam + Clodinafop – propargyl gave the highest onion yield and recorded 6.43 ton / fed increases over weedy check treatment, followed by Metosulam at 20 ml , hand hoeing twice, Metosulam + ammonium sulphate and Metosulam + urea. The superiority of herbicidal treatments and hand hoeing treatment might be attributed to that onion plants exposed to low weed competition as a result of eliminating weed and its negative impacts on onion plants. Weeds compete with onion plants for water, light and nutrients and the feasibility of maintaining high yield with good quality in absence of effective weed control is strongly doubtful. The above results are in agreement with those obtained by Sanjeev *et al.* (2003); Ghosheh (2004); Sharara *et al.* (2006) and Ghalwash, *et al.* (2008).

**C- Some chemical constituents of onion bulbs:****Nitrogen, phosphorus and potassium contents:**

The results in Table (6) indicate that there were significant increases in the contents of N, P and K in onion bulbs due to different herbicide treatments alone or in combination with urea or ammonium sulfate in comparison to the corresponding controls.

Maximum level of N content in bulbs was recorded with Metosulam + Clodinafop – propargyl followed by combined treatment of Clodinafop – propargyl with ammonium sulfate and its single treatment at 70g / fed. Phosphorus content in onion bulb was significantly less in all treatments relative to unweeded check, except in Metosulam + Clodinafop–propargyl treatments. Moreover, the content of K in onion bulbs (Table 6) exhibited the highest value with the combined treatment of Clodinafop – propargyl and ammonium sulfate followed by Metosulam at 40 ml/fed. Significant increment of nutrient contents in bulb onion (Table 6) may be attributed to the reduction of weed competition with onion plant due to the herbicide treatments alone, their combinations with urea or ammonium sulfate (Metwally and Hassan, 2001, El-Metwally, 2002 and

Sharara, *et al.*, 2006), or hand hoeing (Radwan and Hussein, 2001 and El-Sayed *et al.*, 2002).

#### Total carbohydrate contents

Using the herbicides alone as well as their combinations with urea or ammonium sulfate caused significant increase in total carbohydrate contents in onion bulbs (Table 6). Hand hoeing was the most effective in increasing total carbohydrate as compared to control followed by Metosulam + urea (Table 6). On the other hand, the least carbohydrate content was recorded in onion bulbs of that unweeded plots. The results of increasing carbohydrate contents in bulbs of onion due to hand hoeing or herbicide treatments alone or their combination with urea or ammonium sulfate were previously mentioned by Rizk, *et al.* (1995); Metwally and Hassan, 2001 and El-Sayed *et al.* (2002).

**Table (5): Effect of herbicide treatments alone or mixed with urea or ammonium sulphate on bulb criteria and onion yield at harvest (Combined analysis for 2008 / 2009 and 2009 /2010 seasons).**

Treatments	Bulb length (cm)	% of increasing	Bulb diameter (cm)	% of increasing	Bulb weight (g)	% of increasing	Bulb yield (t / fed)	% of increasing
Metosulam at 40 ml / fed	7.5	27.1	6.4	15.04	179.8	40.43	7.19	40.4
Metosulam at 20 ml / fed	9.4	59.3	7.5	36.23	256.0	100.00	10.24	100.0
Metosulam at 20 ml / fed + urea at 2%	9.2	55.9	6.9	24.09	218.0	70.31	8.72	70.3
Metosulam at 20 ml / fed + AMS at 2%	8.6	45.8	7.0	26.09	226.5	76.95	9.06	76.95
Clodinafop – propargyl at 140 g / fed	8.9	50.9	6.7	22.10	214.8	67.77	8.59	67.77
Clodinafop – propargyl at 70 g / fed	8.2	39.0	6.5	18.30	198.8	55.27	7.95	55.27
Clodinafop – propargyl at 70 g / fed + urea at 2%	7.9	33.9	6.5	17.93	195.0	52.34	7.80	52.34
Clodinafop – propargyl at 70 g / fed + AMS at 2%	7.3	23.7	6.0	8.51	168.0	31.25	6.72	31.25
Metosulam at 20 ml / fed + Clodinafop – propargyl at 70 g / fed	9.7	64.4	7.9	43.30	288.8	125.59	11.55	125.59
Two hand hoeing	9.2	55.9	7.2	31.16	238.3	86.13	9.53	86.13
Unweeded (Control)	5.9	—	5.5	—	128.0	—	5.12	—
LSD at 0.05	0.97	—	0.9	—	6.3	—	1.04	—

**Table (6): Effect of herbicide treatments alone or mixed with urea or ammonium sulphate on chemical composition of onion bulbs. (Combined analysis of 2008/2009 and 2009/2010 seasons).**

Treatments	N %	P %	K %	Total carbohydrates (mg / 100 g dry weight)
Metosulam at 40 ml / fed	2.15	0.82	2.80	80.29
Metosulam at 20 ml / fed	2.05	0.69	2.05	72.64
Metosulam at 20 ml / fed + urea at 2%	1.80	0.76	1.62	90.52
Metosulam at 20 ml / fed + AMS at 2%	2.50	1.37	2.43	75.83
Clodinafop – propargyl at 140 g / fed	1.85	0.71	2.07	70.89
Clodinafop – propargyl at 70 g / fed	2.90	0.83	2.44	55.95
Clodinafop – propargyl at 70 g / fed + urea at 2%	1.75	1.11	2.01	78.71
Clodinafop – propargyl at 70 g / fed + AMS at 2%	3.95	0.96	4.15	62.26
Metosulam at 20 ml / fed + Clodinafop – propargyl at 70 g / fed	4.05	1.38	2.42	53.15
Two hand hoeing	2.05	0.69	2.71	91.47
Unweeded (Control)	1.90	1.21	2.00	52.56
LSD at 0.05	0.095	0.054	0.41	2.54

**Corresponding author**

Kowthar G. El-Rokiek  
 Botany Dept., National Research Centre, Dokki,  
 Cairo, Egypt.  
[\\*ahmed\\_eza2000@yahoo.com](mailto:ahmed_eza2000@yahoo.com)

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9/10/2010

# Prevalence of gastrointestinal parasites infections in sheep in the Zoo garden and Sinai district and study the efficacy of anthelmintic drugs in the treatment of these parasites.

Abouzeid. N.Z.<sup>1</sup>; Selim. A. M.<sup>1</sup> and El-Hady K. M.<sup>2</sup>

1. Department of Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, Egypt.

2. Veterinay Clinic Faculty of Veterinary Medicine, Zagazig University, Egypt.

[dr\\_nasser\\_zeidan@yahoo.com](mailto:dr_nasser_zeidan@yahoo.com)

**Abstract:** A survey of the prevalence of gastro-intestinal tract (GIT) parasites in 240 sheep was conducted in different area in the zoo garden (110) and in Sinai district (130) during the period of March 2009 to February 2010. The overall prevalence of infections with nematodes; fasciola and coccidiosis in sheep in Sinai and zoo garden were 66/240 (27.5%); 24/240 (10.0%) and 16/240 (6.7%) respectively. Of the 240 examined sheep, 12.5%; 0.0% and 8.6 % young lambs (1-6 month), 37.7%; 6.9 % and 9.2 % immature sheep (>6-12 months) and 17.1 %; 21.4 % and 1.4 % adult sheep (>one yr) were infested with nematodes, fasciola and coccidia respectively. Most of the animals examined during the present survey had low to moderate infestation. Serum biochemical parameters revealed that serum calcium, inorganic phosphorus, magnesium, copper and iron levels were significantly decreased in all parasitic infested animals. All treated sheep showed significant improvement & disappearance of most clinical signs and significant decrease of egg per gram (EPG) with complete disappearance of eggs in 5<sup>th</sup> day; 4<sup>th</sup> day and 6<sup>th</sup> day post treatment with albendazole (valbazine); doramectin (dectomax) and trichlabendazole (fasinex) respectively. There were gradual increases in the levels of biochemical parameters in 3 groups after one and two weeks post treatment and their levels reached nearly similar to standard levels after 3 week post treatment. Study surveys suggest, appropriate parasitic control approach be explored and tried in order to alleviate the problem of worm burden.

[Abouzeid. N.Z.; Selim. A. M. and El-Hady K. M. Prevalence of gastrointestinal parasites infections in sheep in the Zoo garden and Sinai district and study the efficacy of anthelmintic drugs in the treatment of these parasites. Journal of American Science 2010;6(11):544-551]. (ISSN: 1545-1003).

**Key words:** gastro intestinal parasites, sheep, Zoo garden, Sinai, anthelmintics & biochemical parameters.

## 1. Introduction

Gastrointestinal tract (GIT) parasites are known to be widespread in animals. The direct losses caused by these parasites are attributed to acute illness and death, premature slaughter and rejection of some parts at meat inspection. Indirect losses include the diminution of productive potential such as decreased growth rate, weight loss in young growing animals and late maturity of slaughter stock (**Hansen and Perry 1994**). The infections are either clinical or sub clinical, the latter being the most common and of great economic importance (**Allonby and Urquhart 1972; Msanga 1985; Makundi et al 1998**). Although clinical parasitism has received considerable attention as a result of obvious severity, the study of parasitism in herds without clinical signs of infection has been largely neglected. A review of the literature, however, indicates that only a limited number of studies have been undertaken to provide information on the prevalence, distribution and epidemiology of various species of parasites in sheep . A study was designed to determine the prevalence and intensity of GIT helminthes in sheep in the zoo garden and in

Sinai district and determine the efficacy of anthelmintics in treatment of gastrointestinal nematodes and fasciola.

## 2. Material and Methods

### 2.1. Animals

Sheep used in this study belonged to Sinai district and zoo garden during March 2009 to February 2010. A total of 240 sheep of all sexes and ages, categorized into: young stock (1-6months) [n =40], immature (6-12months) [n =130] and adult (>one yr) [n =70] were used in the study. The animals had not been drenched for at least 8 week prior to sampling. The study herd selection was carried out with the help of veterinarians who were trained to carry out veterinary services in their respective wards or villages in Sinai. Due to the absence of written records, the age of animal was estimated by dentition. Jugular blood for serum collection was also harvested from treated groups in Sinai before and weekly (up to 3 weeks) after treatment. Obtained sera were used to determined serum biochemical parameters.

## 2.2. Sampling and faecal analysis

The faecal samples were collected per rectum and or freshly dropped faeces with new, unused gloves for each animal. Collected samples were put into faecal pots, labelled and kept cool prior to transportation to the laboratory where they were immediately examined or stored at refrigerated temperature ( $4^{\circ}\text{C}$ ) for a maximum of one day before processing. The sedimentation and floatation technique as described by *Soulsby, (1986)* was used to detect the presence of eggs of liver fluke (*fasciola*) and nematodes in the samples. The presence of coccidian oocysts was also recorded. Worm identification through culturing faecal samples were done according to (*Abd El-Gwad, 1974*) and the identification of gastrointestinal nematodes larvae were done according to *Moning (1963)*. The degree of infestation was determined by counting the ova per gram faeces through MC Master Technique according to *Moning (1963)* (100-250 EPG – Not a significant amount ; 250-500 EPG – Low infection level ; 500-1000 EPG – Moderate infection level and >1,000 EPG – High infection level).

The degree of anaemia was determined through The FAMACHA<sup>©</sup> system (system involves checking the color of the mucous membrane of the eye in order to determine the extent of anemia and thus, the level of infestation by internal parasites (*Waller, 2004*). The system categorizes animals on a scale of 1 to 5, with 5 being reserved for the most anemic animals.

## 2.3. Studying the efficacy of valbazine, dectomax and fasinex

Fifteen naturally infested sheep in Sinai were selected and classified into 3 groups.

**1<sup>st</sup> group:** five sheep naturally infested with gastrointestinal nematodes and administered orally with albendazole tablet (Valbazine) at dose level 10 mg/kg B.W.

**2<sup>nd</sup> group:** five sheep naturally infested with gastrointestinal nematodes and administered S/C with doramectin (dectomax) at dose level 200 µg/kg B.W.

**3<sup>rd</sup> group:** five sheep naturally infested with *fasciola* and administered orally with trichlabendazole (fasinex) at dose level 12 mg/kg B.W.

Daily observation of all treated sheep and record any changes of clinical signs. Faeces from each sheep were examined daily to evaluate the efficacy of the used drugs using the following equation (Clearance % =  $a - b/a \times 100$ ). Where  $a$  = mean No. of EPG recorded at zero day;  $b$  = mean No. of EPG recorded at day of observation. Serum samples

of sheep in the 3 groups before and weekly (until 3 week) after treatment were subjected to determine the calcium, inorganic phosphorus, Magnesium, iron and copper by buck scientific atomic absorption spectrophotometer according to *Official Method of Analysis (1974)*.

## 2.4. Statistical analysis

The statistical analysis of data using T. test and ANOVA was carried out according to the method of *Snedecor and Cochran (1989)*

## 3. Results

Regarding to clinical signs due to parasitic infestation which were varied from asymptomatic to adverse signs as diarrhea, paleness or ictric of visible mucous membrane (more than 3 scores), emaciation, shedding of wool and submandibular edema.

### The overall prevalence rates

The overall prevalence of infections with nematodes; *fasciola* and coccidiosis in sheep in Sinai and zoo garden were 66/240 (27.5%); 24/240 (10.0%) and 16/240 (6.7%) respectively (Tabel 1 and Fig. 1&2).

**Table 1.** Farm prevalence of parasites according to localities

Locality	NO	Nematodes		Fasciola		Coccidia	
		+ve	%	+ve	%	+ve	%
Sinai	130	32	24.6	5	3.8	4	3.1
Zoo	110	34	30.9*	19	17.3**	12	10.9**
Total	240	66	27.5	24	10.0	16	6.7

At the column level \*Significant at  $\geq 0.05$  \*\* significant at 0.01

### Prevalances of GIT parasite infection in animals Nematodes

Nematodes formed the most prevalent gastrointestinal infection with average prevalence of 27.5 % (66 out of 240). Of the 40 young stock (lambs) examined, 5 (12.5 %) were positive for nematodes eggs; out of 130 immature sheep, 49 (37.7%) were infected, while out of 70 adult sheep 12 (17.1%). Egg excretion rate was significant high in the zoo garden (30.9%) than in Sinai (24.6%). The observed threshold level of egg numbers in this study may be regarded as low to moderate that mainly manifests as sub-clinical infections (Tabel 2 and Fig. 1&2).

**Table 2.** Prevalence of faecal gastrointestinal nematodes egg counts in lambs (1-6 months), immature (6-12 months) and adult sheep (> one year)

Localities	Lambs (1-6 months)		Immature (6-12 months)		Adult (> one year)		Overall	
	No	+ve & %	No	+ve & %	No	+ve & %	No	%
Sinai	25	3(12.0)a*	60	22 (36.7)a**	45	7(15.6)a	130	32 (24.6)a
Zoo	15	2 (13.3)a*	70	27 (38.6)a**	25	5(20.0)b*	110	34 (30.9)b
Total	40	5(12.5)	130	49 (37.7)	70	12(17.1)	240	66 (27.5)

At the row level \*Significant at  $\geq 0.05$  \*\* significant at 0.01

Column has different letter was significant

**Fasciolasis**

Overall, *Fasciola* eggs were detected 24 out of 240 (10.0%) of examined samples. The highest prevalence rate occurred in adult sheep (21.4%). The prevalence rates of fascioliasis in zoo garden (17.3%) was higher than that in Sinai (3.8%) (Table 3 and Fig. 1&2).

**Table 3.** Prevalence of fasciolaisis in lambs (1-6 months), immature (6-12 months) and adult sheep (> one year)

Localities	Lambs (1-6 months)		Immature (6-12 months)		Adult (> one year)		Overall	
	No.	+ve & %	No.	+ve & %	No.	+ve & %	No.	+ve & %
Sinai	25	0(0)	60	2 (3.3)a*	45	3(6.7)a**	130	5 (3.8)a
Zoo	15	0 (0)	70	7 (10.0)b*	25	12 (48.0)b**	110	19 (17.3)b
Total	35	0 (0)	130	9 (6.9)	70	15 (21.4)	240	24 (10.0)

At the row level \*Significant at  $\geq 0.05$  \*\* significant at  $\geq 0.01$ . Column has different letter was significant**Coccidiosis**

Sixteen samples (6.7%) all 240 collected samples had coccidial oocysts. Coccidial infection was limited to animals of less than one year old. The prevalence rate was significant higher in zoo garden (10.9%) than in Sinai (3.1%) (Table 4 and Fig. 1&2).

**Table 4.** Prevalence of coccida oocysts counts in lambs (1-6 months), immature (6-12 months) and adult sheep (> one year)

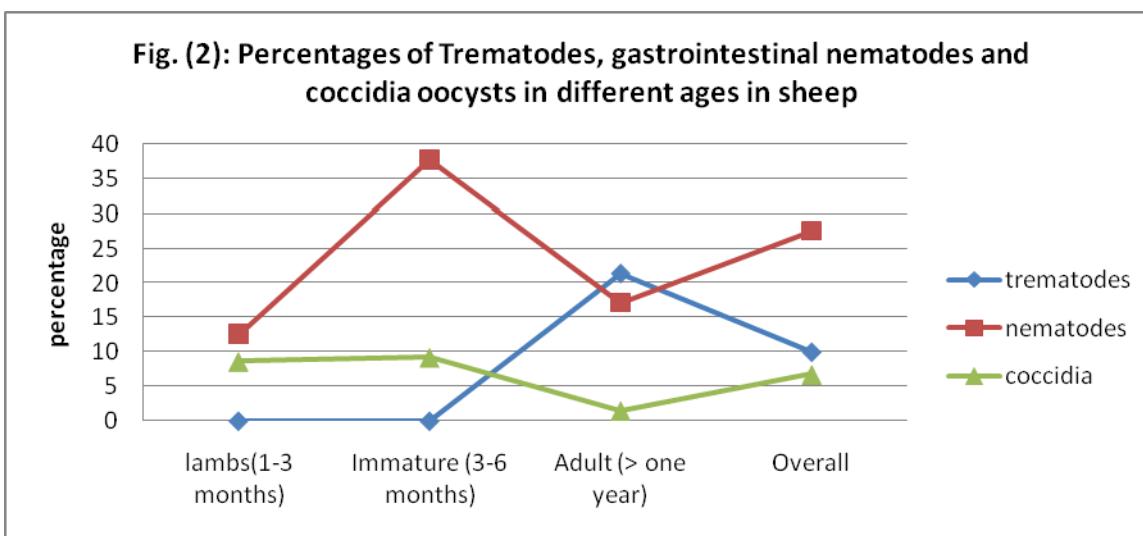
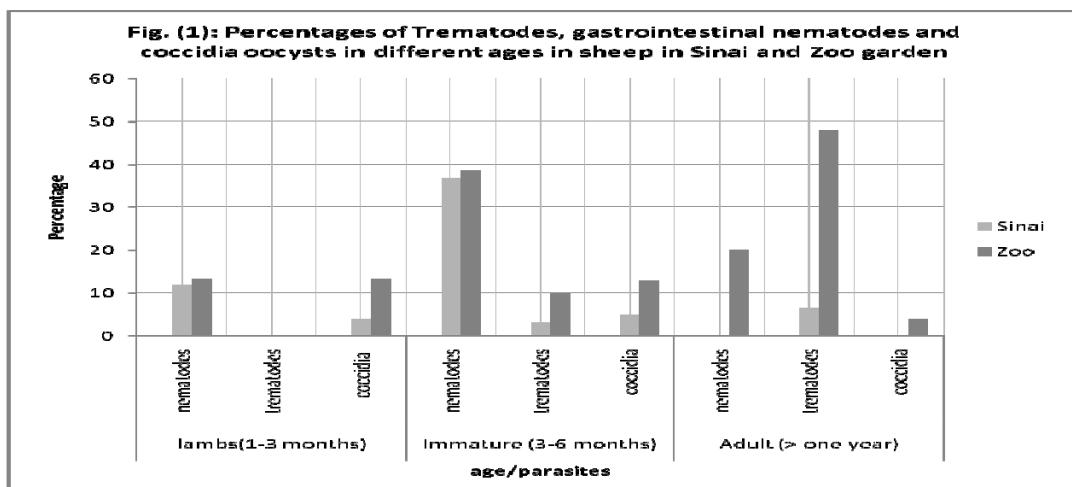
Localities	Lambs (1-6 months)		Immature (6-12 months)		Adult (> one year)		Overall	
	No	+ve & %	No	+ve & %	No	+ve & %	No	+ve & %
Sinai	25	1(4.0) <sup>a</sup>	60	3 (5.0) <sup>a</sup> **	45	0(0) <sup>a</sup>	130	4 (3.1) <sup>a</sup>
Zoo	15	2 (13.3) <sup>b</sup> **	70	9 (12.9) <sup>b</sup> *	25	1 (4.0) <sup>b</sup>	110	12 (10.9) <sup>b</sup>
Total	35	3 (8.6)	130	12 (9.2)	70	1 (1.4)	240	16 (6.7)

At the row level \*Significant at  $\geq 0.05$  \*\* significant at 0.01. Column has different letter was significant**Mixed infection**

There were 10 out of 240 samples had mixed nematodes and fasciola infestations. Whereas mixed nematodes and coccidia spp. was recovered in 15 samples.

**Faecal culture to infested sheep with GIN**

*Haemonchus Spp.*, *Ostertagia Spp.*, *Strongylus Spp.*, *Chabertia Spp.*, *Trichostrongylus Spp.* and *Cooperia Spp.* were recovered in the faecal culture of gastrointestinal nematodes positive samples (66 samples). There were 26 out of 66 (27.5) had single infestation with one nematode parasite (ten had only *Haemonchus Spp*, seven had only *Ostertagia* spp., five had only *Trichostrongylus Spp* and four had only *Strongylus* spp.). Whereas 12 animals had mixed infestation with two nematodes (three had *Haemonchus Spp* and *Ostertagia* spp, two had *Haemonchus Spp.* and *Strongylus* spp., three had *Ostertagia* spp and *Trichostrongylus* spp. and four had *Ostertagia* spp. and *Strongylus* spp.). The others 28 gastrointestinal nematodes positive sheep had mixed infestation with more than 2 nematodes spp. There was significant correlation between FAMACHA score and parasitic infestations especially those infested with *haemonchus* spp.



#### Efficacy of valbazine, dectomax and dasinex on naturally infested sheep

All treated sheep showed significant improvement and disappearance of most clinical signs (diarrhea was decreased; mm became rosy red; improvement of body weight and disappearance of submandibular edema). Parasitological examination revealed that a significant decrease of EPG with complete disappearance of eggs in 5<sup>th</sup> day; 4<sup>th</sup> day and 6<sup>th</sup> day post treatment in the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> group respectively. Serum biochemical parameter revealed that serum calcium, inorganic phosphorus, magnesium, copper and iron levels were significantly decreased in all parasitic infested animals. There were gradual increases in the levels of these elements in 3 groups after one and two weeks post treatment and their levels reached nearly similar to standard levels after 3 week post treatment (Table 5).

**Table 5.** Serum biochemical results before and after treatment with valbazine, dectomax and dasinex.

	Period / Element	Ca	P	Mg	Cu	Fe
	Standard level	11.5-12.8 mg	5.0-7.5 mg	3.6-5.05mg	107-120 $\mu$ g	190.5-254 $\mu$ g
1 <sup>st</sup> group	Before treatment	8.73 $\pm$ 1.44	4.26 $\pm$ 0.85	2.62 $\pm$ 1.08	92.30 $\pm$ 0.19	167.12 $\pm$ 0.25
	One WPT	9.44 $\pm$ 0.85	5.70 $\pm$ 0.26	3.2 $\pm$ 0.6	105.12 $\pm$ 0.04	175.2 $\pm$ 0.12
	two WPT	9.91 $\pm$ 1.47	5.96 $\pm$ 0.15	4.22 $\pm$ 1.15	107.30 $\pm$ 0.45	194.2 $\pm$ 0.65

	three WPT	$11.82 \pm 0.84^*$	$6.94 \pm 0.31^*$	$4.95 \pm 0.35^*$	$110.72 \pm 0.32^*$	$210.14 \pm 0.65^*$
2 <sup>nd</sup> group	Before treatment	$8.59 \pm 1.46$	$4.33 \pm 0.65$	$2.61 \pm 0.08$	$95.23 \pm 0.19$	$166.13 \pm 0.15$
	One WPT	$10.62 \pm 0.85$	$5.50 \pm 0.26$	$3.32 \pm 0.32$	$102.91 \pm 0.14$	$174.41 \pm 0.22$
	two WPT	$11.21 \pm 1.27$	$6.16 \pm 0.05$	$4.42 \pm 0.75$	$105.98 \pm 0.45$	$198.1 \pm 0.25$
	three WPT	$12.02 \pm 1.24^*$	$7.04 \pm 0.53^*$	$4.95 \pm 0.45^*$	$110.32 \pm 0.42^*$	$225.01 \pm 0.95^*$
3 <sup>rd</sup> group	Before treatment	$7.78 \pm 1.54$	$4.66 \pm 0.58$	$2.45 \pm 1.08$	$87.60 \pm 0.29$	$167.12 \pm 0.25$
	One WPT	$8.92 \pm 1.05$	$5.90 \pm 0.16$	$2.92 \pm 0.8$	$98.31 \pm 0.04$	$172.41 \pm 0.32$
	two WPT	$9.91 \pm 0.41$	$6.14 \pm 1.31$	$3.46 \pm 0.75$	$105.2 \pm 0.05$	$185.41 \pm 0.65$
	three WPT	$11.52 \pm 1.74^*$	$7.04 \pm 1.93^*$	$3.94 \pm 0.45^*$	$107.52 \pm 0.42^*$	$95.21 \pm 0.64^*$

\*Significant at level  $\geq 0.05$ . WPT= weeks post treatment.

#### 4. Discussion

Gastrointestinal parasite infections are a world-wide problem for both small- and large-scale farmers. Infection by gastrointestinal parasites in sheep can result in severe losses. Economic losses are caused by gastrointestinal parasites in a variety of ways: they cause losses through lowered fertility, reduced work capacity, involuntary culling, a reduction in food intake and lower weight gains, treatment costs, and mortality in heavily parasitized animals (**Hansen and Perry 1994 and Waller, 2006**).

Regarding to clinical signs due to parasitic infestation were varied from asymptomatic to adverse signs as diarrhea, paleness or ictric of visible mucous membrane, emaciation, shedding of wool and submandibular edema. The clinical finding of parasitic infestation varied depending on the number of infective stage and the time taken after ingestion. A similar signs were observed by **Maingi and Mathenge, (1995) and Anwaar (2000)**.

With regard to the level of parasitic infestation by parasitological examination of 240 faecal samples revealed that gastrointestinal nematodes, fasciola eggs and coccidia oocysts were detected in 66 (27.5 %), 24 (10.0%) and 16 (6.7%) examined faecal samples respectively. The prevalence of gastrointestinal parasites was higher in zoo garden than that in Sinai. This variation may be due to different management system. This result was concordant with that recorded by **Hashem, (1997); Zakaria (2001); Arafa et al., (2007) and Ibrahim et al., (2008)**. But this result was lower than that recorded by **Abd El-Tawab, (1998); Anwaar, (2000) and Al-Gaabary et al., (2007)**. This variation may be attributed to the variation in climatic which necessary for development of infective larvae and to the different methods used in diagnostic, also the

percentage of infestation varies from time to time according animal species, location and season.

Regarding to the relation between gastrointestinal infestation and age, the rate of infestation was highest among young animals and decrease with age. This result was agreement with that recorded by **Dikov and Nekipelova (1984) and Abd El-Salam and Mahran (2004)**. This may be attributed to the development of immunity against gastrointestinal nematodes, while the rate of fasciola increases with the age. This might be due to the fact that young animals have less chance to take the infestation than adults as most breeders keep them indoors fed on concentrate and fresh water (**Abd El-Tawab 1998**). Moreover sheep don't normally develop a protective immune response to re-infection with fascioliasis (**Radostits et al., 2010**).

The observed threshold level of egg numbers in this study may be regarded as low to moderate that mainly manifests as sub-clinical infections (**Waruiru et al 2005**). The effects of these infections can be aggravated by the frequent drought that occurs in some of the study areas (**Anonymous 2005**). This is described as the most economically important form of infection since it occurs in most of the cases leading to unthriftiness and animals are more susceptible to other infections and are continuously contaminating pastures (**Ocaido et al 1996**).

*Haemonchus Spp., Ostertagia Spp., Strongylus Spp. Chaberia Spp., Trichostrongylus Spp. and Cooperia Spp.* were recovered in the faecal culture of positive samples against gastrointestinal nematodes (66 samples). Nearly similar results were reported by **Zaghawa et al., (1992); Abd Rabo, et al., (1993); Costa et al., (2007) and Tariq et al., (2008)**.

There were 26 out of 66 (27.5) had single infestation with one nematode parasite (10 had only *Haemonchus Spp.*, 7 had only *Ostertagia spp.*, 5 had

only *Trichostrongylus Spp* and 4 had only *Strongylus spp.*). Whereas 12 animals had mixed infestation with two nematodes (3 had *Haemonchus Spp* and *Ostertagia*, 2 had *Haemonchus Spp* and *Strongylus*. 3 had *Ostertagia spp* and *Trichostrongylus spp.* and 4 had *Ostertagia spp.* and *Strongylus spp.*). The others 28 gastrointestinal nematodes positive sheep had mixed infestation with more than 2 nematodes spp. There were 10 out of 240 samples had mixed nematodes and fasciola infestations. Whereas mixed nematodes and coccidia spp. was recovered in 15 samples. These results were agreement with **Hashem (1997), Saleh et al., (2006) and Radostits et al., (2010) and Osman, (2008)**.

Coccidial oocysts that were detected in 6.7% of all animals sampled were sporadic and the burden was light. This parasite is probably not an important factor affecting the health of sheep in the study area. Coccidiosis is more important where animals are housed or confined in small areas. The disease is also more important in young animals. However, they are sources of stress and weight loss to animals when they occur in large numbers (**Maingi, et al., 1993**).

With regard to the efficacy of anthelmintics against gastrointestinal nematodes and fasciola revealed that all treated sheep showed significant improvement and disappearance of most clinical signs (diarrhea was decreased; mm became rosy red; improvement of body weight and disappearance of submandibular edema). These results were concordant with those reported by **Marques et al., (1996); Tinar et al., (1997) and Radostits et al., (2010)**. Parasitological examination revealed that a significant decrease of EPG with complete disappearance of eggs in 5<sup>th</sup> day; 4<sup>th</sup> day and 6<sup>th</sup> day post treatment in the 1<sup>st</sup>; 2<sup>nd</sup> and 3<sup>rd</sup> group respectively. These results were nearly similar to those obtained by **Sabry, (1994); Varma and Panda, (1998) and Dorchies et al., (2001)**. While **Waruiru et al., (1998) and Hertzberg et al., (2001)** reported that gastrointestinal nematodes eggs disappeared after 2-3 weeks post treatment with valbazine (5 mg/kg BW) and after 8 weeks post treatment with dectomax respectively. Moreover **Zakaria (2001)** reported that fasciola eggs were disappeared after 2-3 weeks post treatment with fasenix. This variation may be due to lower dose used by those authors.

Serum biochemical parameter revealed that serum calcium, inorganic phosphorus, magnesium, copper and iron levels were significantly decreased in all parasitic infested animals. These results may be due to impaired absorption or increase excretion of concerned elements on consequence to gastrointestinal parasites and fasciola infestation. Similar results were observed by **Aly and El-Gwady, (1991); Ali et al., (1994); Koski and Scott, (2001)**;

**Zakaria, (2001) and Süleyman et al., (2007)**. There were gradual increases in the levels of these elements in 3 groups after one and two weeks post treatment and their levels reached nearly similar to standard levels after 3 week post. These results were in coincidence with **Varma and Panda, (1998); Dorchies et al., (2001)**.

It could be concluded that most of the animals examined during the present survey had low to moderate parasitic infestation, suggesting that the infections were usually sub-clinical. Appropriate GIT parasite control strategy is needed which should be based on cost effective studies to optimise production.

#### Corresponding author

N.Z. Abouzeid

Department of Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, El-Zeraa str. 114; 44511-Zagazig; Egypt

Tel.: +2(055)2081368;

Fax: +2(055)2283683. Mobile: 0108051721

[dr\\_nasser\\_zeidan@yahoo.com](mailto:dr_nasser_zeidan@yahoo.com)

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9/28/2010

## Diet quality in Egyptian Obese Children and Adolescents

Nayera El-morsi Hassan<sup>\*1</sup>, SafaaT. Zaki<sup>2</sup>, Azza Gabr<sup>2</sup> and Hala El gindi<sup>2</sup>

Biological Anthropology Department<sup>1</sup>, Child Health Department<sup>2</sup>. National Research Center, Cairo, Egypt  
[safaa.zaki@hotmail.com](mailto:safaa.zaki@hotmail.com)

**Abstract:** The epidemic increase in the prevalence of obesity is now seen in most countries. Dietary composition, the relative proportions of calories coming from fats, carbohydrates, protein and intake of fiber has been suspected of playing a role in obesity. So, the aim of the present study was to analyze the diet quality and also to determine if an association exists between obesity and the relative percentage of fats, carbohydrates, protein and fiber in the diets of children and adolescent. A cross-sectional survey, comprised 5760 children (2638 boys and 3122 girls) was recruited from 6 public schools. Each child underwent a complete physical examination, including anthropometric measures. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared. Only one thousand and one hundred children of the total sample (19.1%), (417 boys and 683 girls, aged 13.43±2.65 years), with the complaint of obesity, were included .Repeated Twenty-four hour recall method, record food intake for three scattered days (3 recalls), includes one day as a holiday was done to assess the nutritional status of obese children. Nutrient intake were calculated using the computer program World Food Dietary Assessment System<sup>(1)</sup> compared with National Research Council USA1989<sup>(2)</sup>,while vitamins and minerals were compared with USDA, 2005<sup>(3)</sup> . This study highlights the importance of nutritional data that it is not what you eat but rather how much the total number of calories consumed which contributes to obesity. Success in obesity prevention is most likely to be achieved when preventive measures are initiated early and sustained throughout childhood and adolescence. More researches must be done for more evaluation, also, to achieve physical activity and life style for obese children and adolescence.

[Nayera El-morsi Hassan, SafaaT. Zaki, Azza Gabr and Hala El gindi. Diet quality in Egyptian Obese Children and Adolescents. Journal of American Science 2010;6(11):552-558]. (ISSN: 1545-1003).

**Keywords:** Diet quality, obese, children, adolescents.

### 1. Introduction:

The epidemic increase in the prevalence of obesity is now seen in most countries. Several studies have examined relations between nutrients, particularly dietary fat and obesity, but the epidemiological evidence remains controversial<sup>(4)</sup>.

Dietary composition, the relative proportions of calories coming from fats, carbohydrates, protein and intake of fiber has been suspected of playing a role in obesity. However, few studies have examined the association between excess weight and the consumption of these nutrients and the results are inconsistent. Many studies found that higher total energy intake significantly increased the odds of obesity but, the composition of their diets, the relative percentages of carbohydrates, protein, fats, and fiber was generally not a factor. So, it seems that for obesity, quantity (total energy intake) is more important than quality (the balance of nutrients consumed)<sup>(5, 6)</sup>

Dietary pattern analysis; based on the concept that foods eaten together; are as important as a reductive methodology characterized by a single food or nutrient analysis. Dietary pattern analysis is a better method to examine the effect of overall diet: food and nutrients are not eaten in isolation, and the

single food or nutrient approach will not take into account the complex interaction between food and nutrients<sup>(7)</sup>.

The aim of the present study was to analyze the diet quality and also to determine if an association exists between obesity and the relative percentage of fats, carbohydrates, protein and fiber in the diets of children and adolescent.

### 2. Subject and Methods:

This study was conducted by the National Research Centre, Egypt, through a project titled: "Obesity Profile among Egyptian School Children and Adolescents: Early Diagnosis of Metabolic Syndrome and Nutritional Intervention".

It was a cross-sectional survey, comprised 5760 children (2638 boys and 3122 girls). The pupils were recruited from 6 public schools (two Primary , two preparatory and two secondary schools) situated in Giza governorate, during the period of October, 2007 to April 2009. Permission to perform the study was granted by the Ministry of Education, and the directors of the schools. Parents were informed about the purpose of the study and their research permission in the form of written consent was obtained. The

protocol was approved by the "Ethical Committee" of the "National Research Centre".

Each child underwent a complete physical examination, including anthropometric measures. Their pubertal development stages were assessed using the criteria of Tanner stages. The height and the weight were measured. The height was measured to the nearest 0.1 cm on a Holtain portable anthropometer, and the weight was determined to the nearest 0.01 kg on a Seca scale Balance with the subject dressed minimum clothes and no shoes. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared.

Of the total sample, one thousand and one hundred children only (19.1%), (417 boys and 683 girls, aged  $13.43 \pm 2.65$  years), diagnosed as having obesity, were included. These children were required to meet the following inclusion criteria: age, 7–18 years and BMI, greater than the 95<sup>th</sup> percentile for age and gender based on the Egyptian Growth Reference Charts 2002<sup>(8)</sup>. Children were excluded if they had a prior major illness, including type 1 or 2 diabetes, received medications or had a condition known to influence body composition, insulin action or insulin secretion (e.g. glucocorticoids therapy, hypothyroidism and Cushing's disease).

Assessment of the nutritional status of the students included the following:

Repeated Twenty-four hour recall method, record food intake for three scattered days (3 recalls), includes one day as a holiday. Data were collected by qualified dietary staffs, by personal interview. Detailed description of all food and beverages consumed, including cooking methods and the amount of each ingredient, was recorded. The conversion of household measures to grams was achieved through the use of prepared list of commonly used household measures in Egypt. Nutrient intake were calculated using the computer program<sup>(1)</sup>. The daily intakes of calories and protein were compared with National Research Council<sup>(2)</sup>, while vitamins and minerals were compared with USDA<sup>(3)</sup>.

### 3. Results

#### Prevalence of obesity

**Table 1- Distribution of the sample**

	Sex	N	Obese		Overweight	
			N	%	N	%
STUDENTS AGED 7 -12 YEARS	Boys	859	30	3.5	66	7.7
	Girls	1193	92	7.7	151	12.7
SYUDENTS AGED 13-18 YEARS	Boys	1779	144	8.1	177	9.9
	Girls	1929	196	10.2	244	12.4
Total		5760	462	8.02	638	11.07

The Prevalence of obesity of our sample is represented in table (1). In our sample of 2052 children aged 7 to 12 years, we found that 30 boys and 92 girls were obese (BMI>95<sup>th</sup> percentile). Over weight (BMI >85<sup>th</sup> and <95<sup>th</sup> percentile) was represented by 66&151 boys and girls respectively.

The prevalence of obesity in the total sample (3708 children aged 13 to 18 years) was 144 boys(9.9%) and 196 girls(12.4%) , while, 177 boys and 244 girls were overweight.

#### Nutrition

Food quality analysis showed that the obese children consumed more calories than the recommended dietary allowance (RDAs) for the same age. However, they consumed the relative average percentage of calories from carbohydrates, protein and fats

Table (2) represents the obese boys and girls aged 7 to 12 years. They had higher energy intake than RDA, while the % calories from protein are on the low normal % of RDAs. The %calories from fat are within the normal % of RDAs, but the percentage from saturated fat are high. The% calories from CHO are within high normal%. The consumed dietary fibers are higher than RDA.

Table (3) represents the amount of some nutrients of consumed food items. We found deficiency of some vitamins and minerals as E, calcium and, potassium.

Analysis of food of female adolescent aged 13-18 years showed that they are consuming higher% of total energy intake than RDAs. However, the % calories from protein and fats are at low normal RDA %. The percentage calories from CHO are at the high normal RDAs range. The consumed amount of fibers is higher than RDAs (table 4). Vitamin D, E and calcium are deficient in their eating food. While, copper, sodium, manganese and other nutrients are very high than normal (Table 5).

On the other hand, the male adolescents of the same age have a high total energy intake than RDA. The percentage of calories from protein, CHO and fat are within normal range, while dietary fiber intake are low than normal (Table 6).

Table (7) showed the nutrients of their eaten food in relation to RDAs.

**Table 2: Daily intake of selected nutrients by obese boys and girls aged 7 to 12 years**

Nutrient	Minimum	Maximum	Mean	SD	RDAs
<b>Energy intake (kilocalories)</b>	1574	5932	3746.48	1086.44	1400-2000 (kcal)
<b>Carbohydrates (% of energy)</b>	26.5	76.19	57.77	8.83	45-65 (%)
<b>Protein (% of energy)</b>	6.21	18.56	12.18	2.79	10-30 (%)
<b>Total fat (% of energy)</b>	11.97	60.78	30.61	8.85	25-35 (%)
<b>Saturated fat (% of energy)</b>	13.6	87.5	43.01	16.36	< 10 (%)
<b>Fiber (average grams per day)</b>	2.1	121.8	36.69	23.16	31 (%)

**Table (3): Daily intake of some vitamins and minerals by obese boys and girls aged 7-12 years.**

	Mean	SD	RDAs
VITA	1201.48	2719.375	600 ug/d
VITD	4.45	13.561	5 ug/m
VITE	6.63	4.173	11 mgm
VITC	52.03	79.162	45 mgm
Thiamin	1.4887	.68016	0.9 mg/d
Riboflavin	1.6369	.71907	0.9 mg/d
Niacin	18.495	9.0781	12 mg/d
Vit.b6	1.5177	.62135	1 mg/d
Folate	412.08	317.389	300 ug/d
Vit.b12	6.9677	14.88752	1.8 ug/d
Pantothenic acid	6.5245	2.14169	4 mg/d
Calcium	514.74	292.597	1,300 mg/d
Phosphorus	1689.02	599.517	1,250 mg/d
Magnesium	448.23	214.636	240 mg/d
Potassium	2375.55	1089.749	4500 mg/d
Sodium	2433.21	1692.336	1500 mg/d
Iron	14.6871	7.40039	8 mg/d
Zinc	14.4887	4.99131	8 mg/d
Copper	2.5352	1.21921	0.7 mg/d
Manganese	8.3597	4.07729	1.9 mg/d

**Table 4: Daily intake of selected nutrients by obese female students aged 13 to 18 years**

Nutrient	Minimum	Maximum	Mean	SD	RDAs
Energy intake(average in kilocalories)	2025	6710	3267.76	1463.52	1800 (kcal)
Carbohydrates(% of energy)	31.13	83.27	64.17	9.94	45-65 (%)
Protein(% of energy)	9.48	19.59	12.92	2.44	10-30 (%)
Total fat (% of energy)	8.79	48.62	23.88	8.59	25-35 (%)
(%)Saturated fat (% of energy)	9.8	76.6	32.36	15.81	< 10 (%)
Fiber (average grams per day)	8.8	82.6	38.84	20.18	26 (%)

**Table 5: Daily intake of some vitamins and minerals by obese female aged 13-18 years**

	Mean	SD	RDAs
VITA	1137.52	2760.101	700 ug
VITD	3.39	7.306	5 ugm
VITE	5.15	2.874	12 mgm
VITC	117.00	179.276	65 mgm
VITB6	1.6006	.67874	1.2 mgm
Folate	430.97	252.471	400 ugm
VITB12	5.3512	14.14864	2.4 ugm
Pantothenic acid	6.6124	3.17699	5mg
Calcium	598.00	595.231	1300 mgm
Phosphorus	1631.85	756.446	1250 mgm
Magnesium	448.97	206.934	360 mg
Sodium	3187.85	7481.990	2300 mg
Iron	15.1170	6.57190	15 mgm
Zinc	14.4315	6.58555	9 mg
Copper	2297	1.09556	890 ugm
manganese	7.8788	3.70968	1.6 mg

**Table 6: Daily intake of selected nutrients by obese male students aged 13 to 18 years**

Nutrient	Mean	SD	RDAs
Energy intake (kilocalories)	2978.12	1388.48	2200-2400 (kcal)
Carbohydrates(% of energy)	58.66	12.36	45-65 (%)
Protein(% of energy)	12.6	2.35	10-30 (%)
Total fat (% of energy)	29.92	12.73	25-35(%)
Saturated fat (% of energy)	42.64	40.89	< 10(%)
Fiber (average grams per day)	33.29	20.59	38(%)

**Table (7): Daily intake of some vitamins and minerals by obese males aged 13-18 years.**

	Mean	SD	RDAs
VITA	1175.04	1818.643	900 ug
VITD	2.38	3.869	5 ugm
VITE	5.62	3.742	15 mgm
VITC	31.04	33.267	75 mgm
Thiamin	1.3435	.74677	1.2 mg/d
Riboflavin	1.4585	1.02729	1.3 mg/d
Niacin	15.946	8.6574	16 mg/d
Vit.b6	1.2888	.68673	1.3 mg/d
Folate	390.73	236.543	400 ug/d
Vit.b12	4.1735	10.02323	2.4 ug/d
Pantothenic acid	5.6077	2.85355	5 mg/d
Calcium	564.65	684.134	1,300 mg/d
Phosphorus	1487.73	747.284	1,250 mg/d
Magnesium	412.31	233.672	410 mg/d
Potassium	2245.77	1387.113	4700 mg/d
Sodium	1691.96	1618.841	1500 mg/d
Iron	14.1442	8.12319	12 mg/d
Zinc	12.6304	6.28201	11mg/d
Copper	2.0284	1.33606	0.890 mg/d
Manganese	7.3624	3.96358	2.2 mg/d

#### 4. Discussion:

Although obesity was rarely observed among children and adolescent 30 years ago, it is now evident among children in all ages <sup>(9)</sup>.

Childhood obesity is an independent risk factor for adult overweight and obesity <sup>(10)</sup>, with obese children having at least a 25%-50% increased risk of being obese as adults.

A nutrition survey was done to characterize the quality of diets consumed by obese children and adolescent. The analysis of data revealed that our sample of overweight and obese children consumed energy intake higher than RDAs, however, the composition of their diets –the relative percentage of carbohydrates, protein, and fats are within the RDAs for the same age and sex, while the fiber intake was higher than RDAs except in male adolescent.

In our study, the % of calories from protein is at the low normal range in both sexes and in different age group. Langlois,et al <sup>(11)</sup>, found no significant relationship between obesity and the percentage of calories derived from proteins, while a prospective study in 2006, found an inverse relationship between protein intake and five year differences in waist

circumference <sup>(12)</sup>. Ludwig et al <sup>(13)</sup> found a positive relationship between protein intake and body weight.

The percentages of calories derived from carbohydrate are at high normal range value in children and adolescent either male or female. Tucker & Kano, <sup>(14)</sup> and Davis et al <sup>(15)</sup>, showed no association between carbohydrate consumption and excess weight. Other search adjusted that a higher percentage of calories coming from carbohydrate was negatively associated with obesity among men <sup>(16)</sup>.

Many literature studied fat as one of the most nutrient in obesity because of their high caloric count (9 kilocalories per gram versus 4Kcal per gram for each CHO & protein).They assumed that excess consumption may contribute to higher energy intake <sup>(17)</sup>. In our study, we found the % of calories from fat are within normal range of RDAs in both sexes and in different age. Langlois, etal, <sup>(18)</sup> found no association between fat intake and obesity among men and women. Some studies have examined specific types of fat, because it has been suggested that saturated, monounsaturated and polyunsaturated fats might have different effects on adiposity <sup>(19)</sup>. In our results, saturated fat is higher than normal. Moussavi et al <sup>(6)</sup>

found a positive association between saturated fat intake and obesity prevalence, while Bhargava & Gutjrie<sup>(20)</sup> found no relation between saturated fat and BMI.

Dietary fiber delays gastric emptying and thereby, contributes to a sensation of fullness, so, it has been studied as a preventive factor in the developing obesity<sup>(18)</sup>. In the present study the mean average of dietary fiber are higher than RDA in children and in female adolescent, while in male adolescent, we found it less than normal. Newby et al,<sup>(21)</sup> showed no association between dietary fiber intake and annual BMI changes in boys and girls. Hassapidou et al,<sup>(22)</sup> found that fiber intake were significantly lower in overweight boys while no significance among girls.

Concerning micronutrient intake : Calcium intake level among Egyptian adolescents is far below the recommended international figures to prevent osteoporosis, the results of a national survey (DNPCNCD) carried out by the National Nutrition Institute, Egypt<sup>(23)</sup>, reported that, the 25<sup>th</sup> percentile of the daily calcium intake among adolescents aged 10-18 years was 323.5g, while the 50 and 75 percentiles were 494.8g and 704.2 respectively, this is agreed with our results as we found deficiency of calcium in all age groups

Vitamin D deficiency is highly prevalent among children and adolescents worldwide. The high rates of vitamin D deficiency during childhood are of major public health relevance<sup>(24)</sup>. Our results found vitamin D and vitamin E deficiency in all age groups.

Two epidemiologic studies published in the early 1960s noted an association between overweight status among children and adolescents and iron deficiency<sup>(25, 26)</sup>.

Saloojee H,et al,<sup>(27)</sup>. & Bhatia D et al,<sup>(28)</sup>, found that there was a greater prevalence of iron deficiency in overweight and obese children and adolescents. Obese children and especially female adolescents are at risk of increased morbidity starting already in childhood or adolescence. Interestingly, despite their excessive dietary and caloric intake, obese children and adolescents may be at risk of iron deficiency anaemia because they tend to consume unbalanced meals, particularly rich in carbohydrates and fat, this is agreed with our results as we found iron deficiency among adolescent girls (table 5).

This study highlights the importance of nutritional data that it is not what you eat but rather how much the total number of calories consumed which contributes to obesity. Success in obesity prevention is most likely to be achieved when preventive measures are initiated early and sustained throughout childhood and adolescence. More

researches must be done for more evaluation, also, to achieve physical activity and healthy life style for obese children and adolescence.

#### **Corresponding author**

Safaat. Zaki

Child Health Department · National Research Center, Cairo, Egypt

[\\*safaa.zaki@hotmail.com](mailto:safaa.zaki@hotmail.com)

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9/9/2010

# Eusyllinae, Anoplosyllinae, and Exogoninae (Polychaeta: Syllidae) for the Mediterranean Coasts of Egypt, Together the Description of One New Species

F. A. Abd-Elnaby<sup>1</sup> and G. San Martín<sup>2</sup>

<sup>1</sup>National Institute of Oceanography and Fisheries, Alexandria, Egypt

<sup>2</sup> Departamento de Biología (Zoología), Facultad de Ciencias, Universidad Autónoma de Madrid, calle Darwin, 2, 28049 Madrid, Spain.

**Abstract:** In this paper, 18 species of the subfamilies Exogoninae, Anoplosyllinae, and Eusyllinae (Syllidae, Polychaeta) are reported from the Mediterranean Egyptian coasts, 8 of them are new records for the area: *Odontosyllis fulgurans* (Audouin and Milne Edwards, 1833); *Syllides japonicus* Imajima, 1966; *Salvatoria clavata* (Claparede, 1863); *Salvatoria euritmica* (Sardá, 1984); *Sphaerosyllis glandulata* Perkins, 1981; *Parapionosyllis labornica* Cognetti, 1965; *Sphaerosyllis* sp.; and *Prospaerosyllis* sp. Five species were reported previously in the area. Four species are new records for Mediterranean Sea: *Palposyllis prosostoma* Hartmann-Schröder, 1977; *Paraehleria weissmaniodes* (Augener, 1913); *Streptosyllis compoyi* Brito, Núñez and San Martín, 2000; and *Exogone africana* Hartmann-Schröder, 1974; *P. weissmaniodes* and *Exogone africana* are two widely distributed Indo-Pacific species, so they could be considered as Lessepsian migrants. Finally, one new species is described, *Parapionosyllis aegyptia*.

[F. A. Abd-Elnaby<sup>1</sup> and G. San Martín. Eusyllinae, Anoplosyllinae, and Exogoninae (Polychaeta: Syllidae) for the Mediterranean Coasts of Egypt, Together the Description of One New Species. Journal of American Science 2010;6(11):559-566]. (ISSN: 1545-1003).

**Keywords:** *Eusyllinae, Anoplosyllinae, Exogoninae, Taxonomy, Mediterranean, Egypt, New species.*

## 1. Introduction:

Syllidae represent one of the most diverse and systematically challenging families of Polychaeta (Glasby, 2000; Rouse & Pleijel, 2001; San Martín, 2003, 2005; San Martín & Hutchings, 2006; Aguado & San Martín, 2009). It is a widely distributed group, found from the intertidal zone to the abyssal plains all over the world (Glasby, 2000), but less common at depth, with some species symbiotic or parasitic on other marine invertebrates (Martín and Britayev, 1998).

This family is currently divided into 5 subfamilies (Aguado & San Martín 2009): Eusyllinae Malaquin, 1893; Exogoninae Langerhans, 1879; Autolytinae Langerhans, 1879; Syllinae Grube, 1850; and the recently erected Anoplosyllinae Aguado and San Martín , 2009.

To detect newly recorded or new species we will depend on accurate taxonomic identifications and the local biodiversity. The possible existence of complexes of species, whose identity is blurred under one common specific name are present (Aguado and San Martín, 2007).

Knowledge about Polychaetes in the Egyptian waters is still far from complete; as result of less taxonomical studies and less sufficient data about this group. This paper is the second report about

Egyptian Syllids, collected from the Northwestern Coast of Egypt through Salsabeel cruise, Autumn 2008 and Spring 2009, also from Gamasa (Spring 2009), under the frame work organized by National Institute of Oceanography and fisheries branch Alexandria, and from Port Said Harbour (Spring 2008), to study the benthic invertebrates. While the first report about syllid and sabellid species in the Northwestern coast of Egypt, were done by Selim (2008a & b respectively), Abd- Elnaby (2009) also studied polychaetes in Gamasa.

Generally, scarce attention has been given to the polychaetes in Egyptian waters. Fauvel (1927) recorded 8 syllid species from the Suez Canal waters of which 6 belonging to genus *Syllis*. On his work on the polychaetes collected from the fishery grounds near Alexandria, Fauvel (1937) gave a checklist of polychaetes recorded in this area.

Only 16 species of Syllidae were recorded in that paper. More recently, Selim (1978) reported two syllids species in the Eastern Harbour of Alexandria, namely *Syllis* (*Typosyllis*) *variegata* and *Trypanosyllis zebra*. Later, the same author (Selim, 1996) added 6 syllid species from Alexandria coast (*Branchiosyllis exilis*, *Syllis gracilis*, *S. hyalinae*, *S. mediterranea*, *S. prolifera* and *S. variegata*). Finally, Abd-Elnaby (1999) recorded 7 syllid species, and

later (2005) 21 species from Alexandria coast.

The Syllidae of the neighbouring areas were studied by several authors; from Aegean Sea by Çınar & Ergen (2002); Çınar (2003); and Çınar (2005); from Israel and the Gulf of Elat by Ben-Eliah (1977a & 1977b), and anteriorly Fauvel (1955, 1957), from Cyprus by Ben-Eliah (1972), Çınar (2003a&b) and Çınar & Ergen (2003); Lebanon by Aguado & San Martín (2007), and from Turkey by Ergen (1976). A checklist, distribution, and ecological features of Syllidae and other polychaetes from Greece can be reported in Simboura & Nicolaïdou (2001), also from Cyprus by many authors, the most recent one Musco et al. (2005), and the biogeographic revision on Syllidae from the Mediterranean Sea (East and West areas) was carried out by Musco & Giangrande (2005).

During the present study 18 species were recorded, 11 of which are new records for the Egyptian waters. Four species are considered as new species for Mediterranean Sea. Three species are considered as new species, although two of them are under process of description, and one species is described here as new for Science. In this paper, detailed description is given also of some interesting species.

## 2. Materials and methods

Two cruises were carried out on the Northwestern Mediterranean coast of Egypt; on two stations (El Hammam, El Alamein), during Autumn 2008 and Spring 2009, and also one collection Spring 2008. The stations are; Port Said Harbour (station 1), in which samples were collected during Spring 2008, and Gamasa (station2, Spring 2009), depth ranging from 0 .25m to 20 m. (Fig. 1). Sediment samples were collected by a Van Veen grab; while, samples from Port Said Harbour were collected by knife and net used for collecting fauna. Sediment samples were washed up and sieved through 0.3 µm sieve, then sorted under Stereomicroscope. Specimens of Syllidae were extracted and fixed in 10 % formaldehyde in sea water-solution. Examinations and identification were done by using compound microscope. Drawings were made by a camera lucida. The specimens were Preserved in the Marine Reference Collection Center of National Institute of Oceanography and Fisheries, Alexandria, under Code Number (N. Sp. 2/8/3).

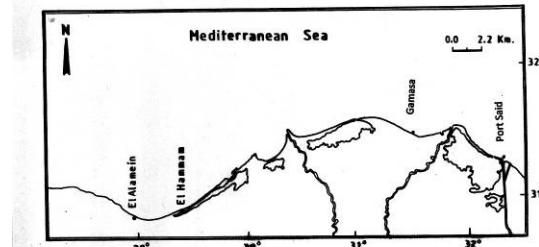


Fig. (1) Map showing the sampling sites, (North western Coast of Egypt, Gamasa and Port Said).

## 3. Results

In the present study, 18 species belonging to the subfamilies Exogoninae, Anoplosyllinae and Eusyllinae (Syllidae, Polychaeta) were recorded and identified from the Mediterranean Egyptian coasts, 8 of them considered new records for the Egyptian Mediterranean waters: *Odontosyllis fulgurans* (Audouin and Milne Edwards, 1833); *Syllides japonicus* Imajima, 1966; *Salvatoria clavata* (Claparede, 1863); *Salvatoria euritmica* (Sarda, 1984); *Sphaerosyllis glandulata* Perkins, 1981; *Parapionosyllis labronica* Cognetti, 1965; *Sphaerosyllis* sp.; and *Prospaerosyllis* sp.; the two later are new species in process of description, although a description of both without any specific name is given by San Martín (2003). Five species were reported previously from different places of Egypt. In addition, four species are considered as a new records for the Mediterranean Sea (*Palposyllis prosostoma* Hartmann-Schröder, 1977; *Paraehlersia weissmanioides* (Augener, 1913); *Streptosyllis compoysi* Brito, Núñez and San Martín, 2000; and *Exogone africana* Hartmann-Schröder, 1974). Finally one species *Parapionosyllis aegyptia* is described as new species. The locations, dates , depth, number of specimens and geographical distribution are presented in Table (1).

**Table(1): Showing the Polychaete species recorded in the present study, Location, Date, Depth, Bottom, Number of specimens, and Distribution..**

Name of the species	Location	Depth (m)	Date	No.	Bottom	Distribution
** <i>Palposyllis prosostoma</i> Hartmann-Schröder, 1977	Gamasa	13.7	Spring 2009	1	S-M	At
** <i>Paraehlersia weissmaniodes</i> (Augener, 1913)	Port Said	0.25	Spring 2008	3	F	At
**** <i>Brevicirrosyllis weismanni</i> Langerhans, 1879	El Alamein	20.0	Autumn 2008	1	C-S	At, Med
*** <i>Odontosyllis fulgurans</i> (Audouin and Milne Edwards, 1833)	Port Said	0.25	Spring 2008	1	F	Cos
** <i>Streptosyllis compoysi</i> Brito, Núñez and San Martín, 2000	Elhammam	20.0	Autumn 2008	1	C-S	At
*** <i>Syllides japonicus</i> Imajima, 1966	Elhammam	20.0	Spring 2009	2	C-S	At, Med, P
*** <i>Salvatoria clavata</i> (Claparède, 1863).	Elhammam	20.0	Spring 2009	1	C-S	Cos
*** <i>Salvatoria euritmica</i> (Sardá, 1984)	Elhammam	8.0	Spring 2009	1	C-S	At, Med, P
**** <i>Salvatoria vieitezzi</i> San Martín 1984	El Alamein	20.0	Autumn 2008	2	C-S	At, Med, P
*** <i>Sphaerosyllis glandulata</i> Perkins, 1981	Gamasa	13.7	Spring 2009	1	S-M	At, Med
**** <i>Sphaerosyllis taylori</i> Perkins, 1981	Elhammam	20.0	Spring 2008	1	C-S	At, Med
*** <i>Sphaerosyllis</i> sp.	El Alamein	20.0	Autumn 2008	1	C-S	At
*** <i>Prospaerosyllis</i> sp.	Elhammam	8.0	Spring 2009	1	C-S	At
** <i>Exogone africana</i> Hartmann-Schröder, 1974	Port Said	0.25	Spring 2008	2	F	At
* <i>Parapionosyllis aegyptia</i> n. sp.	El Alamein	20.0	Autumn 2008	2	C-S	n. sp.
**** <i>Parapionosyllis brevicirra</i> Day, 1954	El Alamein	20.0	Autumn 2008	2	C-S	At, Med
**** <i>Parapionosyllis elegans</i> (Pierantoni, 1903)	El Alamein	20.0	Autumn 2008	2	C-S	At, Med
*** <i>Parapionosyllis labronica</i> Cognetti, 1965	Gamasa	13.7	Spring 2009	1	S-M	At, Med

At= Atlantic Ocean, P= Pacific Ocean, Med= Mediterranean, Cos= Cosmopolitan, S-M= Sandy mud, C-S= Coarse Sand, F= Fouling

\*= New species, \*\*= New record for Mediterranean Sea, \*\*\*= New record for Egyptian waters, \*\*\*\*= Recorded before from Egyptian waters

The most important species will be described in details.

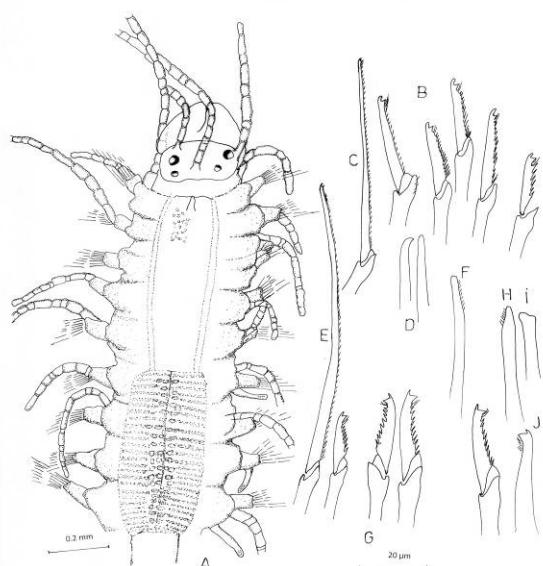


Fig. (2): *Paraehlersia weissmannioides* A: anterior part of body, B: anterior setae, C: spiniger-like compoundseta, anteriorly D: anterior acicula, E: spiniger-like compound seta posterior parapodia F: anterior dorsal simple seta, G: posterior setae, H: dorsal simple seta, posterior parapodia I: acicula, J: ventral simple seta.

Fig. 2 A-J. *Paraehlersia weissmannioides* (Augener, 1913).

*Ehlersia ferrugina* non Langerhans. Böggemann & Westheide, 2004: 418, fig. 6. *Paraehlersia weissmannioides* San Martin & Hutchings, 2006: 312, figs. 43A-C, 47A-I, 48 A-F, 49 D-F.

Material examined. Port Said 0.25 m depth, Spring 2008, one specimen.

Description. Body broad anteriorly, tapered posteriorly, 11 mm long, 0.2 mm wide, with 41 chaetigers (fig. 2 A). Prostomium oval (75 µm), 4 eyes in trapezoidal arrangement, and 2 anterior eye-spots; lateral antenna 162.5 µm long, midian antenna 150 µm long. Palps broad (87.5 µm), basally fused. Dorsal tentacular cirri 147.5-162.5 µm long, ventral tentacular cirri about one third in length of dorsal tentacular cirri. Antennae, tentacular and anterior dorsal cirri; elongated, indistinctly articulated; articulation variable with short and long articles, up to 22 articles; dorsal cirri becoming progressively smoother posteriorly. Infracirral papillae not seen. Parapodia conical, slightly elongate. Ventral cirri digitiform, slightly longer than parapodial lobes. Parapodia with 12-15 falcigerous compound chaetae; blades strongly bidentate, with fine spines on margin (fig. 2 B), 2-3 distalmost ones longer than remaining (30-42.5 µm) (fig. 2 C). Most dorsal compound chaetae, spiniger-like, blades (75 µm long) on midbody, and about 93 µm on posterior parapodia

with fine spines on margin (figs. 2 C, E), absent on most posterior parapodia; indistinctly bidentate. Compound falcigers becoming wider progressively along body, with stronger proximal tooth, slightly hooked (fig. 2G). Dorsal simple chaetae appear from chaetiger 19, truncate, bifid with short spines margin (fig. 2 F, H). Ventral simple chaetae on posterior parapodia, thick, with few long spines on margin, strongly bidentate, proximal tooth large, slightly hooked, and distal one shorter than proximal one (fig. 2 J).

Anterior parapodia with 2-3 slender aciculae, two of them distally rounded with small bending tip and one straight (fig. 2D); from proventricular segments onwards, acicula solitary, with oblique, short tip (fig. 2 I). Pharynx through 6 segments; pharyngeal tooth anteriorly located. Proventricle, rectangular through 4 segments with about 21 muscle cell rows.

Distribution: Australia, Seychelles. New report for the Mediterranean Sea.

Remarks: The Egyptian specimen is similar to Australian ones; it is likely an Indo-Pacific migrant through Suez Canal.

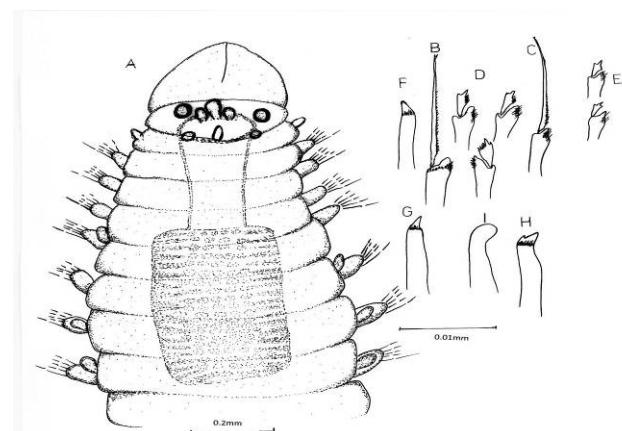


Fig. (3): *Exogone africana* A: anterior part of body, B: anterior spiniger-like seta, C: posterior spiniger-like seta, D: anterior falcigers setae, E: posterior falcigers setae, F: anterior dorsal simple seta, G: posterior dorsal simple seta, H: ventral simple seta, I: acicula.

Fig. 3 A-I. *Exogone africana* Hartmann- Schröder, 1974

*Exogone verugera africana* Hartmann-Schröder, 1974a:137, figs. 164-168; 1979; 108, figs.164-168.

*Exogone africana* San Martín, 2005: 143, fig. 90 a-f.

Material examined. Port Said, 0.25 m depth, on fouling, Spring 2008, 2 specimens.

Description. Body small, slender, relatively broad anteriorly, 3 mm long, 0.23 mm wide, 28 chaetigers. Prostomium oval (fig. 3 A); 4 eyes in trapezoidal arrangement. Antennae short, oval, close to each other, inserted between anterior to eyes; median antenna slightly longer and thicker than lateral one. Palps broad, longer than prostomium, totally fused, with a dorsal furrow (fig. 3A). Peristomium shorter than subsequent segments; one pair of small, papilliform tentacular cirri. Dorsal cirri similar to antennae and tentacular cirri, slightly longer than lateral antennae, present on all segments. Compound chaetae of two types on all parapodia: 1-2 spiniger-like, with long blades 31  $\mu\text{m}$  long (fig. 3 B) on anterior parapodia, slightly short on posterior one (25-27.5  $\mu\text{m}$ ), distally bifid, with short marginal spines (fig. 3 C), and 4 compound chaetae with short falcigerous blades about 7.5  $\mu\text{m}$ , bidentate, subdistal tooth long and distal tooth short, moderate marginal spines (fig. 3 D); posterior falcigers smaller, three in number, blades about 5  $\mu\text{m}$  long (fig. 3 E). Dorsal simple chaetae from anterior segments, with rounded tips (fig. 3 F), subdistally serrated, thicker posteriorly with pointed tip (fig. 3 G). Ventral simple chaetae on posterior parapodia, sigmoid, thick, with some short spines on base of teeth, bidentate, subdistal tooth longer and thicker than distal tooth (fig. 3 H). Acicula solitary, slender, distally rounded (fig. 3 I). Pharynx long, through 4 segments; pharyngeal tooth located on anterior rim. Proventricle occupying 4 segments with 18 muscle cell rows. Pygidium with 2 long anal cirri.

Distribution: Circumtropical. First report to the Mediterranean Sea.

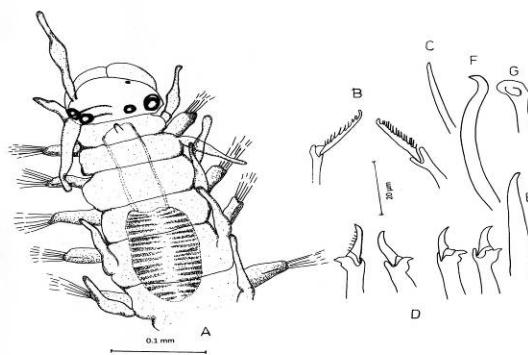


Fig. (4): *Parapionosyllis* n. sp. A: anterior part of body, B: anterior setae, C: anterior dorsal simple seta, D: posterior setae, E: posterior dorsal simple seta, F: posterior ventral simple seta, G: acicula.

Fig. 4 A-G. *Parapionosyllis aegyptia* n. sp.

Material examined. El Alamein 20 m depth, Autumn 2008. Holotype and Paratype, coarse sand.

Description. Holotype 3.5 mm, 0.15mm wide 29 chaetigers (fig. 4 A). Prostomium ovate, wider than long; 2 pairs of eyes, anterior pair larger than posterior ones, arranged in trapezoidal arrangement, and 2 small anterior eye-spots. Antennae spindle-shaped to bowling-pin shaped, longer than prostomium; median antenna (77.6  $\mu\text{m}$ ) slightly longer than lateral ones (67.5  $\mu\text{m}$ ), arising between anterior eyes; lateral antennae arising on anterior margin of prostomium (right one missing on holotype). Palps basally fused, shorter than prostomium. Peristomium with 2 pairs of bowling-pin shaped tentacular cirri, smaller than antennae. Parapodia somewhat elongated (32.5  $\mu\text{m}$ ). Dorsal cirri bowling-pin shaped, from 36- 45 $\mu\text{m}$  in length anteriorly to 65 $\mu\text{m}$  on posterior parapodia. Ventral cirri digitiform, shorter than parapodial lobes.

Anterior parapodia with 7 compound falcigers, unidentate with hooked tips and serrated margin (fig. 4 B); about 10  $\mu\text{m}$  long; shafts becoming posteriorly thick with long curved acute tip, blades with serrated margin on 2 most dorsal ones, and 4-5 unidentate curved, smooth (fig. 4 D). Superior dorsal simple chaetae thin with pointed tip, present in all parapodia, except first one (fig. 4 C), become thicker posteriorly (fig. 4 E). Ventral simple chaetae unidentate, sigmoid (fig. 4 F). Acicula solitary, bent with hollow rounded tip (fig. 4 G). Pharynx extending through 3.5 setigers; mid dorsal tooth on anterior edge. Proventricle extending through 2.5 segments, with 17 rows of muscle cells. Glands small, with granular material, pair on each segment, present from first chaetiger.

Remarks. About 16 species are recognized as *Parapionosyllis*, 6 of them recorded in the Mediterranean Sea. The most similar species is *P. labronica* also found in this collection; both species have posterior compound chaetae with thick shafts, distally curved, and short, unidentate blades, smooth or almost smooth. However, the anterior compound chaetae of *Parapionosyllis aegyptia* are more elongated and provided with somewhat longer spines on margin, and the dorsal simple chaetae are different, being smooth and unidentate in *P. aegyptia* and provided with a sub-distal, thick spine and others shorter, in *P. labronica*. The remaining Mediterranean species are clearly different of these two species, because they have longer compound chaetae (see San Martín, 2003); also, other species of other seas also have more elongated compound chaetae and different dorsal simple chaetae.

Etymology. The species is named after the country in which has been found, Egypt.

#### 4. Discussion

The number of Syllids recorded on Egyptian waters reach about 60 species, a low number when compared with the 190 Syllid species were reported by Musco and Giangrande (2005) from the whole Mediterranean waters, which represents the 31.6 % of the total Mediterranean Syllidae. Also, many other species may possibly remain unreported because of the most coastal area of Egypt are unexplored and many studies are still needed.

Tovar- Hernández *et al.* (2002) referred to the dominance and diversity of syllid members in carbonate sediments, this observation was confirmed by Selim (2008), where El Hammam and El Alamein coasts contain carbonate bottom sediments.

Most of the studied species are well known, common and widely reported for Mediterranean and Atlantic Ocean. The analysis of samples resulted into 18 species, 11 of them new record for the Egyptian Mediterranean waters. Four species were reported previously, (*Brevicirrosyllis weismanni* Langerhans, 1879; *Parapionosyllis elegans* (Pierantoni, 1903); *P.brevicirra* Day, 1954 and *Sphaerosyllis taylori* Perkins, 1981, from many Mediterranean coastal areas and one more (*Salvatoria vieitezii* San Martín 1984) from the Suez Canal. Two of the studied syllids are considered apparently cosmopolitan: *Salvatoria clavata*, and *Odontosyllis fulgurans*. In addition, 11 species were known before from Spanish coasts, only 4 species were previously recorded in Greece, 9 from North West Italian, 9 from Turkish Aegean and 6 from Cyprus. Also four species are considered as a new record for Mediterranean Sea: *Palposyllis prosostoma* Hartmann-Schröder, 1977; *Paraehlersia weissmanioides* (Augener, 1913); *Streptosyllis compoysi* Brito, Núñez and San Martín, 2000; and *Exogone africana* Hartmann-Schröder, 1974.

According to geographic distribution through literature there are 9 species belong to Atlantic-Mediterranean category and one species (*Salvatoria vieitezii*) was recorded before from Suez Canal (Selim, 2009), and four species are new for Mediterranean, that means they are Lessepsian migrants; 3 species are amphi-Atlantic, *Sphaerosyllis taylori*, *Sphaerosyllis glandulata* and *Salvatoria vieitezii*. Two species are considered cosmopolitan species, *Odontosyllis fulgurans* and *Salvatoria clavata* and five species are considered Atlantic-Pacific categories.

In spite of it, many new recorded species usually discovered by way in new researches, still more not recorded until now, more studies requisite to be done along the Mediterranean and Red Sea coasts of Egypt to cover this point.

The present study showed richness of

Eusyllinae, Anoplosyllinae and Exogoninae species inhabiting Egyptian water benthic assemblage.

#### Corresponding author

F. A. Abd-Elnaby

<sup>1</sup>National Institute of Oceanography and Fisheries, Alexandria, Egypt

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9/9/2010

## Re-introduction of Elephant's Infant into Wild Group: First Attempt and Case Study from North-West India

**Ritesh Joshi**

Doon Institute of Engineering and Technology,  
Shyampur, Rishikesh, Dehradun, 249 204, Uttarakhand, India  
E-mail: [ritesh\\_joshi2325@yahoo.com](mailto:ritesh_joshi2325@yahoo.com)

**Abstract:** Elephant's infant is considered extremely difficult to re-introduce into the wild. On 21<sup>st</sup> of November 2009, an eight day old elephant's infant was found strayed from its group at Shyampur forest of the Haridwar forest division. For the first time in the history of Uttarakhand, attempts had been made by forest officials to re-introduce the orphaned baby elephant into the wild. It is noteworthy that during the introduction attempts, group of seven elephants had taken the baby within group, but left her behind after a while. They had responded from all directions to the cries of the baby elephant but the attempts were in vain. Radha – the domesticated elephant at Chilla forest of the Rajaji National Park nurtured the infant for 10 days before infant's death and Radha's behaviour always illuminated something new about elephant's life. It was the first attempt to re-introduce the infant to wild in north-west India in which some lessons came forward and could be helpful in management of elephants and in documentation of conservation-oriented action plan. Additionally, studies on the behaviour of wild elephants are highly required and recommended so that we can ensure the future survival of this endangered species.

[Ritesh Joshi. Re-introduction of Elephant's Infant into Wild Group: First Attempt and Case Study from North-West India. Journal of American Science 2010;6(11):567-570]. (ISSN: 1545-1003).

**Keywords:** Asian elephants, infant, re-introduction, conservation, north-west India

### Introduction

Elephants live in a matriarchal society where the oldest female is the leader. Allomothering in sub-adult and adult females is one of the important factor to survival of elephant's calves, which also decreases the mortality among newly-born calves. In elephant societies, groups have well established home ranges within which all members live in a coordinated manner besides, elephants have strict social bonds, sometimes association among different groups occurring specially during their migration. Elephants are always known for their strong memory, close relationship and cooperativeness. Here, I report a case study of elephant's infant re-introduction / translocation to wild group and on the failure of conservation operation. Such reports are largely absent from the wildlife literature despite their importance in illustrating success and failures of such operations.

On 19<sup>th</sup> of November 2009, merely 7-8 days old female elephant was found strayed from group at Papri Paraw forest of the Shyampur forest of the Haridwar forest division. She was grasped in a nullah (narrow patch of torrential rivulet, natural trench) and was trying to come out from there. After receiving the message from a Gujjar residing near the spot, forest officials started rescue operation and after rescuing her

successfully they shifted orphaned baby to Chilla forest range of the Rajaji National Park, where several other domesticated elephants are kept under the care of mahawats. The calf was healthy and was not suffering from any severe disease or infection. Park officials gave her all the required treatment and facilities along with routine checkups from veterinary officers (Wildlife Institute of India, State Govt. & experts from Delhi). Under the treatment she was fed with lactogen, calcium, coconut oil, olive oil and ORS liquid. In practical it was really a challenge to protect her in artificial environment and under human care.

### Re-introduction of calf into the group

After a day on 20<sup>th</sup> of November 2009, I along with forest officer of Chilla range planned to re-introduce the calf into the group as this was only the appropriate method for her survival. Initials were taken on the morning of 21<sup>st</sup> of November. Forest staff had made an in-depth survey of the area from where the calf had been rescued. At the same duration I collected information about the area and elephant's movement from locals and from Gujjars. As per information collected and field investigation it came to our notice that a group of 07 elephants (02 adult females, 03 sub-adult females, 01 juvenile male and 01 calf) were

moving within the range and during the past two nights (19<sup>th</sup> & 20<sup>th</sup> November) elephant's roaring/vocalization was perceived by Gujjars living near to spot. Only 08 days old infant was on an attempt to go back into the wild. For the first time in the history of Uttarakhand, attempts were been made by forest officials to re-introduce the orphaned baby elephant into the wild.



Figure 1. Forest officials at the site, where re-introduction attempts were made.

At sharp 03 pm after locating the group inside dense forest, we moved to the site where elephant group was moving (07 kilometers faraway) along with the infant. We selected evening hours for the operation because evening hours are the best timings as far elephant's sighting is concerned and during the same hours elephants are known to emerge out from the forest to open areas and near to water sources. When we reached the spot suddenly elephants that were spread randomly started incessant audible communication, besides rumbling sounds were also observed at short interval. At that moment all elephants collected together and tried to approach us speedily. I with a fellow rapidly brought the infant towards group and at the same moment the group started approaching us slowly with very antagonistic behaviour. I realized a very serious threat regarding to our life but a key-view of saving infant's life made us courageous. When barely 20 meters distance remained between us and the elephant's group, I pushed the baby towards them and quickly returned back with other fellows watching the incidence from a distance.

Suddenly and surprisingly group approached the infant, smelled it and after few seconds returned back inside the forest and left the baby alone. A threat was clearly visible among all of them, which might be of human touching or our presence and stench. After few minutes we again made an effort to re-introduce the infant into the group and keeping the view I again

brought up the infant towards the older cow, which was standing to some distance along with two sub-adult cows. When I just came nearer to the cows, I left the spot quickly and watched the incidence from a distance hiding myself behind the tree. I was shocked to see that the same behaviour was repeated by the elephants. They again approached the infant and after giving her greetings returned back speedily inside the forest. But this encouraged the infant and she walked slowly towards the group and joined them but again the group sprinted away. Baby tried to enter within the group several times and this moved her about a kilometer further but the group didn't accept the infant. It is noteworthy that during the introduction attempts, group of seven elephants had taken the baby within the group, but left her behind after a while. They had responded from all directions to the cries of the baby elephant but finally the attempt got unsuccessful.



Figure 2. Constructive efforts: officials brought the infant towards the wild group while re-introducing the infant into the group at Papri paraw area, Haridwar forest division.

All this happened in about three hours and after a while day light became mild and slowly it became too hard for us to see easily with naked eyes but efforts carried out by infant were continued to join the group; it might be she identified her elder ones. Miserably we reallocated the baby into the vehicle and moved away from the spot and infant was again shifted to Chilla forest for care. In that way our attempt to re-introduce the calf with the group had failed, which if had got successful would have become the first ever historical conservation effort in north-west India and could have acted as a milestone in Asian elephant's management and conservation.

### **Love of Radha**

But for the failed effort there was a shining light among the clouds, which was the bond of love between Radha and baby elephant. Radha is a domesticated elephant at Chilla and was brought from Delhi zoo during 2007-2008. She entirely adopted the baby after three days and her behaviour was surprisingly changed. I have kept my observations on both of them as part of my long-term study on Asian elephant's behaviour. Radha nurtured the infant for about 10 days before infant's death and was very serious about it. She always (24 hours) remained close with the baby and doesn't let anyone to move near to the infant. Interestingly she even didn't depart to drink water alone. Unfortunately on 29<sup>th</sup> of November the biased chain, which was in Radha's hind feet, injured the baby while searching the baby and due to this infant felled down in a small trench. After the day baby got distressed and her movements became restricted. Very unfortunately on 2<sup>nd</sup> of December 2009 infant died during night hours.



Figure 3. Bond of Love: Radha with infant at Chilla forest range of Rajaji National Park

#### *Changes in the behaviour of Radha*

Surprisingly and notably the behaviour of Radha was changed just after the death of infant. She was feeling alone and searching for the infant again and again. The baby died near to her forefeet and during early morning when mahawat reached to her to feed the

baby and knew about infant's death, they faced problem in shifting the infant due to abnormal behaviour of Radha. For 2-3 days drastic changes were observed in the behaviour of Radha, which also include the unusualness in feeding.

#### **Current scenario of rescue operations**

After two years, young elephants can begin to look after themselves, but this doesn't bode well for newly-born babies. Infant survival after the rescue is too much controversial. I personally observed that saving a rescued baby elephant is a tough task as wild animals are nature-born and need sensitive parental care besides; the chances of infection during human care are quite large. During September 2005 and May 2008 a 02 years female and a 01 month male baby elephants were rescued from Dehradun (Doiwala forest) and Haridwar (Chiriacpur forest) forest divisions and shifted to Chilla forest for care. Unfortunately, both of them died during care.



Figure 4. Infant feeding on lactogen milk from bottle fixed pipe under care of Radha and mahawat

Mentioned is the third case of such type where we are unable to save the infant. Additionally against to this we have two examples in which elephant's infant had been successfully rescued and shifted to Chilla forest range for care. During 1997 a male elephant (Raja) was separated from group at Haridwar – Dehradun railway track (Motichur – Kansrao rail section) while three members of his group died due to collision with speeding train. Unfortunately after successful capturing he was brought to Chilla forest of the Rajaji National Park. Another such case happened during 2000, when a male baby elephant (Yogi) was shifted to Chilla forest after being rescued from Rishikesh – Dehradun national highway while he strayed from his group.

On the other hand our rescue operations need drastic changes, which include action oriented research on elephant's behaviour. Despite shifting, infant can be re-introduced to possible group on on-spot introduction basis. Additionally, extensive research studies are required on such elephant groups to know more about their behaviour and on the basis of which some recommendations could be given. During June 2000 and January 2001, two elephant's calves died due to collision with train in Haridwar – Dehradun railway track, which is passing in between Rajaji National Park and surprisingly fellows were continuously observed near to their dead bodies for about a day (24 hours), which creates a big problem for forest officials. During May 2007 and March 2008 at Shyampur forest, March 2009 at Chilla forest and October 2009 near to Doiwala forest such type of incidences occurred in which strayed and rescued elephant infants were accepted and brought back by their group members.

### **Recommendations**

1. It was observed during previous year cases that whenever an infant was rescued and shifted to some other place, group was always found wandering near to spot for about two to three days while searching for baby elephant and screaming sounds were always perceived by locals. During 2008 when a one month old male infant was drift down in Rawasan river (Chiriapur forest, Haridwar forest division) and separated from group, forest staff brought the baby to Chilla forest with the view to save him. At that time elephant's movements and vocalizations were continuously observed at Pili forest, just two kilometers away from the spot from where the calf was rescued.

Therefore, based on some previous practical cases and observations it is recommended that during any such case, a small eco-friendly (small wooden pieces, twigs with leaves, fodder species and if possible dung piles may be spread) rough circular fencing of about 20-30 meter (depending upon available space and requirement) should be made and infant should be released inside it. All this will need on-spot operation; for example after releasing the infant, for feeding and care, mahawats, veterinary doctors and wildlife biologists should be deputed for providing extensive care. And during whole of the process a departmental team should be appointed to look after the incidence for example threat from other animals. This can allow the group to bring their baby

safely and is only the method to re-introduce the infant into the group. As elephants are highly social in nature even they were observed to mourn for fellows, besides, their memories are quite strong and therefore, it could save the life of infant.

2. One possibility during the operation is that the selected group may be wrong one wrong, secondly that baby was not rescued before the eyes of her mother and the elephants could smell a lot of human touch, which scared them. Therefore, strong patrolling to search exact group after observing their activities, baby should be touched and feed by only one or two allocated person (mahawats). Even with human touch possibilities of infection may be enhanced.
3. A detailed research is needed to understand the behaviour of elephants in the wild.
4. Despite carnivores several cases of elephants are in front of us and we have learned a lot, therefore, a research oriented action plan is required to be made, which should be entirely practical and could be implemented in field conditions.
5. If not re-introduced and shifted to some other protected area, proper care should be given and precautions should be taken as per the environmental / field conditions.

### **Acknowledgements**

I am thankful to SERC – DST, Govt. of India for providing financial support for studying the elephants of Rajaji National Park. Thanks are due to Dr. Rambir Singh, Director, DST, New Delhi, Mr. C. M. Dobhal, Director and Mr. O. P. Bhatt, Chairman of Doon Institute of Engineering & Technology, Uttarakhand and to Mr. M. S. Negi, Forest Range Officer, Chilla, Rajaji National Park, Mr. Sandeep Rawat, *The Tribune*, Mr. Naveen Pandey and Raju Pushola, *Dainik Jagran* for their cooperation and suggestions during the field work and preparation of this manuscript.

### **Correspondence to:**

Dr. Ritesh Joshi  
Doon Institute of Engineering and Technology,  
Shyampur, Rishikesh, Dehradun, 249 204,  
Uttarakhand, India  
E-mail: [ritesh\\_joshi2325@yahoo.com](mailto:ritesh_joshi2325@yahoo.com)

6/2/2010

# An Approach To Partially Import The Ontologies On Semantic Web Based Upon User Choice

Tayybah Kiren<sup>1</sup>, Muhammad Shoaib<sup>1</sup>, Muhammad Tariq Pervez<sup>2</sup>, Sonia Majid<sup>3</sup>, Qazi Mudassar Illyas<sup>4</sup>

<sup>1</sup>Department of CS & E, University of Engineering & Technology, Lahore Pakistan

<sup>2</sup>Department of CS, Virtual University of Pakistan, Shadman Campus, Shadman Market, Lahore, Pakistan

<sup>3</sup>Lahore College for Women University, Lahore Pakistan

<sup>4</sup>COMSATS Institute of Information Technology, Abbottabad, Pakistan

[tariq\\_cp@hotmail.com](mailto:tariq_cp@hotmail.com)

**Abstract:** With the increase in applications using ontologies to represent semantic information, the issue of partially reusing the ontologies is getting more focus of researchers. Ontology construction from scratch is protracted and labor intensive job. Therefore, it is good to fabricate the ontologies by reusing the existing ontologies. Existing techniques for partially importing the ontology do not consider the user choice while selecting the most relevant ontologies for reusing. Most of the approaches have restriction on the size of ontology that is to be modularized. An approach for partially importing the ontologies has been presented in this paper. The proposed technique selects important keywords from a document by calculating term frequency, IR measure and precision along with class match measure to rank the most relevant ontologies. An algorithm to extract ontology fragments has been presented. This algorithm is independent of the size of ontology being reused.

[Tayybah Kiren, Muhammad Shoaib, Muhammad Tariq Pervez, Sonia Majid, Qazi Mudassar Illyas. AN Approach To Partially Import The Ontologies On Semantic Web Based Upon User Choice. Journal of American Science 2010;6(11):571-581]. (ISSN: 1545-1003).

**Keywords:** semantic web; ontology; partial import; knowledge management; user choice; term frequency

## 1. Introduction

Semantic Web is a new generation of the existing World Wide Web in which contents can be expressed in a way that their meanings are easily explicable by the search engines and it has become easier to access, share and assimilate data (Berners and Handler, 2001). Ontology is a way of formally representing a set of concepts within a domain and the relationships between those concepts. It describes the properties of that domain, and may be used to define the domain (Gruber, 1993). Ontology construction from scratch is regarded as a very protracted and labor-intensive job (Craven et al., 2000; Kietz et al., 2000; Maedche and Volz, 2001; Shamsfard and Barforoush, 2002; Khan and Luo, 2002). A better approach is to build the ontologies by reusing the existing ontologies. Many techniques have been devised so far to reuse the ontologies.

A lot of care is needed to select axioms to be copied because very small negligence in this decision can lead to information loss and even making the structure of resulting ontology awkward (Handle and Schenber, 2002). On the other hand, the technique proposed in (Bezerra et al., 2008) does not give much attention towards combining the modules to construct a partially imported ontology.

The techniques proposed in (Stuckenschmidt and Klein, 2004; Grau et al., 2005) divide all ontologies into modules. This is somehow complicated and computationally expensive task especially in cases when a large number of relevant ontologies are available. These approaches also do not give much consideration to the user's choice while selecting the relevant ontologies and relevant module.

This paper presents an approach, which has already been published in form of dissertation of the first author of this manuscript, for partially importing the ontologies. The proposed technique selects important keywords from a document by calculating Term Frequency (TF). It uses IR measure and precision along with Class Match Measure (CMM) to rank the most relevant ontologies. It also gives an algorithm to extract ontology fragments that is independent of the size of ontology being reused. Thus an ontology is constructed by partial reuse mechanism based on user choice and having no irrelevant details. Partially importing mechanism presented in the paper is very simple and having less computational complexities.

The rest of this paper is structured as follows: In section 2, the previous approaches for reusing the ontologies are described. In section 3, we give materials and methods for the proposed approach. In section 4, we present results and

discussion of the proposed approach. Section 5 presents conclusions. And the last section of paper comprises of the future recommendations.

## 2. Related Work

In this section, we give brief overview of some available approaches to partially import ontologies.

Stuckenschmidt et al., (2004) proposed a method to reuse the ontologies by partitioning the large ontologies into small portions or parts according to their class hierarchy structure. This method firstly creates a weighted graph of the ontology to be reused and then identifies the possible partitions from this dependency graph. Difficulty with this approach is that it is only effective for very large ontologies, and this is actually ontology partitioning algorithm but not a complete methodology to reuse the ontologies (Bezerra et al., 2008).

Bezerra et al., (2008) developed a tool for extorting modules from Ontologies. Their approach was based on OOP standards like encapsulation and information hiding. The extorted module was independent and could be easily exchanged with another module with same interface as the implementation details of the module was hidden from the imported ontology. Their approach concentrates more on digging out the relevant module rather than combining the modules to make ontology.

It is a promising approach but there are some deficiencies; like because of hidden implementation, the imported module might give rise to confusion at the end of person using the partially imported ontology and also their approach does not give much attention towards combining these modules to make a partially imported ontology (Grau et al., 2005).

Grau et al., (2009) introduced a module extraction approach based on logic. This approach allows extracting the modules that are based on locality. They proposed an algorithm to build a locality based module. This algorithm divides the ontology into two parts and initializes one part explicitly as void and second portion as the whole ontology. Then it performs locality test which moves the non local axioms to the first part with respect to the given signature. It performs the same thing until all the axioms in the second module are visited. These modules had the advantage that they are small in size. But at the same time the main flaw with this approach is that relationships among classes are not imported correctly, in other words, the modules are based on syntactic positioning (Grau et al., 2009).

## 3. Materials and Methods

This section describes the proposed technique in detail. We have divided the proposed approach into three modules. Algorithm of each module has also been presented. Figure 1 gives the overview of the proposed technique

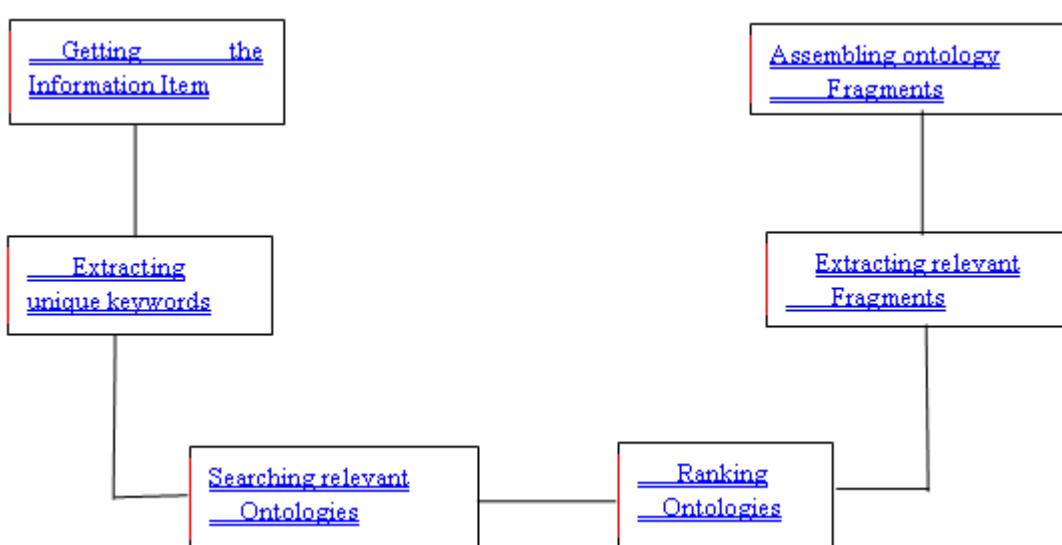


Figure 1. Graphical view of the proposed technique

The proposed technique performs its task in the following three modules.

1. Extracting keywords
2. Retrieval of relevant ontologies

### 3. Partially importing the ontologies

#### 3.1. Extracting Keywords

**Input:** Information item

**Output:** list of keywords

In this module the system takes information item of user's interest. This information item may be a dynamic web page, a web page saved in a local directory or data from some online or offline repository. This document is then passed to a keyword extracting mechanism. The mechanism also sorts the important keywords from the set of extracted keywords.

The steps followed in this module are:

##### 3.1.1. Getting the Information Item

In this step, any information item as per user's interest is received. The information item is received with its full completeness. This information item may be a dynamic/static web page, a web page stored in local directory or a document from an online or offline repository. The document is taken for the purpose of extracting keywords as if a user is exploring/reading some document and he/she wants to search ontology for some keyword (e.g. department, faculty etc). The interest/choice of the user is given much importance because different users have different types of needs, caliber and domain of interest. The benefit of giving the choice to user to select document according to his/her interest is that the user has knowledge about the information item, extracted keywords and thus retrieval of relevant ontologies is easy and comfortable.

##### 3.1.2. Extracting unique keywords

In this step, we extract unique words from the information item. In the extracted unique words the unwanted words like stop words and other signs are not included. As they are neither unique words nor of interest to the user. First the information item is converted to plain text. Then unique keywords are extracted from it and TF of each keyword is computed. The formula for calculating TF as given by (Yates and Neto, 2005)

$$tf_{i,j} = n_{i,j} / \sum_k n_{k,j} \quad (1)$$

Where  $n_{i,j}$  is the number of times a word  $t_i$  appears in the document  $p_j$  and the denominator in the above equation is sum of occurrences of all the

words in the document. After this, the words with TF value above the threshold 0.043 are selected.

#### Algorithm for Extracting Keywords

The processing steps for the Module of extracting keywords are illustrated in an algorithmic form below:

**ALGORITHM:** Generating a set of unique keywords from an information item.

**INPUT:** Information item

**OUTPUT:** Set of keywords.

**STEP1:** /\*getting the Information item

    1.1: Get the document (information item) with its full completeness

**STEP2:** /\* Extracting unique keywords

    2.1: **IF** it is a web page

        2.2: Convert the web page into UTF-8 encoding

        2.3: Perform preprocessing on the page (e.g. Removing HTML tags, Multimedia Contents, links, frames etc)

        2.4: **ELSE GOTO** step 2.5.

        2.5: Convert the document to plain text (Removing stop words etc.)

**End If**

    2.6: Parse the whole document in separate words

    2.8 Compute number of times each word appears in the document

    2.9 Compute the total number of time all the words appear in the document

    2.10 Compute TF (Term frequency) of each word by dividing the results of Step 2.8 by 2.9

    2.11: Save words with TF value above the threshold.

**END**

Algorithm of extracting keywords shows a complete process of extracting keywords from an information item. If an information item is a web page then following two steps are performed: 1) the information item is converted into a UTF-8 encoding document. 2) A preprocessing is performed in which the HTML tags, multimedia contents, links and frames etc. are removed. The purpose of performing preprocessing is to reduce the size and complexity of the information item. Then the information item is converted to a plain text. Now, in the whole information item, each word's occurrence is computed. Then, an aggregation of all word's occurrences in the information item is computed. Finally TF of each word is computed by dividing the occurrence of a single word by the total occurrences

of all words in the information item. Figure 2 shows graphical representation of this algorithm.

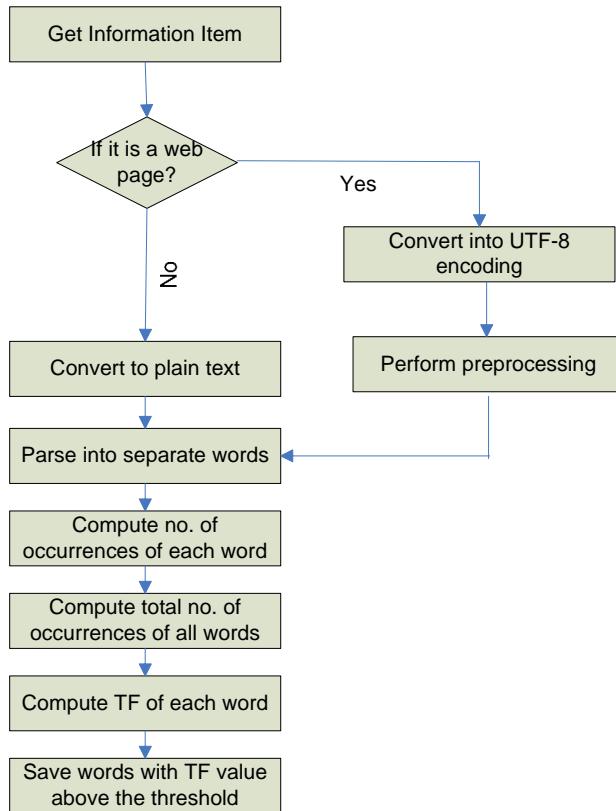


Figure. 2. Graphical view of algorithm of extracting keywords

### 3.2. Retrieval of relevant ontologies

**Input:** Set of keywords

**Output:** List of relevant ontologies

This module uses the list of keywords generated by the module of extracting keywords as an input. After retrieving the ontologies relevant to the set of keywords, this module ranks the ontologies on the basis of their similarity to the keywords set. The module performs the following two steps:

#### 3.2.1. Searching ontologies

This step is concerned with giving a query consisting of keyword(s) to the ontology search engine and saving the set of relevant ontologies retrieved. Output of this step will be a set of retrieved ontologies from the Semantic Web.

#### 3.2.2. Ranking the Ontologies

**Input:** List of ontologies

**Output:** Set of most relevant ontologies

This step of the proposed technique is concerned with re-Ranking of the list of ontologies retrieved using the CMM, IR measure and precision. First we find out that to which extent the class of ontology is matched to the keywords, which is determined by CMM. The formula for CMM is as given by (Alani and Brewster, 2008).

$$\text{CMM } (o, K) = \alpha E(o, K) + \beta P(o, T)$$

The number of classes of ontology is calculated whose labels match with the K. Then this information is used to calculate the CMM. Where  $\alpha$  and  $\beta$  are the measures of exact and partial match. Now the Precision of ontologies selected is calculated. Precision is based upon the user choice, mean, if the user thinks that the document is relevant. Formula for the precision is as given by (Yates and Neto, 2005).

$$P = |R_a| / |A|$$

The ontologies retrieved are ranked according to the decreasing order of precision and CMM.

Algorithm of this module is divided into two parts/algorithms:

- 1 Algorithm for retrieving ontologies
- 2 Algorithm for ranking/retrieving most relevant ontologies

#### ALGORITHM: Retrieving Ontologies

**Input:** A list of keywords

**Output:** A list of ontologies: List\_Ontologies

1. Get a list of keywords
2. Provide the list of keywords to the search engine
3. Save the relevant ontologies in List\_Ontologies

Return List\_Ontologies

End

#### ALGORITHM: Retrieving Most Relevant Ontologies

**Input:** A list of relevant ontologies: List\_Ontologies

**Output:** A list of most relevant ontologies: List\_MostRel\_Ontl

**For** each ontology in List\_Ontologies to length (List\_Ontologies) **do**

Calculate CMM of List\_Ontologies [ontology] as per equation 2

Apply IR measure and precision on List\_Ontologies[ontology] as per equation 3

List\_MostRel\_Ontl[ontology]=List\_Ontologies[ontology]

**End For**

```

Sort List_MostRel_Ontl in decreasing order of
precision and CMM
Return List_MostRel_Ontl
End

```

### 3.3. Partially Importing the Ontologies

**Input:** Output of Module 1 and Module 2

**Output:** Partial ontology

This module of the proposed technique is concerned with actually making it possible to generate the ontology through partial importing from the other ontologies. It performs its task by pursuing the following steps:

#### 3.3.1. Extracting relevant fragments

This step deals with the finding and retrieval of the fragments or portions of the selected ontologies that best match the set of important words. An algorithm is designed to extract relevant fragments from selected ontologies. Figure 3 shows graphical representation of the algorithm.

**ALGORITHM:** Generating fragments of ontologies relevant to the set of keywords.

**INPUT:** An ontology and set of keywords

**OUTPUT:** A relevant ontology fragment

1.1: Create **TobeKept**- A data structure containing nodes included in the retrieved fragment

1.2 Create **TobeChecked**-A data structure containing all the nodes in the ontology

1.3: Current=first element of TobeChecked

1.4: **WHILE** not end of TobeChecked DO

1.5: x = Current

1.6: Compare x with set of keywords

1.7: **IF** matched with any one from the set

1.8:**IF** x  $\notin$  TobeKept

1.9: Insert it and only its direct subclasses and properties into the TobeKept (by Referring to its child nodes)

**END IF**

**END IF**

1.10: Current=next element of the TobeChecked

**END {WHILE}**

**Return** TobeKept

**END**

#### 3.3.2. Assembling the Fragments

This is the final step of our proposed approach where we will finally construct ontology by assembling the ontology fragments resulted from step 1 and by adding the classes and properties that are defined specifically for that ontology.

## 4. Results and Discussion

In this section, we validate the proposed technique and present its result. As an experiment we use a document stored in our local disk as the information item. This document is about a university which is organizing a software exhibition where PhD thesis (S/W Products) is placed. Hence, we construct ontology about the university described in the document. We show that how our proposed technique is used to construct a partially imported ontology of the university described in the document.

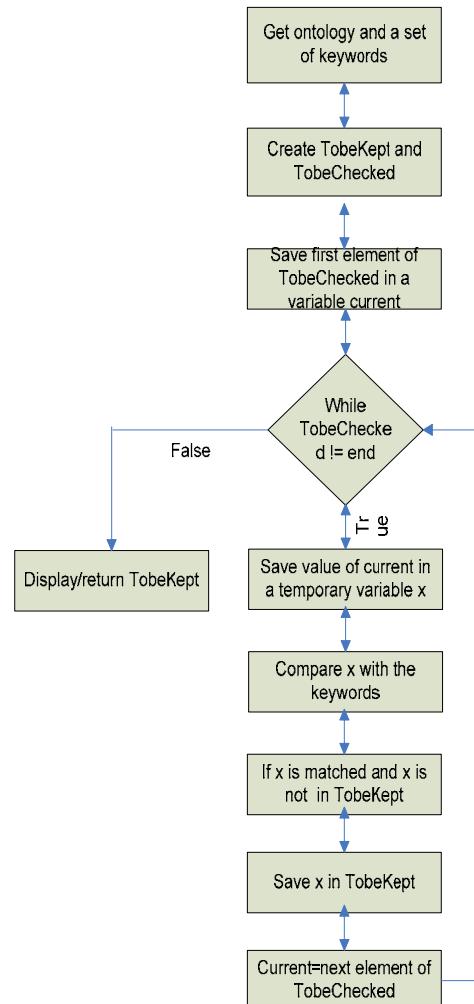


Figure 3. Flow diagram of algorithm of generating fragments of ontologies

### Module 1: Extracting keywords

The document is fed into a keyword extractor (Webseo, 2010). We calculate the TF value of each keyword extracted by the keyword extractor and select the words with TF value above the

threshold value 0.043. Following table shows the list of keywords and their TF values. There are a total of 25 keywords extracted.

As table 1 shows, the set of keywords having TF value greater than 0.043 is K= {University, Department, Thesis, Faculty, Dean, Magazine, Institute, Exhibition, Publisher}.

#### **Module 2: Retrieval of relevant ontologies**

Using the available list of keywords (table 1) related ontologies can be searched from the web. SWOOGLE (Swoogle, 2010) is used for this purpose. Table 2 shows the set of ontologies present on web that are considered to be relevant to the set of keywords according to the professionals.

Once ontologies are retrieved, we re-Rank and then select the most relevant ontologies from the resulting ontologies using the precision measure which is based on the user choice and the CMM value. Table 2 shows re-ranking of the ontologies.

In this experiment we use threshold value of 30% for precision and 4.0 for CMM. Only the ontologies with CMM greater than or equal to 4.0 according to (Tun and Dong, 2008), and precision greater than 30 %, are selected as shown in table 4.

Table 1: Set of keywords and their TF value

S. No	Keywords	n <sub>i,j</sub>	TF
1	University	10	0.083

Table 2. Set of ontologies relevant to K

S. No	Ontology URLs
1	<a href="http://ebiquity.umbc.edu/ontology/contact.owl">http://ebiquity.umbc.edu/ontology/contact.owl</a>
2	<a href="http://www.cs.toronto.edu/semanticweb/maponto/Maponto/Examples/univ-cs.owl">http://www.cs.toronto.edu/semanticweb/maponto/Maponto/Examples/univ-cs.owl</a>
3	<a href="http://svn.mindswap.org/pallet/branches/dlsafe/ontology.owl">http://svn.mindswap.org/pallet/branches/dlsafe/ontology.owl</a>
4	<a href="http://visitology.com/ont/bug/import/clean/academic.owl">http://visitology.com/ont/bug/import/clean/academic.owl</a>
5	<a href="http://owl.cs.manchester.ac.uk/2008/iswc-tones/ontologies/univ-bench.owl">http://owl.cs.manchester.ac.uk/2008/iswc-tones/ontologies/univ-bench.owl</a>
6	<a href="http://swrc.ontoware.org/ontology">http://swrc.ontoware.org/ontology</a>
7	<a href="http://www.cs.man.ac.uk/~rector/Modules/COMP60461-2008/lab-material/Tangled-ontology-from-personnel-dept-01-01.owl">http://www.cs.man.ac.uk/~rector/Modules/COMP60461-2008/lab-material/Tangled-ontology-from-personnel-dept-01-01.owl</a>
8	<a href="http://ontoworld.org/index.php/special:ExportRDF/Mike-Dean">http://ontoworld.org/index.php/special:ExportRDF/Mike-Dean</a>
9	<a href="http://www.mindswap.org/2004/multipointOnt/Factoredontologies/italianUniversities/it-apartition1.owl">http://www.mindswap.org/2004/multipointOnt/Factoredontologies/italianUniversities/it-apartition1.owl</a>
10	<a href="http://iswc2006.semanticweb.org/submission/iswc2006 in use-Allemang-Dean">http://iswc2006.semanticweb.org/submission/iswc2006 in use-Allemang-Dean</a>

Table 3. Table for re-Ranking

S. No	Set of Retrieved ontologies	CMM	Relevancy according to user	P
1	<a href="http://www.cs.toronto.edu/semanticweb/maponto/Maponto/Examples/univ-cs.owl">http://www.cs.toronto.edu/semanticweb/maponto/Maponto/Examples/univ-cs.owl</a>	6.2	T	100.00%
2	<a href="http://downloads.dbpedia.org/3.2/en/dbpedia-ontology.owl">http://downloads.dbpedia.org/3.2/en/dbpedia-ontology.owl</a>	3		50.00%

3	<a href="http://swrc.ontoware.org/ontology">http://swrc.ontoware.org/ontology</a>	4.4	T	66.00%
4	<a href="http://www.aktors.org/ontology/portal">http://www.aktors.org/ontology/portal</a>	2		50.00%
5	<a href="http://www.cs.umd.edu/projects/plus/DAML/onts/univ1.0.daml">http://www.cs.umd.edu/projects/plus/DAML/onts/univ1.0.daml</a>	3.8		40.00%
6	<a href="http://www.mindswap.org/2004/multipointOnt/Factoredontologies/italianUniversities/it-apartition1.owl">http://www.mindswap.org/2004/multipointOnt/Factoredontologies/italianUniversities/it-apartition1.owl</a>	4.2	T	50.00%
7	<a href="http://annotation.semanticweb.org/iswc/iswc.owl">http://annotation.semanticweb.org/iswc/iswc.owl</a>	2.4		43.00%
8	<a href="http://morpheus.cs.umbc.edu/aks1/ontosem.owl">http://morpheus.cs.umbc.edu/aks1/ontosem.owl</a>	1.8		38.00%
9	<a href="http://www.srdc.metu.edu.tr/UBL/ContextOntology/naics.owl">http://www.srdc.metu.edu.tr/UBL/ContextOntology/naics.owl</a>	1		33.00%
10	<a href="http://owl.cs.manchester.ac.uk/2008/iswc-tones/ontologies/univ-bench.owl">http://owl.cs.manchester.ac.uk/2008/iswc-tones/ontologies/univ-bench.owl</a>	4.2	T	40.00%
11	<a href="http://srdc.metu.edu.tr/UBL/ContextOntology/unspe.owl">http://srdc.metu.edu.tr/UBL/ContextOntology/unspe.owl</a>	0.8		36.00%
12	<a href="http://www.apps.ag-nbi.de/makna/semwebexport?language=rdf&amp;model=inferred">http://www.apps.ag-nbi.de/makna/semwebexport?language=rdf&amp;model=inferred</a>			32.00%
13	<a href="http://www.cs.toronto.edu/~yuana/research/maponto/Bibliographic_Data.owl">http://www.cs.toronto.edu/~yuana/research/maponto/Bibliographic_Data.owl</a>	2.8		30.00%
14	<a href="http://www.webkursi.lv/luweb05fall/resources/university.owl">http://www.webkursi.lv/luweb05fall/resources/university.owl</a>	4		28.00%
15	<a href="http://svn.mindswap.org/pallet/branches/dlsafe/ontology.owl">http://svn.mindswap.org/pallet/branches/dlsafe/ontology.owl</a>	4.8	T	34.00%

Table 4. Selected ontologies

S. No	Set of Retrieved ontologies	CMM	Relevancy according to user	P
1	<a href="http://www.cs.toronto.edu/semanticweb/maponto/Maponto/Examples/univ-cs.owl">http://www.cs.toronto.edu/semanticweb/maponto/Maponto/Examples/univ-cs.owl</a>	6.2	T	100.00%
2	<a href="http://swrc.ontoware.org/ontology">http://swrc.ontoware.org/ontology</a>	4.4	T	66.00%
3	<a href="http://www.mindswap.org/2004/multipointOnt/Factoredontologies/italianUniversities/it-apartition1.owl">http://www.mindswap.org/2004/multipointOnt/Factoredontologies/italianUniversities/it-apartition1.owl</a>	4.2	T	50.00%
4	<a href="http://owl.cs.manchester.ac.uk/2008/iswc-tones/ontologies/univ-bench.owl">http://owl.cs.manchester.ac.uk/2008/iswc-tones/ontologies/univ-bench.owl</a>	4.2	T	40.00%
5	<a href="http://svn.mindswap.org/pallet/branches/dlsafe/ontology.owl">http://svn.mindswap.org/pallet/branches/dlsafe/ontology.owl</a>	4.8	T	34.00%

### Module 3: Constructing partially imported ontology

Now, from the above five most relevant ontologies we extract the most relevant fragments using the proposed algorithm for extracting relevant fragments. For example, I use two ontologies (Maponto, 2010; Org, 2010). Ontology in (Maponto,

2010) contains the classes and relationships mentioned in the table 5 in it. This is clear that if we want to reuse this ontology it is not suitable to import the whole ontology because of space complexity. Therefore, instead of importing the whole ontology only a relevant portion of ontology is extracted using the proposed algorithm.

Table 5: Table for TobeChecked

S.No	Classes	Direct classes and properties
1	Work	Research Course, Work Title
2	Journal	
3	School	
4	Faculty	Professor, Lecturer, Post Doc, Teacher of
5	Worker	Faculty, Admin Staff, Assistant
6	Periodical	Journal, Magazine
7	Article	Book article, Journal article, Conference Paper, Technical Report
8	Thesis	Master Thesis, Doctoral Thesis
9	Professor	Full Professor, Associate Professor, Dean, Visiting Professor, Tenured
10	Admin Staff	Director, Dean, System Staff, Clinical Staff
11	University	Master Degree from, Doctoral Degree from
12	Person	Student, Member, Research Interest, email Address, Person Name, Age
13	Student	Undergraduate Student, Graduate Student, Takes course
14	Magazine	
15	Department	
16	Dean	
17	Organization	Department, School, Institute, University, Research group, Affiliated Organization
18	Research Group	
19	Research Assistant	
20	Doctoral Thesis	
21	Publication	Thesis, Book, Manual, Publication author, Publication Research, Pub Title, Periodical
22	Course	Has TAs, has Instructor

Now for extracting the classes that are partially or exactly matched with the set of keywords is done by traversing table 5. In the 1st iteration the ‘Work’ is not matched with any of the keyword, therefore, the algorithm again goes to step 1.4

(Algorithm of generating fragments). Now, the next element is ‘Journal’ which is also not matched with any keyword. This process will be repeated until table 5 is finished. Table 6 shows the contents of TobeKept.

Table 6: Table for TobeKept

S.No	Classes	Direct Subclasses and Properties
1	Faculty member	Professor, Lecturer
2	Thesis	Master Thesis, PhD Thesis, Supervised by
3	University	Has
4	Magazine	
5	Department	Part of, has
6	Dean	Head of

The graphical representation of the fragments extracted shown in table 6 is given in the figure 4.

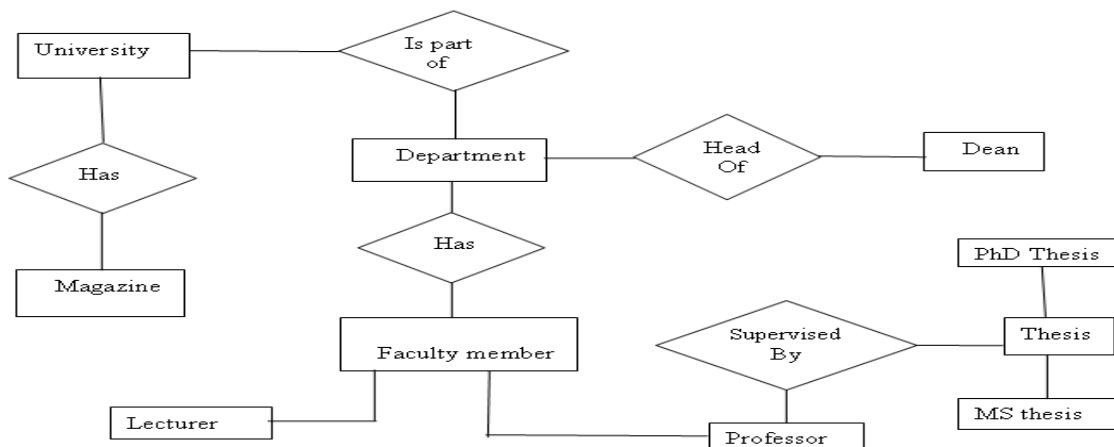


Figure 4. Graphical representation of extracted Fragments

Now if we run the algorithm on the 2<sup>nd</sup> ontology (Maponto, 2010).

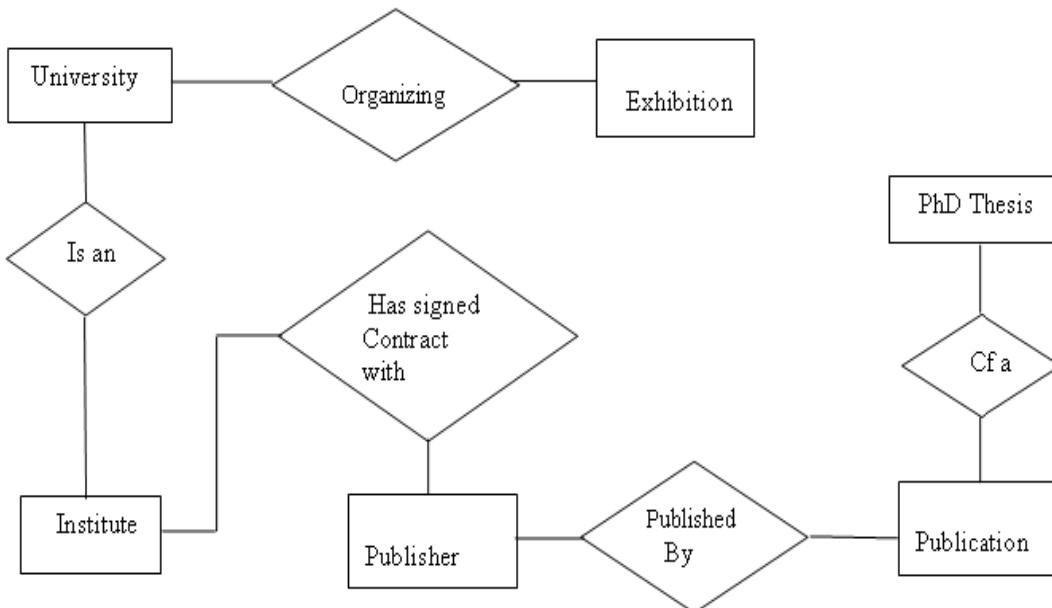


Figure 5. Graphical representation of extracted Fragment

Table 7 shows the relations and classes of the ontology to be constructed.

Table 7: Defined classes and properties

Classes	Relations
University	Has, is a, signed contract with, Organizing
Department	Part of, has
Dean	Head of
Magazine	Published by
Faculty member	Employee of, Lecturer, Professor
Thesis	Published in, Placed in, supervised by, MS Thesis,

	PhD Thesis
Publication	Published by, of a
Publisher	Publishes
Exhibition	Organized by
Software Exhibition	Is an
S/W Product	Presented

Figure 6 shows the graphical representation of the ontology constructed by assembling the above fragments and with adding some new classes and relations.

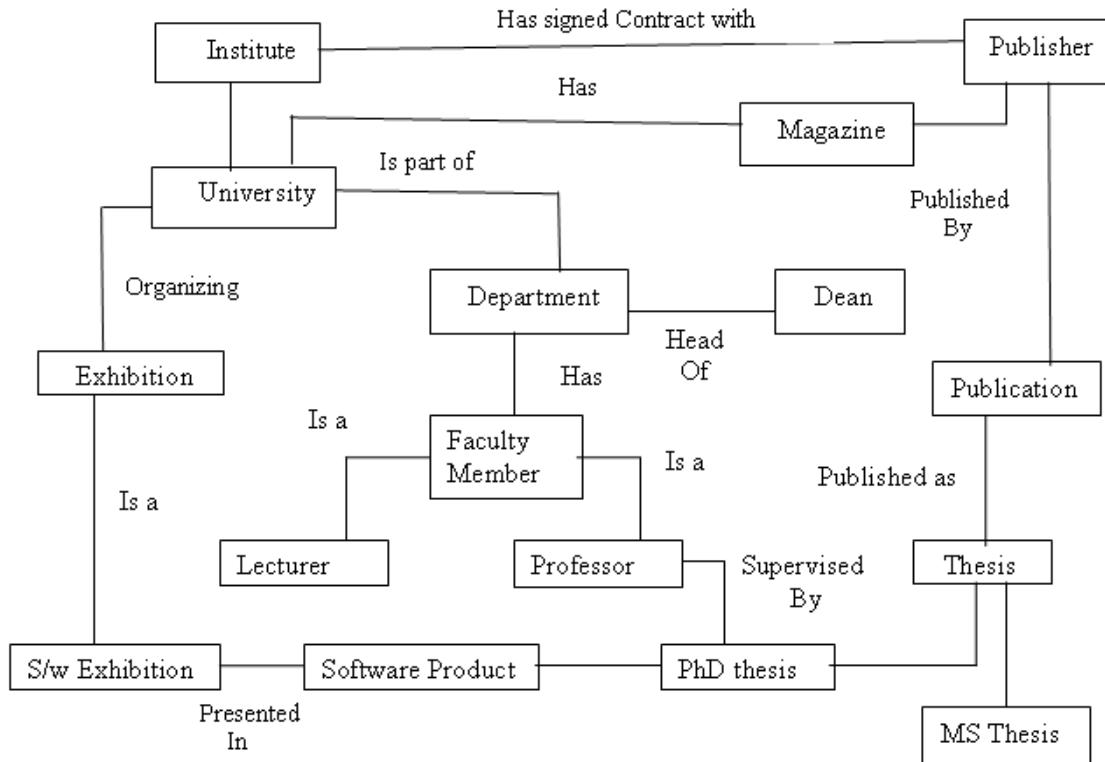


Figure. 6. Graphical representation of Resulting ontology

The resulting ontology is created by reusing the fragments extracted from the existing ontologies. It also contains some other classes and relations that are unique to it. The resulting ontology is the ontology of university that is organizing a software exhibition where the PhD thesis is published as a publication in a magazine. University has department and department has faculty member which include both lecturers and professors and a PhD thesis is supervised by a professor. Department is headed by Dean. Institute has signed a contract with a publisher and institute is a university in our example. Resulting ontology has no irrelevant details, thus reducing the large memory requirement for storing it.

## 5. Conclusions

This paper proposes an approach to partially import the ontologies based upon the user choice. It allows a user to built ontology of any document available at any source. This approach extracts keywords from the document of interest to user by including an important measure term frequency. Then it searches the existing ontologies from the web relevant to these keywords, ranks them and selects the most relevant ontologies with the help of precision measures and CMM, taking into account the user's choice. Then it traverses through the ontology and selects the classes which are matched to

any of the set of keywords along with their direct subclasses and properties, and makes a fragment of it. Then it constructs a partially imported ontology by defining some new classes and properties according to requirement and assembling and including the fragments extracted from the existing ontology. The end result of our approach is an ontology which is constructed by partial reuse mechanism based on user choice and having no irrelevant details. Hence it is concluded that if we partially import the ontologies according to the user choice then we result with the partially imported ontology which do not contain the extra details and also the partially importing mechanism is very simple with very less computational complexities.

## 6. Future Recommendations

The proposed technique has not been implemented. Work can be carried out with the aim to develop a complete application that enables the user to partially import the ontologies. This approach will be applied for knowledge management and knowledge management is an asset for success and survival in an increasingly competitive and global market, so using ontologies for the knowledge management in a good way by partially importing the ontologies is the need of time and this is area where research can be carried out.

It is needed to explore more robust strategies to evaluate the quality of the resulting partially imported ontology by our approach. Fragment ranking can improve the quality of our resulting ontology. The research can be carried out in order to implement ontology fragments by fragment ranking.

## Acknowledgements

We are thankful to the Higher education Commission of Pakistan for her generous financial support. This research paper is extracted from dissertation of the first author. She is very grateful to her supervisor and colleagues who helped her in extracting, writing and finalizing this research paper from her dissertation.

## Corresponding Author:

Muhammad Tariq Pervez  
Department of Computer Science  
Virtual University of Pakistan, Shadman Campus,  
Shadman Market, Lahore Pakistan  
E-mail: [tariq\\_cp@hotmail.com](mailto:tariq_cp@hotmail.com)

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8/31/2010

# The Use of Lemongrass Extracts as Antimicrobial and Food Additive Potential in Yoghurt

Shaaban, M. Abd-El Fattah<sup>\*1</sup>; Abo sree, Yahia Hassan<sup>1</sup>; Hala M. Bayoum<sup>2</sup> and Hesham A. Eissa<sup>3</sup>

Food Toxins and contaminants Department<sup>1</sup>, Dairy Department<sup>2</sup>, Food Technology Department<sup>3</sup>, National Research Centre, Cairo, Egypt.

<sup>\*</sup>shaabanmostafa@yahoo.com

**Abstract:** The following study was conducted to investigate the antifungal and food additive potential of medicinal plants. herbal decoction and essential oil (EO) extracts of *Cymbopogon flexuosus* (lemongrass) leaves and stems were tested for their inhibitory action against spoilage organisms and mycotoxins formation in two separated experiments. In the first experiment, yeast- extract sucrose medium (YES) was used as a basal medium to examine the mold growth and mycotoxin production by three pathogenic fungi: *Aspergillus flavus* (*A. flavus*), *Aspergillus parasiticus* (*A. parasiticus*) and *Aspergillus ochraceus* (*A. ochraceus*). The YES medium was supplemented with four different concentrations of Lemongrass oil, inoculated with 1-mL of a spore suspension containing  $10^5$ - $10^6$  conidia of each test mold and then incubated at 28°C for 14 days. After incubation period, cultures were analyzed for mycelial dry weight and mycotoxin accumulation. In the second experiment, yoghurt medium was used as a basal medium and the same system of study was applied in two different degrees of temperature (5°C and 28°C) for 4 weeks. Evaluation of the Lemongrass oil activity in yoghurt samples focused on the microbial stability of yoghurt, sensory evaluation as well as mold growth and mycotoxin formation. In the 1<sup>st</sup> experiment, the level of 0.1% of the EO extract was effective in inhibition both mold growth and mycotoxin production for all tested molds, and 0.3 % extract completely prevented the growth and toxin production. whereas, 1% of the decoction extract was effective. So, the EO extract was the suitable agent in the second experiment. It is of interest to note that while reduction in mold growth due to increasing extract concentrations was observed, the most striking effect was the reduction of mycotoxin production. The obtained data from the second experiment showed that the EO extract (0.1% concentration) inhibited viable yeasts and preserved yoghurt for over 28 days at 5°C. Also, the inhibitory action of the EO extract against yeasts was concentration dependent. The maximum inhibitory effect was found when the extract level increased above 0.1%. Incubation temperature had an important role in growth and mycotoxin production in yoghurt medium. During cold storage for 28 days at 5°C, the different concentrations of the EO extract added to yoghurt samples displayed different titratable acidity and total bacterial cells and pH than the control yoghurt ( $p < 0.05$ ). Overall sensory acceptability of yoghurt supplemented with the EO extract was higher than that of the control yoghurt prepared without the EO extract. The results indicate that the addition of the appropriate the EO concentration (0.1%, w/v) improved the physicochemical properties as well as sensory characteristics of yoghurt, could be used for decontamination of dairy products such as yoghurt from mycotoxicogenic fungi and mycotoxins formation, beside its beneficial properties as a functional food.

[Shaaban, M. Abd-El Fattah; Abo sree, Yahia Hassan; Hala M. Bayoum and Hesham A. Eissa. The Use of Lemongrass Extracts as Antimicrobial and Food Additive Potential in Yoghurt. Journal of American Science 2010;6(11):582-594]. (ISSN: 1545-1003).

**Key words:** Yoghurt, lemongrass, molds, yeasts, mycotoxins, aflatoxins, ochratoxin A, food additives.

## 1. Introduction:

Yoghurt in addition to its high nutritional value, it possesses antagonistic and therapeutic values. The valuable sensory characteristics of yoghurt are due to its content of carbonyls, mainly acetaldehyde, acetone, diacetyl and ethanol, produced by yoghurt bacteria. Yoghurt provides higher levels of protein, carbohydrate, calcium and certain B vitamins than milk (Gurr, 1987; Deeth and Tamime, 1984). The shelf life of yoghurt is short, i.e., one day under ambient condition (25–30°C) and around five days at 7 °C, which hinders its commercialization (Salji, 1987). Yoghurt defects

due to microbial contamination are widely reported in literature; the most frequent contaminants are yeasts and moulds (Spolaor *et al.*, 1988; and Abdel-Fattah and Abdel-Salam 2004) usually causing the swelling of packs, the presence of superficial coloured spots and abnormal tastes (Ottogalli, 1991). The low pH of yoghurt offers a selective environment for the growth of acid tolerant yeasts and molds (Banaquio *et al.*, 1981, Spillmann and Geiges, 1983). Therefore, it is not surprising that various investigators have found that yeasts are the primary spoilage microorganisms for yoghurt and that fruits, flavors, and colouring agents are frequent

contamination sources (Main, 1984; Weber and Broich, 1986). The spoilage of yoghurt by yeasts has been generally characterized by yeasty offflavors, loss of textural quality due to gas production, and swelling and occasional rupturing of the product containers (Davis, 1974). As a result, there is an apparent need for an effective preservation method to control acid-tolerant spoilage yeasts and molds in yoghurt.

Micotoxigenic Fungi and Pathogenic bacteria can grow at refrigeration temperature to numbers, which can result in an infection. For this reason dairy products should be kept well covered to prevent contamination, should ideally be consumed within two days of opening, or used in cooked foods after that two-day period (Potter and Hotchkiss, 1995). Mycotoxins may be found in milk products, originating from three possible sources: raw milk (such as aflatoxin M1 which present as a consequence of aflatoxin B1 metabolism by the animal); growth of a toxigenic fungal strain on product and mycotoxins synthesis, and production of these toxins in dried milk used to make milk product (Jose *et al.*, 1988).

Hence, it is highly desirable to prevent mould growth or to prevent mycotoxins formation in contaminated food. Several chemicals have been used to detoxify mycotoxins but these chemicals can not be added to foods to prevent mycotoxins formation because of their hazardous effect on human health. In recent years, studies on the natural antifungal agents, herbs and spices, have been reported by numerous investigators (Afrodit, *et al.*, 1995; Hiroshi and Sato, 2002; and Abd-EL Fattah and Abdel-Salam, 2004). They found that some herbs and spices had antifungal effect against some kinds of mycotoxic fungi, such as *A. flavus*, *A. ochraceous* and *A. parasiticus*, in synthetic medium. Among herbs and spices used, were sage, thyme, rosemary, mint, and Lemongrass.

*Cymbopogon flexuosus* (Lemongrass) is an economically important plant that has been used for centuries, as a medicine because of its wide-ranging therapeutic properties included relief of rheumatic and other pain and healing effect on ulcers (Fenwick *et al.*, 1990). Flavonoids extracted from Lemongrass are of considerable interest as natural plant components with antioxidant and antifungal activity (Pratt and Hudson, 1991; Nieto *et al.*, 1993; and Abu-Seif, *et al.*, 2009). Of the flavonoids present in Lemongrass, licochalcone A and licochalcone B which have equal antioxidant activity of vitamin E, and glabrene which is 3 times as active when compared with vitamin E (Okuda *et al.*, 1989).

One objective of the present study was to investigate the inhibitory action of Lemongrass oil

against spoilage organisms and mycotoxins formation in yoghurt under laboratory conditions. The use of Lemongrass herb in this study was due that Lemongrass is naturally occurring material, widely cultivated, cheap, had a medical functions and safe. These properties and the antifungal activity, if possible, make lemongrass oil may be potential multi-functional food additives. The physic-chemical properties, colour characteristics, total phenol content, microorganisms, sensory evaluation and the effect of storage time at 5°C for 2 months of yoghurt were also studied.

## 2. Materials and methods

### 2.1- Experimental design.

Depending on our previous results (Abdel-Fattah, 2002; Abdel-Fattah and Abdel-Salam, 2004 and Abu-Seif, *et al.*, 2009), concerning the antimicrobial effects of herbal extracts, this study was achieved. Two separated experiments were carried out during this study. The first experiment was to examine the best of the tow different extracts of Lemongrass, essential oil (EO) extract and decoction extract as antifungal agents. In the second experiment, the best extract selected from the first study was used on yoghurt medium, to test its antifungal effects and to study the physico-chemical properties, colour characteristics, total phenol content, as well as microbial stability and sensory evaluation of yoghurt during storage time at 5°C for 4 weeks.

### 2.2- Organisms.

*a- Fungal strains:* *Aspergillus flavus* (*A. flavus*), aflatoxigenic local strain; *Aspergillus parasiticus* (*A.parasiticus*) NRRL 2999 and *Aspergillus ochraceus* (*A.ochraceus*) NRRL 3174, were obtained as lyophilized preparation from the Mycotoxin lab., National Research Center, Dokki, Giza, Egypt.

*b- Bacterial strains:* Starter culture of Yoghurt *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (1:1) was obtained from HACCP certified and ISO22000: 2005 Dairy Company, and used as a source of the starter culture.

### 2.3- Milk used for making of lab. Yoghurt:

Raw buffalo's milk used for making yoghurt, the milk was obtained from a dairy farm at Agriculture faculty, Cairo University Governorate. Starter cultures used for making plain yoghurt Old plain yoghurt obtained from HACCP certified & ISO22000: 2005 Dairy Company was used as a source of the starter culture.

#### 2.4- Plant material:

Lemongrass powder (leaves and stems) was purchased from an Egyptian local market (Harraz Co., Cairo, Egypt).

#### 2.5- Mycotoxins standards:

All standards of Mycotoxins (Aflatoxins, B<sub>1</sub>, B<sub>1</sub>, G<sub>1</sub>, G<sub>2</sub>, and ochratoxin A) were purchased from sigma company, USA.

#### 2.6- Analytical methods:

##### 2.6.1- Extract preparations of Plant material:

Lemongrass powder was purchased from a local market and the samples were extracted as follow:

a- Preparation of herbal decoction: Decoctions of lemongrass leaves were prepared by boiling the powder material at solid: liquid ratio 1:10 with distilled water for 5 minutes. The vessel containing the decoction and herb was then covered and removed from the heat and allowed to cool for 5 minutes. The herbal material and liquid was then strained through cheese cloth and the resulting decoction placed into 100 mL reagent bottles which had been kept for use as the test decoction (Abdel-Fattah and Abdel- Salam, 2004).

b- Preparation of herbal Oil: Briefly, 250 gm fresh plant material (leaves and stems) of lemongrass plant was put in a round bottom flask and 1000 mL distilled water was added before subjecting to hydro-distillation (Bankole and Joda, 2004) for 6 hours. The oil was recovered and dried over anhydrous sodium sulphate.

##### 2.6.2- Spore suspension of fungal strains:

These culture strains were grown on (PDA) slants for 10 days at 25 °C Until will sporulated . the spores were washed from the slants with a sterile 0.01% solution of tween 80 as a spore dispersal agent. The final spore preparations were resuspended in the appropriate volume of sterile saline to yield a direct microscopic count of approximately 10<sup>5</sup>-10<sup>6</sup> spores /mL, of each tested fungus.

##### 2.6.3- Preparation of yoghurt containing Lemongrass essential oil:

Raw buffalo's milk was subjected to a heat treatment at 72°C for 2 seconds to kill microorganisms followed by cooling to 40 – 45°C. Oil extract of Lemongrass was added to milk before processing with different concentrations. As starter culture yoghurt (*L.bulgaricus* and *S. thermophilus*) was added (1.5%) to the milk, followed by mixing,

and packed in sterilized glass capped cups 100 mL capacity, followed by incubation at 40°C for 3 hours till gel forms (pH 4.5). Freshly yoghurt was cooled and stored at refrigeration at 5°C till examination to slow down the physical, chemical and microbiological analysis.

##### 2.6.4- Antifungal assay:

2.6.4.1- With yeast extract sucrose medium (YES): Yeast extract- sucrose (YES) broth (2% yeast extract, 15% sucrose) was used in the 1st experiment as a basal medium for mould growth and mycotoxin production in stationary cultures (Davis *et al.*, 1966). Each medium (50 mL) in 250 mL Erlenmeyer flasks was sterilized at 121°C for 15 min. For each organism used, the appropriate amounts of lemongrass EO and lemongrass decoctions were added into sterile YES to obtain the concentrations of 0.05, 0.1, 0.3, 0.5 and 1.0 %. YES without any herbal extract added served as control. Each flask with or without appropriate extract was inoculated with 0.1 mL spore suspension, then cultures were incubated at 28°C in the dark for 14 days. Each concentration of tested extracts was tested twice, sometimes three times.

2.6.4.2- With yoghurt medium: The same antifungal assay on YES, was applied on yoghurt medium except that extract used was the EO extract, added to milk before processing and the culture media were incubated at two different temperature, 5 and 28 °C for 35 days. Yoghurt medium were inoculated with mycotoxicogenic fungi and stored for 35 days at 5 and 28 °C. Semi quantitative assay of the tested oil extract was conducted according to Harboure (1973).

##### 2.6.4.3- Evaluation of antifungal properties of lemongrass extracts.

a- *With YES broth media:* Contents of flasks, with and without (serve as the control) lemongrass extracts, were analyzed in triplicates for dry weight of mycelium and mycotoxin accumulation. Dry weight of mycelium was determined according the method of Davis *et al.*, (1966). Aflatoxins were extracted and determined according to the AOAC methods, (1980). Ochratoxin A (OA) was extracted and determined according to the method of Scott *et al.*, (1971). Percent reduction or accumulation over control, in growth or mycotoxin production was calculated by the following equation:

$$\text{Percent reduction} = 100 - \{(A_1 / A_0) * 100\}$$

$$\text{Accumulation over control} = \{(A_1 / A_0) * 100\} - 100$$

Were:  $A_1$  = The amount obtained by treatment.

$A_0$  = The amount obtained by control.

b- *With yoghurt medium:* Fungal growth of all tested molds were visually assessed using a semi-quantitative scale, Viz.(0) no growth; (1) very little growth covered the surface of the plate; (2) 25 % of the plate surface covered; (3) 50% of the plate surface covered; (4) 75 % of the plate surface covered and (5) 100% of the plate surface covered. Yoghurt medium were examined for the presence of aflatoxin production as described by the AOAC methods, (1980). For ochratoxin A, cultures were extracted, and OA levels were determined as described by Valenta and Michael, (1996).

#### 2.6.5- Microbiological stability of yoghurt:

The microbial stability of yoghurt containing the EO extract of lemongrass during storage at refrigerator (5°C) for 28 days were investigated. The populations of total bacteria, yeast and molds were determined by the method of Sadler, *et al.*, (1992). The counts of total bacteria (TPC), yeast and molds (Y&M) calculated per one gram of all yoghurt slices using plate count agar and malt extract agar (Merck KGaA, Darmstadt, Germany). The number of colonies (TPC or Y&M) that appeared on the plates was counted and expressed as Colony Forming Unit (CFU/g).

#### 2.6.6- Determination of pH:

The pH of fruit sample was measured using a combination pH electrode with a digital pH meter (HANNA, HI 902 meter, Germany) standardized with stirring as described in (AOAC, 2000).

#### 2.6.7- Determination of total soluble solids (TSS):

The percent total soluble solids, expressed as oBrix, were determined with a refractometer (ATAGO, Japan).

#### 2.6.8- Determination of Titratable acidity (TA):

Titratable acidity were determined as described by (Tung *et al.*, 1995) by using approximately 10 g portion of yoghurt sample blended with 100 mL distilled water for 30 sec in blender and was titrated to pH 8.0 with a 0.1N NaOH solution. The end point was determined with a pH-meter. Titratable acids in the sample were calculated as percent of lactic acid.

### 2.6.9- Viscosity measurements:

The viscosity measurements were carried out using a HAAKE viscometers (HAAKE, Mess-Technik GmbHu. Co., Germany) with thermostatic bath to control the working temperature within the

temperature of 25°C. Results of viscosity were expressed in centipoise (cP) according to the method of Ibarz *et al.*, (1994).

#### 2.6.10- Total phenol determination:

Total phenol content of the untreated and treated samples was measured by the method of Amerine and Ough (1980), the absorbance was measured at 765nm using Spectrophotometer, UVD-3500, Labomed, USA and the results were expressed as milligram of garlic acid as standard equivalent per gram.

#### 2.6.11- Colour characteristics determinations:

Colour is one of the more important quality parameters in processed products. Undoubtedly, possible colour changes would influence the Organolytic properties of samples and would limit their potential applications.

Hunter  $a^*$ ,  $b^*$  and  $L^*$  parameters were measured with a colour difference meter using a spectrophotometer (Tristimulus Colour Machine) with the CIE lab colour scale (Hunter, Lab Scan XE - Reston VA, USA) in the reflection mode. The instrument was standardized each time with white tile of Hunter Lab Colour Standard (LX No.16379):  $X=72.26$ ,  $Y=81.94$  and  $Z=88.14$  ( $L^*=92.46$ ;  $a^*=-0.86$ ;  $b^*=-0.16$ ) (Sapers and Douglas, 1987). Colour difference, Delta E, was calculated from  $a^*$ ,  $b^*$  and  $L^*$  parameters, using Hunter-Scotfield's equation (Hunter., 1975) as follows.

Delta E = ( $\delta a^2 + \delta b^2 + \delta L^2$ )<sup>1/2</sup> ----(1)  
 where : a -  $a_o$ , b -  $b_o$  and L -  $L_o$ ; subscript "o"  
 indicates colour of control or untreated sample.

The Hue-Angle ( $H^*$ ), Chroma ( $C^*$ ) and Browning Index ( $B_I$ ) was calculated according to the method of Palou *et al.*, (1999) as follows:

$$C^* = \text{square root of } [a^{2*} + b^{2*}] \quad \dots \dots \dots \quad (3)$$

Where:  $X = (a^* + 1.75L^*) / (5.645L^* + a^* - 3.012b^*)$

#### 2.6.12- Sensory Evaluation:

Sensory evaluation of the studied was carried out included 20 experienced panelists. The attributes such as: flavour intensity, body, texture and colour were organolptically assessed at stated by (Crandall, *et al.*, 1990). The all tested samples subjected to sensory evaluation after 28 days in yoghurt samples.

## 2.7- Statistical Analysis:

Analyses for experiments were performed in duplicated, and results were averaged. A Duncan Multiple Range Test was carried out by means of the "shortest significant ranges SSR" (Larmond, 1974)

to determine the differences between the treatments using HDSS statistical analysis program.

### 3. Results and Discussion:

#### 3.1- Antifungal effect of the two different extracts of lemongrass on YES borth medium:

Data presented in Table (1) clearly indicate that mould growth by all tested strains, were suppressed by lemongrass oil extract or decoctionextract. The inhibitory effect of these extracts was proportional with their concentrations. Slightly effect on fungal growth was observed when low concentration (0.05% and 0.1%, respectively) of the EO extract and decoction extract were applied, whereas high concentrations of these extracts inhibited fungal growth and, consequently, mycotoxin formation. The maximum inhibitory effect of these extracts were recorded at the level 1 % and 0.3% for decoction extract and EO extract, respectively (Table, 1).

In regard to Table (2), *A. ochraceus* was more sensitive one for the two lemongrass extracts than the two other molds. The EO extract was more effective agent on mycelial growth than decoction extract. The inhibitory effect of the two different extracts on mycelial growth according to the mold type, may be rankled as follow: *A.ochraceus* > *A.flavus* > *A. parasiticus*, for the EO extract. However, for decoction extract were: *A.ochraceus* > *A. parasiticus* > *A.flavus*. These differences in the inhibitory effect may be mainly due to interfering some factors: the mold type, incubation temperature and type of extract and subsequently the differences in the chemical composition for each extract. In this respect, many publications indicated that the compositions and concentrations of compounds within the distinct types of herbal extract preparations would differ and play an important role in its antifungal activity action (Buchanan and Shepard, 1981; Lienert *et al.*, and Nass *et al.*, 1998; and Abdel-Fattah and Abdel-Salsm, 2004). Also, El Gendy and Marth, 1980, reported that temperature is one factor affects mold growth and mycotoxin production by toxigenic aspergilli and penecillus and in the presence of lactic acid bacteria. Abdel-Fattah, (2002), found that mold type and incubation temperature were important factors affecting mold growth and aflatoxin production by *A. flavus* and *A. parasiticus* in media contained solvent extracts of licorice.

Data represented in Table (2) clearly indicate that increasing levels of the extract, in YES broth media, resulted in detection of decreasing levels of mycotoxin production. At the lowest level (0.05% extract), reduction in mycotoxin production was slightly decreased in the media supplemented

with decoction extract compared to those supplemented with oil extract. Increasing extract concentrations caused a linear depression in mycotoxin formation by the all tested molds, but the maximum inhibitory effect was differ, and this may be referred that organism was more variable in its reaction to the extract. This trend indicates that extract inhibited mycotoxin formation by inhibiting the mould growth. In a similar study, Masood and Ranjan (1991) reported that extracts of *Argemone mexicana* and *cyperus rotundus* inhibited aflatoxin production by inhibiting the growth of *A. flavus*. Also, Mahmoud, (1994), found that extracts of *lupinus* and *xanthium punens* inhibited aflatoxin production by inhibiting the growth of *A. flavus*.

Reduction of fungal growth and mycotoxin production by the EO extract in our study was due to interference by active principles of these extracts. Such interference may be at the biosynthetic levels. In this respect, Kumar and Prasad (1992) suggested that growth and aflatoxin production by *A. flavus* are proportionate processes. However, Bhatnagar and McCromick (1987) reported that the growth and aflatoxin production by *A. parasiticus* are independent phenomena. On the other hand, Abu-Seif, *et al.*, (2009) reported that oil extract of lemongrass leaves and stems, completely inhibited mycelial growth and mycotoxin production of *A. flavus*, *A. parasiticus* and *A. ochraceus* at level (0.3 %) of YES broth medium.

Data represented in Table (1) showed that there were a considerable differences in mold or mycotoxin inhibition in YES medium supplemented with the EO extract or the decoction extract, in trend to EO extract. Therefore, the EO extract of Lemongrass leaves and stems was selected, as the best, to examine its antimicrobial effect on yoghurt medium.

#### 3.2- Antifungal effect of lemongrass oil on yoghurt medium:

Results obtained from Table (3) showed that increasing levels of EO extract added to yoghurt either incubated at 5 or 28 °C for 28 days, an inhibitory effect was noted on the growth of the all tested molds. At the lowest concentrations of extract (0.05%), the mold growth by the tested molds, were comparatively no changed over control. However, increasing concentration level up to 0.1% completely prevented the mold growth, either when the media incubated at 5or 28 °C. These results also indicate that growth was influenced by both mold type and incubation temperature. Also, these results revealed that 28 °C was the optimum incubation temperature for growth and consequently, mycotoxin formation occurred in this study.

**Table (1): Mold growth (mg/50 mL media) and percent reduction of the tested molds as affected by the two different extracts of lemongrass on YES broth media for 14 days at 28° C.**

Extract level, %	Mold growth and percent reduction for different toxigenic strains					
	<i>A. flavus</i>		<i>A. parasiticus</i>		<i>A. ochraceus</i>	
	Mould growth	percent reduction	Mould growth	percent reduction	Mould growth	percent reduction
<b>Control, 0.0%</b>	410±13.4 <sup>F</sup>	0.0	305±14.8 <sup>F</sup>	0.0	840±10.5 <sup>F</sup>	0.0
<b>Decoction extract</b>						
0.05	325±16.5 <sup>E</sup>	20.73	251.5±19.5 <sup>E</sup>	17.54	599.6±16.3 <sup>E</sup>	28.62
0.1	304±8.3 <sup>E</sup>	25.85	251.5±8.3 <sup>E</sup>	17.54	538±22.4 <sup>D</sup>	35.95
0.3	259±13.0 <sup>D</sup>	36.83	217.5±14.7 <sup>D</sup>	28.70	315±16.5 <sup>C</sup>	62.50
0.5	192±21.5 <sup>C</sup>	53.17	176.5±9.5 <sup>C</sup>	42.13	110±8.7 <sup>B</sup>	86.90
1.0	175±13.5 <sup>C</sup>	57.32	170.0 ±7.3 <sup>C</sup>	44.26	80±7.2 <sup>B</sup>	90.48
<b>EO extract</b>						
0.05	180±11.6 <sup>C</sup>	56.10	65±0.9 <sup>B</sup>	78.69	113±11.4 <sup>B</sup>	86.55
0.1	112±5.8 <sup>B</sup>	72.68	0.0 <sup>A</sup>	100	0.0 <sup>A</sup>	100
0.3	0.0 <sup>A</sup>	100	0.0 <sup>A</sup>	100	0.0 <sup>A</sup>	100
0.5	0.0 <sup>A</sup>	100	0.0 <sup>A</sup>	100	0.0 <sup>A</sup>	100
1.0	0.0 <sup>A</sup>	100	0.0 <sup>A</sup>	100	0.0 <sup>A</sup>	100
<b>LSD at (p ≤ 0.05)</b>	28.8	-	21.2	-	36.5	-

Each value represents the mean ± SE of three replicates.

**Table (2): Mycotoxin production and percent reduction of the tested molds as affected by the two different extracts of lemongrass on YES broth media for 14 days at 28° C.**

Extract level, %	Mycotoxin production (µg per 50 mL YES) and percent reduction for different toxigenic strains.					
	<i>A. flavus</i>		<i>A. parasiticus</i>		<i>A. ochraceus</i>	
	Total aflatoxins	percent reduction	Total aflatoxins	percent reduction	Ochratoxin A	percent reduction
<b>Control, 0.0%</b>	265	0.0	345	0.0	270	0.0
<b>Decoction extract</b>						
0.05	180	32.07	295	14.49	150	44.44
0.1	135	49.06	210	39.13	103	61.85
0.3	55	79.24	73	78.84	45	83.33
0.5	56	79.24	31	91.01	0.0	100
1.0	0.0	100	0.0	100	0.0	100
<b>EO extract</b>						
0.05	125	52.83	113	67.25	28	89.63
0.1	105	60.38	93	73.04	0.0	100
0.3	0.0	100	0.0	100	0.0	100
0.5	0.0	100	0.0	100	0.0	100
1.0	0.0	100	0.0	100	0.0	100

Each value represents the mean of three replicates.

The mold growth was higher by *A. flavus* and *A. ochraceus* than *A. parasiticus*. Also, growth was influenced by mold type and temperature degree of incubation. Temperature is one factor which affects mold growth and mycotoxin production. Other publications supported our results (EL-Gendy and Marth, 1980; Maschaly and El-Deeb, 1982, Abdel-Fattah, 2002, and Abdel-Fattah and Abdel-Salam, 2004). When mycotoxin production was

determined in the media incubated at 5 and 28°C, the effect of EO extract was even more striking (Tables 4 and 5). Increasing concentration of extract resulted in detection of decreasing levels of mycotoxin production. At the lowest level of extract (0.05%), a great reduction mycotoxin production were found, and the reduction was especially pronounced with aflatoxins and ochratoxin A. No toxin was detected when the extract level increased to 0.1%.

**Table (3): Effect of various concentrations of lemongrass essential oil on molds incubated for 28 days at 5 and 28°C.**

Extract , %	<i>A. flavus</i>		<i>A. parasitics</i>		<i>A. ochraceus</i>	
	5 °C	28 °C	5 °C	28 °C	5 °C	28 °C
<b>Control, 0.0%</b>	3	5	3	5	2	4
<b>EO extract</b>						
0.05	2	4	3	4	1	3
0.1	0	0	0	0	0	0
0.3	0	0	0	0	0	0
0.5	0	0	0	0	0	0
1.0	0	0	0	0	0	0

(0) no growth; (1) very little growth ; (2) 25 % of the plate surface covered with mycelia ; (3) 50% of the plate surface covered with mycelia; (4) 75 % of the plate surface covered with mycelia and (5) 100% of the plate surface covered with mycelia.

Regarding results represented in Tables (3, 4 and 5), the lack of mycotoxin production on yoghurt raises questions concerning the reasons for this phenomenon. The possibility that ingredients contained in yoghurt, but not in YES, might be inhibitory to growth and toxin production was considered. These results may be supported by those obtained by Montagna *et al.*, (1998), Velluti *et al.*, (2003), and Abdel-Fattah and Abdel-Salam, (2004).

It is possible that antifungal activity of the used lemongrass EO in this study is due to an unidentified component of the antioxidants extracted (perhaps phenols, flavonoids, flavones, etc). In this respect, Rosenthal *et al.*, (1997) reported that phenols play an important role as antifungal agents. They found that

ferulic acid, p-coumaric acid and other plant cell wall phenols, had antifungal actions in microorganisms isolated from dairy products. Also, Abu-seif *et al.*, (2009), found that phenolic compounds extracted from Lemongrass leaves had antifungal effects on *A. flavus* and *A. parasiticus*. In the present investigation, the lemongrass EO and its components exhibited efficacy against *A. flavus*, *A. parasiticus*, *A. ochraceous* and mycotoxin production, emphasising their significance in quantitative as well as qualitative control of herbal raw materials because tropical and subtropical countries are chiefly affected by mycotoxins (Singh *et al.*, 2008; Abu-Seif, *et al.*, 2009).

**Table (4): Effect of various concentrations of lemongrass essential oil on mycotoxin production in yoghurt medium for 28 days at 5 and 28°C.**

Extract level	Mycotoxin production (µg per 50 mL YES)						
	At 5°C				At 28°C		
	Total Aflatoxins		Ochratxin A	Total Aflatoxins		Ochratxi n A	
	A. Flavus	A. parasiticus		A. Flavus	A. parasiticus		
<b>Control, 0.0%</b>	35.3	84.0	116.0	108.6	142.5	185.0	
<b>EO extract</b>	0.05	0.0	0.0	65	56.5	0.0	
	0.1	0.0	0.0	43	40.15	0.0	
	0.3	0.0	0.0	0.0	0.0	0.0	
	0.5	0.0	0.0	0.0	0.0	0.0	
	1.0%	0.0	0.0	0.0	0.0	0.0	

- Each value represents the mean of three replicates.

**Table (5): percent reduction in mycotoxin production over control by mold with lemongrass essential oil in yoghurt media for 28 days at 5 and 28° C.**

Extract level	Percent reduction						
	At 5°C			At 28°C			
	Total Aflatoxins		Ochratoxin A	Total Aflatoxins		Ochratoxin A	
	A. Flavus	A. parasiticus		A. Flavus	A. parasiticus		
<b>Control,</b> 0.0%	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Oil extract</b>							
0.05	100	100	100	40.14	60.35	100	
0.1	100	100	100	60.40	71.82	100	
0.3	100	100	100	100	100	100	
0.5	100	100	100	100	100	100	
1.0%	100	100	100	100	100	100	

Each value represents the mean of three replicates.

3.3- The effect of essential oil of lemongrass on the log (CFU/gm) of growth yeast and molds (Y&M) and bacteria in yoghurt during storage at refrigerator for 4 weeks.

The microbial stability of yoghurt supplemented with extracts of lemongrass during storage at refrigerator (5°C) for 4 weeks were investigated. Tropical spices (lemongrass) may prove useful in preservation of yoghurt by hurdle technology (Ejechi, *et al.*, 1998). Total microbial count of different yoghurt treatments with 0.0, 0.05, 0.1, and 0.3 % of lemongrass EO, and of untreated yoghurt were followed up through 4 weeks at 5°C. The effect of treating yoghurts with the studied various volatile or essential oil extract and stored at 5°C for 4 weeks on inhibiting the microbial counts are seen in Table (6). It can be observed that the yoghurt treated with low concentration of lemongrass EO have the highest inhibition of yeast and molds (Y&M) and bacteria (B) followed by those treated with high concentration of lemongrass EO after 4 weeks at 5°C. Untreated yoghurt was 6.75 log (CFU/gm) of B compared to 0.00 log (CFU/gm) in case of those Y&M. Whereas, the yoghurt treated with 0.5% lemongrass was 0.00 log (CFU/gm) of Y&M and 6.10 log (CFU/gm) of B.

The results from Table (6) showed that the yoghurt treated with high concentration of lemongrass has also the highest reduction of Y&M and B followed by low concentration of lemongrass, but untreated samples were the lowest reduction of Y&M and B for 4 weeks stored at 5°C.

These results are partially confirmed by those of Kanako *et al.*, (1998). They found that Lemongrass and clove exhibiting strong anti-fungal activity for 30 days. Whereas no colonies were seen for 30 days and fungal growth was inhibited for more than 30 days. On the other hand, Sebti and Tantaoui, (1994) showed that cinnamon powder

although very efficient at inhibiting the fungi, imported a dark colour to the papers and therefore is not recommended. While, cinnamon water extract did not inhibit fungal growth up to concentration of 80 g/kg (8%). Also, results from Table (6) showed that refrigeration temperature 4 °C of yoghurt could enhance the inhibitory effect of lemongrass EO. These results nearly in consistent with results given by Eissa *et al.*, (2003a, b), Eissa *et al.*, (2008) and Ting and Deibel, (1992) who appeared that refrigeration temperature (5°C) could enhance the inhibitory effect of some spices extracts but not others. When 0.5 or 1.0 % cloves were tested, the organism died more rapidly in tryptic soy broth at 24°C than at 5°C. Whereas, > 5 log reduction in CFU was observed after 7 days of incubation at 5°C and after 3 h incubation at 24°C in tryptic soy broth.

In general, the refrigeration of yoghurt effects increased the inhibition of bacteria, yeast and mold counts. Also, the results showed that the yoghurt treated with different concentrations of lemongrass EO were observed no browning and lowest microbial count (T & M and B) during storage at 5°C for 4 weeks. There are only a few reports on antimicrobial activity of lemongrass EO against some food borne bacteria (Oussalah *et al.*, 2006) and fungi (Anthony *et al.*, 2003). Lemongrass EO as preservatives may be due to it contain aldehydes and volatile compounds that have efficient on inhibition of browning and inhibition of growth microorganisms. Zaika, (1988) reported that food product safety and shelf life depend in some part on the type, quantity, and character of volatile oil spices extracts added to the products. Then, our results showed that refrigerating at 5°C and 0.05% volatile or essential oil extracts treatments caused a marked reduction in yeast and bacteria populations with acceptable taste and extension shelf life of yoghurt up to 5 weeks.

**Table 6. Effect of lemongrass essential oil on the number of colonies (TPC or Y&M) that appeared on the plates and expressed as the log (CFU/gm) in yoghurt during storage at refrigerator (5 °C) for 4 weeks.**

Extract level,%	Zero time		1 week		2 weeks		4 weeks	
	*M&Y	TPC	M&Y	TPC	M&Y	TPC	M&Y	TPC
0.0	0	6.1	0	6.50	0	6.50	0	6.75
0.05	0	5.95	0	6.20	0	6.30	0	.506
0.1	0	5.90	0	6.00	0	6.10	0	5.95
0.3	0	5.90	0	5.90	0	6.00	0	6.10

- Each value represents the mean of three replicates

### 3.4- Effect of lemongrass EO on physico-chemical content in yoghurt during storage time:

#### Quality evaluation of yoghurt products.

The following discussion of the chemical characteristics for fresh, products and lemongrass EO pre-treated yoghurt is based on the data given in table (7). The pH of fresh and treated yoghurt ranged from 4.04 to 4.49 showing a increase in pH values. The increase in pH was directly related to increase lemongrass EO concentrations in yoghurt. TSS (g/Kg) of yoghurt products after 28 days storage was lower than the fresh yogurts. Whereas, the increase of TSS was obvious with increasing of lemongrass EO concentration. This increase of TSS was attributed to the greater degree of tissue breakdown, releasing more components that contribute to soluble solids (Tung *et al.*, 1995). The TSS / acid ratio is the major analytical measurement for quality in fresh

and treated yoghurt. The TSS / acid ratio of fresh, and treated yoghurt was increased by increasing of lemongrass EO concentration. TSS / acid ratio was shown to be correlated with sweetness but not so closely with flavour (Guyer, *et al.*, 1993).

Titratable acidity of yoghurt products was lower than fresh yoghurt (Table 7), which may due to enzymatic desertification and degradation of pectin resulting in an increased of total acid.

The viscosity (cP) was selected as a measure of yoghurt quality. However, the viscosity between fresh and treated yoghurt samples were decreased from 2.92 cP to 2.64 by increasing the lemongrass EO concentration in yoghurt than fresh sample (2.86 cP), respectively as seen in Table (7). Total phenol content were decreased also by increasing of lemongrass EO concentration in all yoghurt samples, as seen in table (7).

**Table (7): Effect of Concentrations on physico-chemical properties in yoghurt at zero time and after 28 days at 5 °C.**

Extract level,%	TSS		pH		% acidity		TSS/acidity		Viscosity (cP)		Total Phenols	
	at zero time	after 28 days	at zero time	after 28 days	at zero time	after 28 days						
0.0	7.50	6.00	4.04	4.39	0.94	0.94	7.8	6.38	2.86	2.60	1342.85	1342.85
0.05	7.00	6.00	4.00	4.30	0.96	0.92	7.4	6.51	2.92	2.80	1345.97	1345.97
0.1	7.50	7.00	4.08	4.39	0.97	0.94	7.7	7.44	2.75	2.60	1338.17	1338.17
0.3	8.00	7.00	4.11	4.49	0.98	0.95	8.17	7.36	2.64	2.52	1317.87	1317.87

### 3.5- Effect of lemongrass EO concentrations and storage on Colour characteristics of yoghurt:

#### a- Colour parameters during storage time of yoghurt:

Tristimulus Reflectance Colourimetry (TRC) measuring the reflectance L\*, a\* and b\* values) was used to follow the extent of browning in yoghurt and change of colour in foods (Sapers and Douglas., 1987). The results in Table (8) showed change of colour in yoghurt during storage time up to 28 days at 5°C. These results illustrated the changes in colour of yoghurt in terms of redness a\*, yellowness b\* and lightness L\* during 28 days of storage at 5°C. In addition to determination of the lightness L\*, redness a\* and yellowness b\*, for

experimented yoghurt. Hue angle (H\*) as well as the chromaticity (C\*) were determined. Hue is the aspect of colour that we describe by words such as green, blue, yellow or red. The chroma refers to reflection at given wavelength and indicates how much a colour differs from gray (Eissa and Moharram, 2001). The equations No. 1, 2, 3 and 4 are showed the DE, B<sub>1</sub>, H\* and C\*.

The H\* values were closely stable in all samples with increasing of storage time up to 28 days at 5°C. The chromaticity (C\*) increased by the increasing of stoarge time in yoghurt up to 28 days. Thereafter, no relation was noticed. It can be observed that the a\*-value of the fresh yoghurt was -2.22 compared to -2.27 after 7 days, -2.39 after 14

days and increased -2.18 after 28 days, as seen in Table (8).

Regarding the lightness L\* and the yellowness b\*. It is clear that the lightness L\* as well as the yellowness b\* were decreased as a result of increasing the time of storage up to 28 days. The effect of storage time on increasing the a\*-value from -2.22 at zero time days to -2.18 after 28 days was noticed. The change in colour may be referred to chemical changes occurred during storage (Kumar *et al.*, 2006).

The analysis of variance identified the significant ( $p<0.05$ ) effect of storage time on Hunter values of yoghurt. Although the a-value showed a definite increased trend throughout storage, the L-value decreased and the b-value increased as the yoghurt storage aged, as seen in Table (8).

It can be concluded that the storage of yoghurt slightly inhibited the changes in colour yoghurt. The total colour differences (DE) increased by the increasing of storage time in yoghurt up to 28 days as presented in Table 1, total colour differences of yoghurt were small, which almost correspond to the sensory difference threshold (Rohm and Jaros, 1996b). However, greatly different values of DE were found for yoghurts at 7, 14 and 28 days of storage and at different concentrations lemongrass

EO treated samples. The almost identical colour values found in yoghurt could be attributed to their similar structure. The browning index (B<sub>I</sub>) increased by the increasing of storage time in yoghurt up to 28 days, especially in high concentration treated yoghurt (0.3%). Thereafter, no relation was noticed.

#### b- Non-enzymatic browning of yoghurt samples:

Non-enzymatic browning in yoghurt is only one component that determines overall colour and might not be a problem at low levels. The effects of heat treatment of milk and yoghurt products in the inhibition of the browning reaction are listed in Tables (8). It is obvious that the yoghurt product treated and untreated yoghurt samples inhibits the development of A 420 nm and red colour a\*. For example, the A420 nm and a\*-value of fresh yoghurt was 64.34 compared to 64.11, 64.36 and 61.56 in case of the different concentrations of lemongrass EO yoghurt samples, respectively. Crandall *et al.*, (1986) concluded that two measures of browning were used, colour a\* or L\* and absorbance at 420nm where the higher numbers indicate increased absorbance due to the formation of brown pigments. Browning is also indicated by a decrease in the colour L\* toward black and an increase in the colour a\* toward brown or red.

**Table (8): Effect of on colour characteristics in yoghurt during storage at 5 °C for 28 days.**

Extract level%	L*	a*	b*	Delta E	C*	H*	B <sub>I</sub>	OD 420 nm
At zero time								
0.0	92.22	-2.22	11.38	11.65	11.59	78.96	20.15	64.34
0.05	91.89	-2.14	11.04	11.31	11.25	79.03	19.60	64.11
0.1	92.19	-2.25	11.38	11.65	11.60	78.82	20.11	64.26
0.3	91.33	-2.27	11.91	12.22	12.12	79.21	21.46	61.56
Storage after 7 days								
0.0	93.68	-2.27	11.75	12.04	11.97	79.07	20.54	65.83
0.05	93.41	-2.24	11.08	11.35	11.30	78.57	19.20	66.3
0.1	93.97	-2.09	10.21	10.52	10.42	78.43	17.44	68.88
0.3	93.51	-2.38	10.32	10.63	10.59	77.01	17.34	68.34
Storage after 14 days								
0.0	92.4	-2.39	11.94	12.19	12.18	78.68	21.08	64.3
0.05	92.33	-2.09	11.12	11.34	11.31	79.36	19.75	64.77
0.1	92.09	-2.53	13.01	13.27	13.25	79	23.33	61.71
0.3	92.97	-2.23	12.12	12.35	12.32	79.57	21.57	63.56
Storage after 28 days								
0.0	93.2	-2.18	10.47	10.74	10.69	78.24	18.02	67.83
0.05	92.6	-2.25	11.92	12.17	12.13	79.31	21.20	64.47
0.1	91.69	-2.04	11.25	11.52	11.43	79.72	20.26	63.63
0.3	92.4	-2.59	13.75	14.03	13.99	79.33	24.82	60.63

#### 3.6- Sensory evaluation of Yoghurt:

The results of sensory evaluation of the products based on colour, odour, taste, texture and

appearance are shown in Table (9). Sensory evaluation of the yoghurt samples was carried out during 4 weeks of samples at 5°C by 20 experienced

panels using 10 points scales. Difference in sensory properties of yoghurt samples due to the effect of different concentrations of lemongrass EO was determined by analysis of variance (ANOVA). Sensory attributes are of great importance to measure consumer attitudes and their influence on food choice and acceptability. The colour of yoghurt is the first quality attribute used to judge acceptability of yoghurt products. The change in colour may be referred to chemical changes occurred during storage of yoghurt samples (Young-Hee, and Song Sun, 2009). The mean value of flavor or odour scores as well as the overall mean score for the texture of yoghurt for all treatments of the tested yoghurt samples were affected by refrigeration and by increasing concentration of lemongrass EO up to 0.3 % (Table, 9). These levels of score indicate the importance of the lemongrass EO in keeping a texture for the tested yoghurt. However, there were significant differences between the individual ripening. Samples pre-treated for 0.3% lemongrass EO showed the highest score (8.25) in texture stored at 5°C. In general, the lemongrass EO pretreated yoghurt samples received higher sensory scores than

untreated sample, but the differences were nonsignificant ( $P<0.05$ ) for all samples (Table, 9). Samples treated with 0.05 and 0.1 % EO extract generally had better score for all sensory characteristics at all samples. On the contrary, the sample treated with 0.3% EO extract had lower score for all sensory characteristics at all samples especially in taste characteristics. However, concentration of lemongrass EO had a positive influence on acceptability of colour and flavor of yoghurt samples. Also, increased concentration of lemongrass EO showed that the same odour, colour texture and acceptability characteristics in hoghurt samples. However, all sensory scores were in the acceptable range, which greater than 5 scores.

It is clear that the the lemongrass EO pretreated yoghurt gave higher mean panel scores (8.0- 8.5) than the untreated yoghurt sample, which were the most preferred in all the studied characteristics. The lemongrass EO pretreated yoghurt samples with different concentrations had a non-significant difference ( $P<0.05$ ) between these samples.

**Table (9): effect of lemongrass EO concentrations on sensory evaluation of youghurt stored at 5°C for 28 days.**

Extract level, %	Appearance	Texture	Colour	Taste	Odour
0.0	8.13 <sup>A</sup>	8.13 <sup>A</sup>	8.75 <sup>A</sup>	8.50 <sup>A</sup>	8.13 <sup>A</sup>
0.05	8.02 <sup>A</sup>	8.03 <sup>A</sup>	8.50 <sup>A</sup>	7.13 <sup>B</sup>	7.85 <sup>A</sup>
0.1	8.50 <sup>A</sup>	8.13 <sup>A</sup>	8.50 <sup>A</sup>	7.13 <sup>B</sup>	7.79 <sup>A</sup>
0.3	8.63 <sup>A</sup>	8.25 <sup>A</sup>	8.38 <sup>A</sup>	6.88 <sup>B</sup>	7.65 <sup>A</sup>
LSD	NS	NS	NS	S	NS

LSD = least significant difference at 0.05 level.

NS = non significant

S = significant

#### 4- Conclusion:

While the primary function of Lemongrass may not be preservative in nature, it has preservative properties, which may useful in built-in safety systems in food. In addition, Lemongrass herb is cheap, safe, and had a medical functions. Our results show that water extract from Lemongrass, at level 0.1% was effective agent to inactivate mold growth and mycotoxin formation and increasing level to 0.3% completely prevented both mold growth and mycotoxin production , for the all tested molds, in YES broth medium. However, in yoghurt medium its inhibitory effect was different according to mold type, supplemented concentration and constituents of the used medium. Its evident from our data that, if possible, a sufficient amount of lemongrass EO to prevent mold growth needs to be used if one wishes to prevent mycotoxin production. It can be concluded from the results of this work that

of lemongrass EO treatments with refrigeration at 5°C may serve as alternative to conventional chemical preservatives in the preservation of yoghurt by hurdle technology.

#### Corresponding author

Shaaban, M. Abd-El Fattah  
Food Toxins and contaminants Department National Research Centre, Cairo, Egypt.  
[shaabanmostafa@yahoo.com](mailto:shaabanmostafa@yahoo.com)

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8/21/2010

# The Assessment Of Mycotic Settlement Of Freshwater Fishes In Egypt

Refai, M.K.<sup>1</sup>, Laila, A. Mohamed<sup>2</sup>, Amany, M. Kenawy<sup>2</sup>, and Shima, El-S.M.A.\*<sup>2</sup>

<sup>1</sup> Microbiology Dept., Faculty of Vet.Medicine, Cairo University, Giza, Egypt.

<sup>2</sup> Hydrobiology Dept., National Research Center. Dokki, Giza, Egypt.

\* [shimaakhalifa2003@yahoo.com](mailto:shimaakhalifa2003@yahoo.com)

**Abstract:** This study was carried out on 360 freshwater fishes (240 *Oreochromis* species and 120 *Clarias gariepinus*). They were collected from different governorates and during different seasons. Naturally infected fishes showed clinical abnormalities such as skin darkening, exophthalmia, corneal opacity, abdominal distention, ulceration of the skin and cotton wool like growths on various parts of the body. Fishes were then subjected to post mortem examination which revealed many abnormalities. Mycological examination revealed the isolation of 2081 fungal isolates from 150 diseased and 210 apparently healthy fish samples (1658 mould and 423 yeast isolates), of which 1334 were isolated from *Oreochromis* species and 747 isolates from *Clarias gariepinus*. Isolated moulds belonged to the following genera: *Saprolegnia* (4.2%), *Aspergillus* (43.0%), *Fusarium* (14.1%), *Mucor* (14), *Penicillium* (17.2), *Rhizopus* (4.8%), *Scopulariopsis* (1.2%), *Paecilomyces* (1%) and *Curvularia* (0.4%). Yeasts isolated also from both fish species had the following incidence: *Candida albicans* (35.9 %), other *Candida* species (19.1%), *Rhodotorula* species (31.4%) and *Torulopsis* species (13.5%). Experimental infection with the most predominant fungi (*Aspergillus flavus*, *Fusarium* species and *Candida albicans*) was conducted to evaluate the pathogenicity of these isolates. Clinical pictures of experimentally infected fish were similar to those of natural infection. Inoculated fungi were re-isolated from different organs. Results were confirmed with histopathological examination, which revealed the presence of fungal hyphae and spores in different organs.

[Refai, M.K., Laila, A. Mohamed, Amany, M. Kenawy, and Shima, El-S.M.A. The Assessment Of Mycotic Settlement Of Freshwater Fishes In Egypt. Journal of American Science 2010;6(11):595-602]. (ISSN: 1545-1003).

**Keywords:** Mycotic infection, *Oreochromis* species, *Clarias gariepinus*, Moulds, Yeasts, *Aspergillus*, *Fusarium*, *Candida*, *Penicillium*.

## 1. Introduction

Many of the fungi that affect fishes are considered opportunists, attacking the fishes when they are stressed or immunocompromised because of unfavorable environmental conditions, or secondary to bacterial or viral infections, or when they have lost their mucus protection because of trauma or excessive handling (Roberts 1989 and Quiniou *et al.*, 1998). Mycotic infections of fishes by Oomycetes are wide spread in freshwater and represent the most important fungal group affecting wild and cultured fishes. The *Saprolegniaceae*, in particular members of the genus *Saprolegnia*, are responsible for significant infections involving both living, dead fishes and eggs. Oomycetes are classical saprophytic opportunities, multiplying on fishes that are physically injured, stressed or infected (Pickering and Willoughby, 1982). Members of this group are generally considered agents of secondary infection arising from conditions such as bacterial infections, poor husbandry, and infestation by parasite and social interaction. However, there are several reports of Oomycetes as primary infectious agents of fishes (Pickering and Christie, 1980) and their eggs (Walser

and Phelps, 1993). Moreover, there are other fungi that have been implicated in fish diseases. Some of the genera involved include *Aspergillus* (Salem *et al.*, 1989b), *Fusarium* (Bisht *et al.*, 2000), *Ichthyophonus* (Faisal *et al.*, 1985), *Branchiomyces* (Easa 1984), *Phoma* (Hatai *et al.*, 1986), *Paecilomyces* (Lightner *et al.*, 1988), *Exophialia* (Langdon and MacDonald 1987), *Phialophora* (Ellis *et al.*, 1983), *Rhizomucor* (Wolf and Smith 1999) and *Candida* (Neish and Hughes 1980). Most of these are multiple case reports or single, and causing systemic disease with high mortality rates in fishes. The objective of this study was to determine the types of fungal pathogens affecting freshwater fishes specially those causing high mortality rates, elucidation of the incidence and distribution of such pathogens in *Oreochromis* species and *Clarias gariepinus*, studying the seasonal variations enhancing fungal diseases of fishes and determination of the pathogenicity of the most prevalent isolated fungi.

## 2. Material and Methods

A total number of 360 fish were observed for their behavior, external lesions prior to autopsy. Then they were killed and examined. The examination included external changes as well as examination of internal organs. Wet mount preparation of fish samples were commonly made in 10% KOH. A simple stain such as lactophenol cotton blue was used. The preparation was examined microscopically after about 30 minutes for fungal elements. Mycological examination was done according to and (1993 Noga). Identification of moulds was carried out according to Refai (1987). Identification of yeasts: Plates were examined microscopically for the presence of chlamydospores, arthrospores and blastospores (Refai, 1987) and the scheme of identification of yeasts given by Terrence (1971). Urease test (Cruickshank *et al.*, 1975). Suspected *Candida* species were scratched onto rice or corn meal agar for pseudohyphae and chlamydospores production (Larone, 1987) and a confirmatory identification was carried out by germ tube test (Martin, 1979).

### Histopathological examination:

Tissue samples were prepared according to Roberts 1989. and stained by periodic acid Schiff's (PAS) and GMS (Sheehan and Hrapchak 1980).

### Experimental infection:

A total of 120 *Oreochromis* species with 30-40 g average body weight were used. They were divided into four equal groups (each one contained 30 fish). Each group were subdivided into three sub-groups, each contained 10 fish.

**Preparation of spores suspension for experimental infection:** Inocula were prepared by spreading 5 ml of sterile phosphate buffer saline over the plates containing 7- 10 day old pure cultures of *Aspergillus flavus* and *Fusarium* sp. The spores were harvested by gentle washing of the surface of the colonies with sterile loop, then transferred aseptically to sterile flasks. Two drops of tween 80 were added to avoid clumping of spores in case of *Aspergillus flavus* group. Spores were counted by aid of haemocytometer and suspension was diluted to reach  $9 \times 10^4$  spores/ml for both *Aspergillus flavus* and *Fusarium* sp.

**Preparation of yeast suspension for experimental infection:** A loopfull of one day old pure yeast culture of *Candida albicans* was added to test tube containing 5 ml of sterile phosphate buffer saline and mixed gently to reach equal distribution. Spores were counted by using haemocytometer then

suspension was adjusted to reach  $2 \times 10^3$  *Candida* spores per ml.

## 3. Results and Discussion

Mycological examination revealed the isolation of 2081 fungal isolates from 150 diseased and 210 apparently healthy fish samples, of which 1334 were isolated from *Oreochromis* species and 747 isolates from *Clarias gariepinus*. Identification of fungi into yeasts and moulds revealed that the percentage of moulds was slightly higher in *Oreochromis* species (80.5%) in comparison to that in *Clarias gariepinus* (78.2 %). On other hand, the rate of yeast isolates per fish was slightly higher in *Clarias gariepinus*. Isolated moulds belonged to the following genera: Saprolegnia, Aspergillus, Fusarium, Mucor, Penicillium, Rhizopus, Scopulariopsis, Paeciliomyces and Curvularia. The same fungal isolates were reported by Abdel Alim (1992) and Khalil (1993).

The Incidence of moulds in diseased and apparently healthy fishes were recorded in (Fig.1&2), also the incidences of isolated moulds from different organs of *Oreochromis* species (Fig.3) and *Clarias gariepinus* (Fig.4) were detected. Seasonal incidences were seen in (Fig. 5). As these isolates were recovered from apparently healthy and clinically diseased *Oreochromis* species and *Clarias gariepinus* This was expected, as almost all these fungi were categorized by Shaheen (1986) as normal mycoflora. This does not mean that they cannot produce disease. They can better be considered as opportunistic fungi (Refai, 1987) as many of them possess virulence factors, which enable them to cause diseases (Refai *et al.*, 2004), particularly under favourable predisposing condition. Regarding to seasonal incidence *Saprolegnia* species were isolated with high incidence in Winter, followed by early Spring and late Autumn. This result agrees with Naguib (1994), who stated that the seasonal variations play an important role in spreading of the *Saprolegnia* infection among freshwater fishes especially during late Autumn, Winter and early Spring, where the water temperature was low.

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Clinical findings of *Oreochromis* species inoculated with *Aspergillus flavus*, *Fusarium* species and *Candida albicans* revealed that exophthalmia (Photo.21), skin darkening (Photo.22), cotton wool-like growth on various parts of the body (Photo.23&24), moderate abdominal distention (Photo.25) and corneal opacity and haemorrhages all over the body surface (Photo.26). These results are supported by Marzouk *et al.*(2003).

Postmortem finding revealed congestion and ulceration of gills (Photo.27), haemorrhagic abdominal fluids, necrotic foci within liver and distention of gall bladder (Photo.28), multiple nodules within spleen (Photo.29) and severe intestinal congestion (Photo.30) were also observed. On the other hand, no clinical or postmortem changes

were detected on fish groups maintained at 18°C. These findings are in agreement with those of Refai *et al.* (1987).

It can be concluded from the results obtained in the present work that, though most fungi isolated from fishes are considered by several authors as normal mycoflora, yet we could prove in the present study that many fungi can cause natural infections. This was confirmed by histopathological reactions characteristic of fungal infection in naturally infected fishes, and the presence of fungal elements in the lesions. This was substantiated also by experimental infection of fish that induced similar findings as the natural infection, i.e. a clear application of Koch's postulate. This conclusion should direct our attention to the possible role of fungi in affecting fishes industry.

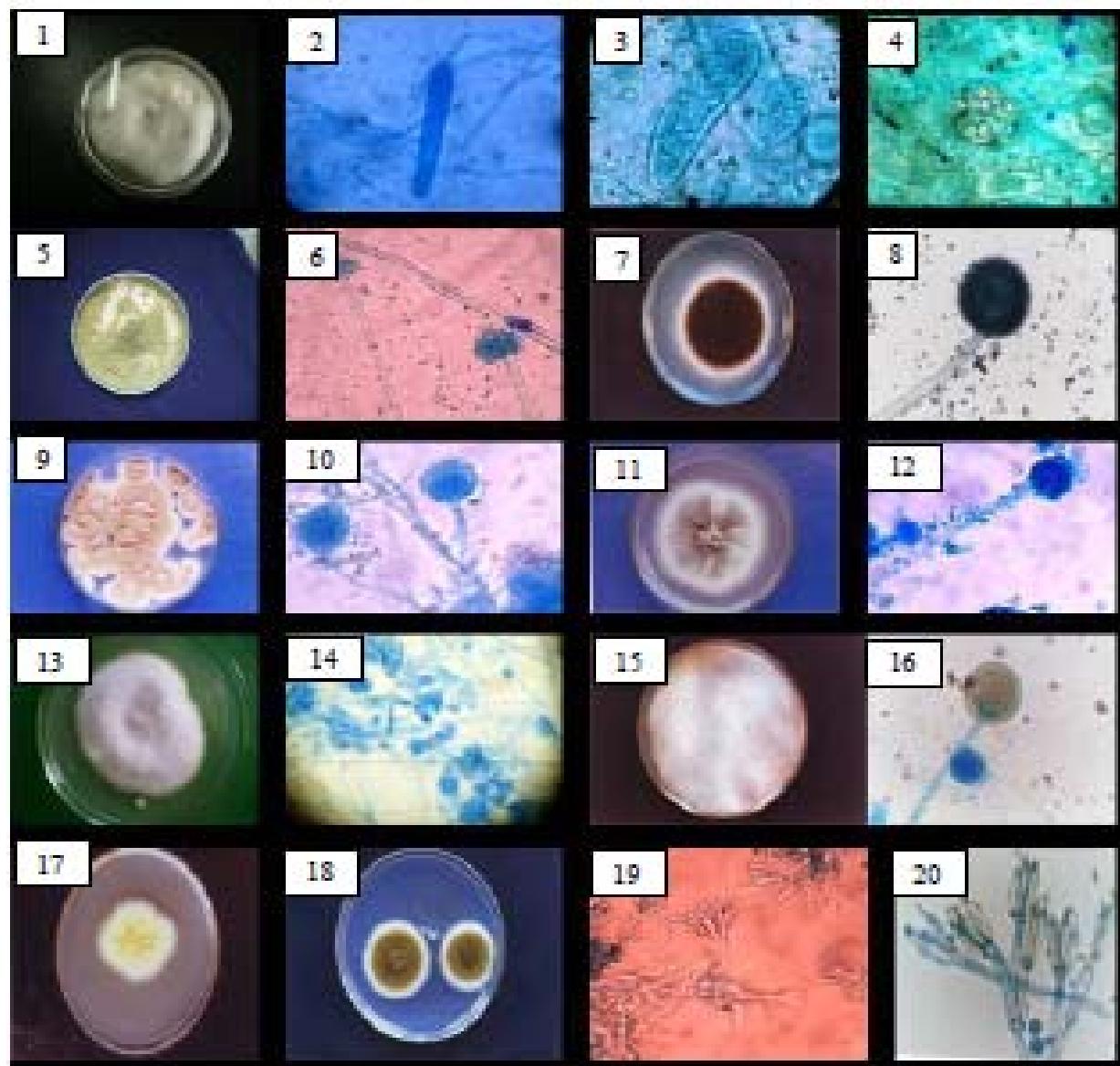
The pathological changes and the fungal elements in tissue sections in naturally infected fishes of various organs are described under each of the following photos (31-39.), stained by either PAS or GMS stains.

#### Corresponding Author:

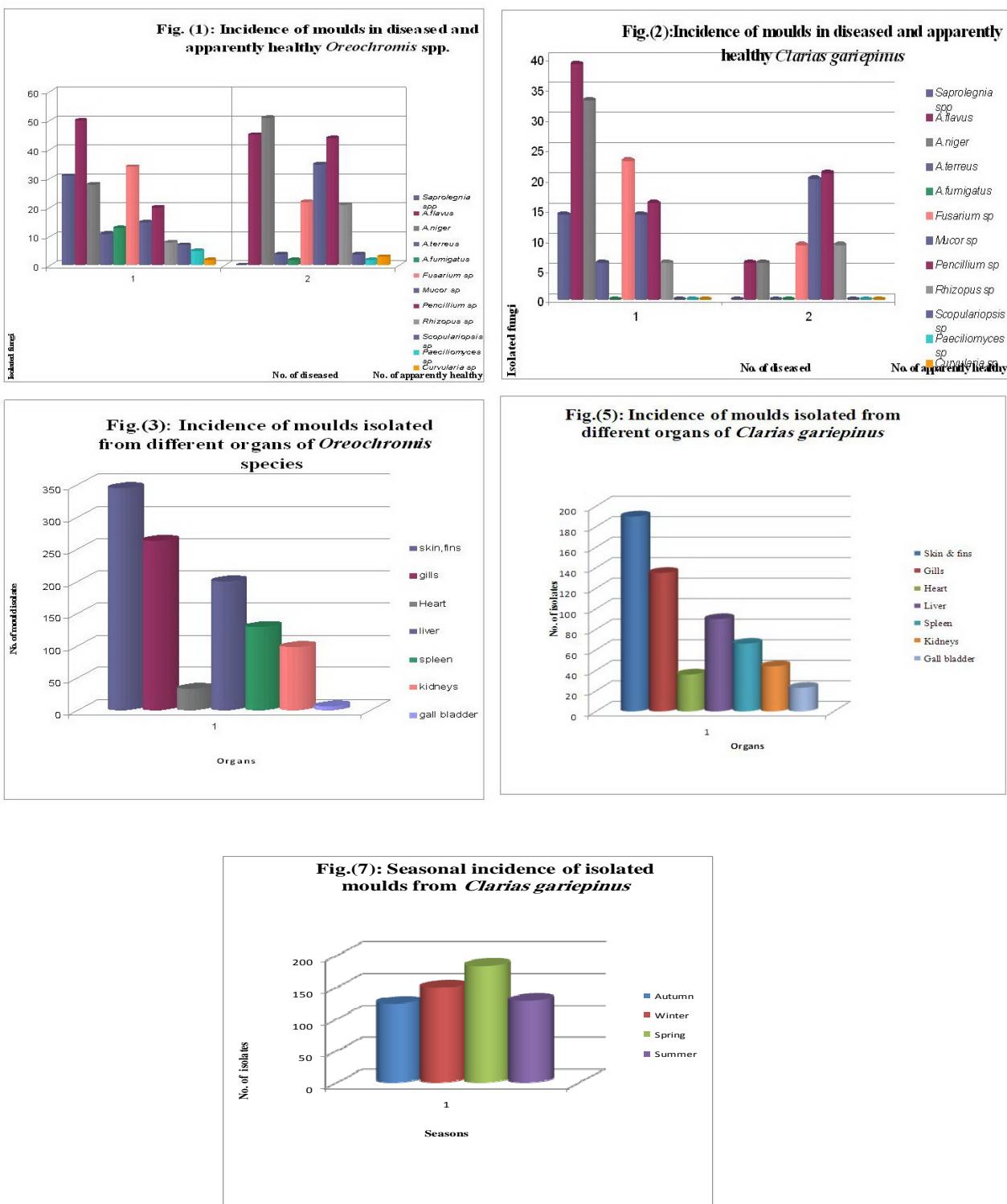
Shimaa, Khalifa  
Dept. of Hydrobiology, veterinary research division,  
National Research Center.Dokki, Giza,Egypt. Tel.:  
+202-3371728- Fax: +202-3370931  
E-mail: [shimaakhalifa2003@yahoo.com](mailto:shimaakhalifa2003@yahoo.com)

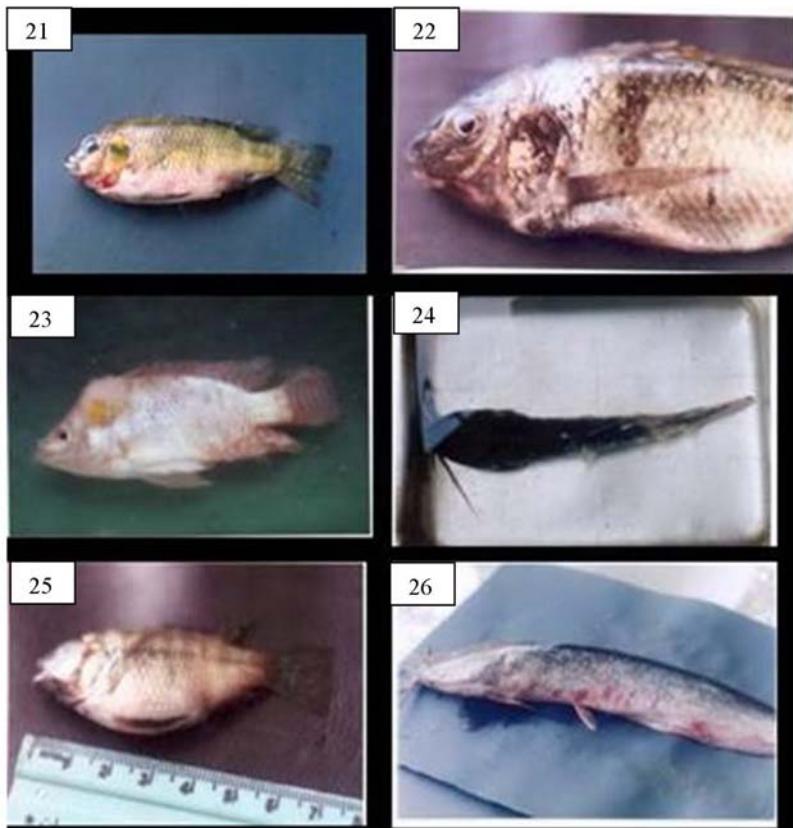
**Table (3): Type, average body weight of fish, spores concentration per ml, dose, route of inoculation and temperature.**

Fish	Body weight	Number of fish in each subgroup	Inoculated fungi	Dose	Conc.	Route	Temp.	References	
Tilapia sp.	30-40 g	10	<i>Aspergillus flavus</i>	0.2ml	$9 \times 10^4$	I.P	18°C	Olufemi <i>et al.</i> (1983)	
		10	<i>Aspergillus flavus</i>	0.2ml	$9 \times 10^4$	I.M			
	30-40 g	5	Normal saline	0.2ml	—	I.P	26°C		
		5	Normal saline	0.2ml	—	I.M			
Tilapia sp.	30-40 g	10	<i>Aspergillus flavus</i>	0.2ml	$9 \times 10^4$	I.P	22°C	Muvhich <i>et al.</i> (1989)	
		10	<i>Aspergillus flavus</i>	0.2ml	$9 \times 10^4$	I.M			
	30-40 g	5	Normal saline	0.2ml	—	I.P	22°C		
		5	Normal saline	0.2ml	—	I.M			
Tilapia sp.	30-40 g	10	<i>Candida albicans</i>	0.2ml	$2 \times 10^3$	I.P	22°C	Faisal <i>et al.</i> (1986)	
		10	<i>Candida albicans</i>	0.2ml	$2 \times 10^3$	I.M			
	30-40 g	5	Normal saline	0.2ml	—	I.P	22°C		
		5	Normal saline	0.2ml	—	I.M			

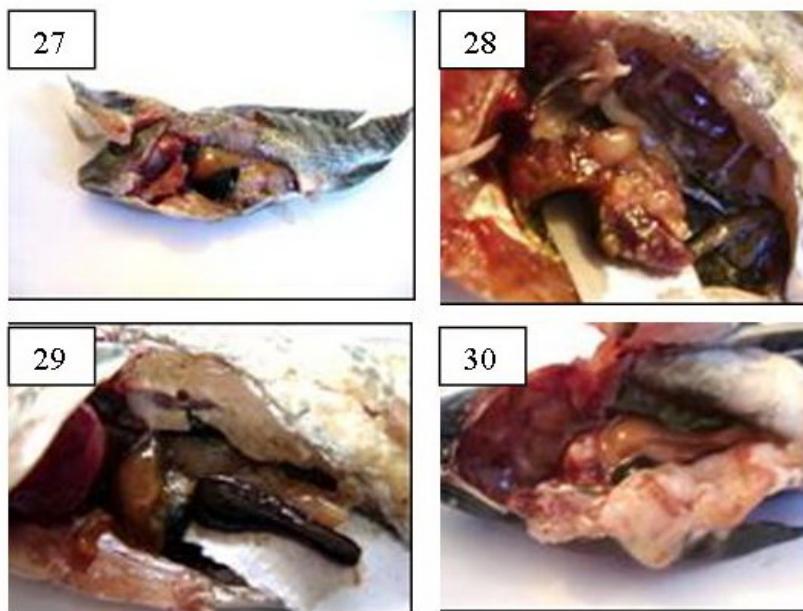


**Photo. (1):** A colony of *Saprolegnia* species with the characteristic cotton-wool like growth. **Photo. (2):** Non-septated broad hyphae of *Saprolegnia* species (X 200). **Photo. (3&4):** Different stages of reproductive structures of *Saprolegnia* species on hemp seeds (X 400). **Photo. (5):** Colonies of *Aspergillus flavus* on SDA, one weak old. **Photo. (6):** Typical heads *Aspergillus flavus* (X 400). **Photo. (7):** A colony of *Aspergillus niger* on SDA. **Photo. (8):** *Aspergillus niger* showing characteristic round head with black conidia (X 400). **Photo. (9):** Colonies of *Aspergillus terreus* on SDA. **Photo. (10):** *Aspergillus terreus* with small hemispherical vesicle (X 400). **Photo. (11):** A colony of *Aspergillus fumigatus* on SDA. **Photo. (12):** *Aspergillus fumigatus* with columnar head (X400). **Photo. (13):** A colony of *Fusarium* species on SDA with rose pigments on the center. **Photo. (14):** *Fusarium* species with characteristic slender, multicelled conidia (X 200). **Photo. (15):** Colonies of *Mucor* species showing spread over the surface of SDA. **Photo. (16):** Round sporangia of *Mucor* species containing sporangiospores (X 400). **Photo. (17):** *Penicillium* species on SDA with different colour and texture. **Photo. (18):** *Penicillium* species showing brush-like arrangement of fruiting head "A" (X400) and "B" (X 200). **Photo. (19):** *Rhizopus* species colony on SDA showing dens woolly mycelia. Sporangia are seen as small black dots. **Photo. (20):** *Rhizopus* species showing long, branched Sporangioophores and terminate with rhizoids (X200).

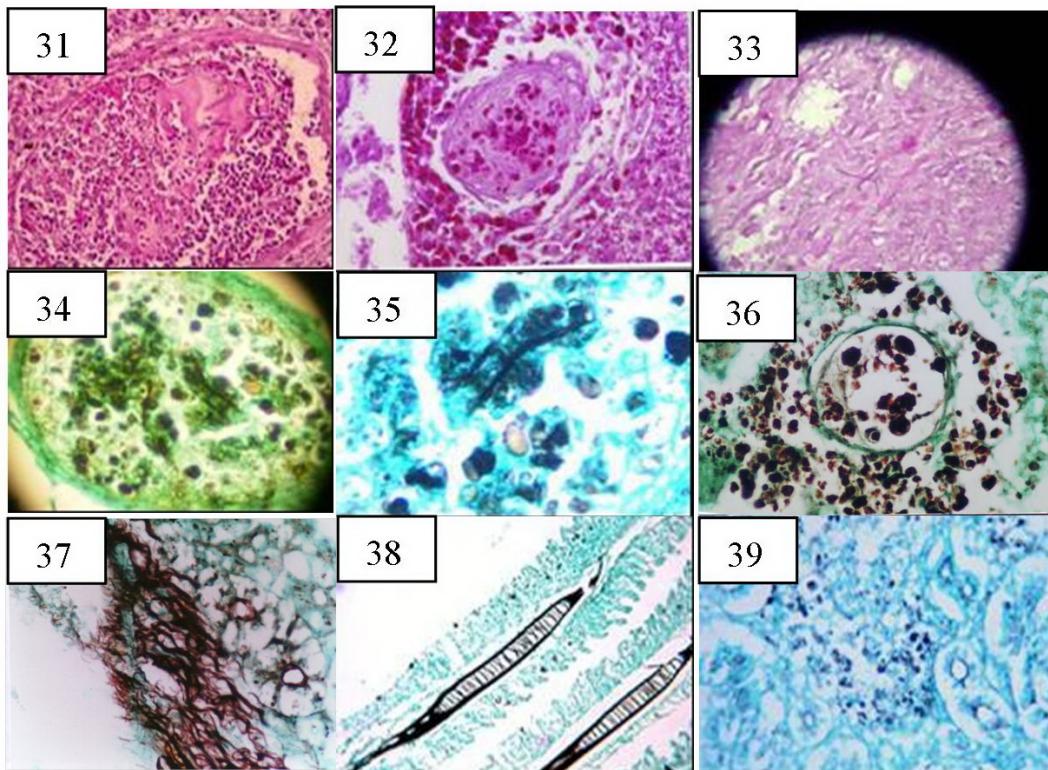




**Photo. (21):** *Oreochromis* species showing exophthalmia. **Photo. (22):** *Oreochromis* species showing skin darkening. **Photo. (23&24):** *Oreochromis* species and *Clarias gariepinus* showing cotton wool-like growth on various parts of the body. **Photo. (25):** *Oreochromis* species showing ascitis. **Photo. (26):** *Clarias gariepinus* showing haemorrhages all over the body surface.



**Photo. (27):** Liver of *Oreochromis* species showing necrotic foci with distention of gall bladder. **Photo. (28):** Spleen of *Oreochromis* species showing multiple nodules. **Photo. (29):** *Oreochromis* species showing severe enteritis. **Photo. (30):** *Oreochromis* species showing severe enlargement of spleen.



**Photo. (31):** Spleen section stained with PAS (X400) showing a granuloma formed of epithelioid cells and macrophages surrounded with fibroblasts and fibrous connective tissue capsule. Fungal hyphae appear within the granuloma. **Photo. (32):** Spleen section stained with PAS (X400) showing granuloma consists of epithelioid cells, macrophages and surrounded with connective tissue capsule. Large number of fungal spores appear within and surrounding granuloma. **Photo. (33):** Liver section showing fungal hyphae between the hepatocytes stained with PAS (X200). **Photo. (34):** Liver section stained by GMS (X400) showing granuloma consists of aggregation of epithelioid cells, macrophages and fibrous connective tissue capsule. Fungal hyphae and spores appear within granuloma. **Photo. (35):** Liver section stained by GMS (X 1000) showing fungal hyphae and spores between the hepatic tissue. **Photo. (36):** Spleen section stained by GMS (X 400) showing focal aggregation of spores surrounded with proliferating fibroblasts and fibrous connective tissue in between. **Photo. (37):** Kidney section stained by GMS (X 400) showing hyphal threads in between the interstitial tissues with marked severe degenerative changes in the tubular epithelium. **Photo. (38):** Gills section stained by GMS (X 400) showing yeast cells investing necrosed areas of epithelial lining the secondary lamellae. **Photo. (39):** Kidney section stained by GMS (X 400) showing yeast cells investing the interstitial tissues.

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9/1/2010

## Bacterial Infections Affecting Marine Fishes in Egypt

M. Moustafa<sup>1</sup>, Laila. A. Mohamed<sup>2</sup>, M.A. Mahmoud<sup>3</sup>, W.S, Soliman<sup>2</sup>, and M.Y. El-gendy<sup>\*2</sup>

<sup>1</sup> Department of Fish Diseases and Management, Faculty of Veterinary Medicine, Cairo University. Giza, Egypt

<sup>2</sup> Department of Hydrobiology, National Research Center, Dokki, Egypt

<sup>3</sup> Department of Pathology, Faculty of Veterinary Medicine, Cairo University. Giza, Egypt

[\\*mamduhousif@yahoo.com](mailto:mamduhousif@yahoo.com)

**Abstract:** Some fish species are suffering from continuous depletion due to devastating environmental changes at their native aquatic environment. Qarun Lake and Suez Gulf are among the most vulnerable areas. Thus, representative fish samples from those areas were inspected for the presence of any fish pathogenic bacteria. The inspected samples included six different species; *Epinephelus tukina*, *Siganus rivulatus*, and *Dedalechilus labiosus* native to Suez Gulf at Suez governorate; *Tilapia zilli*, *Mugil capito* and *Solea vulgaris* native to Qarun Lake at El-Fayoum governorate. A total of 600 samples were examined throughout the different year seasons. Gram positive and negative fish pathogenic bacteria were isolated from a total of 245 fish sample. Among those samples, the following bacteria were retrieved in the following percentages respectively, 17.55% (*V. anguillarum*), 16.73% (*V. alginolyticus*), 15.51% (*P. piscicida*), 15.91% (*Ps. fluorescens*), 13.46% (*S. enterica*), 11.02% (*A. hydrophila*), 6.12% (*A. sobria*) and 3.67% were infected with *Staph. aureus*. The *Siganus rivulatus* was the highest infected fish species with a prevalence of 8.33%, while *Mugil capito* was the lowest infected species (5.67%). The highest total prevalence of bacterial infection was recorded in summer season (40.81%) while the lowest was recorded in winter (15.91%). The aforementioned bacterial isolates were successfully re-isolated from experimentally infected fish. The retrieved isolates were matched against standard isolates as well as confirmed to be positive using semi-automated (API 20 E) and conventional biochemical tests.

[M. Moustafa, Laila. A. Mohamed, M.A. Mahmoud, W.S, Soliman and M.Y. El-gendy. Bacterial Infections Affecting Marine Fishes in Egypt. Journal of American Science 2010; 6(11):603-612]. (ISSN: 1545-1003).

**Keywords:** Marine fishes, Bacterial infections, Diagnosis, Seasonal variation.

### 1. INTRODUCTION

Fisheries represent an important sector in the Egyptian national income structure. In the fisheries economy, marine fishes represent the major investment choices for the national fishermen. Marine fishes are liable to variable number of environmental stressors, including chemical, natural and biological invaders. Such stressors are the main predisposing factors for the chronic immune-suppression of marine aquatic animals in the affected marine habitat. As an ultimate fate for the staggering immuno-suppression of fishes, bacterial invasion will be the most probable event (Ellis, 1999). Further, the bacterial invasion of any marine species could possibly exacerbate under the triggering effect of the fluctuating climatic changes (Wedemeyer, 1996).

The prevalence of bacterial pathogens have been well documented in several cultured and wild freshwater fish species, however; only a few bacteriological surveys have involved the marine species disease outbreaks (Alicia, et al 2005).

On the long run water resources will be the most limiting factor of aquaculture development in Egypt. Therefore, marine fisheries are the immediate

alternative for water needed for mariculture (Sadek, 2000).

The present work was planned to isolate and identify the most predominant bacterial pathogens in some marine fishes, native to both Suez Gulf and Lake Qarun. Further, work aimed to evaluate the seasonal variation of bacterial isolates among the examined fishes.

### 2. MATERIALS AND METHODS

#### 2.1. Sampling and processing

Six hundred (600) marine fishes of six different species were freshly captured from two localities in Egypt, (Suez Gulf and Lake Qarun) through the different seasons of the year.

On each season, twenty-five fish of each species were collected and examined. Fish species, numbers of fishes, average body weights and localities are shown in table (1). Clinical and P.M examination were carried out using the methods described by (Buller, 2004).

Samples from gills, liver, spleen, kidney and external lesions from fishes were cultured on general

and selective media; tryptic soy agar and tryptic soy broth (Difco) supplemented with 1.5% (w/v) NaCl, marine agar ( Difco), and thiosulphate–citrate–bile salt–sucrose agar (TCBS, Difco). Aeromonas agar base medium supplemented with ampicillin, pseudomonas agar base medium supplemented 2 % NaCl and Azide blood agar supplemented with 2 % NaCl. All the inoculated media were incubated at 22 °C for 48 hours.

## 2.2. Identification of the isolates

Pure cultures of the isolates were identified by biochemical characterization following the criteria proposed by those described in the Bergey's Manual of Determinative Bacteriology, (Garrity, 2001). Final confirmation of each strain was achieved using the analytical profile index of API20-E system (Buller, 2004).

## 2.3. Experimental infection

A total of 70 apparently healthy *O. niloticus* fish, weighting  $50 \pm 5$  gram were selected for determination of the pathogenicity of the most prevalent bacterial isolates. Fishes were divided into seven groups each contained 10 fish. The inocula prepared for bacterial isolates as I/P injections were prepared according to (Austin & Austin, 2007). Fish were observed daily for 15 days. Six groups were consistently inoculated I/P with the bacterial suspension of (*Aeromonas hydrophila*, *Pseudomonas fluorescens*, *Vibrio anguillarum*, *Pasteurella piscicida*, *Streptococcus fecalis* and *Staphylococcus aureus*) at a dose of  $0.2 \text{ ml}$  of ( $3 \times 10^7 \text{ CFU}$ ) while the control group (group 7) were injected I/P with  $0.2 \text{ ml}$  of sterile tryptic soy broth according to Hussain (2002).

## 3. RESULTS

### 3.1. Clinical examination:

Clinical signs included generalized hemorrhages (Fig 1). Some fishes showed bilateral exophthalmia and opacity (Fig 2), scale detachment and skin darkening. Gills were congested (Fig 3) while in others appeared to be pale with accumulation of excessive amount of mucus. Ascitis was observed in some fishes. Vent inflammation as well as prolapse were also observed.

Necropsy findings of naturally infected marine fishes exhibited sero-sanguinous fluid in the abdominal cavity. Liver was pale in some fishes (Fig 4), while in others was congest, haemorrhagic with numerous randomly scattered whitish nodules (Fig 5). Spleen and kidneys were congested and enlarged in some fishes. The intestines were haemorrhagic, inflamed with congestion of blood vessels. In other samples, the intestines were seen filled with gases.

### 3.2. Isolation and identification of the bacterial isolates:

Biochemical characteristics of the Gram negative bacterial spp. isolated from examined fishes are shown in Table (2).

Results indicated that 245 naturally collected fishes out of 600 were found to be infected with different types of bacteria. The culture results demonstrated that (203) fishes were found to be infected with Gram-negative bacteria and only (42) fishes were infected with Gram-positive bacteria. 17.50 % of the infected fishes were positive for *V. anguillarum*, (16.73%) for *V. alginolyticus*, (15.51%) for *P. piscicida*, (15.91%) for *Ps. fluorescens*, (13.46%) for *S. fecalis*, (11.02%) for *A. hydrophila*, (6.12%) for *A. sobria* and (3.67%) of the surveyed fishes were infected with *Staph. aureus*. The total prevalence of bacterial isolates is shown in table (3). Moreover, the results revealed that *Siganus rivulatus* was the most infected fish spp (50 %), followed by *E. tuvina* (42 %), *S. vulgaris* (41 %), *Tilapia spp.* (40 %), *M. sahlae* (38 %), while *M. capito* was the lowest infected spp (34 %).

Prevalence of bacterial infections for fishes collected from both Suez Gulf and Lake Qarun was illustrated in table (4). The results revealed that: The total prevalence of bacterial infections for fishes collected from the Gulf of Suez (53.06%) was higher than that recorded for those collected from Lake Qarun (46.93%).

### 3.3. Seasonal prevalence:

Results indicated that, the highest total prevalence of bacterial infections among the naturally infected marine fishes was recorded in the summer season (40.81%), followed by autumn (25.71%), then spring (17.14%). On the other hand the minimal prevalence of infection was recorded in winter (15.91%). table (5).

The highest prevalence of bacterial infection among the naturally infected marine fishes in winter season, was recorded for *Ps. fluorescens* (7.75%) while the lowest one (0.40%) was recorded for *V. anguillarum*. on the other hand *P. piscicida*, *S. fecalis* and *Staph. aureus* were not recorded. For spring season, the highest prevalence of bacterial infection (3.67%) was recorded for *A. sobria* and *V. anguillarum*, while the lowest (1.22%) was recorded for *Ps. fluorescens* and *S. fecalis*. The highest prevalence of bacterial infection (9.38%) in summer season was recorded for *V. anguillarum*, while the lowest (0.40%) was recorded for *A. sobria*. The highest prevalence of bacterial infection (5.30%) in autumn season was recorded for *V. alginolyticus* and *P. piscicida* while the lowest (0.40%) was recorded

for *A. sobria*. The Prevalence of different types of bacterial infections in the different seasons is illustrated in table (5).

### 3.4. Results of experimental infection:

Mortality patterns in experimentally infected *O. niloticus* with the different bacterial isolates (Table 6). Experimentally infected *O. niloticus* with the different isolates were characterized by septicemic lesions nearly similar to those of naturally infected fishes. Experimentally infected fish showed haemorrhagic patches distributed on different parts of the body surfaces , fin and tail rot (Fig. 6). Some fish exhibited typical ulcers (Fig.7). Some cases showed inflammation of the vent (Fig. 8). Necropsy findings showed, congestion of Liver. Spleen and kidneys were congested and enlarged. Gall bladder was distended. The gut was haemorrhagic and filled with yellowish content. Serous to sero-sanguinous

fluid in the abdominal cavity was noticed in some cases.

Re-isolation of all inoculated bacterial isolates was obtained from dead and sacrificed fish. Moreover, the results of the culture and biochemical characteristics of the re-isolated bacterial isolates revealed the same morpho-chemical characteristics of the inoculated bacterial isolates.

Table (1): Fish species, locality, number and weight of examined fish

Fish Species	Locality	Number	weight
<i>E. tuvina</i>	Suez Gulf	100	95±20
<i>S. rivulatus</i>	Suez Gulf	100	70±10
<i>M. sahla</i>	Suez Gulf	100	50±5
<i>S. vulgaris</i>	Lake Qarun	100	75±15
<i>M capito.</i>	Lake Qarun	100	85±10
<i>Tilapia zilli</i>	Lake Qarun	100	65±5

Table (2): Biochemical characteristics of the bacterial isolates retrieved from naturally infected marine fishes.

	<i>A. hydrophila</i>	<i>A. sobria</i>	<i>Ps. fluorescens</i>	<i>V. anguillarum</i>	<i>V. alginolyticus</i>	<i>P. piscicida</i>
B –Galactosidase production (OPNG)	+	+	-	+	-	-
Arginine dihydrolase production (ADH)	+	+	+	+	-	+
Lysine decarboxylase production(LDC)	-	+	-	-	+	-
Ornithine decarboxylase production(ODC)	-	-	-	-	-	-
Citrate utilization (CIT)	-	V	-	V	+	-
H2S production(H2S)	-	-	-	-	-	-
Urease production(URE)	-	-	-	-	-	-
Tryptophane deaminase production (TDA)	-	-	-	-	-	-
Indole production(IND)	+	+	-	+	+	-
Acetoin production(VP)	+	+	+	+	V	+
Gelatinase production(CEL)	+	+	-	+	+	-
Acid from glucose(GLU)	+	+	V	+	+	+
Acid from manitol(MAN)	+	+	-	+	+	-
Acid from inositol(INO)	-	-	-	-	-	-
Acid from Sorbitol(SOR)	-	-	-	+	-	-
Acid from rhamnose(RHA)	+	-	-	-	-	-
Acid from sucrose(SAC)	+	+	-	+	+	-
Acid from Melibiose (MEL)	-	-	V	-	-	-
Acid from amygdalin (AMY)	V	-	-	-	V	-
Acid from arabinose (ARA)	V	-	V	V	-	-
Cytochrome oxidase (OX)	+	+	+	+	+	+

V: variable result.

Table (3): Prevalence of bacterial infections in the examined marine fishes.

Type of M.O Fish spp	No. Of Exam fish	NO Inf. fish	<i>A. hydrophila</i>		<i>A. sobria</i>		<i>Ps. fluorescens</i>		<i>V.anguillarum</i>		<i>V.alginolyticus</i>		<i>P.piscicida</i>		<i>S. fecalis</i>		<i>Staph. aureus</i>	
			No. inf	%	No inf	%	No inf	%	No inf	%	No inf	%	No inf	%	No Inf	%	No .inf	%
<i>E. tuvina</i>	100	42	2	4.76	4	9.52	7	16.66	10	23.8	8	19.04	6	14.28	2	4.76	3	7.14
<i>S. rivulatus</i>	100	50	4	8	5	10	6	12	7	14	9	18	10	20	8	16	1	2
<i>S. vulgaris</i>	100	41	6	14.63	0	0	5	12.19	8	19.51	7	17.07	4	9.75	9	21.95	2	4.87
<i>M capito.</i>	100	34	3	8.82	2	5.88	4	11.76	5	14.7	3	8.82	9	26.47	6	17.64	2	5.88
<i>M. sahlae</i>	100	38	7	18.42	1	2.63	8	21.05	6	15.78	10	26.31	3	7.89	3	7.89	0	0
<i>Tilapia zilli</i>	100	40	5	12.5	3	7.5	9	22.5	7	17.5	4	10	6	15	5	12.5	1	2.5
Total	600	245	27	11.02	15	6.12	39	15.91	43	17.55	41	16.73	38	15.51	33	13.46	9	3.67

•Percentage was calculated according to the total number of infected fish.

Table (4) Prevalence of bacterial infections in Lake Qarun and Suez Gulf.

M.O	Locality	Lake Qarun		Prevalence %		Suez Gulf		Prevalence %	
		No inf	%	No inf	%	No inf	%	No inf	%
<i>A. hydrophila</i>		14		5.71		13		5.30	
<i>A. sobria</i>		5		2.04		10		4.08	
<i>Ps. fluorescens</i>		18		7.34		21		8.57	
<i>V. anguillarum</i>		20		8.16		23		9.38	
<i>V. alginolyticus</i>		14		5.71		27		11.02	
<i>P. piscicida</i>		19		7.75		19		7.75	
<i>S. fecalis</i>		20		8.16		13		5.30	
<i>Staph. aureas</i>		5		2.04		4		1.63	
Total		115		46.93		130		53.06	

Table (5): Seasonal prevalence of bacterial infections in the examined marine fishes.

Type of M.o season	<i>A.hydrophila</i>		<i>A.sobria</i>		<i>Ps.fluorescens</i>		<i>V.anguillarum</i>		<i>V.alginolyticus</i>		<i>P.piscicida</i>		<i>S.fecalis</i>		<i>Staph.aureus</i>		Total
	No inf	%	No inf	%	No inf	%	No inf	%	No inf	%	No inf	%	No inf	%	No inf	%	
Winter	14	5.71	4	1.63	19	7.75	1	0.40	2	0.81	0	0	0	0	0	0	15.91
Spring	7	2.85	9	4.08	3	1.22	9	3.67	5	2.04	6	2.44	3	1.22	0	0	17.55
Summer	3	1.22	1	0.40	6	2.44	23	9.38	21	8.57	19	7.75	20	6.16	7	2.85	40.81
Autumn	3	1.22	1	0.40	11	4.48	10	4.08	13	5.30	13	5.30	10	4.08	2	0.81	25.71
Total	27	11.02	15	6.12	39	15.91	43	17.55	41	16.73	38	15.51	33	13.46	9	3.67	



Fig. 1: Naturally infected *S. vulgaris* with *V. anguillarum* showing haemorrhages on the ventral surface of the body.



Fig. 2: Naturally infected *E. tuvina* with *P. piscicida* showing bilateral exophthalmia and corneal opacity.



Fig. 3: Naturally infected *M. capito* with *A. hydrophila* showing severe congestion of gills.



Fig. 4: Naturally infected *S. rivulatus* with *S. fecalis* showing paleness of liver.



Fig. 5: Naturally infected *E. tuvina* with *P. piscicida* showing enlargement of the liver with numerous scattered whitish nodules.



Fig. 6: *O. niloticus* I/P inoculated with *Ps. fluorescens* showing tail and fin rot.



Fig. 7: *O. niloticus* I/P inoculated with *A. hydrophila* showing skin ulcers.



Fig. 8: *O. niloticus* I/P inoculated with *S. fecalis* showing inflammation of the vent.

Table (6): Mortality patterns of *O. niloticus* experimentally infected with different bacterial isolates.

Bacterial isolates	No of mortality /day												Mortality (%)
	1	2	3	4	5	6	7	8	9	10	11	12-15	
<i>A. hydrophila</i>	-	1	-	2	-	-	2	1	-	1	1	-	80
<i>Ps. fluorescens</i>	-	2	-	-	1	1	2	1	1	-	1	1	100
<i>V. anguillarum</i>	1	1	2	1	2	-	-	1	-	1	-	1	100
<i>P. piscicida</i>	2	0	-	1	1	-	-	2	-	-	1	1	80
<i>S. fecalis</i>	1	-	1	-	-	1	1	1	1	2	-	1	90
<i>Staph. aureas</i>	-	1	-	2	-	-	1	1	-	-	2	-	70
Control	-	-	-	-	-	-	-	-	-	-	-	-	0

N.B. The dose of bacteria inoculated per fish were 0.2 ml of  $3 \times 10^7$  CFU

Number of I/P inoculated fishes per each group were 10.

#### 4. DISCUSSION

Septicemic bacterial infections such as vibrios, aeromonads, pseudomonads, photobacteria, streptococci and staphylococci were recorded in several fingerlings, juveniles, adults and brood stocks of some marine fish species (Alicia *et al.*, 2005 and Samuelsson *et al.*, 2006).

In regards to bacterial pathogens that have been isolated, results came in this study revealed that Gram-negative bacteria prevailed the Gram-positive isolates with *Vibrio anguillarum*, *Vibrio alginolyticus*, *Pasteurella piscicida* (*photobacterium damsella* subspp *piscicida*), *Pseudomonas fluorescens*, *Streptococcus fecalis*, *Aeromonas hydrophila*, *Aeromonas sobria* and *Staphylococcus aureus* were the most common isolated spp. Results are supported by those reported by Zorrilla *et al.* (2003) who declared that the main pathogenic microorganisms isolated from diseased gilt-head seabream in the marine water at south western Spain were; *Vibrio* spp, *Pseudomonas* spp, *P. piscicida*, *Flavobacteria maritimus*, *Aeromonas* spp and Gram positive bacteria were also isolated but in low prevalence.

In concern to the total prevalence of bacterial infections in the naturally infected marine fishes (40.83 %) which may appear to be lower than those reported by some authors for freshwater fishes as Soliman (1999) who noticed that the total bacterial prevalence was (65%). This difference could be due to the unfavorable effect of the salinity of marine water on the viability of bacterial pathogens.

In regard to the localities of isolation, results revealed that the prevalence of bacterial infections was higher (53.06%) in fishes collected from the Gulf of Suez than that (46.93%) recorded for Lake Qarun. This may be explained by the fact that Lake Qarun is the largest reservoir of agricultural and sewage drainage of Fayoum province as well as the

drainage from fish farms established around the lake (Mansour & Sidky, 2003).

The high prevalence of isolation recorded from the Gulf of Suez may in part be attributed to the stress induced by high crude oil pollution at the Gulf water which is maintained by the low water flow, low water exchange rates and daily crowded ship traffic crossing the gulf as well as industrial effluents from oil refineries. All these factors are compromising to the fish immune system ending up with marked increase in the magnitude of bacterial infections.

Study declared that marine fish can succumb MAS, as supported by Larsen & Jensen (1977) who isolated *A. hydrophila* from ulcer disease in Cod, *Gadus morhua* L., a strictly marine fish. Authors added that motile aeromonas group especially *A. hydrophila* is considered as one of the most important pathogen responsible for haemorrhagic septicemia in a wide variety of marine water fish. Moreover, Vethaak (1992) isolated *A. hydrophila* from ulcers, lesions, and blood of ulcerated European flounder

Results pointed out that the highest prevalence of *A. hydrophila* was recorded in winter season (5.71%) followed by spring (2.85%), while in summer and autumn the results were the same (1.22%). These results were supported by Pathak *et al.* (1988) who suggested that the highest isolation rates of *A. hydrophila* occurred during late winter followed by a progressive decline in density during summer and monsoon seasons. Moreover, Popovic *et al.* (2000) mentioned that there was clear seasonality in the prevalence of *A. hydrophila* as there were no isolates recovered in the summer months. On contrast, Meyer (1970) reported that the most epizootics of motile aeromonads were generally reported in spring and early summer.

In regards to the seasonal prevalence of *A. sobria*, our study recorded that the highest prevalence e of *A. sobria* septicemia was recorded during the spring (3.67%) followed by winter (1.63%) while the minimal prevalence e of infection (0.40%) was recorded during the summer and autumn. Results are in accordance with those obtained by Wahli *et al.* (2005) who noticed that mortalities due to *A. sobria* peaked during the low water temperatures of winter time and reached levels. On contrary, the results are not in concordance with those obtained by Kooj *et al.* (1988) who demonstrated that the highest prevalence of Aeromonads in marine water have been obtained in the warmer months.

The pathogenicity of *A. hydrophila* for experimentally infected *Oreochromis niloticus* fishes may be attributed to toxins and extracellular enzymes produced by *A. hydrophila* (Saavedra *et al.*, 2004).

In regards to the total prevalence of pseudomonas septicemia, the study recorded that (15.91%) of infected fish were positive for pseudomonas infection. These results are in concordance with those obtained by Hussain (2002) who reported that (15.27 %) of naturally infected marine fishes were positive for *Ps. fluorescens* septicemia. on contrast, results are lower than those reported by Khan *et al.* (1981) who reported that *Pseudomonas* spp accounted for (72 %) of the mortalities recorded in captive Atlantic cod, *Gadus morhus* L associated with fin rot disease.

The highest prevalence of *Ps. fluorescens* septicemia was recorded during the winter season (7.75%) followed by autumn (4.48%) summer (2.44%) and spring (1.22%), this reveals that *Ps. fluorescens* has certain affinity to low temperature for propagation and wide spreading infection (El-Moghazy, 2004). Results were supported by Golomazou *et al.* (2006) who demonstrated that the Pseudomonads were isolated mainly in cold months of winter. On the contrary, results are not in accordance with those obtained by Hoda *et al.* (1999) who revealed that the prevalence of pseudomonads was lower in winter than summer. This may also be attributed to amplified activity of proteinases produced by pseudomonads at the low temperature (10-25°C) that play significant role in the pathogenesis of pseudomonas septicemia (Hoshino *et al.*, 1997).

The pathogenicity of *Ps. fluorescence* for experimentally infected *Oreochromis niloticus* may be attributed to the production of extracellular enzymes and lethal toxins El-Attar & Moustaf (1996).

In regards to the total prevalence of vibriosis recorded (34.28%), result are in accordance with those reported by Khan *et al.* (1981) who recorded that vibrios accounted for (28%) of mortalities in captive Atlantic cod, *Gadus morhus* L associated with fin rot disease. the results are lower than those recorded by Zorrilla *et al.* (2003) who recorded that the prevalence of vibrios among diseased gilthead sea bream, *Sparus aurata* L in southwestern Spain was (69.90%).

*V. anguillarum* in this study was the *Vibrio* spp most frequently isolated as (17.55%) of the infected cases were positive for *V. anguillarum*. this is in accordance with Zorrilla *et al.* (2003). On the other hand *V. alginolyticus* was the cause of (16.73%) of the recorded cases. Such high prevalence of *V. alginolyticus* could explain its importance in mariculture as supported by Zhu *et al.* (2000) who suggested that *V. alginolyticus* causes great harm to a wide variety of marine fishes.

The highest prevalence of *V. anguillarum* infection was recorded during the summer (9.38%), followed by autumn (4.08%), spring (3.67%), and only (0.4%) were recorded in winter. On the other hand the highest prevalence e of *V. alginolyticus* infection was recorded in summer (8.57%), autumn (5.30%), spring (2.04%) and (0.81%) in winter. The results of the seasonal prevalence of *Vibrio* spp are in concordance with those reported by Roberts (2001) who demonstrated that in wild, vibriosis normally occurs in fish in late summer when the temperatures are high. On the other hand, (Golomazou *et al.* (2006) reported that *V. alginolyticus* were not associated with a particular season.

The pathogenicity of *V. anguillarum* for experimentally infected *O. niloticus* may be attributed to extracellular toxins and enzymes produced by the bacterium (Nottage & Birkbeck, 1987).

In respect to the total prevalence of *P. piscicida* in this study recorded that (15.51%) of diseased fish were positive for *P. piscicida*. The results are higher than those recorded by Balebona *et al.* (1998) who declared that (6.7%) of diseased gilt-head sea bream, *Sparus aurata* L. in southwestern Spain were infected with *P. piscicida*. On the other hand results are lower than those recorded by Athanassopoulou *et al.* (1999) who recorded that the prevalence of *P. piscicida* in diseased Cuvier, *Puntazzo puntazzo* L. collected from marine aquaculture systems in Greece was (80%).

Seasonally, the highest prevalence e of *P. piscicida* was recorded during summer season (7.75%) , autumn (5.30%) followed by the spring

(2.44%) on the other hand, it was not recorded in winter. Results are in concordance with those reported by Magarinos *et al.* (1996) who declared that *P. piscicida* causes high fish mortality only when the water is warm. On the other hand, Mladineo *et al.* (2006) suggested that temperature has no strong influence on the course of pasteurellosis.

The pathogenicity of *P. piscicida* for experimentally injected *O. niloticus* may be attributed to extracellular products of the bacterium (Nakai *et al.*, 1992).

In regards to the total prevalence of streptococcal septicemia, results indicated that (13.46 %) of infected fishes were positive for streptococcal infection. Results were higher than that recorded by Zorrilla *et al.* (2003) who recorded (7%) of bacterial infection affecting cultured gilthead sea bream, *Sparus aurata* L was attributed to Gram-positive bacteria. Hussain (2002) recorded that (6.25 %) of naturally infected marine fish were positive for streptococcal septicemia.

In regards to the seasonal prevalence of streptococcal septicemia, the highest prevalence of the streptococcal infection was recorded in summer season (6.16%) followed by autumn (4.08%) and spring (1.22 %) on the other hand it was not recorded during the winter. These results are in accordance with those obtained by Varvarigos (1997) who revealed that *Streptococcus* spp cause septicemia to all farmed species mainly during late spring and early summer when sea water temperatures are high.

In concern to the experimental infection of *O. niloticus* with *S. fecalis*, the pathogenicity of streptococci may be attributed to the effect of exotoxins produced by the bacterium (Kimura & kusuda, 1979 and Woo, 1999).

The total prevalence of *Staph. aureus* infections was (3.67%). Results were lower than those recorded by Athanassopoulou *et al.* (1999), who recoded that the total prevalence of *Staph. epidermidis* among diseased *Puntazzo puntazzo* in marine aquaculture systems in Greece was (10 %). Moreover, Zorrilla *et al.* (2003) recorded that (7%) of bacterial infections affecting gilthead sea bream *Sparus aurata* L. were attributed to Gram-positive bacteria.

Seasonally the highest recorded prevalence of Staphylococcal infection was recorded in the summer season (2.85%) followed by autumn (0.81%) with no records in spring or winter. Results are supported by Varvarigos (2001) who declared that *Staphylococcus* spp causing septicemia to all farmed species during high temperature of sea water in late spring and early summer.

Results of experimental infection of *O. niloticus* with *Staph. aureus* were in accordance with Huang (2000) who indicates that staphylococci can be a possible cause of mortalities and losses among fishes.

From the present study it was concluded that bacterial pathogens are the most significant microbial agents affecting marine fishes and climatic changes may plays a great role in modulating the occurrence of bacterial fish diseases.

#### **Corresponding author**

M.Y. El-gendy

Department of Hydrobiology, National Research Center, Dokki, Egypt

\*[mamdouhyousif@yahoo.com](mailto:mamdouhyousif@yahoo.com)

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10/1/2010

# Effect of Antioxidant on Lead-Induced Oxidative Damage and Reproductive Dysfunction in Male Rabbits

**\*El-Tohamy, M.M., and El-Nattat,W.S.**

Department of Animal Reproduction and A.I., National Research Centre, Cairo, Egypt.

**\*eltohamymagda@yahoo.com**

**Abstract:** The objective of this study was to characterize the Lead toxicity syndrome, to assess biomarkers that may be most useful for detecting toxicant-induced reproductive dysfunction, and to determine whether supplemental vitamin C would tend to alleviate the lead toxicity in rabbits. To test the hypothesis that the level of lead exposure is associated with an adverse effect on semen quality, in terms of sperm concentration, morphology, motility to assess antioxidant as important markers of disease using total antioxidant status. Adverse effects of lead on the testes may be mediated by oxidative damage and subsequent lipid peroxidation. The effect of lead acetate administration on testicular, hepatic and renal functions and the biomarker of effect for them were investigated in the present study with a trial of treatment by vitamin C. 35 male rabbits were divided into five groups. One control group and four groups received orally low and high doses of lead acetate (10.8 and 15 mg/kg b.wt., respectively). One low and one high received, in addition, 1 g vitamin C / L in drinking water. SOD,  $\gamma$ -GT, AST, ALT, cholinesterase, acid phosphatase, and LDH activities were measured in both serum and semen. Also semen characteristics were measured. Results concerning all the enzymes were promising. SOD, LDH, ALT and acid phosphatase activities in serum and semen were obviously affected by lead. Vitamin C was a good antioxidant that recuperates from the normal enzymatic status in both serum and semen. In conclusion, lead levels led to testicular hypo function, which is supported by the results of semen picture. The hazardous effect of lead led to disturbance in the activities of enzymes under investigation such as SOD,  $\gamma$ -GT, LDH, AST, ALT, Cholinesterase, Acid phosphatase. Vitamin C proved its antioxidant effect on recuperating from the normal status of enzymes in semen and serum. LDH and prostatic acid phosphatase are shown to be biomarkers of testicular dysfunction, while LDH, ALT may be used as biomarkers for hepatic and renal dysfunction. This study established the principle that lead toxicity can be prevented and makes it worthwhile to establish an acceptable treatment or preventive regimen in the light of the present results. [El-Tohamy, M.M., and El-Nattat,W.S. Effect of Antioxidant on Lead-Induced Oxidative Damage and Reproductive Dysfunction in Male Rabbits. Journal of American Science 2010;6(11):613-622]. (ISSN: 1545-1003).

**Keywords:** Effect; Antioxidant; Lead; Oxidative Damage; Reproductive Dysfunction; Male; Rabbit

## 1. Introduction:

Lead is a male reproductive toxicant (Winder, 1989), the primary mechanism of the toxic action of lead appears to be a disruption of the hypothalamic control of pituitary hormone secretion and in turn, spermatogenesis (Sokol, 1987). Since males do not possess accessory reproductive organs, reproductive potential relates to three factors; sperm availability, quality and quantity (Tsuiji and Karagatzides, 2001).

Reactive oxygen species (ROS) have been shown to have an important role in the normal functioning of a reproductive system and in the pathogenesis of infertility. ROS may also play a role in other reproductive organ diseases. Oxidative stress develops when there is an imbalance between the generation of ROS and the scavenging capacity of antioxidants in the reproductive tract. It affects both natural and assisted fertility. Because assisted reproductive techniques are used extensively in the treatment of infertility, it is critical to understand the in-vitro conditions that affect fertilization and embryo

development. Treatments that reduce oxidative stress may help infertility that is caused by this imbalance. Such strategies include identifying the source of excessive generation of ROS, treating the primary cause, and in-vitro and in-vivo supplementation of antioxidants. Research is in progress to identify the mechanisms that are involved in the etiology of reproductive diseases caused by ROS, and to create effective strategies that can counteract oxidative stress (Agarwal and Allamaneni, 2004).

This effect is associated with indicators of oxidative stress.. The present study showed that rabbits subjected to lead and cadmium had increased serum levels of LPO. This may be a result of either overproduction of ROS or accumulation of ROS resulting from dysfunction of antioxidants/antioxidants during lead exposures.

Exposures of experimental rabbits to lead induce oxidative stress, but to date, no examination of this phenomenon has been reported. Exposure to lead and cadmium results in decreased nitric oxide production in rabbits. And inhibition of NO synthesis

leads to a marked decrease in GSH synthesis through down regulation of the rate-limiting enzyme. Elevated serum (LPO) some possible mechanisms for the lead induced -oxidative stress is discussed. Pb exerts at a dose encountered exerts adverse effects on the male reproductive system, and this effect is associated with indicators of oxidative stress.

The imbalance between (ROS) production and (TAC) in seminal fluid indicates oxidative stress and is correlated with male infertility. A composite ROS-TAC score may be more strongly correlated with infertility than ROS or TAC alone recent scientific evidence revealed that a condition known as "oxidative stress" may, in fact, is a common factor in some of the causes of male infertility. Oxidative stress in the semen occurs when the level of ROS (reactive Oxygen Species) is greater than the TAC (Total Antioxidant Capacity). Although low levels of ROS are needed to normal sperm function, high levels of ROS clearly impair fertility. Antioxidants are substances that protect cellular components from damaging oxidative reactions by reaction with the free radicals and other reactive oxygen species.

The specific aims of the present study are to determine the efficacy of Vitamin C to improve reproductive performance of rabbits. There is growing evidence that oxidative stress significantly impairs sperm function, and plays a major role in the etiology of defective sperm function.

This may lead to the onset of male infertility via mechanisms involving the induction of per oxidative damage to the plasma membrane; both spermatozoa and seminal plasma possess antioxidant systems capable of counteracting the harmful effects of ROS. Studies have demonstrated that infertile male is more likely than fertile ones to have depressed (TAC) and lower levels of individual antioxidants (Smith *et al.*, 1996Lewis *et al.*, 1995). ROS is thought to be involved in many aspects of male infertility, where spermatozoa are rendered dysfunctional by lipid peroxidation and altered membrane function, together with impaired metabolism, morphology, motility, and fertility.

On the other hand, there is another category of enzymes, which act on superoxide radicals to eliminate its hazardous effects. The inhibition of these enzymes by lead causes the increase of superoxide radicals  $O_2^-$  and its accumulation inside the cell leading to its death (Mylroie *et al.*, 1986). Vitamins A, E, D and C were recorded to have antioxidant activities. Their antagonistic effects to superoxide accumulation varied from one vitamin to the other.

In this study, Vitamin C is used to investigate its antioxidant activity for decreasing the inhibitory effect of lead acetate on serum and semen

enzymes in New Zealand rabbits and monitoring the biomarker of effect on the vitality of the male.

## 2. Materials and methods

### Experimental design:

35 mature male rabbits (2.5 kg average) were raised in the animal house at National Research Centre (NRC), they were divided into 5 equal numbered groups (n=7). The first group was adopted as a control group (distilled water treated). The second and third were dosed 10.8 mg lead acetate/kg b.wt. Orally and considered as the low dose group. The fourth and fifth groups were dosed 15 mg lead acetate/kg b.wt. and considered as the high dose group. All the groups received their dosage orally dissolved in distilled water using animal gavages. The four latter groups were treated 5 consequent days/week, and the treatment expended for 8 weeks. The third and fifth groups obtained in addition to the lead acetate doses, vitamin C in concentration of 1 g / litre drinking water every day without disturbance. All lived in animals were sacrificed on the day after the last dose.

### Semen collection:

Semen was collected from bucks using a rabbit AV and a teaser female (Hafez, 1970). The collection was achieved from the end of the fourth week until the end of the experiment for the routine evaluation of both live sperm and sperm abnormalities percentage using eosin aniline stain and before the slaughter immediately for enzyme determination.

### Blood sampling:

Blood was collected while sacrificing animals in sterilized capped tubes and sterilized heparinized tubes (for SOD determination). The tubes were incubated at 37°C for 10 minutes in a slope position, and then centrifuged at 3500 rpm for 10 minutes. Serum was collected and immediately tested for the enzymes.

The whole heparinized blood was treated according to the following method: 0.5 ml of whole blood was centrifuged for 10 minutes at 3000 rpm and then the plasma was aspirate off. Then erythrocytes were washed four times with 3 ml of 0.9% NaCl solution and centrifuged for 10 minutes at 3000 rpm after each wash. The washed and centrifuged erythrocytes were made up to 2.0 ml with cold redistilled water, mixed and left to stand at +4°C for 15 minutes. The lysate was diluted with 0.01 mmol/l phosphate buffer pH 7.0 (Randox, Cat. No. SD 124). so that the % inhibition falls between 10% and 60%. A 25 folds dilution of lysate is recommended (final dilution factor = 100).

Enzymatic analysis in blood and semen:

- 1- In erythrocytes:
- i- Superoxide dismutase (SOD) in whole blood according to Wooliams et al. (1983) using *kits purchased from Randox, UK.*
- 2- In serum and whole semen
- i- Aspartate amino transferase (AST), alanine amino transferase (ALT) according to Bergmeyer (1978) and lactate dehydrogenase (LDH) according to Buhl and Jackson (1978) using *kits purchased from Stanbio, Texas, USA.*
- ii- Cholinesterase according to Den Blawen et al. (1983), Acid phosphatase according to Moss (1984) and □□GT according to Szasz (1969) using *kits purchased from Quimica Clinica Aplicada S.A., Spain.*
- iii- The MDH 586 method was used to determine MDA activity as described by Gerard et al., 1998) and measured at 586.
- iv- All enzymes were measured using Shimadzu Spectrophotometer with different wavelengths specific for each enzyme.

Statistical analysis:

Data were analyzed using one-way ANOVA to determine whether the effect of lead (Pb) and vit.

**Table 1- The effect of lead acetate alone or in the addition of vitamin C on semen characteristics in the male rabbits.**

Treatment Biomarkers	Group I (Control) (n=12)	Group II Low Lead (n=36)	Group III (lowLead+C) (n=12)	Group IV (high Lead) = (n=7)	Group V (high Lead+C) (n=28)
<b>Mass motility score (1-5)</b>	4.5 <sup>b</sup> ± 0.12	3.44 <sup>a</sup> ± 0.13	4.00 <sup>ab</sup> ± 0.25	3.57 <sup>a</sup> ± 0.12	3.86 <sup>a</sup> ± 0.22
<b>Individual motility (%)</b>	91.25 <sup>b</sup> ± 0.90	76.25 <sup>a</sup> ± 1.33	81.25 <sup>a</sup> ± 3.09	79.64 <sup>a</sup> ± 1.31	80.71 <sup>a</sup> ± 2.32
<b>Volume (ml)</b>	0.48 <sup>a</sup> ± 0.056	0.69 <sup>b</sup> ± 0.055	0.47 <sup>a</sup> ± 0.014	0.39 <sup>a</sup> ± 0.021	0.52 <sup>ab</sup> ± 0.063
<b>Concentration (x10<sup>6</sup>/ml)</b>	120.00 <sup>b</sup> ± 6.89	87.50 <sup>a</sup> ± 5.59	77.00 <sup>a</sup> ± 2.71	70.40 <sup>a</sup> ± 3.99	106.86 <sup>b</sup> ± 6.71
<b>Total concentration (x10<sup>6</sup>)</b>	53.80 <sup>bc</sup> ± 3.62	58.23 <sup>c</sup> ± 5.24	36.30 <sup>ab</sup> ± 2.22	25.83 <sup>a</sup> ± 1.27	62.53 <sup>c</sup> ± 9.33
<b>Live %</b>	94.33 <sup>c</sup> ± 0.25	82.53 <sup>b</sup> ± 0.32	90.26 <sup>d</sup> ± 0.50	74.21 <sup>a</sup> ± 0.51	88.83 <sup>c</sup> ± 0.46
<b>Primary spr abnormalities %</b>	15.33 <sup>a</sup> ± 0.55	25.16 <sup>c</sup> ± 0.25	21.27 <sup>b</sup> ± 0.41	34.31 <sup>d</sup> ± 1.42	19.35 <sup>b</sup> ± 0.78

Same superscript are non significantly different within row (P<0.05) Duncan test

The results of present study showed that lead acetate significantly affected the semen characteristic in both group II and IV, while; vitamin C corrected these adverse effects (table 1). This agrees with the finding of El-Nattat et al. (2000). Low lead exposure was more consistently associated with indicators of sperm production than was semen lead. Measurement of semen leads may not be a valuable

C gave a significant difference than control group or not ( $H_0$ ). The results were accepted at a level of 95% confidence. Statistical analysis using SPSS (Statistical package for social science) version 12, software package for data analysis (Saeys et al., 2007) was done.

### 3. Results and Discussion:

We previously reported that dietary exposure to lead resulted in suppressed spermatogenesis and testosterone levels without significant changes in luteinizing hormone.(El-tahamy 2003). The result showed that exposure to concentrations of inorganic lead ( micrograms/dl in blood impaired male reproductive function by reducing the sperm count, volume, density or changing sperm motility and morphology. Semen analysis (Table 1) revealed that the lead treated groups were significantly ( $P<0.01$ ) lower in mass motility, individual motility, sperm concentration / ml semen, live sperm %, while, they were significantly ( $P<0.01$ ) higher in the total primary sperm abnormalities %, then the control group. Vitamin C has ameliorated the hazardous effect of lead in both groups (III and V).

adjunct to conventional blood lead monitoring for investigations of male reproductive system toxicity.

The results also showed that vitamin C supplementation reduced ROS generation and improved semen quality. The beneficial effect of vitamin C in improving fertilization rate was possibly due to a reduction in lipid per oxidation potential. Seminal plasma confers some protection against ROS

damage because it contains enzymes that scavenge ROS.

High lead exposure induces oxidative stress in animal. Oxidative stress is an imbalance between the free radical production and antioxidant defense systems of the body (Yao et al., 2006 and Urso et al, 2003).

Antioxidants present in the seminal plasma are the most important form of protection available to spermatozoa against (ROS) (Aitken, 1999). They provide a defense mechanism through 3 levels of protection, prevention, interception and repair. A growing body of evidence suggests that low seminal (TAC) is related to male infertility (Sikka, 2001). Thus, it is important to ensure that any measurement of seminal TAC is accurate and reliable and easy as a diagnostic tool in the evaluation and follow up of male infertility. In a normal situation, the cellular antioxidant mechanisms present in almost all tissues and their secretions are likely to quench those ROS and protect against oxidative damage (Jones, et al., 1979). Antioxidant supplementation can theoretically protect and prevent such per oxidative damage. To evaluate this oxidative stress and determine the role of antioxidants have a great potential in therapeutic practice. Studies on how these cellular changes caused by LPO effect seminal parameters and sperm function and whether they could be reversed by antioxidants are open to further investigations.

Antioxidants, in general, are free radical scavengers that suppress the formation of ROS and/or

oppose their actions. SOD is well known biological antioxidants that convert superoxide (O<sub>2</sub>) and peroxide (HO) radicals to form O<sub>2</sub> and H<sub>2</sub>O. SOD protects against spontaneous O<sub>2</sub> toxicity and LPO (Fridovich, 1985). SOD and catalase also remove O<sub>2</sub> and play an important role in protecting spermatozoa. In spermatozoa, production of (MDA) an end-product of LPO induced by ferrous ion promoters, has been reported (Bell et al., 1993). Seminal plasma possesses major antioxidant defenses, including enzymatic and non enzymatic antioxidants. Chain-breaking antioxidants trap ROS directly to prevent amplification of radical formation and subsequent damage to sperm.

The results (Table 2). showed that ROS combined with total antioxidants capacity could predict fertility in male. Oxidative stress in the semen occurs when the level of ROS is greater than the TAC. Although low levels of ROS are needed to normal sperm function, high levels of ROS clearly impair fertility. Supplementation of vitamin C improved sperm motility and reproductive efficiency, and reduced the production of free radicals which can improve rabbit semen quality. The conclusion is that the antioxidants used are effective in combating cell damaging free radicals, which are known to contribute towards testicular function. Lipid peroxidation considered to be the key mechanism of ROS-induced sperm damage, which leads to loss of sperm motility.

**Table 2. The effect of lead acetate alone or in the addition of vitamin C on MDA and TAC in the semen of male rabbits.**

parameters Groups	Control	Low dose lead acetate(10.8mg/Kg b.wt)	High dose lead acetate(15.mg/Kg b.wt)	Low dose lead acetate + vit. C (1g/L drinking water)	High dose lead acetate + vit. C (1g/L D water)
MDA serum Nmol/ml	10.98 ±0.37 <sup>b</sup>	14.79±0.21 <sup>ac</sup>	18.28±0.25 <sup>ac</sup>	11.62±0.14 <sup>b</sup>	10.36±0.15 <sup>b</sup>
MDA semen Nmol/ml	11.34±0.35 <sup>b</sup>	16.06±0.36 <sup>ac</sup>	18.30±0.37 <sup>ac</sup>	12.36±0.39 <sup>b</sup>	11.83±0.37 <sup>b</sup>
TAC serum mmol/L	0.61±0.24 <sup>bc</sup>	0.47±0.01 <sup>ac</sup>	0.28±0.02 <sup>ac</sup>	0.70±0.02 <sup>a b</sup>	0.93±0.02 <sup>a b</sup>
TAC semen mmol/L	1.25±0.10 <sup>bc</sup>	0.83±0.02 <sup>ac</sup>	0.60±0.02 <sup>ac</sup>	1.92±0.09 <sup>a b</sup>	2.74±0.17 <sup>a b</sup>

Data were recorded as Mean ± S.E.

Different superscript in rows are significantly different at P<0.05.

a: Significant change when comparing all groups with the control group

b: Significant change when comparing all groups with lead acetate

c: Significant change when comparing all groups with lead acetate + vit.C

The results showed that supplementation of vitamin C had beneficial effects on semen characteristics. The beneficial effects of vitamin C can be attributed to the fact that vitamin C is a very

efficient antioxidant and a scavenger of oxygen free radicals who are toxic byproducts of many metabolic processes. Vitamin C is important in maintains the physiological integrity of the testes. Supplementation

of vitamin C improved sperm motility and reproductive efficiency and reduced the production of free radicals which can improve rabbit semen quality. The study required to determine whether vitamin C and other naturally occurring compounds can function as effective free radical trapping antioxidants, thereby preventing testicular peroxidative injury.

Vitamin C is essential to the body as both an antioxidant and as a nutritional supplement. Numerous antioxidants have proven beneficial in treating male infertility, such as Vitamin C and E, glutathione, Zinc and Selenium (Sinclair, 2000). Antioxidants can protect against the damaging effect of leukocyte-derived reactive oxygen species on sperm movement and may be of clinical value (Baker et al., 1996). The production of ROS is a normal physiological event in various organs, including the testes. Overproduction of ROS, can be detrimental to sperm, which associated with male infertility (Akiyama, 1999).

The results of the present study showed that lead acetate significantly affected the semen characteristic in both group and IV, while vitamin C corrected these adverse effects (table 1). The role of some enzymes markers are greatly influencing by heavy metals. Most of them contain a sulphydryl group. Such group is a target for metals. Others like some metal-enzymes have a prosthetic group that may be replaced by these metals leading to an inhibition of enzyme activity (WHO, 1993). These suppression masks their physiological actions specially those enzymes related to superoxide radicals  $O_2^-$  that accumulates inside the cells leading to the deterioration and death of the cells.. SOD is one of these enzymes (Patra et al., 1999). The results indicated that lead acetate induced an exaggerated inhibitory effect for the SOD. As lead mimics Cd in replacing Zn in its sites, this suggested the interaction of lead with the Cu, Zn and Mn , moieties such as interaction has been demonstrated where lead replaces Zn to form Cu-Pb-SOD (Bauer et al., 1980) Vitamin C ameliorate the inhibitory action of lead by removing ROS once formed, thus preventing radical chain Reactions. This observation supports the hypothesis that SOD activity is stimulated by an increased superoxide radical generation associated with the decline of SOD and glutathione peroxides (GSH-Px) Allen and Balin, (1989) generated by the inhibitory action of lead; on the other hand, the antioxidants refresh the enzyme's activity and antagonize the inhibitory effect of lead.

Lactate dehydrogenase plays an important role in the intermediary metabolism as a link between

amino acid metabolism and the citric acid cycle where it converts lactate into pyruvate(Table 3).. The serum lactate dehydrogenase is cytosolic, and in cellular damage the liver, lung, muscle, kidney, testicles or heart releases it into systemic circulation (Bhargava et al., 1978). In the present study, there was a decrease in the activity of the LDH in serum due to the lead intoxication. This agrees with the findings of Yagminas et al. (1990), while, it recuperates its activity values around the control LDH activity due to the treatment with vitamin C in both semen and serum. This decrease in LDH activity may be due to an inhibitory effect induced by the dose levels of lead, although an increase in the enzyme leakage may be present. On the other hand, in the semen the leakage from testicular and glandular tissues was significant, approximately twice to four times than the treated groups with vitamin C and triple the control group. Gulvik (1989) recorded a reduced activity of LDH in the testicular tissue. This result agrees with our findings that LDH is a sensitive and convenient biosensor for detection of heavy metal salts (Fennouh et al., 1998).

The role of some enzyme markers is greatly influenced by heavy metals. Most of them contain sulphydryl group (-SH) at their site of the action (Keogh, 1992), such group is a target for metals. Others like some metal-enzymes have a prosthetic group that may be replaced by these metals leading to an inhibition of enzyme activity (WHO, 1993). These suppression masks their physiological actions, especially those enzymes related to superoxide radicals  $O_2^-$  that accumulate inside the cells leading to the deterioration and death of the cells. SOD is one of those enzymes (Patra et al., 1999).

The results indicated that lead acetate induced an exaggerated inhibitory effect for the SOD. As lead mimics Cd in replacing Zn in its sites, this suggested the interaction of lead with the Cu, Zn and Mn moieties. Such interaction has been demonstrated where lead replaced Zn to form Cu-Pb-SOD (Bauer et al., 1980). Vitamin C ameliorates the inhibitory action of lead by removing ROS once formed, thus preventing radical chain reactions. This observation supports the hypothesis that SOD activity is stimulated by an increased superoxide radical generation associated with the decline of SOD and glutathione peroxidase (GSH-Px) (Allen and Balin, 1989) generated by the inhibitory action of lead, while, on the other hand, the antioxidant refreshes the enzyme activity and antagonizes the inhibitory effect of lead.

**Table 3- The effect of lead acetate alone or in the addition of vitamin C on some enzymes biomarkers in blood of male rabbits.**

Treatment Biomarkers	Group I (Control) (n=5)	Group II (Low Lead) (n=7)	Group III (Low lead+C) (n=6)	Group IV (high Lead+) (n=7)	Group V (high Lead+C) (n=4)
<b>Superoxide dismutase (units SOD/ml blood)</b>	115.43 <sup>a</sup> ± 18.27	210.64 <sup>bc</sup> ± 25.89	187.58 <sup>b</sup> ± 7.82	262.81 <sup>c</sup> ± 25.89	109.78 <sup>a</sup> ± 10.53
<b>□-GT (U/L)</b>	9.65 <sup>ab</sup> ± 0.51	11.39 <sup>bc</sup> ± 1.95	7.78 <sup>a</sup> ± 0.80	14.09 <sup>c</sup> ± 0.47	10.26 <sup>ab</sup> ± 0.76
<b>Lactate dehydrogenase (U/L)</b>	518.66 <sup>b</sup> ± 22.87	349.19 <sup>a</sup> ± 12.30	559.71 <sup>b</sup> ± 21.04	355.32 <sup>a</sup> ± 7.68	532.61 <sup>b</sup> ± 20.60
<b>AST (U/L)</b>	81.33 <sup>a</sup> ± 3.55	95.37 <sup>b</sup> ± 2.95	80.82 <sup>a</sup> ± 3.07	81.86 <sup>a</sup> ± 4.47	72.79 <sup>a</sup> ± 1.44
<b>ALT (U/L)</b>	88.04 <sup>bc</sup> ± 2.84	100.09 <sup>c</sup> ± 11.50	78.30 <sup>ab</sup> ± 2.60	133.78 <sup>d</sup> ± 9.06	61.46 <sup>a</sup> ± 4.25
<b>Choline esterase (U/L)</b>	1049.44 <sup>b</sup> ± 109.82	838.04 <sup>ab</sup> ± 162.24	1051.23 <sup>b</sup> ± 128.86	479.25 <sup>a</sup> ± 67.69	1090.89 <sup>b</sup> ± 166.16
<b>Total acid phosphatase (U/L)</b>	59.51 <sup>a</sup> ± 2.40	66.72 <sup>b</sup> ± 1.97	84.91 <sup>d</sup> ± 2.16	66.68 <sup>b</sup> ± 1.26	76.84 <sup>c</sup> ± 2.83
<b>Prostatic acid phosphatase(U/L)</b>	1.82 <sup>a</sup> ± 0.33	3.31 <sup>a</sup> ± 0.43	8.00 <sup>b</sup> ± 0.56	3.00 <sup>a</sup> ± 0.43	7.45 <sup>b</sup> ± 1.56

Same superscript are insignificantly different within row (P<0.05) Duncan test.

Certain enzymes, proenzymes, and their substrates are present at all times in the circulation of normal individuals and perform a physiological function in blood. Pseudo cholinesterase is one of these enzymes (functional enzymes). They are synthesized in the liver but present in blood in equivalent or higher concentrations than in tissues (table 4). While, nonfunctional plasma enzymes

which have the unknown physiological function in blood, only provide valuable diagnostic and prognostic clinical evidence in the case of dysfunction and diseases. These nonfunctional plasma enzymes include those in exocrine secretions and true Intracellular enzymes. Prostatic acid phosphatase is one of those categories (Rodwell, 1991).

**Table 4- The effect of lead acetate alone or in the addition of vitamin C on some enzymes biomarkers in the semen of male rabbits.**

Treatment Biomarkers	Group I (Control) (n=5)	Group II (Lead) (n=7)	Group III (Lead+VitC) (n=6)	Group IV (high Lead) (n=7)	Group V (high Lead+C) (n=4)
<b>Lactate dehydrogenase (U/L)</b>	954.75 <sup>a</sup> ± 52.98	3850.83 <sup>c</sup> ± 39.45	1646.90 <sup>b</sup> ± 30.45	4817.65 <sup>d</sup> ± 390.60	1788.63 <sup>b</sup> ± 5.07
<b>AST (U/L)</b>	420.79 <sup>a</sup> ± 62.50	1614.77 <sup>c</sup> ± 104.17	247.13 <sup>a</sup> ± 26.13	773.99 <sup>b</sup> ± 1.49	330.81 <sup>a</sup> ± 48.23
<b>ALT (U/L)</b>	43.32 <sup>b</sup> ± 2.12	44.20 <sup>b</sup> ± 7.56	38.60 <sup>b</sup> ± 3.15	23.18 <sup>a</sup> ± 4.87	16.50 <sup>a</sup> ± 0.93
<b>Choline esterase (U/L)</b>	195.50 <sup>c</sup> ± 25.60	119.91 <sup>a</sup> ± 4.93	156.40 <sup>b</sup> ± 3.41	101.66 <sup>a</sup> ± 3.41	106.87 <sup>a</sup> ± 7.88
<b>Total acid phosphatase (U/L)</b>	124.74 <sup>a</sup> ± 5.40	113.86 <sup>a</sup> ± 12.24	107.01 <sup>a</sup> ± 9.53	171.20 <sup>b</sup> ± 14.70	99.76 <sup>a</sup> ± 10.84
<b>Prostatic acid phosphatase (U/L)</b>	12.63 <sup>a</sup> ± 0.95	16.57 <sup>a</sup> ± 2.74	16.06 <sup>a</sup> ± 1.19	24.88 <sup>b</sup> ± 2.37	15.48 <sup>a</sup> ± 3.28

Same superscript are insignificantly different within row (P<0.05) – Duncan test.

The  $\gamma$ -glutamyl transferase, an enzyme that supports the transfer of certain amino acids into the GSH, which reduces, peroxides accumulation into the

RBCs and other cells. It is presented in abundant in the plasma membrane of renal tubular cells and in the endoplasmic reticulum of the hepatocytes (Murray,

1991). In the present study, the results showed a significant increase in the enzyme activity in case of high dose of lead acetate (14.09 U/l) than the control group. This indicates that there is a destruction of the enzyme in the store cells, and release in the blood serum (Murray, 1991). Therefore, the activity in the store cells like hepatocytes and renal tubular cells is decreased (Sivaprasad et al., 2002). Murray (1991) reported that high malonylaldehyde levels (a compound indicator on lipid peroxides) along with lowered activities of catalase, SOD, glutathione peroxidase, glutathione metabolizing enzymes (glutathione reductase, glucose-6-phosphate dehydrogenase, glutathione-S-transferase) indicate that the cells are under deteriorating stress factors including heavy metals. He recorded that a powerful antioxidant can reverse the oxidative damage by bringing about an improvement in the reductive status of the cell. The present study proved that vitamin C has stored the integrity of cells and reduced the leakage of enzymes outside the damaged cells, in addition, to its antioxidant effect via an indirect way. Moreover, vitamin C may help in decreasing the superoxide radical's accumulation inside the cells, although the SOD activity is inhibited by the hand make of lead, , it conserves the soundness of the cells in return, no leakage of enzymes and no increased enzyme activity in the serum than the normal. This is in agreement with the proposal of Sivaprasad et al. (2002). Upasani and Balaraman (2001) concluded that vitamin C had a significant antioxidant activity thereby protecting the organs from the lead-induced toxicity.

Lactate dehydrogenase plays an important role in the intermediary metabolism as a link between amino acid metabolism and the citric acid cycle where it converts lactate into pyruvate. The serum lactate dehydrogenase is cytosolic, and in cellular damage the liver, lung, muscle, kidney, testicles or heart releases it into systemic circulation (Bhargava et al., 1978). In the present study, there was a decrease in the activity of the LDH in serum due to the lead intoxication. This agrees with the findings of Yagminas et al. (1990), while, it recuperates its activity values around the control LDH activity due to the treatment with vitamin C in both semen and serum. This decrease in LDH activity may be due to an inhibitory effect induced by the dose levels of lead, although an increase in the enzyme leakage may be present. On the other hand, in the semen the leakage from testicular and glandular tissues was significant, approximately twice to four times than the treated groups with vitamin C and triple the control group. Gulvik (1989) recorded a reduced activity of LDH in the testicular tissue. This result agrees with our findings that LDH is a sensitive and

convenient biosensor for detection of heavy metal salts (Fennouh et al., 1998).

Many authors have discussed the effect of lead on AST and ALT in the serum (Randhawa et al., 1995; and El-Nattat, 1997). While, in liver cell culture Gutierrez et al. (1992) found that LC<sub>50</sub> of lead acetate (100 micromole) caused significant leakage of ALT and AST into the medium. This concludes that leakage cytoplasmic enzymes appear to be a sensitive indicator of cellular injury produced by heavy metals. In the current study, lead acetate executed the leaked enzymes leading to increase in activity in the serum and semen, while, on offering vitamin C the hazardous effect was removed, and the leakage was decreased. This was obvious via the decrease in ALT activity in the serum and semen.

Cholinesterases are enzymes, which hydrolyze esters of choline to give choline and the acid. Two types have been distinguished, true and pseudo. True cholinesterase is thought to be responsible for the destruction of acetylcholine.

The neuromuscular junction and is found in nerve tissue and in the red blood cells. Pseudocholinesterases are found in various tissues such as liver, heart muscle and intestine and also present in plasma or serum (Varley, 1976). The heavy metal ions (mercury, lead, cadmium, arsenic and some others) and their organic compounds belong to noncompetitive inhibitors of enzymes. They may block the -SH groups that make part of the catalytic site of the enzyme (Stroev, 1989). A significant reduction in the activity of cholinesterase was found in the serum of bucks received a dose of 15 mg/kg b.wt. This indicates that lead had reduced the cholinesterase in the serum. This may affect the role of pseudocholinesterase in the serum and liver in case of hypnotic's administration, e.g. succinylcholine (muscle relaxants). The same results were obtained in the semen, which indicates that the enzyme behaves like that in the serum and liver.

The acid phosphatase is present in high concentration in the prostate gland, erythrocytes, platelets, reticuloendothelial cells, liver, spleen and kidney. Its increase in the serum may be due to a carcinoma in the prostate, particularly if the cancer has spread beyond the capsule of the gland or has metastasized (Krupp et al., 1987). In the present study, the total and prostatic acid phosphatase activity, in the serum of lead and lead + vitamin C treated groups, were significantly increased than in the control serum. On the other hand, the lead groups treated with vitamin C showed a significantly higher level of the enzyme activity than the lead groups alone, which indicates that a tumor is present in the prostate gland or in the hemopoietic system as the vitamin could not cure it or ameliorate the case.

Moreover, the semen of those groups didn't show significant change only in the high lead dose. This agrees with the findings of Othman and El Missiry (1998).

In conclusion, lead exposure led to testicular disturbed hypo function, which is supported by the results of semen picture. The hazardous effect of lead led to disturbance in the activities of these enzymes under investigation, SOD,  $\text{GOT}$ , LDH, AST, ALT, cholinesterase, acid phosphatase, Vitamin C proved its antioxidant effect on recuperating from the normal status of enzymes in semen and serum thus counteracts the hazardous oxidant effect of lead inside the different organs. LDH and the prostatic acid phosphatase are shown to be biomarkers for testicular dysfunction, while the ALT and LDH may be used as biomarkers for hepatic and renal dysfunction. Lead exposure may acutely induce oxidative stress and enhance antioxidant defenses and decreased lipid peroxidation in animal.

#### **Corresponding author v**

El-Tohamy, M.M.

Department of Animal Reproduction and A.I., National Research Centre, Cairo, Egypt.

\*eltohamymagda@yahoo.com

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9/1/2010

# Tara Gum Carbamate: A New Thickening System for Cotton Printing Using Vat Dyes

**A. Hebeish<sup>1</sup>, A.A. Ragheb<sup>1</sup>, S.H.Nassar<sup>1</sup>, E.Allam<sup>2</sup> and J.I. Abd El -Thalouth<sup>2</sup>**

<sup>1</sup>Textile Research Division, National Research Center Dokki , Cairo Egypt

<sup>2</sup> Faculty of Applied Arts, Helwan University, Cairo, Egypt

**Abstract:** Green technology-based textile thickeners, namely, glactomannan was isolated from tara seeds and harnessed to vat printing of cotton fabrics before and after being carbamated. Carbamation was effected through reaction with urea at 160 °C for 15 and 90 min. to produce tara carbamate derivatives having 1% N and 3.12 % N respectively. These derivatives are soluble in water at room temperature and characterized by non-Newtonian pseudoplastic behaviour. However, for a given rate of shear, tara carbamate derivative having 1% N exhibits lower apparent viscosity than the derivative with 3.12 % N. On the other hand the apparent viscosity of pastes prepared from these two derivatives increases by storing for 24 or 48 hours before commence measuring. Prints could successfully be achieved using either of the two new tara carbamate derivatives in single use or in admixture with conventional thickener viz. Lameprint A6. Colour strength (K/S) values of prints prepared using the new tara carbamate –based thickeners are higher than those obtained with the conventional thickener, meanwhile the overall fastness properties of all prints are equal, irrespective of the thickener used.

[A. Hebeish, A.A. Ragheb, S.H.Nassar, E.Allam and J.I. Abd El -Thalouth. Tara Gum Carbamate: A New Thickening System for Cotton Printing Using Vat Dyes. Journal of American Science 2010;6(11):623-631]. (ISSN: 1545-1003).

**Keywords:** Tara Gum Carbamate; System; Print; Vat Dye

## 1. Introduction:

Tara gum is obtained from the kernels (seeds) of the tara shrub *Caesalpinia*, sometimes referred to as *Caesalpinia tinctoria* (H.B.K.) or *Caesalpinia pectinata* (Cav.)<sup>(1)</sup>. This legume is native to northern regions of Africa and South America. It is mainly distributed in the valley area in the middle of the east Andes and in the sea shore desert along the Pacific ocean<sup>(2)</sup>. Peru is the primary commercial source of tara<sup>(3)</sup>.

Vat dyes give a wide range of bright colours resistant to wet treatment and weather conditions; hence they occupy one of the prominent places among the other classes of dyes. They are used for printing cellulosic fabrics and also proteinic fabrics. At any event, however, although this group of dyes embrace a wide range of molecular structure types which are often complex yet all contain one or more carbonyl (=C=O) groups<sup>(4,5)</sup>. When they are treated with reducing agents, the carbonyl groups combine with hydrogen to form the leuco compounds, which contain secondary hydroxyl group. These compounds do not dissolve in water but form soluble sodium derivatives in the presence of alkalis. On oxidation the sodium compounds are converted to the insoluble coloured derivatives<sup>(6)</sup>.

Urea, on the other hand is one of the most important materials used in the field of textile finishing and printing. In the latter it acts as a solvent for dye and it promotes the dye migration from the printed film to the fibre interior. It has been reported<sup>(7)</sup> that urea reacts with alcohol and polyhydric alcohols to bring about the corresponding carbamate derivatives. A temperature range from 160 – 190 °C was reported as optimal for carbamate derived from urea and alcohols. Furthermore, it was shown that under such high temperature urea decomposes with loss of ammonia to form cyanic acid. The latter reacts with the alcohol to yield carbamates.

In a very recent study we have reported on the synthesis, characterization and application of carboxymethyl tara derivatives in reactive printing of cotton fabrics. Results were so promising that they stimulate current work especially after the search revealed that no systematic study was so far carried out on carbamation of tara gum.

Obviously, then, current work is undertaken with a view to carbamate tara gum by reacting it with urea and harness the obtained derivatives in printing cotton fabrics with vat dyes. The work involves technical applications in textile chemical technology evaluation of the tara carbamate derivatives through monitoring the nitrogen content as a measure for the

extent of carbamation, the rheological properties of the derivatives –based thickeners, printability of the tara carbamate derivatives alone as well as in admixture with the universe commercial thickener (Lame Print A6) and, properties of the prints. A comparison is also made between Vat prints obtained with the new tara carbamate derivatives and prints obtained using Lame Print A6.

## 2. Experimental

### 2.1. Materials

2.1.1. Cotton fabric: Cotton fabric: Mill desized, Kier boiled and bleached poplin cotton fabric ( $140 \text{ g/m}^2$ ) produced by Misr/helwan for Spinning and Weaving Company was used throughout the present work.

2.1.2. Plant seeds: Dry clean seeds of tara were obtained from tara shrub. They were kindly supplied by EL-Khawaga Farm at EL-Khatatba, Menufia. The seeds are composed of three components namely hull, endosperm, and germ obtained from ripe pods. The latter is rich in pyrogallol tannin. The gum is collected in the endosperm and composed mainly of galactomannan; the molar ratio of mannose to galactose is  $3:1^{(8,9)}$ .

2.1.3. Thickening agent: Lameprint A6, commercial plant seed gum ether, manufactured by Grunau, Germany, was kindly supplied by Misr/El-Mahala for spinning and weaving. It is resistant to metal salts, non-ionic, no residues. It is recommended for printing with Vat dyes and Rapidogen and Neutrogen dyes, fast colour salts and aniline salt.

2.1.4. Vat dyes: Two different Novatic microperle Vat dyes manufactured by Atul LTD, Gujarat, India were employed. These dyes were Novatic Orange 3G Microperle (C.I. Vat Orange 15) and Novatic Blue RS Microperle (C.I. Vat Blue 4).

2.1.5. Other chemicals: Urea and Sodium carbonate were of laboratory grade chemicals whereas ethyl alcohol was of technical grade. Sodium sulphoxalate formaldehyde (Rongalite C) as a reducing agent. Hostapal CV-ET (non -ionic detergent).

### 2.2. Methods:

2.2.1. Preparation of carbamate derivatives: After being isolated from the tara seeds, galactomannan gum was allowed to react with urea using the solid state technique as follows:

The gum and urea (1:1) were mixed well in the solid state using a laboratory mixer. The mixture was transferred to a porcelain crucible then subjected to high temperature ( $160^\circ\text{C}$ ) for different periods of time (15, 30, 45, 60, and 90 min). At this end the

thermally treated mixture was dissolved in distilled water at room temperature, precipitated with commercial ethyl alcohol, filtered on a sintered glass funnel and washed several times with 75% ethyl alcohol then purified.

2.2.2. Purification: The prepared carbamate derivatives prepared as described above were purified by extraction in soxhlet using 75% ethyl alcohol, and the purified derivatives were dried in a desiccator containing calcium chloride.

2.2.3. Preparation of the printing paste: This is done according to the popular potash / Rongalite process. The following is the recipe which has been employed:

#### Preparation of the thickening agent:

Thickening Agent*	X	g
Water	1000 - X	g
Total	1000	g

\*The thickening agent used were Tara carbamate derivative, 2.5 g, or commercial thickening agent namely Lameprint A6 6g.

#### Preparation of stock thickener:

Thickening agent	-----	600	g
Glycerine	-----	80	g
Potassium carbonate	-----	150	g
Rongalite C	-----	150	g
Water	-----	20	g
Total	-----	1000	g

#### Preparation of the printing paste:

Novatic microperle dye	-----	20	g
Water	-----	180	g
Stock thickener	-----	800	g
Total	-----	1000	g

The required amount of dyestuff was pasted with warm water and stirred well to make homogenous suspension paste. The previously prepared stock thickening was added into the paste and mixed thoroughly.

2.2.4. Printing technique: All the printing pastes were applied to cotton fabric as per flat screen printing.

2.2.5. Steaming: After printing and drying, the printed goods were subjected to steaming at  $100-102^\circ\text{C}$  for 10 minutes.

2.2.6. Washing: After steaming the printed goods were washed with cold water to remove the thickening agent and alkali and the vat dye was oxidized with dilute sodium perborate 5 g/l followed by washing thoroughly, soaping at the boil in a solution containing 2 g/l non-ionic detergent namely Hostapal CV, and cold water, air dried and assessed for K/S and overall fastness properties.

### 2.3. Analysis and Measurements:

#### 2.3.1. Determination of nitrogen content:

The nitrogen content of the purified tara gum carbamate derivatives was determined at Micro Analytical Laboratory, NRC, Egypt.

2.3.2. Determination of the rheological properties<sup>(10)</sup>: The rheological properties of the printing pastes were measured using Rheomat-15 at 25°C and the apparent viscosity ( $\eta$ ) at various rates of shear was calculated from the shearing stress ( $\tau$ ) and rates of shear (D) as follows:

$$\eta = \frac{\tau}{D}$$

#### 2.3.3. COLOUR MEASUREMENTS<sup>(11, 12)</sup>:

The colour strength, expressed as K/S and the overall fastness properties (washing, perspiration and crocking) were assessed according to the standard methods.

### 3. Results and Discussion:

#### 3.1. Tentative Reaction Mechanism of Carbamation of Tara Gum:

**Table I: Effect of time of carbamation of tara gum on the nitrogen, carbon, and hydrogen contents of the obtained products\*.**

Time of reaction in minutes	%N	%C	%H	Solubility in	
				Water	Ethyl alcohol
0	0	38.67	6.55	Swelled only	Insoluble
15	1	38.69	4.81	Soluble	Insoluble
30	1.63	37.46	4.86	Soluble	Insoluble
45	2.08	37.25	5.4	Soluble	Insoluble
60	2.28	37.85	5	Soluble	Insoluble
90	3.12	36.67	6.22	Soluble	Insoluble

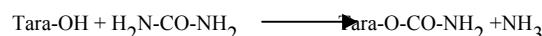
\* The reaction was carried out at 160 °C; the ratio of urea: gum is 1:1

#### 3.2. Dependence of Carbamation on Reaction Time:

It is seen (Table I) that, the percent nitrogen increases significantly by prolonging the duration of carbamation from 15 to 90 minutes. A nitrogen content of 1 % could be achieved with the derivative when urea was allowed to react with the tara gum for 15 minutes. This is against a nitrogen content of 3.12 % for a reaction time of 90 minutes. Trials have been made to increase the duration of carbamation more than 90 minutes. Allowing the carbamation reaction to proceed for 120, 150 or 180 minutes brings about tara gum derivatives which are insoluble in water. Longer durations cause charring of the reaction mixture.

Table I shows the element analysis, namely carbon and hydrogen of the derivatives under

To start with, tara galactomannan gum was isolated from tara seeds according to the procedure described elsewhere<sup>(13)</sup>. The obtained dry gum was allowed to react with urea using the solid state technique<sup>(14)</sup>. The reaction was conducted as detailed in the experimental section, at 160 °C for different intervals of time (15 to 90 minutes). The reaction may be drawn as follows:



Previous reports<sup>(7)</sup> have also disclosed that urea decomposes at high temperature and loses ammonia to form cyanic acid (HN = C = O) which react with the hydroxyl groups of the carbohydrate to give the carbamate derivative. Thus:



After the necessary purification, obtained tara gum carbamate derivatives were analyzed for nitrogen which is an indication of the degree of carbamate substitution in the molecule. The results obtained are given in Table I.

Time of reaction in minutes	%N	%C	%H	Solubility in	
				Water	Ethyl alcohol
0	0	38.67	6.55	Swelled only	Insoluble
15	1	38.69	4.81	Soluble	Insoluble
30	1.63	37.46	4.86	Soluble	Insoluble
45	2.08	37.25	5.4	Soluble	Insoluble
60	2.28	37.85	5	Soluble	Insoluble
90	3.12	36.67	6.22	Soluble	Insoluble

investigation. It also shows the solubility of these derivatives. While the results of carbon and hydrogen are self explanatory, the derivatives are soluble in water but are insoluble in ethyl alcohol. Insolubility in water is encountered when these derivatives were prepared under the influence of reaction time longer than 90 minutes as indicated above.

#### 3.3. Rheology:

The scientific study of the mechanical properties such as flow, ductility and plasticity of concentrated colloidal system has been termed rheology<sup>(15)</sup>. Textile printers have long been aware of the empirical relationship between the characteristic flow properties of the various thickening agents, their

use, and their suitability for printing various types of design.<sup>(15,16)</sup>

It is, therefore, of interest to investigate the rheological characteristic of pastes of the prepared carbamate tara gum derivatives. Hence aqueous pastes at a concentration of 2 % were prepared and their rheological properties were measured using Rheomat-15 immediately after preparation and also after storing of their pastes for 24 and 48 hours.

The investigation performed permits to obtain experimental rheograms (up and down flow curves), in which the ordinate represents the values of X proportional to the shearing stress ( $\tau$ ), and the abscissa represents the values of Y proportional to the rate of shear (D) as shown from figures 1,2 and 3 for the freshly prepared pastes and after storing of their pastes for 24 and 48 hours respectively.

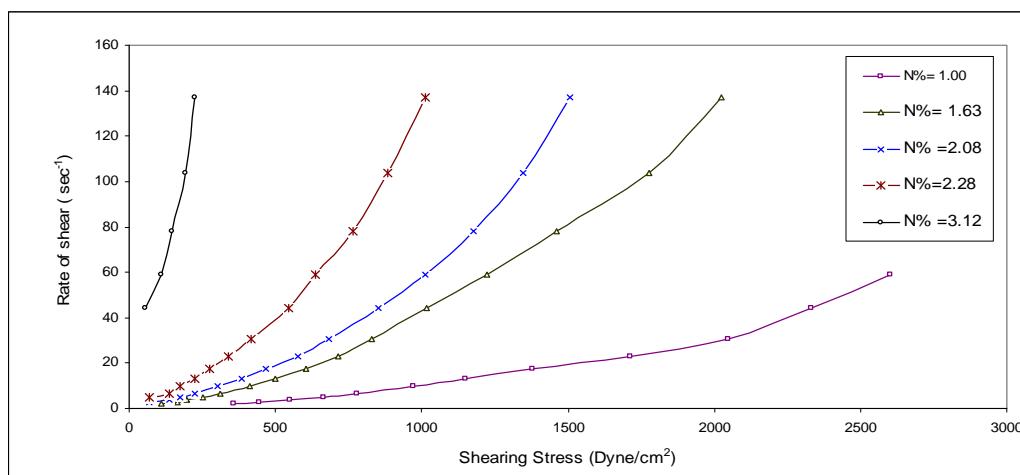
It is obvious (figure 1) that all the examined samples, of % N equal to 1.63, 2.08 , 2.28 and 3.12 are characterized by a non-Newtonian pseudoplastic behaviour, since the up and down flow curves are coincident. This means that, if the viscosity (resistance to flow) of these pastes is measured using a large applied force (shearing stress) which causes a high velocity of flow (shear rate), the apparent viscosity is less than that of the same paste determined with smaller force and at lower rate of flow<sup>(16)</sup>. In this system, i.e. pseudoplastic, no time dependent effects are observed, i.e. as soon as the applied force is removed, the paste rebuilds itself and retains its original viscosity immediately.

It is also clear from (figure 1) that, in spite of the fact that all the pastes are characterized by pseudoplasticity, yet the degree of carbamation, (i.e. % N) plays a dominant role on the viscometric properties of these pastes. As the degree of

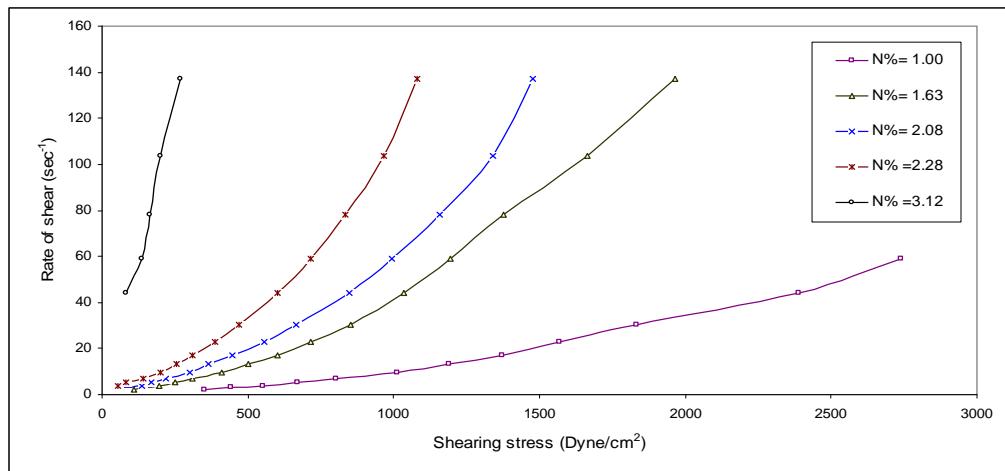
carbamation increases, the rheogram is shifted regularly near the rate of shear axis which indicates a decrease in the apparent viscosity at a constant rate of shear. This will be discussed later.

Figures 2 and 3 depict the effect of storing of carbamated tara gum pastes on their rheological properties. On comparing figures 2 and 3 with figures 1, one would realize that storing of these pastes has no effect on the rheological properties of these pastes, since they are still characterized by non-Newtonian pseudoplastic properties after storing. However, a close examination of these rheograms would reveal that, the location of the rheogram with respect to the rate of shear depends on the time of storing. As the latter increases the location of the rheogram is shifted far from the rate of shear axis indicating an increase in apparent viscosity by storing at a constant rate of shear. This state of affairs is more clarified in Tables II, III and IV.

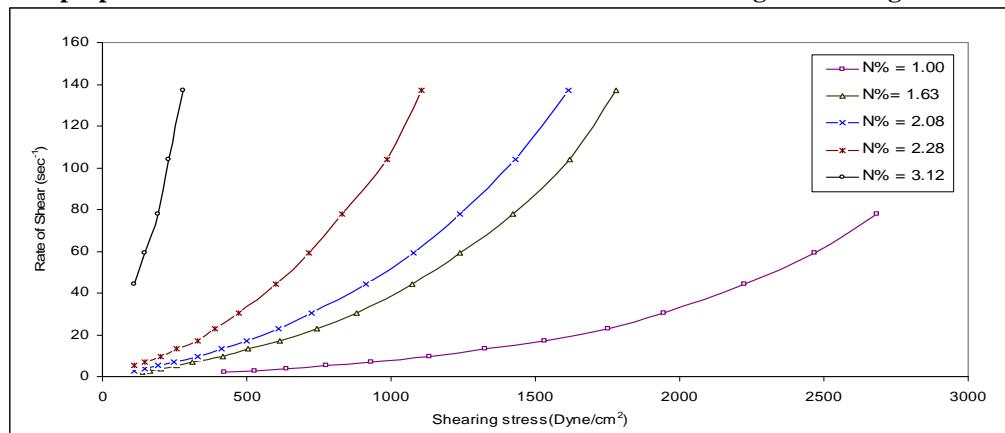
Table II shows that as the degree of carbamation (expressed as % N) of tara gum increases, the apparent viscosity at a given rate of shear decreases. For example at a rate of shear  $5.139 \text{ sec}^{-1}$ , the apparent viscosity decreases from 128.9 to 13.32 poise by increasing the nitrogen content from 1 % to 2.28 %. This indicates that higher extents of carbamation of tara gum are accompanied by higher thermal molecular degradation of the gum. It is understandable that carbamate derivatives with higher nitrogen content could be achieved at relatively longer reaction duration at a temperature as high as  $160^\circ\text{C}$ . Under such conditions thermal hydrolytic decomposition of the derivative inevitably occurs. Once this is the case, the molecular weight and therefore, apparent viscosity decreases.



**Figure 1: Effect of degree of carbamation of tara gum expressed as %N on the rheological properties of pastes freshly prepared thereof.**



**Figure 2:** Effect of degree of carbamation of tara gum expressed as %N on the rheological properties of pastes prepared thereof then stored for 24 hours before commencing the rheological measurements.



**Figure 3:** Effect of degree of carbamation of tara gum expressed as %N on the rheological properties of pastes prepared thereof then stored for 48 hours before commencing the rheological measurements.

**Table II:** Effect of nitrogen content of tara gum carbamate derivatives on the apparent viscosity of their freshly prepared pastes.

Rate of Shear (Sec <sup>-1</sup> )	Apparent viscosity in poise for carbamated tara gum acquire:				
	%N = 1	%N = 1.63	%N = 2.08	%N = 2.28	%N = 3.12
2.180	164.531	50.239	-	-	-
2.927	151.540	56.126	23.386	-	-
3.851	142.197	51.191	35.549	-	-
5.139	128.935	49.017	33.566	13.320	-
6.779	114.706	45.640	33.119	20.195	-
9.771	99.197	42.313	31.104	17.934	-
13.120	87.650	38.190	29.216	17.113	-
17.260	74.716	35.058	27.126	16.022	-
23.030	65.508	31.030	24.967	14.742	-
30.380	70.478	27.218	22.531	13.699	-
44.100	52.898	23.097	19.309	12.355	1.242
59.220	43.923	19.974	17.107	10.773	1.849
77.920	-	17.289	14.828	9.804	1.862
103.900	-	17.682	12.965	8.512	1.845
137.100	-	14.778	10.984	7.389	1.638

\* The concentration of the pastes was 2%.

**Table III: Effect of nitrogen content of tara gum carbamate derivatives on the apparent viscosity of their pastes after storing for 24 hours\*.**

Rate of Shear (Sec <sup>-1</sup> )	Apparent viscosity in poise for carbamated tara gum acquire:				
	%N=1	%N=1.63	%N=2.08	%N=2.28	%N=3.12
2.180	162.020	50.239	-	-	-
2.927	151.540	50.513	18.709	-	-
3.851	142.908	51.191	35.549	14.220	-
5.139	130.000	49.017	33.033	15.984	-
6.779	118.341	45.640	32.312	21.003	-
9.771	103.680	42.033	30.824	20.456	-
13.120	90.571	38.190	27.756	19.408	-
17.260	79.475	34.899	26.016	18.084	-
23.030	68.123	31.149	24.134	16.882	-
30.380	58.581	28.029	21.900	15.502	-
44.100	55.878	24.338	19.247	13.659	1.863
59.220	46.234	20.436	16.783	12.067	2.312
77.920	-	17.675	14.864	10.682	2.108
103.900	-	15.100	12.913	9.302	1.924
137.100	-	15.378	10.784	7.869	2.796

\* The concentration of the pastes was 2%.

**Table IV: Effect of nitrogen content of tara gum carbamate derivatives on the apparent viscosity of their pastes after storing for 48 hours\*.**

Rate of Shear (Sec <sup>-1</sup> )	Apparent viscosity in poise for carbamated tara gum acquire:				
	%N=1	%N=1.63	%N=2.08	%N=2.28	%N=3.12
2.180	193.419	62.798	-	-	-
2.927	179.602	56.126	37.417	-	-
3.851	165.659	51.902	37.682	-	-
5.139	150.779	49.549	37.828	21.312	-
6.779	137.324	46.044	36.350	21.810	-
9.771	116.010	42.593	33.906	20.456	-
13.120	101.006	38.399	31.303	19.617	-
17.260	88.834	35.534	28.871	19.036	-
23.030	76.089	32.219	26.393	17.001	-
30.380	63.989	28.930	23.793	15.502	-
44.100	50.476	24.338	20.675	13.659	2.483
59.220	41.657	20.944	18.170	12.113	2.450
77.920	34.436	18.272	15.883	10.682	2.460
103.900	-	15.601	13.756	9.487	2.187
137.100	-	12.981	11.244	8.048	2.057

\* The concentration of the pastes was 2%.

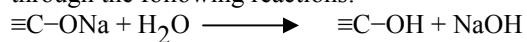
For the sake of verifying the effect of storing of the pastes under investigation on their apparent viscosity at specific rate of shear, comparison is made among Tables II, III and IV. For instance, at a rate of shear of 9.771 sec.<sup>-1</sup>, freshly prepared paste exhibits apparent viscosity value of 99.197 poise. This value increases to 103.68 poise and 116 poise after storing the paste for 24 hours and 48 hours respectively. This is, indeed, the case regardless of the extent of carbamation.

The increase in viscosity of carbamated tara gum by storing calls for formation of crosslinks between adjacent galactomannan tara gum chains. It is also likely that, besides exerting cross linking, storing acts in favour of inducing higher swellability of the derivative leading ultimately to increased viscosity.

### 3.4. Printing:

Vat dyes are found amongst the oldest natural colouring materials used for textiles<sup>(5)</sup>. They

include a wide range of structural types and often the molecules are complex, but they all contain one or more carbonyl groups ( $=\text{C}=\text{O}$ ), which, when treated with reducing agents combine with hydrogen to form leuco compounds containing ( $\equiv\text{C}-\text{OH}$ ) groups. These secondary alcohols do not dissolve in water but form soluble sodium derivatives ( $\equiv\text{C}-\text{ONa}$ ) in the presence of alkalies. On exposure to air, or oxidation with a suitable oxidizing agent, the sodium compound is converted to the insoluble coloured derivatives through the following reactions:



Hence printing with these dyes need a thickening agent which is not affected by the necessary auxiliaries added to the vat dye printing paste, i.e. reducing agent and alkali.

This work's objective is to introduce tara gum carbamate derivative as a new thickener for printing cotton fabrics with vat dyes.

Hence, different printing pastes thickened by tara gum carbamate derivative and containing Novatic Orange 3G (C.I. Vat orange 15) were prepared. Tara gum carbamate derivatives having

different nitrogen contents viz. 1, 1.63, 2.08, 2.28 and 3.12 % N were used.

Another two printing pastes containing the same Vat dye and thickened by a conventional commercial thickening agent namely Lameprint A6 were also prepared. This conventional thickener is recommended, in practice, for vat printing. Furthermore, a series of printing pastes thickened by a mixture of tara carbamate derivatives and the commercial Lameprint A6 thickener at a ratio of 1:1 (wt/wt) was prepared.

The so prepared pastes were employed for screen printing of cotton fabrics. This was done immediately after the preparation of the printing paste or after storing of the pastes for 24 or 48 hours before commence printing. After printing and drying the printed goods were subjected to steaming at 100-102 °C for 10 minutes followed by washing with cold water, oxidation with 5 g/l sodium perborate solution followed by a thorough wash, soaping at the boil, washing thoroughly and finally drying at room temperature. After being conditioned, the printed goods were assessed for measuring K/S and overall fastness properties. The results obtained with Novatic Orange 3G are set out in table V. Printing was performed using the freshly prepared pastes and after the latter were stored for 24 or 48 hours before commence printing.

**Table V: Colour strength, expressed as K/S of cotton fabric samples printed using pastes thickened by tara gum carbamate derivative, Lame Print A6 or mixture of both when printing was performed using freshly prepared pastes, 24 hours and 48 hours stored pastes containing Vat dye namely Novatic Orange 3G. Fastness properties are also shown when only freshly prepared thickeners were used as example.**

Thickening agent	K/S			Washing fastness		Rubbing fastness		Perspiration fastness			
	a	b	c	Alt.	St.	Dry	Wet	Acidic		Alkaline	
								Alt.	St.	Alt.	St.
Lameprint A6	1.48	1.35	0.77	4-5	4-5	3-4	3-4	4-5	4-5	4-5	4-5
Carbamate gum (%N = 1)	2.06	1.37	0.78	4	4	4	4	4-5	4-5	4-5	4-5
Carbamate gum (%N = 1.63)	1.94	1.35	0.75	4-5	4-5	4	4	4-5	4-5	4-5	4-5
Carbamate gum (%N = 2.08)	1.96	1.27	0.72	4-5	4-5	4	4	4-5	4-5	4-5	4-5
Carbamate gum (%N = 2.28)	1.87	1.58	0.95	4-5	4-5	4	3-4	4	4	4-5	4-5
Carbamate gum (%N = 3.12)	1.58	1.16	0.99	4-5	4-5	3-4	3-4	4-5	4-5	4	4
Lameprint / Carbamate (% N = 1) mixture	1.52	1.24	1.05	4-5	4-5	4	4	4-5	4-5	4-5	4-5
Lameprint / Carbamate (% N = 1.63) mixture	1.39	0.97	0.83	4-5	4-5	4	3-4	4-5	4-5	4-5	4-5
Lameprint / Carbamate (% N = 2.08) mixture	1.16	0.96	0.83	4-5	4-5	4	4	4	4	4-5	4-5
Lameprint / Carbamate (% N = 2.28) mixture	1.08	0.86	0.74	4-5	4-5	4	3-4	4-5	4-5	4-5	4-5
Lameprint / Carbamate (% N = 3.12) mixture	1.11	1.09	1.09	4	4	3-4	3-4	4-5	4-5	4	4

(a) Freshly prepared pastes (b) 24 hours pastes (c) 48 hours stored pastes. St: Staining; Alt: Alteration

### 3.4.1. Colour strength (K/S):

Results of table V disclose that the K/S value of the printed goods depends on: (1) % N of the tara gum carbamate thickener which, in turn, speaks of its nature and (2) the time elapsed before commence printing. In combination with this is the nature of the dye. When we have used different vat dyes under identical conditions, different K/S values were obtained.

Table V reveals that the K/S values of Novative Orange 3G on cotton fabric samples printed using tara gum carbamate are generally higher than their corresponding samples printed using the commercial thickener (Lameprint A6). The highest K/S value (2.06) is obtained upon using the carbamate thickener with 1% N, while the lowest K/S value (1.58) is obtained when a carbamate thickener having 3.12 % N was used. This is against K/S value of 1.48 obtained on using the commercial thickening agent. Similar trend was observed when other Vat dyes were used.

The superiority of tara gum carbamate with the lowest nitrogen content (1 %) within the range studied suggests that subjecting tara gum to carbamation for only 15 minutes at 160 °C is sufficient to convert the gum into a stable thickener with high ability to transfer the dye from the printing paste to the fabric. That is, this particular tara gum carbamate is capable of swelling, dispersing and jumping in an aqueous medium without complete elimination of intermolecular forces between themselves and water. Similar observation was reported<sup>(17)</sup> for cyanoethyl starch derivative having 1.75 % N.

Tables V shows the colour strength (K/S) of fabric samples printed using the pastes after being stored for 24 and 48 hours before commence printing respectively. As is evident storing decreases the K/S values and, the longer the storing the higher is the decrease in K/S. This suggests that the reducing agent undergoes partial decomposition under the influence of alkali and long storing at temperature of ca 30 °C as storing was carried out at ambient conditions. Storing of the pastes, before commence printing was performed during summer time in Cairo when a temperature as high as 40 °C could be encountered.

### 3.4.2. Fastness properties:

Table V shows the overall fastness properties including colour fastness to rubbing, to washing and to perspiration of the cotton fabric samples printed using tara gum carbamate derivatives. It is clear that these properties are almost identical to their corresponding samples printed using the commercial thickening agent (Lameprint A6). In

all cases rubbing fastness is ranging from 3-4 to 4 whereas, the washing and perspiration fastness properties range from 4 to 4-5.

### 3.4.3. Thickener Mixture:

Printing pastes containing the said vat dye and thickened by a mixture (1:1) of tara gum carbamate thickener and Lameprint A6 conventional thickener were prepared and used for printing cotton fabrics. The printed goods were monitored for colour strength (K/S) and overall colour fastness properties. Results obtained are set out in Tables V.

It is clear (Table V) that the highest K/S is obtained when the paste is thickened by a mixture containing the carbamate derivative having the lowest nitrogen content (1 % N). Increasing the degree of carbamation is accompanied by a decrease in K/S. This holds good for all derivatives with one exception; carbamate derivative having 3.12 % in the mixture brings about prints with marginally higher K/S than carbamate derivative having 2.28 % N in the mixture.

The effect of storing before commence printing of pastes thickened by tara gum carbamate / Lameprint A6 mixture may be realized from Table V. As is evident storing is accompanied by a decrease in K/S values. This is rather in accordance with the above results obtained upon using pastes thickened by only tara gum carbamate derivative and, therefore, could be interpreted on similar lines.

## 4. Conclusion

Tara gum carbamate derivatives can be used successfully in printing cotton fabrics with vat dyes. In most cases the lower the nitrogen content, the higher the K/S. Samples printed using carbamated tara gum, in most cases, acquire higher K/S values than those obtained using Lameprint A6 commercial thickening agent. The overall fastness properties of the prints are nearly, identical, and in all cases ranging between good to very good for rubbing and very good to excellent for washing and perspiration.

## Corresponding author

A. Hebeish<sup>1</sup>

<sup>1</sup>Textile Research Division, National Research Center Dokki , Cairo Egypt

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9/2/2010

# Use of GIS and Remote Sensing for Environmental Sensitivity Assessment of North Coastal Part, Egypt.

**Ahmed A. Afifi<sup>\*1</sup>; Gad, A<sup>2</sup>. and Refat, A.<sup>1</sup>**

<sup>1</sup> Soils and water use dept., National Research Centre, Dokki, Giza, Egypt.

<sup>2</sup> National Authority for Remote Sensing and Space Sciences, Egypt.

[\\*a.afifinrc@gmail.com](mailto:a.afifinrc@gmail.com)

**Abstract:** Desertification is considered as an important problem facing arid and semi-arid regions, as Egypt. These processes are resulted either from human activities or adverse natural conditions. However, the combination of both is often applicable. The aim of this study is the identification of areas sensitive to desertification in the north coast of Egypt. Based on the MEDALUS approach and the characteristics of the study area regional model developed using GIS. Three main indicators of desertification, including: soil, vegetation and climate were considered. The several sub-indicators affecting the quality of each main indicator were identified. Based on the MEDALUS approach, each sub-indicator was quantified according to its quality and given a weighting of between 1 and 2. Arc-GIS 9.2 was used to analyze and prepare the layers of quality maps using the geometric mean to integrate the individual sub-indicator. ETM and SRTM satellite images, geologic and soil maps were used as main sources for calculating the Environmental Sensitivity Areas Index (ESAI) for desertification. The results show that the soil of the north coast is characterized mainly by high sensitive areas for desertification (44.01 % of the total area), distributed mostly in the north western coast and the northern part of Sinai, where the soil quality, climatic quality and vegetation quality are low, while, 9.37 % of the total area exhibit are sensitive. The areas of moderate sensitive to desertification revealed in the studied area, representing an area of 3834.577 Km<sup>2</sup> (11.04 %) of the total area. The low sensitivity areas for desertification exhibit the whole area of the Nile Delta, as they represent 27.17 % of the total area (i.e. 9434.928 Km<sup>2</sup>). The low sensitivity for desertification is due to the good vegetation cover and soil quality. It can be concluded that implementing the maps of sensitivity to desertification is rather useful in the arid and semi arid areas as they give a more likely quantitative trend for frequency of sensitive areas. The integration of different factors contributing to desertification sensitivity may lead to plan a successful combating. The usage of space data and GIS proved to be suitable tools to rely on estimation and to fulfill the needed large computational requirements. They are also useful in visualizing the sensitivity situation of different desertification parameters.

[Ahmed A. Afifi; Gad, A. and Refat, A. Use of GIS and Remote Sensing for Environmental Sensitivity Assessment of North Coastal Part, Egypt. Journal of American Science 2010;6(11):632-646]. (ISSN: 1545-1003).

**Keywords:** Remote sensing, GIS, Environment, Desertification, Egypt

## 1. Introduction:

Desertification is defined in the first art of the convention to combat desertification as “land degradation in arid, semiarid and dry sub-humid areas resulting from climatic variations and human activities”. Its consequence includes a set of important processes, which are active in arid and semi arid environment, where water is the main limiting factor of land use performance in such an ecosystem (UNEP, 1992). Desertification sensitivity can be defined, in this context, as the response of the environment, or part of it, to a change in one or more external factors (Batterbury and Warren, 2001). Environmental systems are generally in a state of dynamic equilibrium with external driving forces. Small changes in the driving forces, such as climate or imposed land use tend to be accommodated partially by a small change in the equilibrium and partially by being absorbed or buffered by the system (Tucker et al. 1991). Desertification of an area will

proceed if certain land components are brought beyond the specific threshold, beyond which further change produces irreversible changes (Nicholson et al. 1998).

The MEDALUS method (Kosmas et al. 1999) identifies regions that are an environmentally sensitive area (ESAs). In this model, different types of ESAs to desertification can be analyzed in terms of various parameters such as landforms, soil, geology, vegetation, climate and human actions. Each of these parameters is grouped into various uniform classes and weighting factor is assigned to each class. Then four layers are evaluated soil quality, and management quality. After determined indices for each layer, the ESAs to desertification are defined by combining the four quality layer. All the data defining the four main layers are introduced in a regional geographical information system (GIS), and overlain in accordance with the developed algorithm

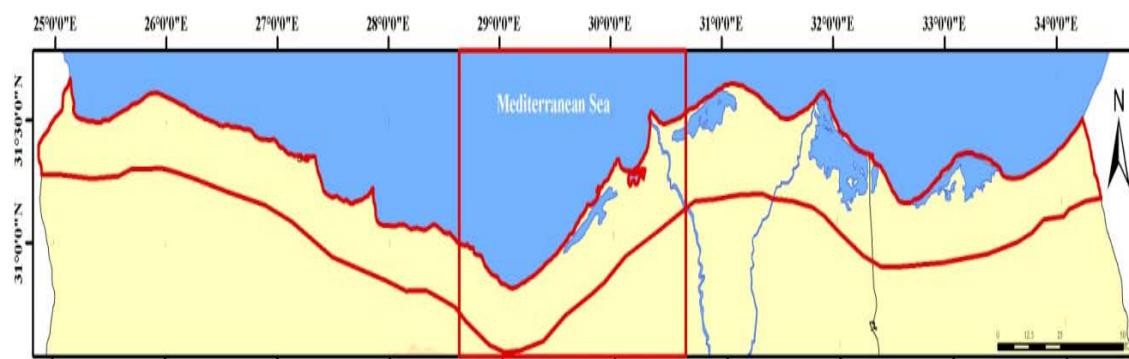
which takes the geometric mean to compile maps of ESAs to desertification.

In order to implement the UNCCD (United Nation Convention to Combat Desertification, 1996) plane, it requires reliable and up-to-date information. Not only the real-time information, but also, the analysis and technical methodologies that integrate this information to generate environmental, soil, water geo-spatial database together with environmental sensitivity maps. In this context, the Egyptian government prepared the national action plane (NAP) in 2005 in order to identify the sensitive area to desertification. It was possible to subdivide

the territory into four geographical areas on a basis of environmental farm Agro Ecological Zones characterized. Each of the Agro ecological Zones is characterized by specific environmental conditions. The Agro-ecological regions include the following;

1. North Coastal Zone
2. Nile Valley and Delta
3. The Inland Sinai and Eastern Desert
4. The Western Desert

The main aim was the assessment of most important factors affecting desertification in the study area (north coastal zone) by modifying MEDALUS system.



**Figure (1). The area of study**

## 2. Materials and methods

### 2.1. Study area

The study area represents the northern coastal zone of Egypt in a buffer zone of 20 Km from the coast line with the Mediterranean Sea. It extends from Rafah in the east on the border with Palestine to Al-Saloum in the west on the border with Libya (Figure, 1), representing an area of 32242.43 km<sup>2</sup>. The mean annual temperature of the representative metrological stations (19 stations distributed in the whole area) reaches its maximum during July and August, then decreases gradually to their minimum in December and January, enjoys a typical Mediterranean climate, being strongly influenced by the presence of the sea. The rainy season starts in October to January are the rainiest months, and the dry season extends for seven months. The study area is suffering mostly from high to moderate relative humidity. The maximum relative humidity occurs at July and August. Different investigations indicate that the wind speed, at an altitude of 10 meters, range between 3.8 to 5.2 m/sec. The prevailing wind is mostly from the north however, 25% of windy day record southerly dusty warm storms. The later harm

cultivation and causes soil water loss through its influence on increasing evapo-transpiration.

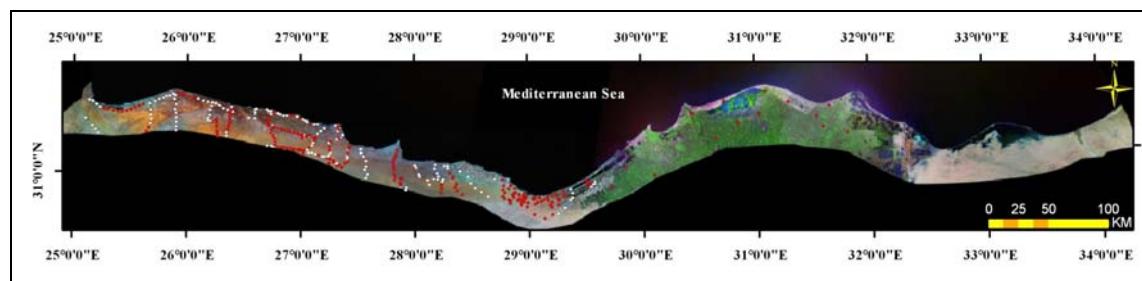
### 2.2. Methods

This study is based on multi concept data, thus the materials of different nature such as satellite data, thematic maps, ground truth geography and topographic data were used. Hybrid classification of ETM Landsat image (figure, 2) was the main mapping tools. Image analysis was made accordance with field observation. A number of 162 soil profiles representing the mapping units were studied. The soil sample was collected, analysed and classified to the level of the sub-great group according to (USDA, 2004). Arc-GIS, version 9.2 has been used as the main GIS software for producing geo-referenced maps.

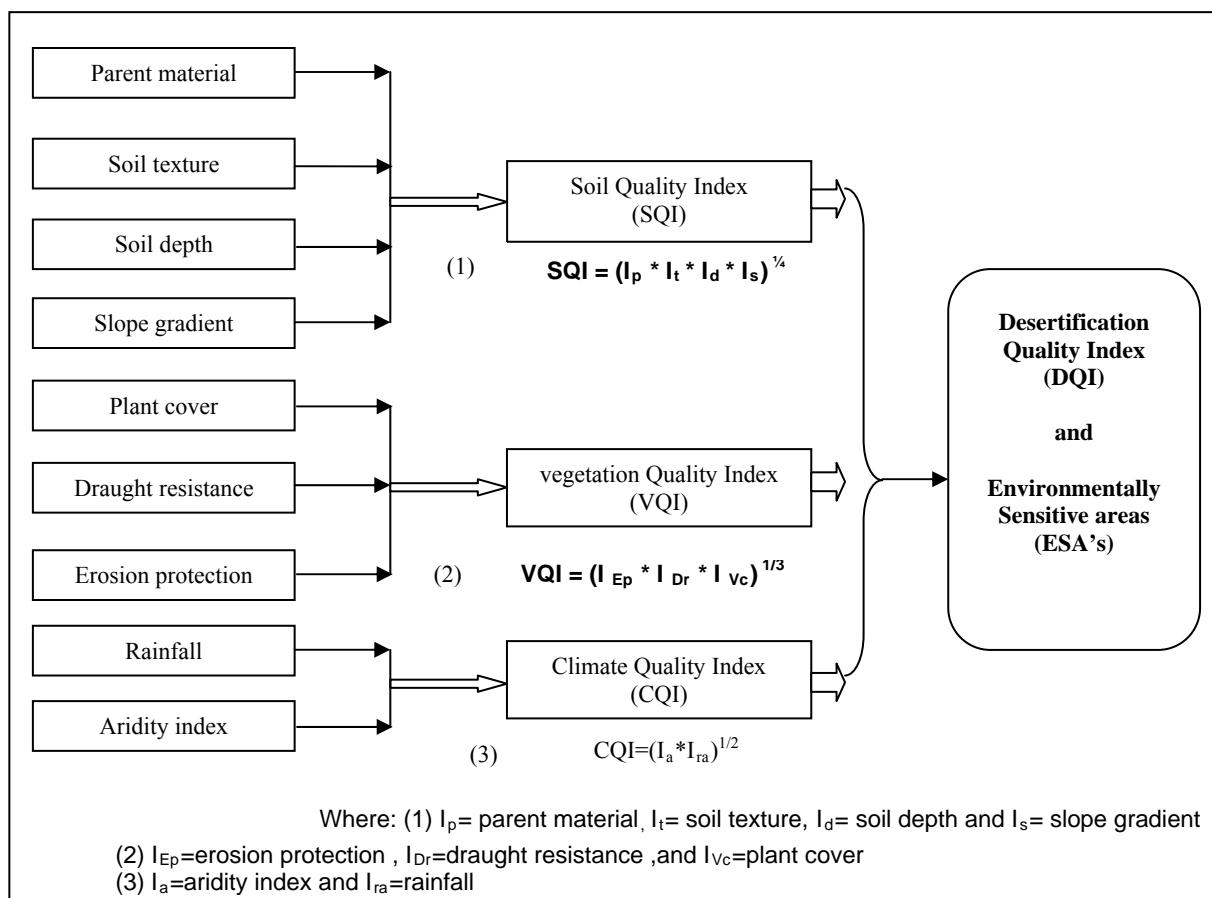
This study used spatial analyses in a Geographic Information System (GIS) to assess and map the environmental sensitivity to desertification in the north coastal zone of Egypt depending upon the soils, climate, vegetation and management quality indices. Concerning the data required for estimating the Environmental Sensitivity to desertification, the indices of soils, vegetation,

climate and management were computed. The main input data for calculating these indices include land surveying and laboratory analyses, Landsat ETM+ image (path 177 / row 39), Digital Elevation Model (DEM), climatic data and geological map of the studied areas (CONOCO, 1989). The satellite images were processed using the ERDAS

IMAGINE 8.3 system. Different enhancement and classification techniques were tried to specify the optimal ones for the study purposes. Computational and map editing functions were performed using Arc-GIS 9.2 to find out the environmental sensitivity areas (ESA's) (figure, 3).



**Figure (2) Location of the observation points plotted over Landsat satellite image**



**Figure (3) Flow chart of mapping Environmentally Sensitive Areas (ESA's)**

The hypotheses used by the MEDALUS model for the identification of sensitive areas derive from research and field experiments activities. The model applies a geometrical average of the quality indices used in order to provide sensitivity diagnosis. The model implicitly assumes that each of the indices taken individually has only a limited capacity to influence the final value of the ESAs index and that only when several parameters have a high score, an area can be assigned to a high sensitivity class (Kosmas et al. 1999). The quality indices used for assessing the desertification sensitivity were calculated and displayed as GIS ready maps from which class areas were deduced, and then the Desertification Sensitivity Index (DSI) was calculated in the polygonal attribute tables linked with the geographic coverage using the spatial analyst tool in Arc GIS 9.2 software.

#### 2.2.1. Mapping Soil Quality Index (SQI)

Soil is the dominant factor of the terrestrial ecosystems in the arid and semi arid and dry zones, particularly through its effect on biomass production (Basso et al, 1998). Four soil parameters, related to water availability and erosion resistance, were considered (I.e. parent material, soil texture, soil depth and slope gradient) following Medalu's project methodology (European Commission, 1999). Weighting factors were assigned to each category of the considered parameters, based on (Gad and Lotfy, 2007). The soil Quality Index (SQI) was computed based on the following equation:

$$SQI = (I_p * I_t * I_d * I_s)^{1/4}$$

Where  $I_p$  index of parent material,  $I_t$  indexed of soil texture,  $I_d$  index of soil depth,  $I_s$  indexed of the slope gradient). (Tables, 1 to 4) demonstrate the assigned indexes for different categories of each parameter. The soil Quality Index (SQI) was calculated based on the following equation, and classified according to categories shown in (table, 5).

**Table (1) Classes and assigned weighting index for parent material**

Class	Description	Score
Coherent: Limestone, dolomite, non-friable sandstone, hard limestone layer.	Good	1.0
Moderately coherent: Marine limestone, friable sandstone	Moderate	1.5
Soft to friable: Calcareous clay, clay, sandy formation, alluvium and colluvium	Poor	2

**Table (2) Classes and assigned weighting index for soil depth**

Class	Description	Score
Very deep	Soil thickness is more than 1 meter	1
Moderately deep	Soil thickness ranges from <1m to 0.5 m	1.33
Not deep	Soil thickness ranges from <0.5m to 0.25 m	1.66
Very thin	Soil thickness 0.15 m	2.00

**Table (3) Classes and assigned weighting index for soil texture**

Classes	Texture	Description	Score	
			Areas dominated by water erosion	Areas dominated by wind erosion
Not very light to average	Loamy sand, Sandy loam, Balanced	1		1
Fine to average	Loamy clay, Clayey sand, Sandy clay	1.33		1.66
Fine	Clayey, Clay loam	1.66		2
Coarse	Sandy to very Sandy	2		2

**Table (4) Classes and assigned weighting index for Slope gradient**

Classes	Description	Score
< 6%	Gentle	1
6 – 18 %	Not very gentle	1.33
19 – 35 %	Abrupt	1.66
> 35 %	Very abrupt	2

**Table (5) Classification of soil quality index**

Class	Description	Range
1	High quality	>1.13
2	Moderate quality	1.13 to 1.45
3	Low quality	> 1.46

### 2.2.2. Mapping Vegetation quality index (VQI)

Vegetation quality was evaluated according to (Basso et al 2000) in terms of three aspects (i.e. erosion protection to the soils, drought resistance and plant cover) (table, 6). The mosaiced landsat satellite image (Fig. 2) is the main material used to map vegetation and plant cover classes. Rating values for erosion protection, drought resistance and vegetal cover classes were adapted based on (OSS, 2004). Vegetation Quality Index was calculated according to the following equation, while VQI was classified based on the ranges indicated in the (European Commission, 1999) (table, 7).

$$VQI = (I_{Ep} * I_{Dr} * I_{Vc})^{1/3}$$

Where:  $I_{Ep}$  index of erosion protection,  $I_{Dr}$  index of drought resistance and  $I_{Vc}$  index of vegetation cover).

#### c) Mapping Climatic quality index (CQI)

Climatic quality is assessed by using parameters that influence water availability to plants such as the amount of rainfall, air temperature and aridity, as well as climate hazards, which might inhibit plant growth (Thornes, 1995). Table (8) reveals the classification categories of climatic quality index according to (OSS, 2004). The Climate quality index is evaluated through the Aridity Index (AI), using the methodology developed by FMA in accordance with the following formula. In the current study, rainfall and evapotranspiration data on a number of 19 metrological stations were used to calculate the CSI as follows;

$$CQI = P/PET$$

Where: P is average annual precipitation, and ETP is average annual Potential Evapotranspiration.

### 2.2.3 Mapping Environmentally Sensitive Areas (ESA's) to Desertification

ArcGIS9 software was used to map ESA's to Desertification (Kosmas et al, 1999) by integrating all data concerning the soil and vegetation. Different quality indices were calculated and displayed as GIS ready maps from which class areas were deduced. The Desertification Sensitivity Index (DSI) was computed in the polygonal attribute tables linked with the geographic coverage based on the following equation;

$$DSI = (SQI * VQI * CQI)^{1/3}$$

Classification of (DSI) was done according to the values of Medalus project Mediterranean desertification and land use Manual (table, 9) (European Commission, 1999).

**Table (6) Classes and assigned weighting index for different vegetation parameters**

Class	Description	$I_{Ep}$	$I_{Dr}$	$I_{Vc}$
1	Perennial cultivation	1	1	1
2	Halophytes	1.33	1	1.33
3	Temporal and orchards, mixed with crop land	1.66	1.33	1.66
4	Saharan vegetation < 40%	2	1.66	1
5	Saharan vegetation > 40%	2	1	1

**Table (7) Classification of vegetation quality index (VQI)**

Class	Description	Range
1	Good	< 1.2
2	Average	1.2 to 1.4
3	Weak	1.4 to 1.6
4	Very weak	> 1.6

**Table (8) Classification of Climatic quality index (CQI)**

Class number	Climatic zone	P/PET	CQI
1	Hyper-Arid	< 0.05	2
2	Arid	0.05 – 2.0	1.75
3	Semi-Arid	0.20 – 0.50	1.50
4	Dry Sub-Humid	0.50 – 0.65	1.25
5	Humid	> 0.65	1

**Table (9) Ranges and classes of desertification sensitivity index (DSI)**

Classes	DSI	Description
1	> 1.2	Non affected areas or very low sensitive areas to desertification
2	1.2 < DSI < 1.3	Low sensitive areas to desertification
3	1.3 < DSI < 1.4	Medium sensitive areas to desertification
4	1.3 > DSI < 1.6	Sensitive areas to desertification
5	DSI > 1.6	Very sensitive areas to desertification

### 3. Results and Discussion:

The environmental sensitivity area to desertification is a complex concept to rationalize since, depending on the context. It can be caused by many different factors operating in isolation or in association (Rubio, 1995, Thernes, 1995, UNEP, 1992 and Basso et. al., 2000). ESAs can be considered, in general, as a specific and delimited entity in which environmental and socio-economical factors are not balanced or are not sustainable for that particular environment. The various types of ESA's to desertification can be distinguished and mapped by using certain key indicators for assessing the land capability to withstand further degradation, or the land suitability for supporting specific types of land use. The key indicators for defining ESA's to desertification, which can be used at a regional or national level, can be divided into four broad categories defining the qualities of soil, climate, vegetation, and land management (Kosmas et. al. 1999). The Environmental Sensitivity Index (ESI) to desertification of an area can also be seen as the result of the interactions among elementary factors (information layers) that are differently linked to direct and indirect degradation or desertification phenomena (Basso et. al. 1998). Severe, irreversible environmental degradation phenomena could be resulted from a combination of poor management quality together with various combinations of critical environmental factors (soil, climate, and vegetation).

#### 3.1 Soil Quality Index (SQI)

Soil is an essential factor in evaluating the environmental sensitivity of an ecosystem, especially in the arid and semi-arid zones. Soil properties related to desertification phenomena affect the water storage and retention capacity and erosion resistance.

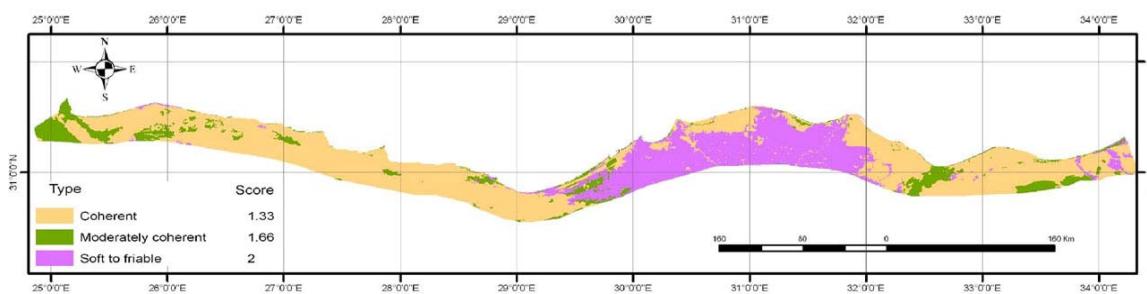
##### 3.1.1. Parent material

Parent material is considered a very important factor for determining the susceptibility to desertification. (Table, 10 and Figure, 4) represent the types, scores and areas of the parent materials of the northern coast of Egypt.

The results show that 61.79 % of the study area ( $21454.32 \text{ Km}^2$ ) originated from coherent parent material. It dominates the eastern and western part of the study area. This type of parent material is the least susceptible to desertification and takes a score of 1.00 on the desertification sensitivity index. The moderately coherent parent material such as marine limestone and friable sandstone covers  $3852.66 \text{ Km}^2$  representing 11.1 % of the study area. This type of parent material is moderately susceptible to desertification, and it takes a score of 1.5 on the desertification sensitivity index. The soils originated from soft to friable covers 27.12% ( $9415.03 \text{ Km}^2$ ). It dominates the northern part of Nile delta of the study area. This type of parent material is the most susceptible to desertification and takes a score of 2.00 on the desertification sensitivity index.

**Table (10) Types and scores of the parent materials in the studied area**

Type	score	Area Km <sup>2</sup>	Area %
Coherent: Limestone, dolomite, non-friable sandstone, hard limestone layer	1.00	21454.32	61.79
Moderately coherent: Marine limestone, friable sandstone	1.50	3852.66	11.10
Soft to friable: Calcareous clay, clay, sandy formation, alluvium and colluvium	2.00	9415.03	27.12
<b>Total</b>		<b>34722.01</b>	<b>100.00</b>

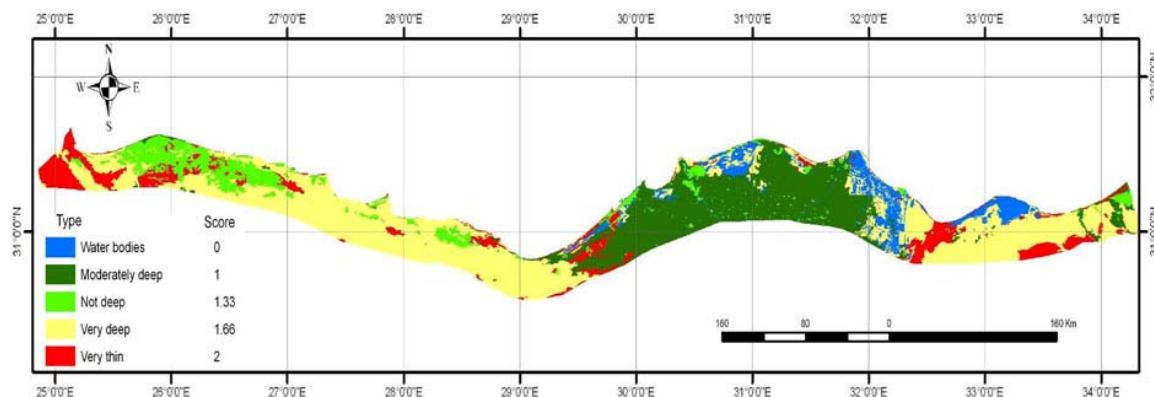
**Figure (4) soil parent types and scores of the north coast of Egypt**

### 3.1.2. Soil depth

Soil depth is a very important factor for determining the susceptibility to desertification, the deeper the soil the lesser sensitive to desertification and vice versa. As illustrated in (table, 11 and figure, 5) the very deep soil covers an area of  $15295.11 \text{ Km}^2$  representing 44.05 % of the total area. It dominates in the eastern and western part of the study area and represented by a score of 1.00 on the desertification sensitivity index. The moderately deep soil covers 27.12 % ( $9415.03 \text{ Km}^2$ ) of the study area. This class dominates in the northern Nile delta part of the study area. The moderately deep soil takes a score of 1.33 on the desertification sensitivity index. Not deep soil is more susceptible to desertification than very deep and moderately deep soils. It covers an area of  $3679.62 \text{ Km}^2$ , representing 10.60 % of the study area. It is located in eastern Sinai and the most western part of the study area. The not deep soil takes a score of 1.66 on the desertification sensitivity index. The very thin soil is the most susceptible to desertification. It covers 11.10 % ( $3852.66 \text{ Km}^2$ ) of the study area. It is located in western Sinai, western Nile delta and western most part of the study area. The very thin soil takes a score of 2.00 on the desertification sensitivity index.

**Table (11) distribution of soil depth classes and assigned scores in the studied area**

Type	score	Area Km <sup>2</sup>	Area %
Water bodies and urban	0.00	2479.58	7.14
Very deep	1.00	15295.11	44.05
Moderately deep	1.33	9415.03	27.12
Not deep	1.66	3679.62	10.60
Very thin	2.00	3852.66	11.10
<b>Total</b>		<b>34722.01</b>	<b>100.00</b>



**Figure (5) soil depth classes and scores of the northwestern coast of Egypt**

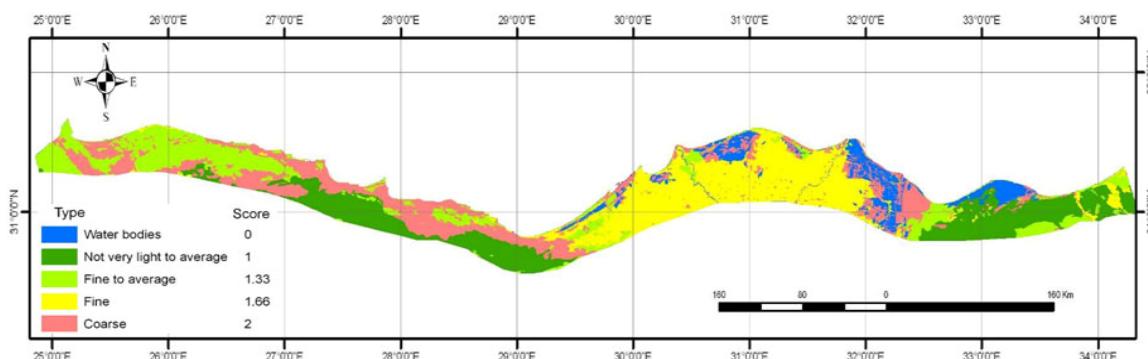
### 3.1.3. Soil texture

Soil texture is a very important factor for determining the susceptibility to desertification, (table, 12 and figure, 6) represents the types, scores and areas of the soil texture the north coast of Egypt.

The soil is divided into four classes according to its texture not very light to average is least susceptible to desertification. It represents 21.29 % of the north coast of Egypt, and it covers an area of 7393.43Km<sup>2</sup>. It dominates Nile delta part of the study area. It takes a score of 1.00 on the desertification sensitivity index. While, 20.48% (7111.32 Km<sup>2</sup>) of the studied area is moderately susceptible to desertification, which classified as Fine to average soil texture. It dominates the northern Nile delta part of the study area and takes a score of 1.33 on the desertification sensitivity index. Nevertheless, the fine soil texture covers 27.12 % (9415.03Km<sup>2</sup>) of the studied area. It is located in the north western part of Sinai and takes a score of 1.66 on the desertification sensitivity index. The rest of the studied area is classified as coarse soil texture, which is the most susceptible to desertification. It covers 23.97 % of the study area (8322.65 Km<sup>2</sup>). It is located in the eastern Sinai and western coast of the study area and takes a score of 2.00 on the desertification sensitivity index.

**Table (12) Distribution of soil texture classes and assigned scores in the studied area**

Type	score	Area Km <sup>2</sup>	Area %
Water bodies and urban	0.00	2479.58	7.14
Not very light to average	1.00	7393.43	21.29
Fine to average	1.33	7111.32	20.48
Fine	1.66	9415.03	27.12
Coarse	2.00	8322.65	23.97
<b>Total</b>		<b>34722.01</b>	<b>100.00</b>



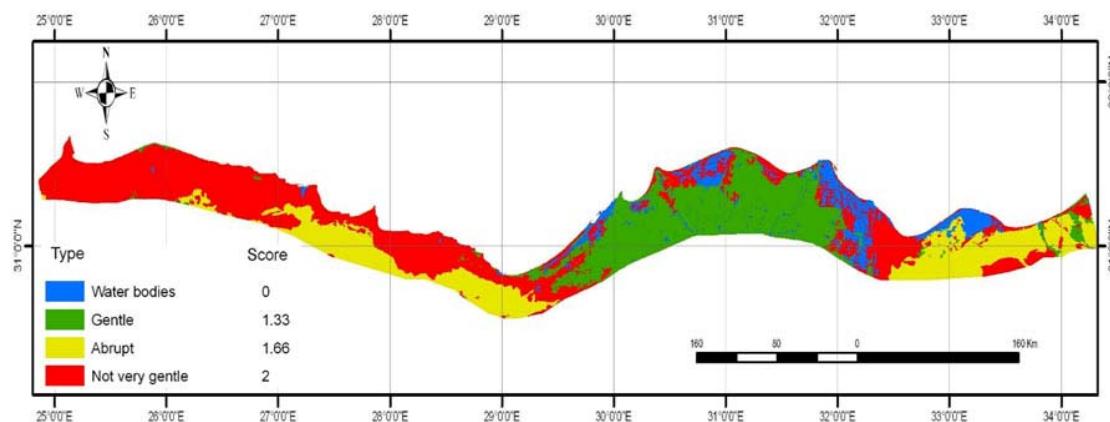
**Figure (6) soil texture classes and scores of the northwestern coast of Egypt**

### 3.1.4. Surface slope

Soil surface slope is very important factor for determining the susceptibility to desertification, (table 13 and figure, 7). The soil surface slope is divided into three classes. The Gently slope soil with surface slope of less than 6 % which is the least susceptible to desertification. It represents 27.12 % (9415.03.11Km<sup>2</sup>) of the studied area. It dominates in the Nile delta part of the studied area and takes a score of 1.00 on the desertification sensitivity index. While, the majority 43.24 % of the studied area slope is located in the Gently undulating class where surface slope between 6 and 18 % which is moderately susceptible to desertification. It dominates the northern western coastal part and takes a score of 1.33 on the desertification sensitivity index. The rest of the studied area is classified as undulating soil with surface slope between 19 and 35 % which is the most susceptible to desertification. It covers 21.29 % of the study area covers 7393.43 Km<sup>2</sup>. It is located in the western part of Sinai and eastern part of the northwestern coastal part and takes a score of 1.66 on the desertification sensitivity index.

**Table (13) Distribution of slope classes and assigned scores in the studied area**

Type	score	Area Km <sup>2</sup>	Area %
Water bodies and urban	0.00	2900.54	8.35
< 6%	1.00	9415.03	27.12
6 – 18 %	1.33	15013.00	43.24
19 – 35 %	1.66	7393.43	21.29
<b>Total</b>		<b>34722.01</b>	<b>100.00</b>



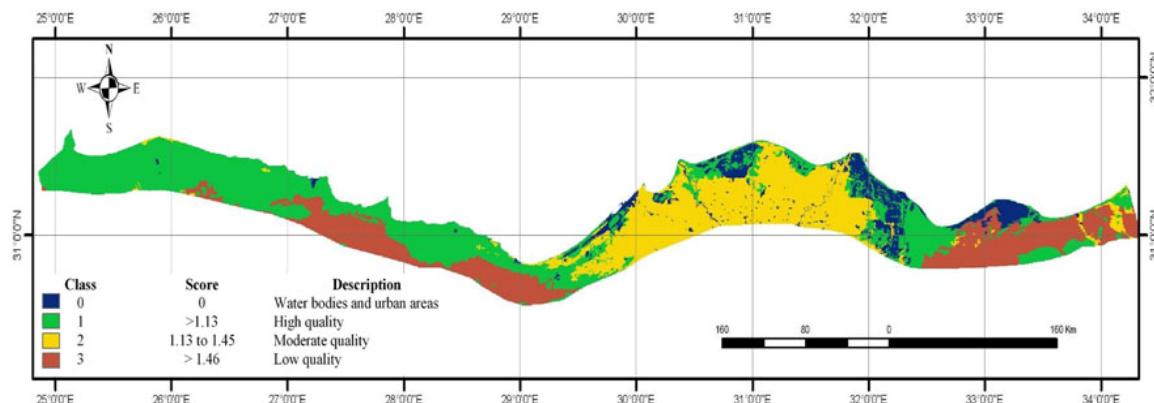
**Figure (7) Surface slope classes and scores of the northwestern coast of Egypt**

### 3.1.5. Soil Quality index

The soil quality index (SQI) was evaluated depend upon the drainage condition, rock fragments (%) slope gradient (%), soil texture class, soil depth (cm) and the parent material (Figure, 8 and table, 14). The layer of soil quality index of the studied area, indicate that the areas of high soil quality index (value <1.13) is found in large areas especially in the north western coast as it dominates an area of 14982.47 km<sup>2</sup> i.e. 43.15 % of the total area. The areas of moderate quality index (value = 1.13 – 1.45) represents 27.17% of the total area i.e. 9434.93Km<sup>2</sup> as it found mainly in the north coast of the Nile Delta. The areas of low soil quality index (value >1.45) represents 21.27 % of the total area i.e. 7386.66Km<sup>2</sup>, it found mainly in the north coast of Sinai. The low soil quality dominates the areas which characterized by sandy texture, shallow depth and poor drainage soils.

**Table (14) Soil Quality Index of the northwestern coast of Egypt**

Class	Score	Description	Area%	Area Km <sup>2</sup>
0	0	Water bodies and urban areas	8.40	2917.95
1	>1.13	High quality	43.15	14982.47
2	1.13 to 1.45	Moderate quality	27.17	9434.93
3	> 1.46	Low quality	21.27	7386.66
<b>Total</b>			<b>100</b>	<b>34722.01</b>



**Figure (8) Soil Quality Index of the northwestern coast of Egypt**

### 3.2. Vegetation Quality layers

The ETM satellite images were classified, and field validation was performed to convert the unsupervised classes to vegetation type. Different vegetation types were given score values evaluating vegetation cover type, erosion protection and drought resistance, and hence calculating the vegetation quality index (VQI).

#### 3.2.1. Plant cover

Vegetation cover plays an important role in mitigating the effects of desertification and land degradation phenomena. The percentage of vegetation is a function of both man-made agriculture and natural vegetation coverage. The percentage of vegetation cover is a necessary input in a multi-criteria model to assess the vegetation quality index.

Hyperid classification of ETM images resulted in identifying a number of four vegetation classes. Each of these classes was given a score evaluating vegetation cover, erosion protection and drought resistance (Figure, 9 and table, 15).

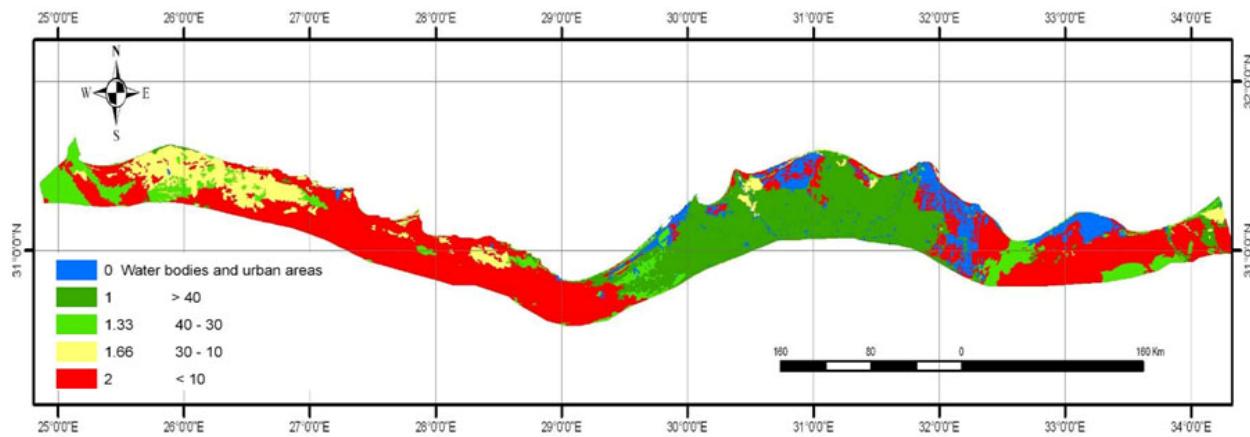
**Table (15) List of the vegetation covers categories and their area percentage**

Vegetation cover %	Value	Area Km <sup>2</sup>	Area %
Water bodies and urban areas	0.00	2900.54	8.35
Vegetation cover > 40%	1.00	9415.03	27.12
Vegetation cover (40 – 30 %)	1.33	3852.66	11.10
Vegetation cover (30 – 10%)	1.66	3258.66	9.39
Vegetation cover <10%	2.00	15295.11	44.05
<b>Total</b>		<b>34722.00</b>	<b>100.00</b>

As illustrated in (table, 15) 44.05% of the vegetation cover is very weak and sensitive to desertification. This category is distributed along the whole study area with higher concentration in the western section (northwestern part). Whilst, distributed as a few scattered areas in the middle section (northern Nile delta region) particularly in the sand dune areas and sabkhas. Nevertheless, the dense vegetation cover > 40% is representing a small area of 9415.03 km<sup>2</sup> (8.35%). This category occupies most of the middle section (northern part of the Nile Delta region) while it represents a minor area in the western section (northern western desert) and occupies a very minor area in the eastern section (northern Sinai). The rest of the study area has vegetation cover between 40 and 10%, distributed in the whole study area.

**Table (16) Vegetation resistance to drought**

Type	Value	Area km <sup>2</sup>	Area %	Drought Resistance
Water bodies and urban areas	0.00	2900.54	8.35	
Evergreen trees; Bedrocks; Bare soils	1.00	13267.69	38.21	Very high
Orchards; Deciduous trees	1.33	3258.66	9.39	High
Shrubs	1.66	7901.68	22.76	Moderate
Annual crops; Very low vegetated	2.00	7393.43	21.29	Low
<b>Total</b>		<b>34722.01</b>	<b>100.00</b>	

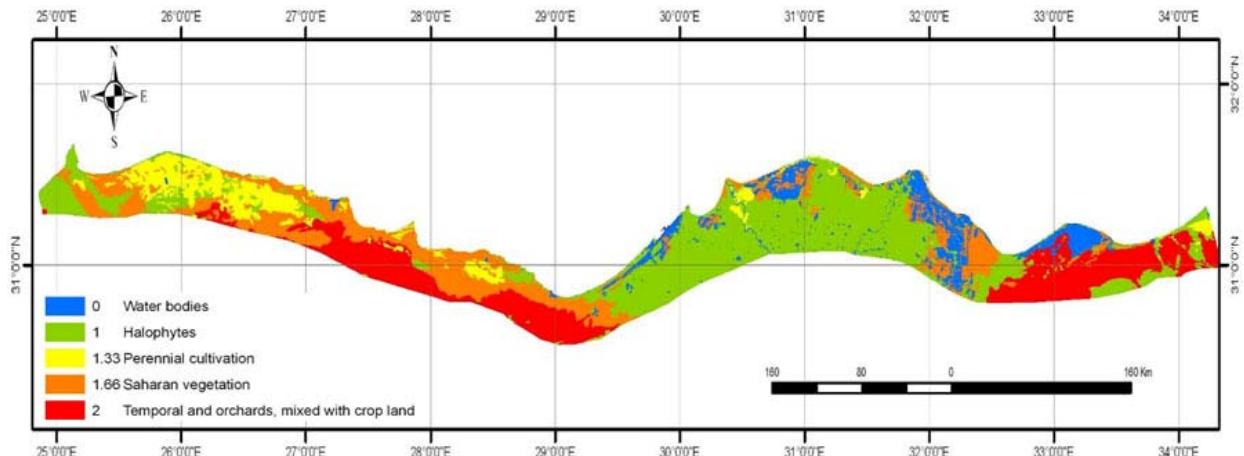


**Figure (9) vegetation cover percentage of the northwestern coast of Egypt**

### 3.2.2. Vegetation drought resistance

According to the universal standard classification for the susceptibility of vegetated land to drought and therefore its resistance, the study area in reflection to vegetation cover classified into five categories of drought resistance (Figure,10 and Table, 16).

Evergreen trees, bedrocks and bare soils; this category represents a significant area of about 38% of the study area. This category shows very high vegetation drought resistance with value of 1.0. It occupies most of the middle section (northern part of the Nile Delta region) and some areas in the western section (northern western coast). There are some scattered areas in the eastern section (northern Sinai) particularly the area of El-Tina plain and Wadi Al-Arish. Orchards and deciduous trees; this category occupies just 9% of the study area which is distributed in all the study area. This category shows high drought resistance with value of 1.33. The majority of this category is located in the western section (northern western desert) and few areas in the middle section around Lake El-Borouls and few areas near Rafah on the eastern border with Palestine. Shrubs; this category represents about 23% of the study area with moderate drought resistance of value of 1.66. The majority of this category occupies the western section (northern western desert). There are some areas of this category in the middle section around the coastal lagoons of Lake Manzala and lake Borouls. However, in the eastern section (northern Sinai) El-Tina plain and east of Suez Canal shows this moderate of drought resistance category. Annual crops and very low vegetated land; this category is the lowest category of drought resistance and it occupies 21% of the study area. This category is distributed in both the eastern and western sections (i.e. northern Sinai and north western desert). The terrain and geomorphological landforms in these two sections are the key constrains of this category to be low drought resistance.



**Figure (10) vegetation resistant to drought in the north coast of Egypt**

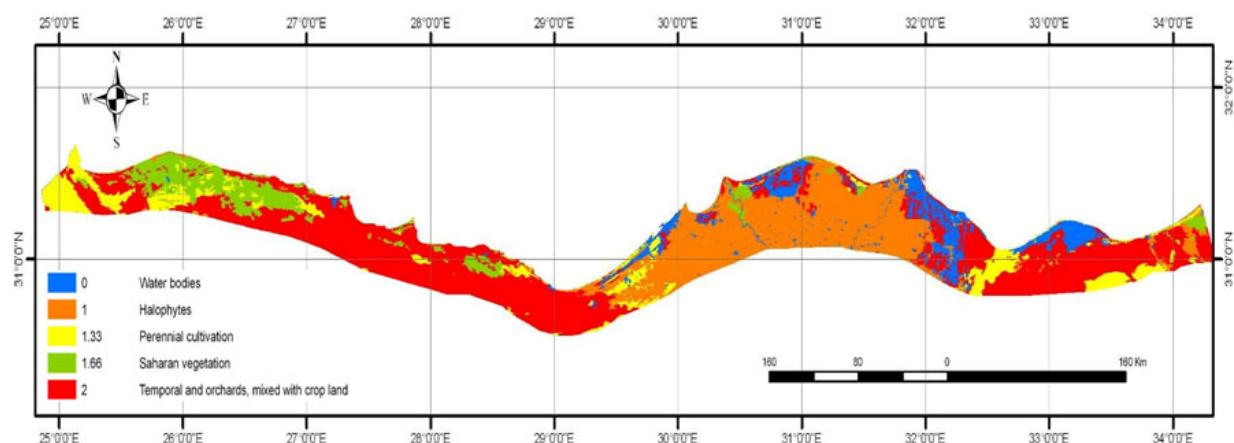
### 3.2.3. Vegetation erosion protection

Vegetation erosion resistance shows how much land, and its vegetation cover is resistant to erosion. The study area in reflection to the vegetation cover shows five categories of erosion resistance (Figure, 11 and Table 17).

Evergreen trees and bedrocks; this category represents a significant area of about 27% of the study area. This category shows very high vegetation erosion resistance with value of 1.0. It occupies most of the middle section (northern part of the Nile Delta region) and some areas in the western section (northern western desert) particularly the area close to the border. There are some scattered areas in the eastern section (northern Sinai) particularly the area of El-Tina plain and Wadi Al-Arish. Shrubs; this category occupies just 11% of the study area which is distributed in all the study area. This category shows high erosion resistance with value of 1.33 which located in the eastern section (northern Sinai) and western section (northern western desert). There are minor areas in the middle section around Lake El-Borouls and in the area west Gamasa. Annual crops; this category represents about 9% of the study area with moderate drought resistance of value of 1.66. The majority of this category occupies the western section (northern western desert) and as some patches in the eastern section (northern Sinai) close to Rafah. Bare soils; this category is the lowest category of erosion resistance, and it occupies 44% of the study area. This category occupies most of the eastern and western sections (i.e. northern Sinai and north western desert). The terrain and geomorphological landforms in these two sections are the key constraints of this category to be low erosion resistance. There are a few scattered areas in the middle section (northern Nile Delta region) particularly, in the sand sheets and sand dune areas.

**Table (17) erosion resistance of each vegetation category**

Type	Value	Area km <sup>2</sup>	Area %	Erosion resistance
Water bodies and urban areas	0.00	2900.54	8.35	
Evergreen trees; Bedrocks	1.00	9415.03	27.12	Very high
Shrubs	1.33	3852.66	11.10	High
Annual crops	1.66	3258.66	9.39	Moderate
Bare soils	2.00	15295.11	44.05	Low
<b>Total</b>		<b>34722.01</b>	<b>100.00</b>	



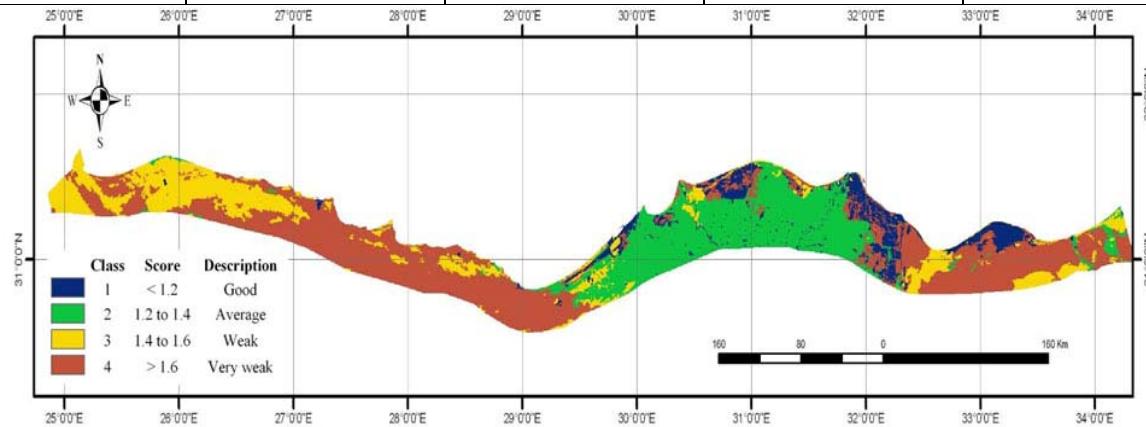
**Figure (11) vegetation erosion protection in the north coast of Egypt**

### 3.2.4. Vegetation Quality index

The plant cover (%), erosion protection, and drought resistance parameters were used for assessing the vegetation quality index (VQI). As illustrated in (figure, 12 and table, 18) the areas of low vegetation quality (Value <1.20) dominate coastal parts near the northern lakes, it represents 8.40 % of the total area (i.e. 2917.95 Km<sup>2</sup>), the moderate vegetation quality index (Value 1.2 – 1.4) dominates the north parts of the Nile Delta, it represents 27.17 % of the total area (i.e. 9434.93 Km<sup>2</sup>). The weak (Value 1.4 – 1.6) and very weak sensitive vegetation index (Value >1.60) dominates the rest of the coast representing 11.04 and 52.38 % of the total area (i.e. 3834.58 and 18534.56 Km<sup>2</sup> respectively). The low vegetation index is due to the low density of plant cover.

**Table (18) Vegetation Quality Index of the studied area**

Class	Score	Description	Area %	Area Km <sup>2</sup>
1	< 1.2	Good	8.40	2917.95
2	1.2 to 1.4	Average	27.17	9434.93
3	1.4 to 1.6	Weak	11.04	3834.58
4	> 1.6	Very weak	53.38	18534.56
<b>Total</b>			<b>100</b>	<b>34722.01</b>

**Figure (12) Vegetation Quality Index of the north coast of Egypt**

### 3.2.5. Climatic Quality Index

Climate quality index (CQI) is assessed depend upon the amount of rainfall, aridity and slope aspect parameters. The amount of rainfall and aridity are the same in the studied area, but the microclimate is differ from place to another depend on the surface slope and slope aspect. The digital elevation model (DEM) of the depression was established and used for extracting the slope and aspect. The climatic quality index layer of the area refer that the northwestern coast of Egypt is characterized by a low (>1.80) climatic quality index.

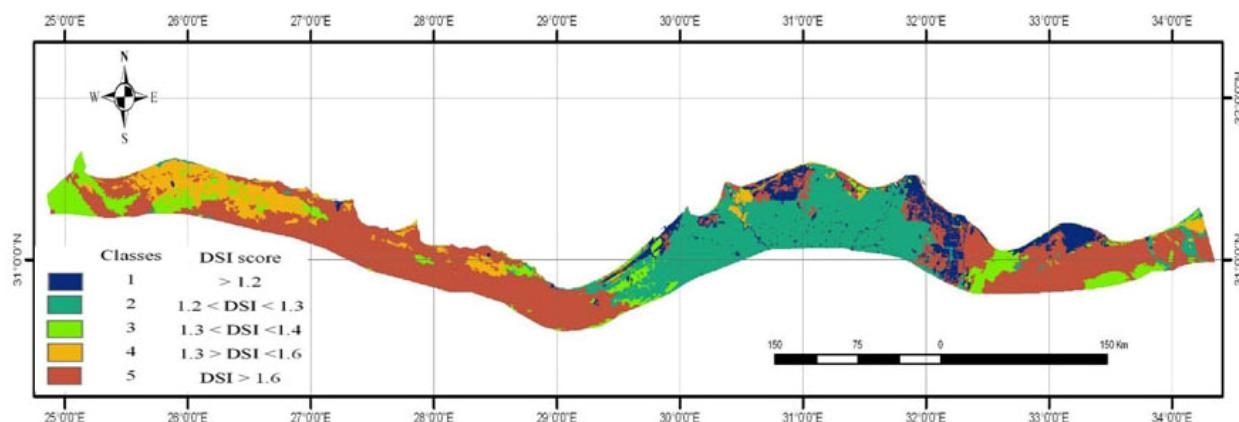
### 3.3. Desertification sensitivity index of the north coast of Egypt

The abovementioned layers (soil, vegetation and climate indices) were driven together to assess the environmentally sensitive areas (ESA's) to desertification, on basis of the calculated desertification sensitivity index (DSI). As revealed from (figure, 13 and table 19) the high sensitive areas for desertification in the area (44.01 % of the total area) are found in north western coast and the northern part of Sinai, where the soil quality, climatic quality and vegetation quality are low.

The sensitive areas are found where the vegetation cover is low, it found in Sidi Barrani and El Salum areas where, the sensitive areas represent 9.37 % of the total studied. The areas of moderate sensitive to desertification, revealed in the studied area, representing an area of 3834.577 Km<sup>2</sup> (11.04 %) of the total area. The low sensitivity areas for desertification exhibit the whole area of the Nile Delta, as they represent 27.17 % of the total area (i.e. 9434.928 Km<sup>2</sup>). The low sensitivity for desertification is due to the good vegetation cover and soil quality.

**Table (19) Environmentally Sensitive Areas to desertification in the north coast of Egypt**

Classes	DSI score	Description	area %	area Km <sup>2</sup>
1	> 1.2	Non affected areas or very low sensitive areas to desertification	8.403747	2917.95
2	1.2 < DSI < 1.3	Low sensitive areas to desertification	27.17276	9434.928
3	1.3 < DSI < 1.4	Medium sensitive areas to desertification	11.04365	3834.577
4	1.3 > DSI < 1.6	Sensitive areas to desertification	9.371228	3253.879
5	DSI > 1.6	Very sensitive areas to desertification	44.00862	15280.68
Total			100	34722.01



**Figure (13) Environmentally Sensitive Areas to desertification in the north coast**

#### 4. Conclusions and Recommendations

It can be concluded that the assessment of desertification sensitivity is rather important to plan combating actions and to improve the employment of natural resources. Remote sensing, in addition to thematic maps, may supply valuable information concerning the soil and vegetation quality at the general scale. However, for more detailed scales, conventional field observation would be essential.

Remote sensing, in addition to thematic maps, may supply valuable information concerning the soil and vegetation quality. However, field validation is rather important for reliable information. The Geographic Information System (GIS) is a valuable tool to store, retrieve and manipulate the huge amount of data needed to compute and map different quality indices to desertification.

It can be recommended that mathematical modeling should be developed for the operational monitoring of different elements contributing in desertification sensitivity.

Multi scale mapping of ESA's are needed to point out the risk magnitude and causes of degradation in problematic areas.

The Egyptian north coast is susceptible to very high-to-high desertification sensitivity, however the Nile Valley is moderately sensitive because of its vegetation cover.

#### Corresponding author

Ahmed A. Afifi

Soils and water use dept., National Research Centre, Dokki, Giza, Egypt.

\*[a.afifinrc@gmail.com](mailto:a.afifinrc@gmail.com)

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9/3/2010

# Efficiency of Natural Minerals in Presence of Different Nitrogen Forms and Potassium Dissolving Bacteria on Peanut and Sesame Yields

Gehan H. Youssef, Wafaa M. A. Seddik and Mona A. Osman

Soil, Water and Environ. Research Institute, agricultural Research Center (ARC), Giza, Egypt

**Abstract:** A field experiment was carried out for two summer seasons at Ismailia Agric. Res. Station to study the effect of some natural minerals combined with potassium dissolving bacteria inoculation in the presence of different nitrogen forms on chemical properties of soil, nutritional status and yield of peanut-sesame. Each experiment was designed in a split-split design with three replications. Three forms of nitrogen fertilizer were included along with two natural minerals, in a presence of potassium dissolving bacteria inoculation, as well as one mineral fertilizer as source potassium fertilizer. Furthermore, data show high significant increases in available N due to the application of ammonium nitrate in combination with feldspar, and calcium nitrate in combination with potassium sulfate in a presence of inoculation for peanut and sesame, respectively. However, application of calcium nitrate combined with potassium sulfate, and ammonium nitrate in combination with feldspar, in a presence of inoculation, led to significant increases in K available in soil for peanut and sesame, respectively. Oppositely, the pH values, different to those of EC, decreased either for inoculation or non-inoculation as compared to control. In spite of that, the values of EC and pH of soil were higher with application of either bentonite or bentonite + feldspar in a presence of all nitrogen fertilizer forms. Generally, the highest EC values in soil, after the two studied seasons were encountered with calcium nitrate fertilizer as well as bentonite mineral. Moreover, applying feldspar mineral and ammonium nitrate treatments had recorded the highest values of yield components as well as nutrient (N and K) uptake by either peanut or sesame crops, particularly in the presence of inoculation as compared to those given by other treatments. [Gehan H. Youssef, Wafaa M. A. Seddik and Mona A. Osman. Efficiency of Natural Minerals in Presence of Different Nitrogen Forms and Potassium Dissolving Bacteria on Peanut and Sesame Yields. Journal of American Science 2010;6(11):647-660]. (ISSN: 1545-1003).

**Keywords:** Efficiency; Natural Mineral; Nitrogen; Potassium; Bacteria

## 1. Introduction:

Sandy soils represent the most desert area in Egypt, and they are usually deficient in organic matter and plant nutrients (Abdel Wahab et al, 2003). Potassium is one of the three essential element viz., NPK, for the growth and reproduction of plants; it plays vital roles in its nutrition. The crop production in Egypt relies completely on imports to meet its annual requirement of potash fertilizers; besides the high cost of conventional, water-soluble K fertilizers constrains their use by most of the farmers in the country. In order to reduce the dependence on imported potash, feldspar potash mineral contains 11.25 % K<sub>2</sub>O and therefore, it could be a potential K-source for crop production. New approaches are needed to unlock K from the silicate structure of this mineral in order to render K more available for plant nutrition (Badr, 2006). Many researchers showed that microbes can accelerate weathering of minerals and rocks by producing organic acids, phenolic compounds, siderophores and possibly other metabolites, which influence pH and redox conditions. In addition, direct contact between bacteria and minerals may be important in mineral

alteration reaction, as microbial surfaces can be complex with metal ions. Some recent reports showed that silicate dissolving bacteria played a promotion role in the release of Si, Fe and K from feldspar (Badr, 2006).

According to Abdel Wahab et al., (2003), the highest values of growth and green yield of pea were obtained in case of organic compost application in combination with chemical or natural sources of P and K. Also, the applied natural sources of P and K gave an almost similar trend to that obtained with the chemical ones combined with the organic compost. The concentrations of phosphorus and potassium in plant tissues increased with increasing compost levels irrespective of their sources. In addition, the combined treatment of organic compost with both sources of P-K achieved the highest values of P and K concentrations in plant tissues.

Significant increases were obtained in height of main stem, branch stem length, number of branches, main stem diameter and leaf area of olive seedlings treated with compost fortified with plant guard and feldspar compared with the control treatment. The same trend was also observed

concerning the application of compost and feldspar on micro and macronutrient contents in leaves of the mentioned olive seedlings (Abd El-Motty et al., 2009).

Biofertilizers have been used as sources to improve the status of plant nutrients in sustainable agriculture. Inoculation with bacterial strain *Bacillus edaphicus NBT* was found to increase root and shoot growth of cotton and rape. Strain NBT could mobilize potassium efficiently in both plants when illite was added to the soil. In cotton and rape growing on soils treated with insoluble potassium and inoculated with strain NBT, the potassium content was increased by 30 and 26 %, respectively. Bacterial inoculation also resulted in higher N and P contents of above ground plant components (Sheng, 2005).

Rock P and K applied either singly or in combination did not significantly enhance soil availability of P and K, indicating their unsuitability for direct application. PSB (phosphate solubilizing bacteria) was a more potent P-solubilizer than KSB (potassium solubilizing bacteria), and co-inoculation of PSB and KSB resulted in consistently higher P and K availability than in the control without bacterial inoculum and without rock material fertilizer. Combined together, rock material and both bacterial strains consistently increased further mineral availability, uptake and plant growth of pepper and cucumber plants, suggesting their potential use as fertilizer (Han et al., 2006).

Phosphorus and potassium nutritional status in the soil were markedly improved through inoculation with solubilizing bacteria (*Bacillus mucilaginosus*); groundnut plant dry matter increased by 125 % and the oil content 35.41 % were increased through bacterium inoculation (Sugumaran and Janarthanam, 2007).

Recently, the treatment of *Bacillus circulans* + rock phosphate + feldspar was superior in plant height, number of branches, number of nodules per plant and fresh yield (ton/fed.) of snap bean plants when compared with control and the un-inoculated plants. The NPK analysis of shoot dry matter of snap plants showed that as a result of addition of alternatives and the viability of *B. circulans*, there was marked increases in phosphorus and potassium solubilization (Massoud et al., 2009).

The objective of this study was to determine the efficiency of using different natural mineral as alternative potassium fertilizer in the presence of nitrogen forms and potassium dissolving bacteria inoculation adopted for peanut and sesame plants grown on sandy soil.

## 2. Materials and methods

Two summer successive field experiments were carried out on peanut (*Arachis hypogaea*) – sesame (*Sesamum indicum*) cropping sequence in a sandy soil under drip irrigation system at Ismailia Agric. Res. Station (A.R.C).

Some physical and chemical characteristics of the studied soil before cultivation are shown in Table (1).

**Table (1): Some physical and chemical properties of soil samples representing the studied location.**

Soil characteristics	Values
<b>Particle size distribution %</b>	
Coarse sand	50.4
Fine sand	40.4
Silt	3.20
Clay	6.0
Texture class	Sandy
<b>Chemical properties</b>	
CaCO <sub>3</sub> %	1.4
pH (suspension 1:2.5)	7.92
EC dS/m (saturated paste extract)	0.37
Organic matter %	0.40
<b>Soluble cations and anions (meq L<sup>-1</sup>)</b>	
Ca <sup>++</sup>	0.95
Mg <sup>++</sup>	0.89
Na <sup>+</sup>	1.51
K <sup>+</sup>	0.45
CO <sub>3</sub> <sup>2-</sup>	-
HCO <sub>3</sub> <sup>-</sup>	1.42
CL <sup>-</sup>	1.02
SO <sub>4</sub> <sup>2-</sup>	1.36
<b>Available nutrients (mg L<sup>-1</sup>)</b>	
N	40.0
P	15.0
K	55.6

**Table (2): Analysis of natural mineral constituents.**

Determination	Bentonite	Feldspar
EC dS m <sup>-1</sup>	2.89	0.44
pH	8.08	8.56
<b>Available nutrients (mgL<sup>-1</sup>)</b>		
N	166	216
P	2.10	5.76
K	151	400

Peanut and sesame were cultivated in a randomized split-split plot design, each treatment being replicated three times. The main plots were

either inoculated or un-inoculated with potassium dissolving bacteria (*Bacillus pasteurii*) as (Biopotash). The sub main plots were three nitrogen sources, including ammonium sulfate, calcium nitrate and ammonium nitrate, added at the rate of 30 kg N/fed. The sources of nitrogen were added in four equal split doses after sowing. The sub- sub plots represented the natural minerals (feldspar and bentonite), which were added individually or in combination (50% bentonite and 50% feldspar, as compared to mineral fertilizer (potassium sulfate)) at the rate of 50 kg K<sub>2</sub>O/ fed. Phosphorus fertilizer was added at the recommended dose (200 kg/fed.) for both peanut and sesame in the form of superphosphate 15.5 % P<sub>2</sub>O<sub>5</sub>. Both potassium and phosphorus fertilizers were completely added to soil before cultivation.

Plant and soil were sampled at 150 days and 120 days after sowing for peanut and sesame respectively, which represent the harvesting stage.

Surface soil samples (0-15 cm depth) were taken after harvesting stage to evaluate soil pH, EC and available nutrients (N and K) were determined according to Page et al. (1982).

Peanut and sesame plant samples were taken at harvesting stage to determine the nutrients status and yield components (straw and grain yield). Plant samples were oven dried at 70 °C for 48 hrs up to constant dry weight, then ground and wet digested using H<sub>2</sub>SO<sub>4</sub>:H<sub>2</sub>O<sub>2</sub> method described by Page et al. (1982). The digests were then subjected to the measurement of nutrients (N and K) according to procedures described by cottenie et al. (1982).

Obtained results were subjected to statistical analysis using STATISTICA 6.0 (statSoft, Inc, Tusla, USA) according to Hill and Lewicki (2007). Analysis of variance (ANOVA) was employed to examine the independent and interacted effects of inoculation with potassium dissolving bacteria, nitrogen and potassium sources. Also, treatments were compared by using L.S.D. at 0.05 level of probability according to Snedecor and Cochran (1980).

### **3. Results and Discussion:**

1- Influence of nitrogen fertilizer, natural mineral and inoculation with bacteria on some soil chemical characteristics.

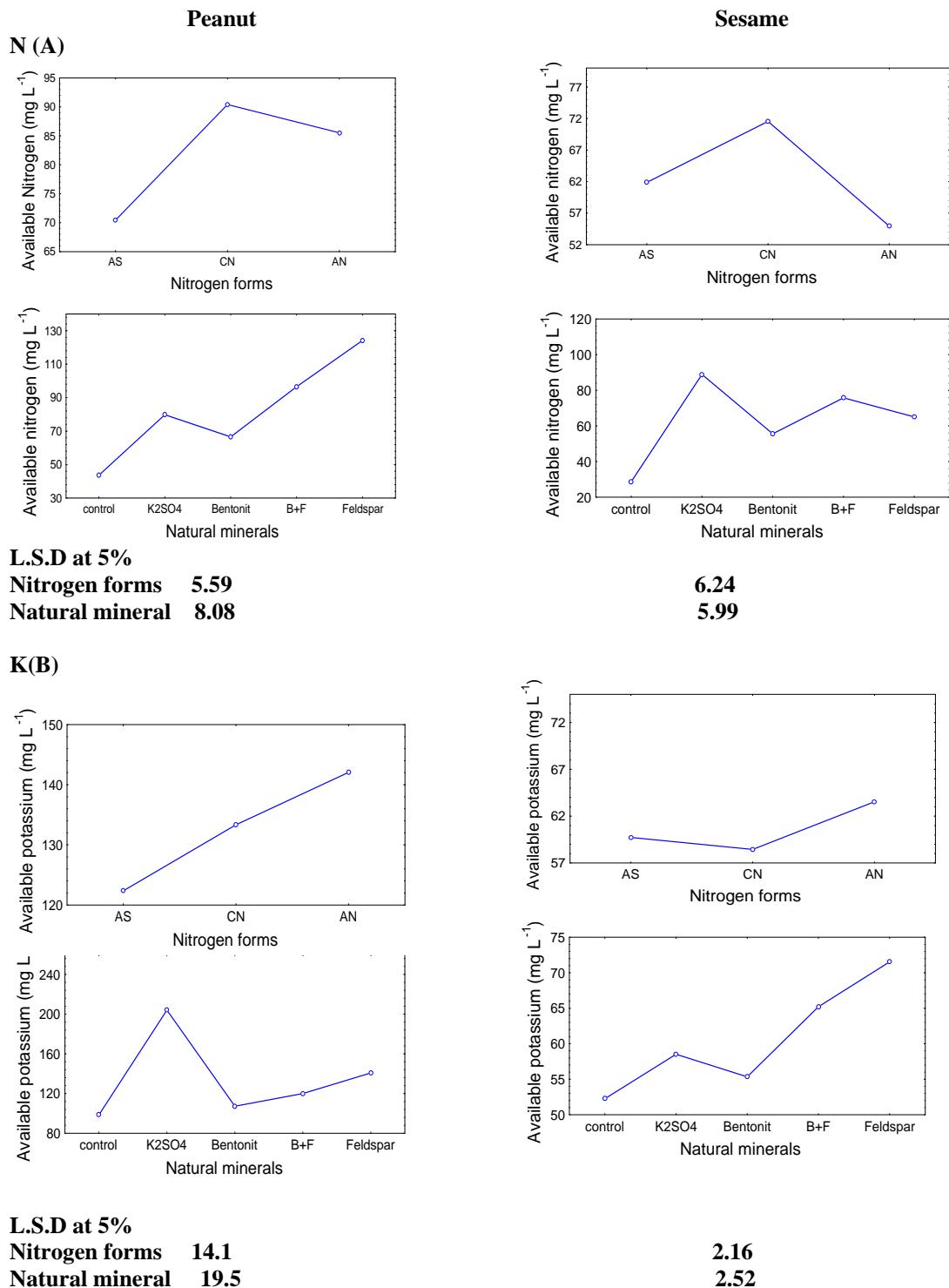
As for the effect of nitrogen forms and natural mineral on nitrogen and potassium availability, at the two studied seasons, results in Fig (1 A, B) reveal that calcium nitrate treatment was

superior at available N while ammonium nitrate being superior at available K.

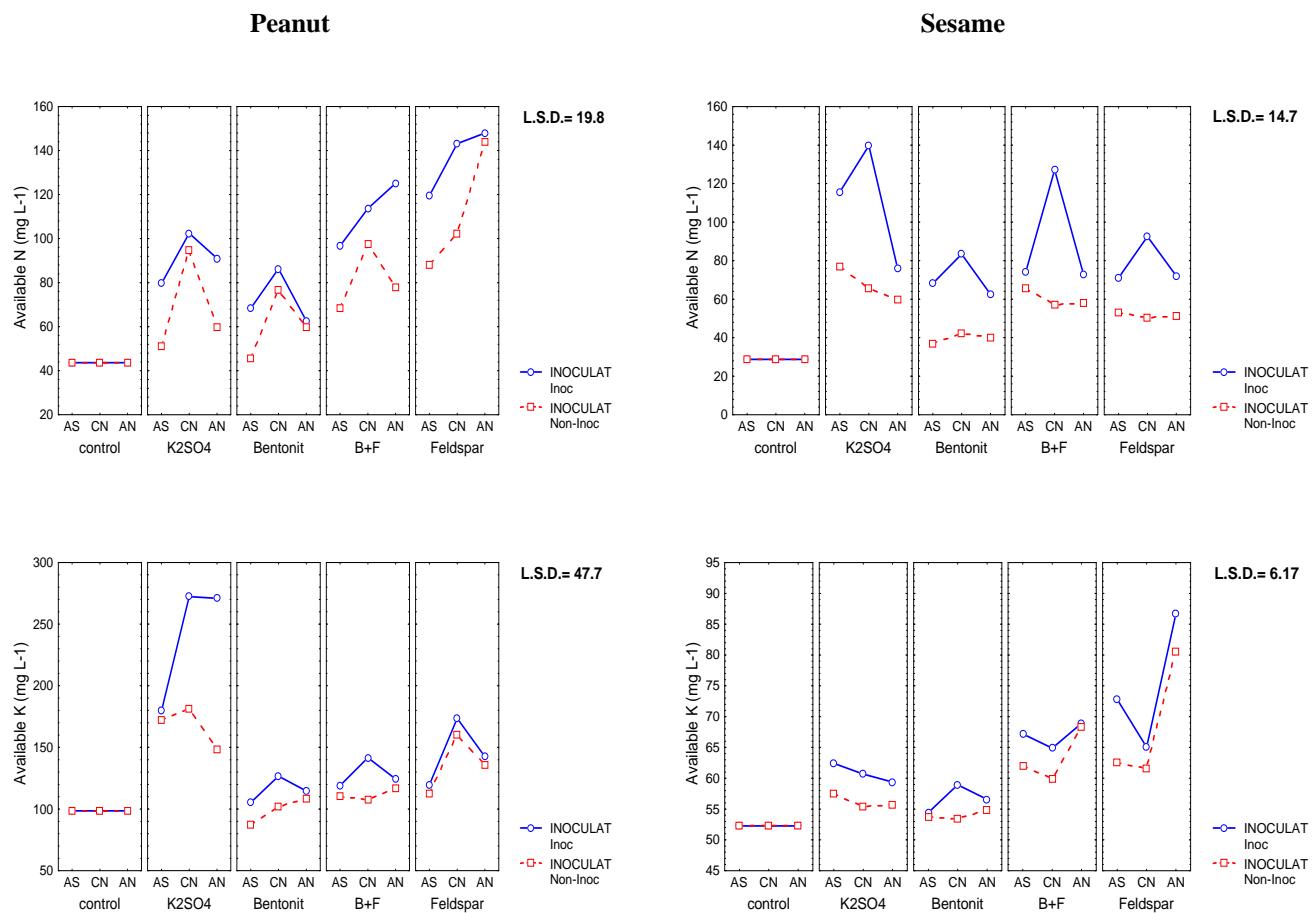
Furthermore, results showed that the highest values of available nitrogen and potassium exist in case of feldspar and potassium sulfate treatments, respectively at the first studied season. Such results are confirmed by Hagin and Shaviv (1990) who reported that the adequate supply of potassium enhances ammonium utilization and thus improves yields. An opposite trend was obtained at second season, which appeared to be highly significant with applied potassium sulfate for available nitrogen with feldspar being highly significant for available potassium. This obtained data could be due to the application of K solubilizing bacteria, which may produce bacterial acids, alkalies or chelates to enhance solubility and release of elements from potassium containing minerals in soil ( Lin Qi-mei , et al., 2002 and Seddik, 2006).

To make the picture clearer, it was thought usefully to express the obtained results as interactions between the influences of both natural mineral and nitrogen fertilizer forms; such interactions are shown in Fig (2). For available nutrients (N and K) in soil, at the two studied seasons, values were significantly increased as a consequence of applied natural mineral in the presence of nitrogen fertilizer and inoculation as compared to control. A previous study (Barker, et al., 1997 and Badr, et al., 2006) confirmed that this bacterial strain produces several organic acids such as acetate, butyric, pyruvic, lactic and formic acid during their biological activities. Such acids can increase mineral dissolution rates; carboxylic acid groups, which were shown to promote dissolution of silicate, are also common in extra cellular organic materials. Moreover, some microorganisms in the soil environment contain enzymes that function in ways analogous to chitinase and celluloses. i.e. they specifically break down mineral structures and extract elements required for metabolism or structure purposes (e.g., mineralization).

Also, data in Fig (2) show high significant increases in available N due to the application of ammonium nitrate in combination with feldspar, and calcium nitrate in combination with potassium sulfate in the presence of inoculation for peanut and sesame, respectively. However, application of calcium nitrate combined with potassium sulfate, and ammonium nitrate in combination with feldspar, in the presence of inoculation, led to significant increases in K available in soil for peanut and sesame, respectively.



**Fig (1): Effect of one factor either nitrogen fertilizer or natural mineral on both nitrogen (A) and potassium (B) availability for the tested soil after peanut and sesame harvesting.**

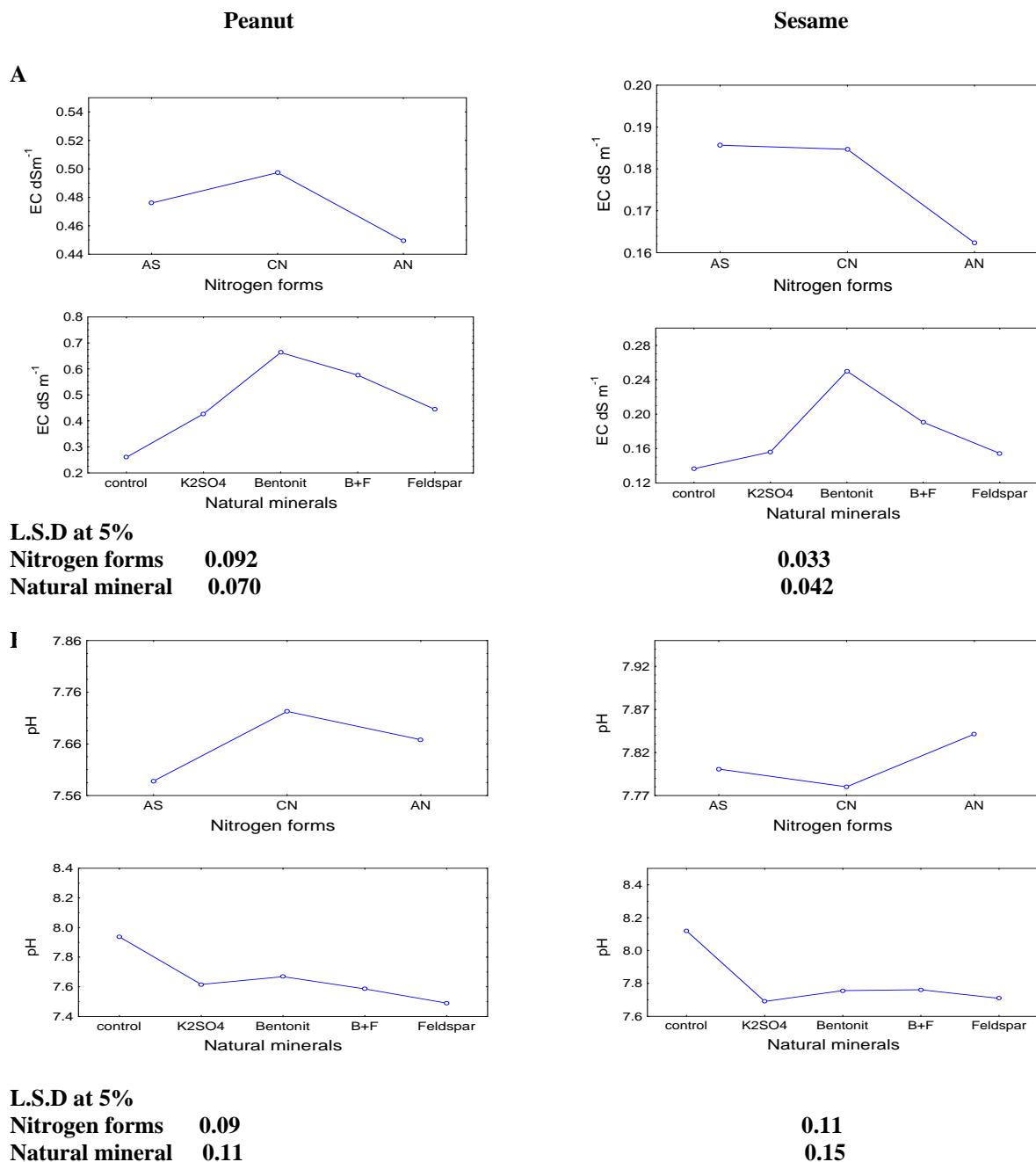


**Fig (2): Effect of different sources of natural mineral, nitrogen fertilizer and/ or inoculation with potassium dissolving bacteria on available nutrients (N and K) for the tested soil after peanut and sesame harvesting.**

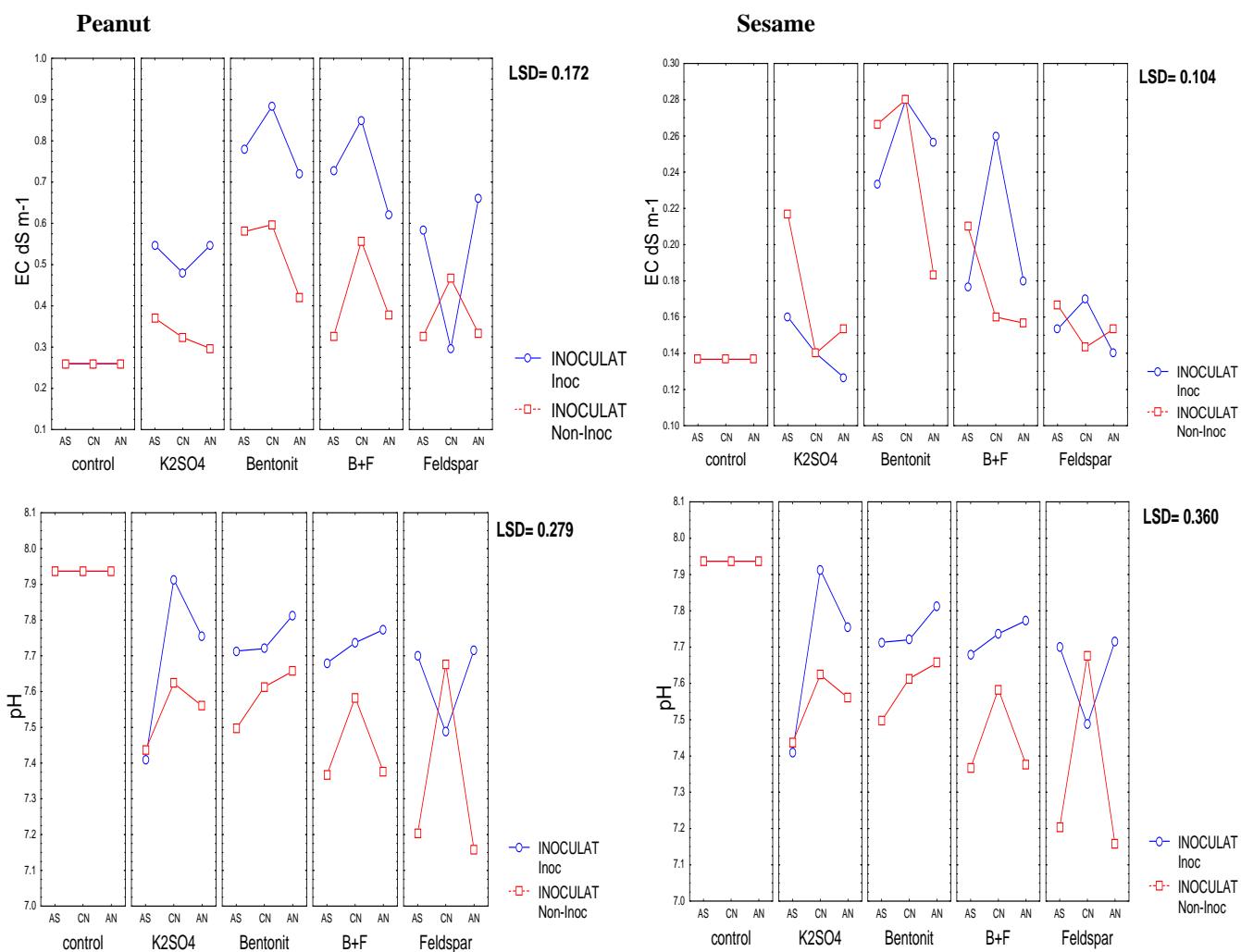
Concerning EC and pH values, the highest EC values in soil following the two studied seasons were reported for calcium nitrate fertilizer as well as bentonite mineral as a source of potassium fertilizer (Fig 3.A). With respect to pH values, the highest values in soil were reported for calcium nitrate at the first season (peanut) and for ammonium nitrate at the second season (sesame). An opposite trend was generally encountered with natural mineral, particularly for applied feldspar, which led to a decrease in pH values at the two seasons as compared to control (Fig 3.B).

Again, to make the picture clearer, it was thought useful to express the obtained results as

interactions between the influences of both natural mineral and nitrogen fertilizer forms; such interactions are shown in Fig (4). Data indicated that, for both studied seasons, application of different natural mineral significantly increased the EC values but decreased the pH values of the studied soil in the presence of nitrogen fertilizer forms as compared with control, whether inoculation or non-inoculation was performed. Moreover, the indicated values of EC and pH were higher in case of applying either bentonite or bentonite + feldspar ratio in the presence of all nitrogen fertilizer forms. Soil salinity increase could be due to the relatively high content of salts in bentonite (Gouda, 1984).



**Fig (3): Effect of either nitrogen fertilizer or natural mineral on both electrical conductivity (EC) (A) and soil reaction (pH) (B) for the tested soil after peanut and sesame harvesting.**



**Fig (4): Effect of different sources of natural mineral, nitrogen fertilizer and/ or inoculation with potassium dissolving bacteria on both electrical conductivity (EC) and soil reaction (pH) for the tested soil after peanut and sesame harvesting.**

2- Influence of nitrogen fertilizer, natural minerals and inoculation with bacteria on yield components at harvesting stage.

With respect to yield of straw and yield of grains or seeds for both peanut and sesame crops, data shown in Fig (5. A and B) revealed that the highest significant yield components of peanut and sesame crops were reported for ammonium nitrate fertilizer as compared to other treatments. Treatments of nitrogen fertilizer may be arranged as follows: ammonium nitrate > calcium nitrate > ammonium sulfate. Also, feldspar mineral gave the highest values of yield components. Treatments of natural mineral may be arranged as follows: feldspar > potassium sulfate > bentonite + feldspar > bentonite and feldspar > bentonite + feldspar > potassium

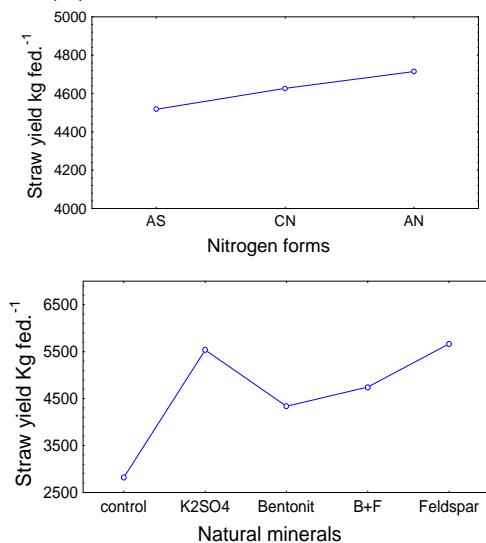
sulfate > bentonite for peanut and sesame yield, respectively.

In general, to make the picture clearer, it was thought usefully to express the obtained results as interactions between the influences of both natural mineral and nitrogen fertilizer forms; such interactions are shown in Fig (6). Data show that natural mineral was favorable to yields either in the presence of nitrogen fertilizer forms or inoculation with potassium dissolving bacteria as compared to control. Furthermore, results indicate that yield components of peanut and sesame increased in the presence of inoculation with potassium dissolving bacteria as compared to non-inoculation. Such results are confirmed by those of Han and lee (2005) who reported that the inoculation of potassium solubilizing bacteria synergistically solubilized the K

materials which were added into the soil and made them more available to the plant. This led to the promotion of their uptake and plant growth. Growth enhancement by *Bacillus* may be also related to its ability to produce hormones, especially IAA. In short,

### Peanut

#### Straw (A)

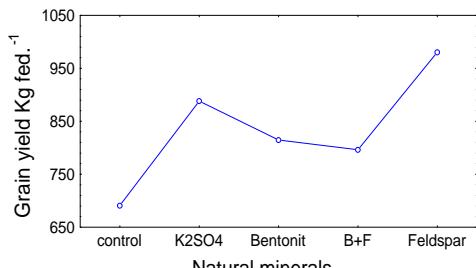
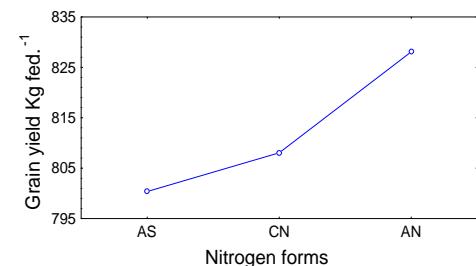


#### L.S.D at 5%

**Nitrogen forms** 315

**Natural mineral** 317

#### Grain and Seeds (B)



#### L.S.D at 5%

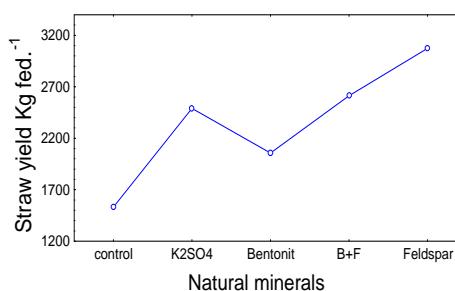
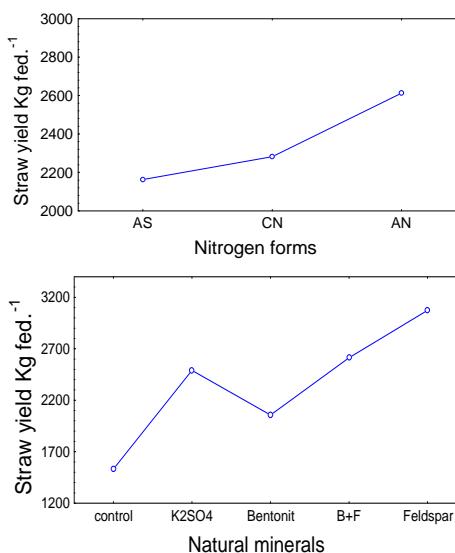
**Nitrogen forms** 63.9

**Natural mineral** 77.7

**Fig (5): Response of yield components, straw (A) and grain or seeds (B), (Kg fed⁻¹) for both peanut and sesame at two seasons to one factor either nitrogen fertilizer or natural mineral at harvest stage.**

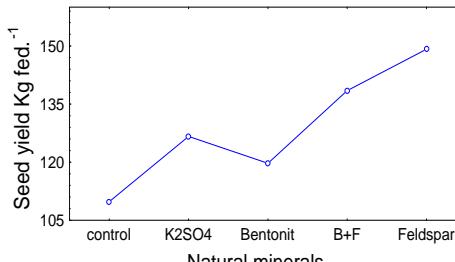
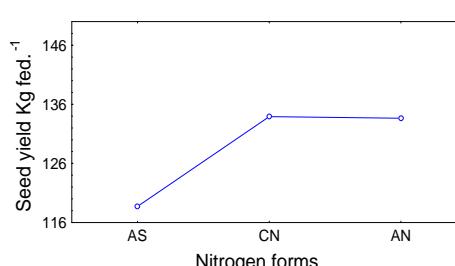
co-inoculation of plant growth promoting rhizobacteria (PGPR) with different beneficial properties may be the future trend for bio-fertilizer application to enable sustainable crop production.

### Sesame



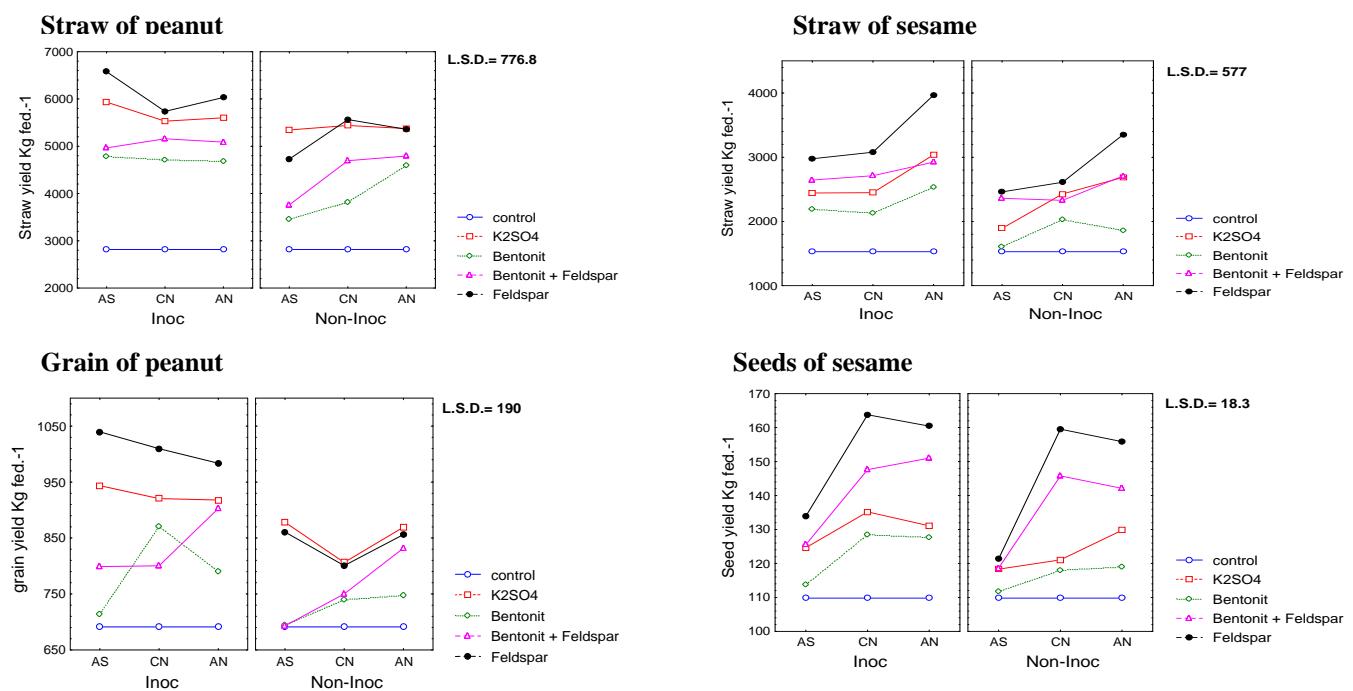
**205**

**236**



**7.93**

**7.49**



**Fig (6): Response of yield components (Kg fed.<sup>-1</sup>) for both peanut and sesame at two seasons to application of different sources of natural mineral, nitrogen fertilizer and/ or inoculation with potassium dissolving bacteria at harvest stage.**

Feldspar treatment had recorded the highest values of yield components for either peanut or sesame, particularly in the presence of inoculation as compared to those given by other treatments. These increases in yield components of peanut crop, recorded 117 % and 46.2 % against 118 % and 39.4 % for sesame straw and grains or seeds as compared to control, respectively. These data agreed with the results reported by Badr (2006) who found that the better performance of feldspar-compost plus silicate dissolving bacteria could be attributed to better maintenance of soil nutrient status in the root zone, which in turn helped the plants to utilize nutrients more efficiently; release of potassium took place frequently, and thus favorably affects growth of the crop. Locascio and Hochmuth (1997) reported that potassium supply by the soil is an extremely important factor in yield production, and the high yield depends on the level of K available to the plants. Recently, Massoud et al. (2009) reported that AM-fungi inoculation combined to *B.circulans* is highly beneficial to the growth of plants. This combination optimizes the K solubilization from feldspar and increased microbial activity in the rhizosphere of plants. So, the weathering of feldspar by AM- mycorrhizal fungi and *B. circulans* bacteria enhance the release of K ions that led to encouragement for the growth and consequently, the diverse of rhizospheric microflora.

3- Influence of nitrogen fertilizer, natural mineral and inoculation with bacteria on nutrients uptake at harvesting stage.

#### A- Nitrogen uptake

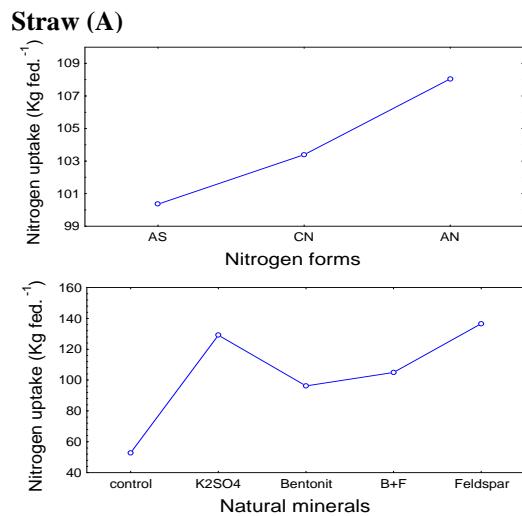
With respect to nitrogen uptake of peanut and sesame plants, generally, the highest significant increases for nitrogen uptake of straw and grain or seeds for either peanut or sesame yields were reported for ammonium nitrate fertilizer and feldspar mineral compared to other treatments (Fig 7., A and B). Treatments of nitrogen fertilizer may be generally arranged as follows: ammonium nitrate > calcium nitrate > ammonium sulfate for the two studied seasons. On the other hand, treatments of potassium fertilizer may be arranged as follows: feldspar > potassium sulfate > bentonite + feldspar > bentonite at the first season while arranged as follows: feldspar > bentonite + feldspar > potassium sulfate > bentonite for the second season. Also, behavior of nitrogen uptake followed the same trend of those obtained for yield components at the two studied seasons. In fact, Katai et al. (2010) indicated that the large bentonite doses reduced the nitrate- N content along with available phosphorus and potassium contents of soil, which reflected on nutrients uptake by plants.

To make the picture clearer, it was thought usefully to express the obtained results as interactions between the influences of both natural mineral and

nitrogen fertilizer forms; such interactions are shown in Fig (8). Data show that values at the two studied seasons were positively affected by application of natural mineral in the presence of nitrogen forms either of inoculation or non-inoculation compared to control.

Moreover, results showed that the nitrogen uptake was significantly increased with inoculation by potassium dissolving bacteria. These results are in agreement with those of Han et al. (2006) who reported that the soil inoculation with potassium solubilizing bacteria significantly increased nutrients uptake in pepper and cucumber plants, especially

#### Peanut

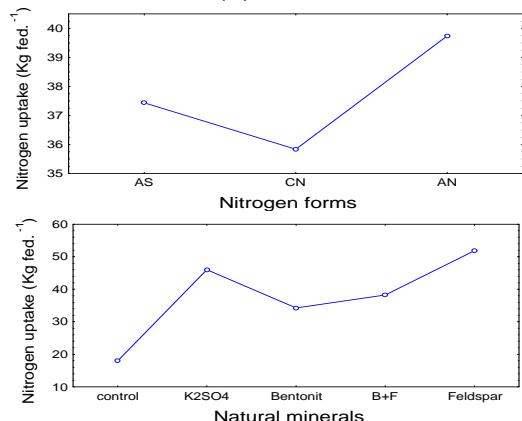


#### L.S.D at 5%

Nitrogen forms 11.9

Natural mineral 11.5

#### Grains and Seeds (B)



#### L.S.D at 5%

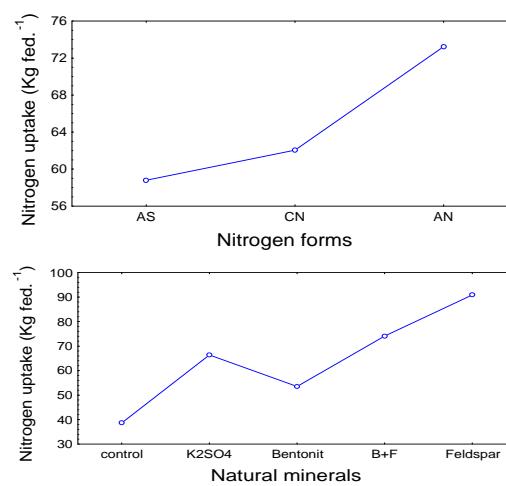
Nitrogen forms 3.08

Natural mineral 4.03

when the respective rock potassium were added. Generally, pattern of nitrogen uptake followed the same trend of those obtained with yield components of both crops (peanut and sesame).

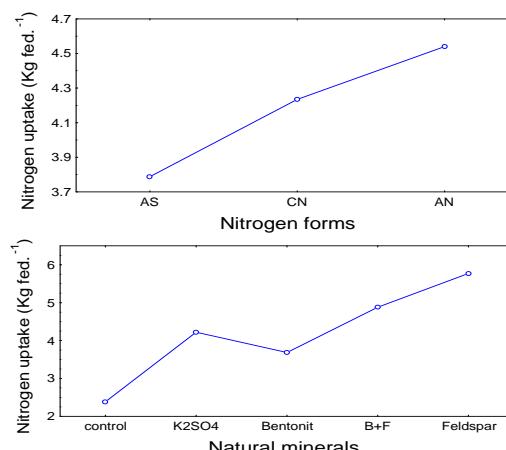
Application of feldspar with ammonium nitrate as a source of nitrogen fertilizer generally improved the uptake of nitrogen in plants, especially in the presence of inoculation compared to control. These increases in nitrogen uptake of peanut crop, recorded 214 % and 301 % against 219 % and 176 % for sesame straw and grains or seeds compared to control, respectively.

#### Sesame



5.62

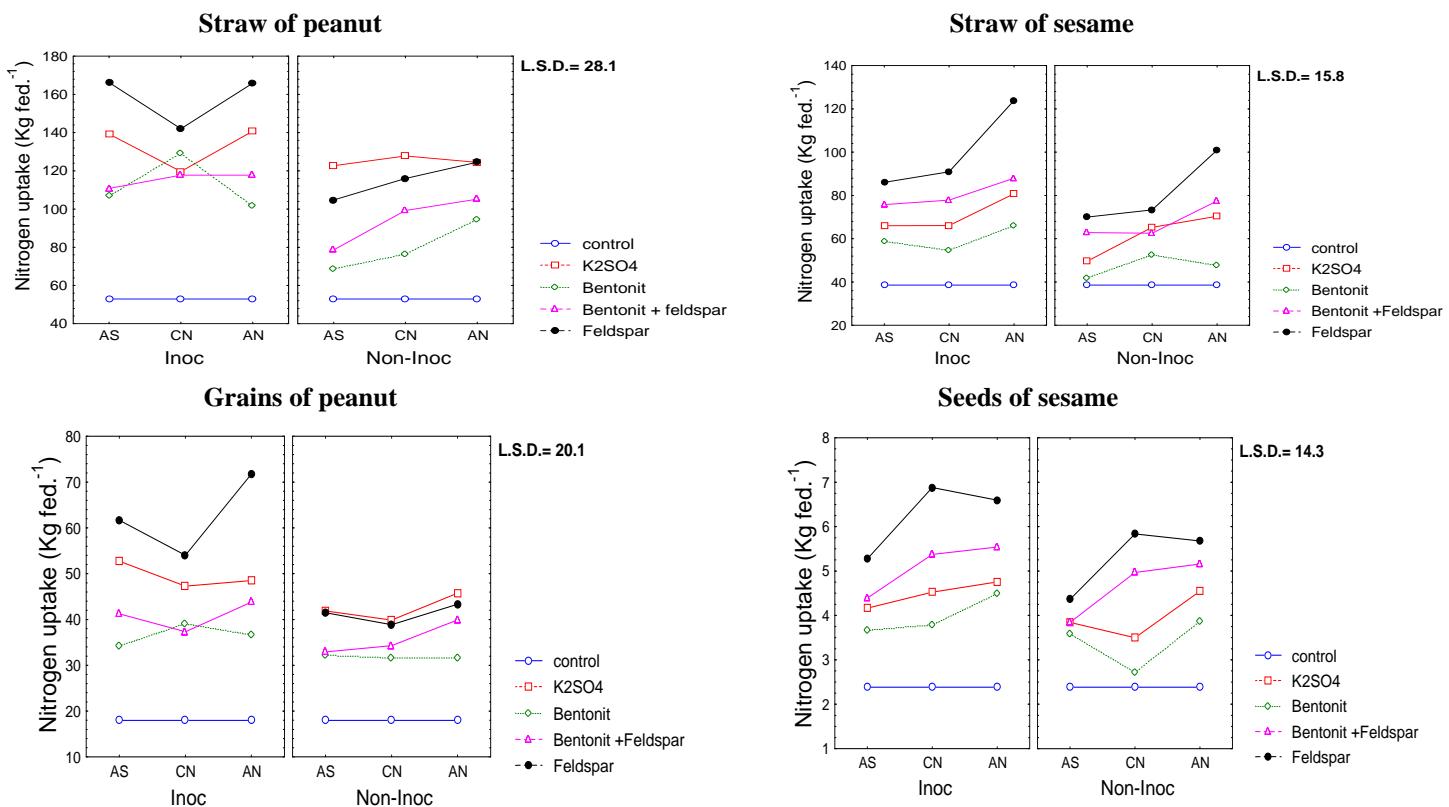
6.44



0.45

0.49

**Fig (7): Response of nitrogen uptake, straw (A) and grain or seeds (B), ( $\text{Kg fed}^{-1}$ ) for both peanut and sesame at two seasons to one factor either nitrogen fertilizer or natural mineral at harvest stage.**



**Fig (8): Response of nitrogen uptake ( $\text{Kg fed}^{-1}$ ) for both peanut and sesame at two seasons to application of different sources of natural mineral, nitrogen fertilizer and/ or inoculation with potassium dissolving bacteria at harvest stage.**

#### B- Potassium uptake

However, the highest response for potassium uptake of straw and grains or seeds at the two studied seasons were recorded for ammonium nitrate fertilizer as a source of nitrogen fertilizer with potassium sulfate and feldspar mineral as sources of natural mineral for peanut and sesame yield, respectively. (Fig. 9, A and B).

Treatments of nitrogen fertilizer seemed to follow a trend for potassium uptake similar to those obtained for nitrogen uptake. Regarding to treatments of natural minerals, they could be arranged as follows: potassium sulfate > feldspar > bentonite + feldspar > bentonite for the peanut yield while arranged as follows: feldspar > bentonite + feldspar > potassium sulfate > bentonite for the sesame yield. The last behavior of potassium uptake, again, seemed to follow a trend similar to those obtained for nitrogen uptake for the two studied seasons. In the same concern, Badr (2006) found that the total uptake was greater when feldspar- compost plus silicate dissolving bacteria was applied followed by potassium sulfate while the lower was recorded for feldspar. This may indicate that a major portion of K present in feldspar mineral as well as in the organic

materials became available for uptake and contributed considerably towards the nutritional requirements of the crop. Further, loses due to drainage, leaching and percolation of potassium from feldspar charged compost are negligible as compared to soluble potassium salts. Hence, use of feldspar in a biological form should be, further, more economical than imported potash fertilizer.

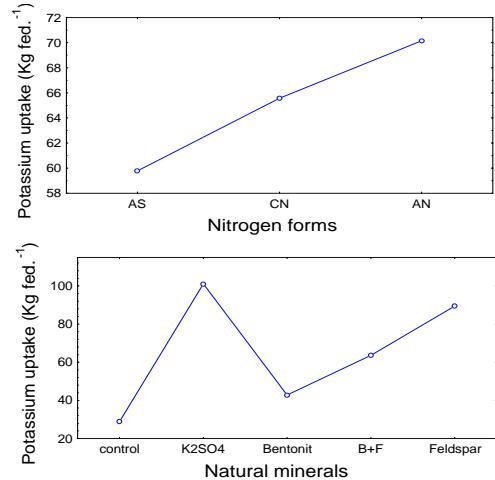
The obtained results as interactions between the influences of both natural mineral and nitrogen fertilizer forms (Fig 10) reveal positive responses for potassium uptake to application of natural mineral treatments in the presence of either nitrogen forms or inoculate as compared to control.

Application of ammonium nitrate with feldspar was generally superior, particularly in the presence of dissolving potassium bacteria. However, an exception being obtained in case of applying calcium nitrate with potassium sulfate for straw at first season compared to control. These increases in potassium uptake of peanut crop, recorded 336 % and 78.3 % against 352 % and 180 % for sesame straw and grains or seeds as compared to control, respectively.

From our obtained results, it could be concluded that, the feldspar mineral and ammonium nitrate as a source of potassium and nitrogen recorded the highest values of yield components as well as nutrient (N and K) uptake for either peanut or sesame particularly in the presence of inoculation. Moreover, the effects of both feldspar and feldspar + bentonite

### Peanut

#### Straw (A)

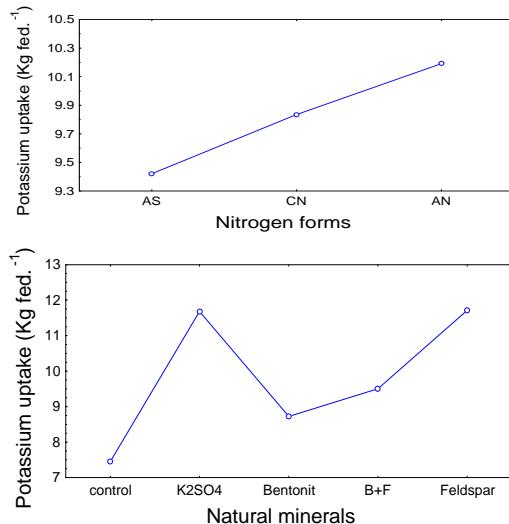


L.S.D at 5%

Nitrogen forms **7.68**

Natural mineral **8.18**

#### Grains and Seeds (B)



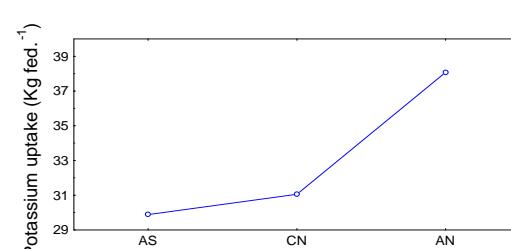
L.S.D at 5%

Nitrogen forms **1.01**

Natural mineral **1.01**

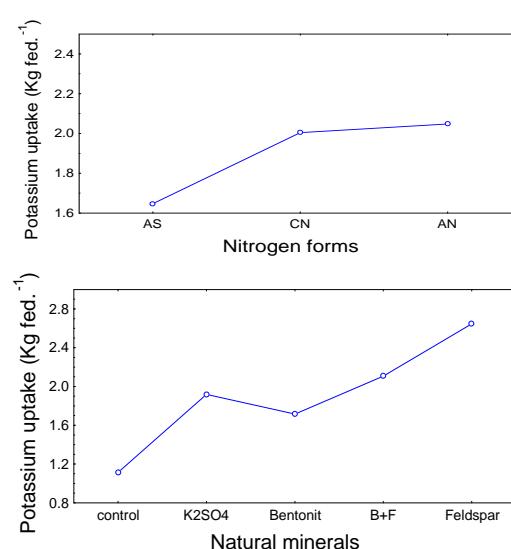
was generally similar to that of potassium sulfate, particularly in the presence of inoculation. So, this biofertilizer is highly efficient to achieve the economy of potash fertilizer and reduce the cost of cultivation through the use of cheap and locally potash source.

### Sesame



**2.81**

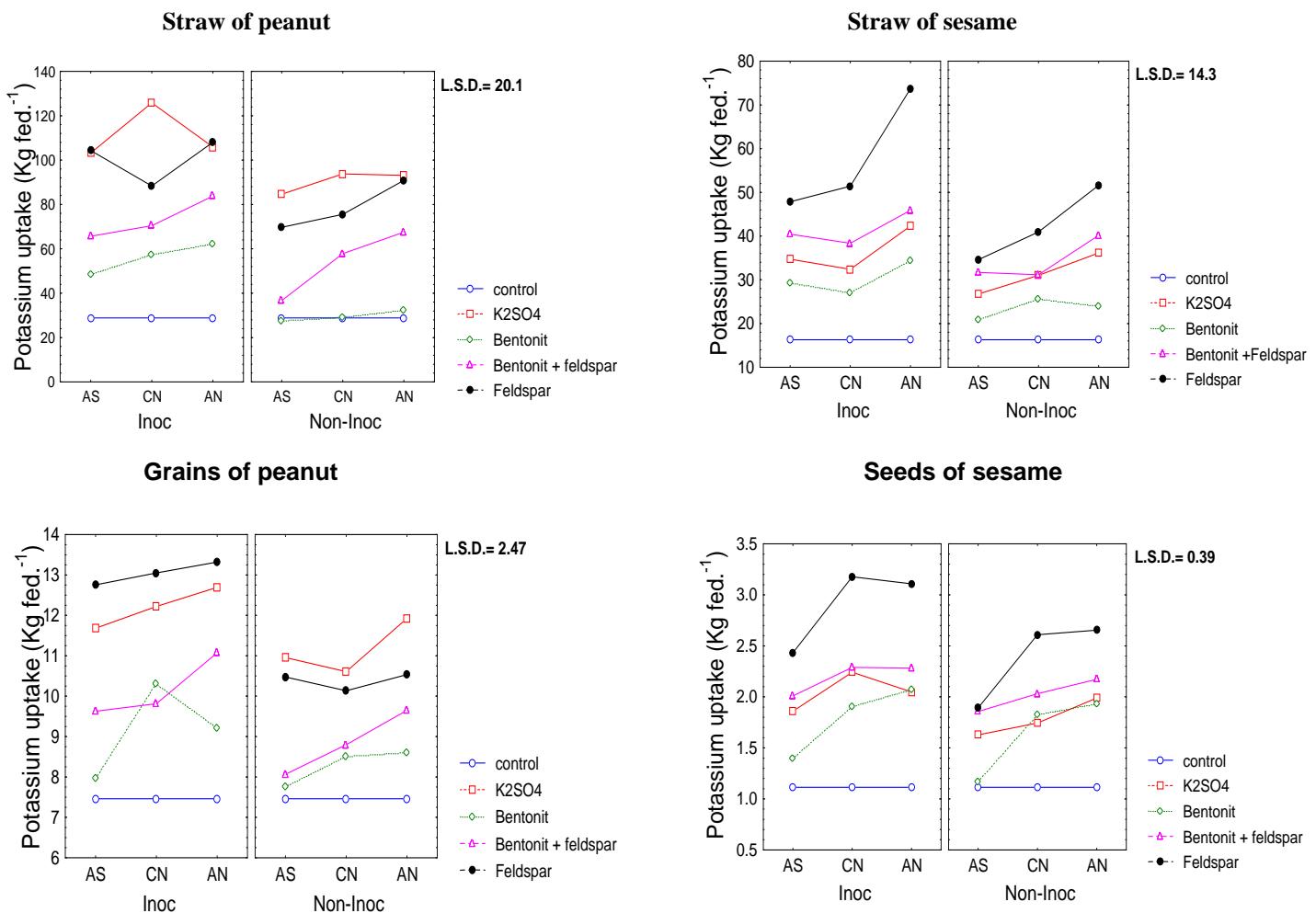
**5.85**



**0.18**

**0.16**

**Fig (9): Response of Potassium uptake, straw (A) and grain or seeds (B), (Kg fed.<sup>-1</sup>) for both peanut and sesame at two seasons to one factor either nitrogen fertilizer or natural mineral at harvest stage.**



**Fig (10): Response of Potassium uptake ( $\text{Kg fed}^{-1}$ ) for both peanut and sesame at two seasons to application of different sources of natural mineral, nitrogen fertilizer and/or inoculation with potassium dissolving bacteria at harvest stage.**

#### Acknowledgment

The authoresses wish to express their sincere gratitude and appreciation to the Development of Soil Conditioners Project, Dept. of Chemistry and Physics of Soil, Soils, Water and Environ. Res. Inst., Agric. Res. Center (ARC), Giza, Egypt, for introducing all facilities needed to accomplish this study.

#### Corresponding author

Gehan H. Youssef  
Soil, Water and Environ. Research Institute,  
agricultural Research Center (ARC), Giza, Egypt

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9/9/2010

## Clinical Perspective of Repeat Breeding Syndrome in Buffaloes

Ahmed W.M.<sup>\*</sup>, El-khadrawy H.H., Emtenan M. Hanafi , Amal H. Ali , Shalaby S.A.

Department of Animal Reproduction and Artificial Insemination, National Research Center Dokki,Cairo, Egypt.

<sup>\*</sup>[wahidmma@hotmail.com](mailto:wahidmma@hotmail.com)

**Abstract:** Local meat production in Egypt is in continuous decrease and can not meet the local market requirement. So this study was designed to throw light on true repeat breeding syndrome (RBS) as one of the reproductive disorders that hinders the buffalo meat and milk production. A field survey was carried out on 1358 female buffaloes which were subjected to clinical and gynecological examination , and blood samples were collected for carrying out some relevant analyses. Treatment trials were practiced using different ways to control the condition and the economic impact of this syndrome has been studied. Results revealed that the incidence of clinical repeat breeding (RB) in the examined buffalo cows was 4.34 %. Typical repeat breeders represented 7.25 % of total reproductive disorders in female buffaloes. Serum progesterone level was  $1.44 \pm 0.39$  and  $3.66 \pm 0.84$  in RB and normal buffaloes (NB), respectively. Oxidant/antioxidant markers in RB buffalo-cows showed increased malondialdehyde (MDA) and nitric oxide (NO) and decreased catalase (CAT), superoxide dismutase (SOD), ascorbic acid (ASCA), reduced glutathione (GSH-R) and total antioxidant capacity (TAC). Serum zinc, copper,iron and selenium values were lower in repeat breeder cows compared to normal animals. Repeat breeder buffalo-cows responded to the treatments with mineral mixture, GnRH and Lugol's solution with recovery rates; 63.64, 61.54 and 60.00%, respectively. The study concluded that special care should be paid for food additives to control this syndrome.

[Ahmed W.M., El-khadrawy H.H., Emtenan M. Hanafi , Amal H. Ali , Shalaby S.A. Clinical Perspective of Repeat Breeding Syndrome in Buffaloes. Journal of American Science 2010;6(11):661-666]. (ISSN: 1545-1003).

**Key words:** Repeat breeding buffaloes - progesterone - oxidant/antioxidants and trace elements

### 1. Introduction:

Currently, the incidence of infertility becomes relatively increased with consequent reduction of productivity of farm animals. According to global Agricultural Information Network report for 2010, the total number of Egyptian cattle and buffalo decreased from 6.256 million head in 2008 to 6.248 million in 2009 and it is expected to decline in 2010 to level lower than 2009 because of many problems that continue to limit the growth of the animal production industry [1]. The price of domestic beef increased dramatically and the imported frozen meat and liver are still important sources of protein in domestic market. The average per capita consumption of red meat including variety meats is estimated at 8.5 kilograms per year, which is quite low compared to consumption levels in other countries. The lower consumption is mainly due to limited local production combined with lower per capita income [1].

Although buffalo constitutes 49% of the above mentioned number, it is the source for high quality milk (65% of milk production), lean meat (33.9% of meat production) and preferred by most of Egyptians. However, in Egypt and most of developing countries having buffalo population, these animals are mostly raised in small holder farms under hard socioeconomic circumstances [2].

Reproductive disorders, poor nutrition, parasitic infection are the main constraints of buffalo development. Ovarian inactivity, silent heat, endometritis and repeat breeding are the main reproductive disorders in buffaloes in Egypt [3].

Typical repeat breeding (RB) is defined as the animal that did not conceive after three or more consecutive inseminations, despite, it comes normally in heat and shows clear estrous signs with no clinical detectable reproductive disorders [4].

The objective of this study was to throw light on typical repeat breeding syndrome in Egyptian buffaloes with emphasis on the oxidative status and application of some field treatment trials. Also, economic impact of this syndrome has been investigated.

### 2. Materials and Methods

#### Animals

The current study was conducted on 1358 mature polyparous buffalo cows randomly selected from small-scale holders at Al Sherkia governorate, lower Egypt during 2008-2010. These animals were fed on Barseem (*Trifolium alexandrinum*), wheat or rice straw and a few amount of concentrate mixture. Based on owner complains, case history, general health condition and the gynaecological examination, animals were categorized into two groups; the first group (G1) bred and conceived normally after no

more than 3 inseminations. The second group (G2) was those animals which did not conceive after three or more inseminations, despite no clinically detectable reproductive disorders were observed (Typical repeat breeders). Gynaecological examination was carried out through rectal palpation aided by ultrasonography machine (PiaMedical Falc e`Saote, Netherlands) with an endorectal linear array of 8.6 M hertz to register the reproductive status and/or disorders.

#### Sampling and Analysis:

Blood samples were drawn from the jugular vein of each animal, in tubes with and without EDTA. Serum was separated after centrifugation and stored at -20 °C until analysis. Serum progesterone level was assayed by ELISA microwell technique using kits from DIMA (Germany). The kit had a sensitivity of 2.0 pg/ml with inter- and intra- run precision coefficient of variations of 2.9 and 4.85, respectively [5]. The concentrations of malondialdehyde (MDA) [6], nitric oxide(NO) [7], catalase (CAT) [8], ascorbic acid (ASC) [9], superoxide dismutase (SOD) [10] and total antioxidants(TAC) [11] in the serum and glutathione reduced (GSH-R ) [12] in the whole blood were determined by colorimetric methods using chemical kits (Biomed Egypt) and Shimadzu UV 240 spectrophotometer. Zinc, copper, iron and selenium concentrations were determined using atomic absorption spectrophotometry (Perkin Elmer, 2380) as outlined by Varley et al.[13]

#### Treatment trials:

A total number of 34 repeat breeder buffalo cows was subjected to one of the following treatments:

- 1- No treatment at all and kept as the control group (n=5).
  - 2- Lugol's Iodine solution (0.5 – 2%) as a vaginal wash for 3 successive weeks (n=5).
  - 3- Receiving 20 g from a mixture of minerals, vitamins and Lasalocids® in their ration for 10 successive days. This mixture was prepared in the laboratory by through mixing of 20 g of zinc sulphate , 6.25 g of copper sulphate, 1.5 g potassium iodide, 30 mg sodium selenite, 200 g AD3E and 5 g Lasalocids®,(F-Hoffman-LaRoche,Basle, Switzerland) and sodium phosphate dibasic up to 1 kg [2] ( n=11)
  - 4- Receiving an intramuscular injection of GnRH (Receptal, Hoechst Roussel Vet GmbH) (n=13)
- Treatments were carried out according to the instruction of manufacturing companies. Animals were followed up during the next weeks for conception.

#### Economic evaluation:

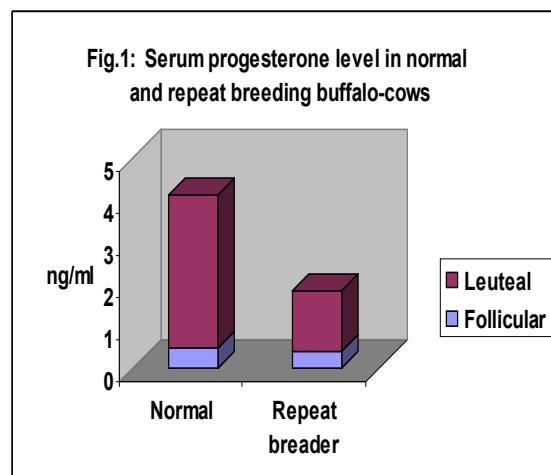
Economic losses were calculated on light of decreased calf crop, prolonged calving intervals, decreased milk production and veterinary intervention services, cost of the used drugs as well as cost of repeated AI.

#### Statistical analysis:

The data were computed and statistically analyzed using PSS-10.5 software package [14].

### 3. Results

The studied buffalo cows came normally in heat inseminated in the proper time with normal proven fertile bulls and came in heat again after 20 – 22 days during the breeding season of buffaloes (September– March). Ultrasonographic examination revealed that such animals showed no detectable clinical reproductive disorders with the corresponding normal physiological structure during the different stages of the estrous cycle. Serum progesterone level (Fig. 1) was significantly ( $P < 0.05$ ) low ( $1.44 \pm 0.39$  ng/ml) during the mid -luteal phase of the estrous cycle in repeat breeder animals compared to normal animals ( $3.66 \pm 0.84$  ng /ml).



#### 3.3. Oxidative status:

Table 1 show the oxidant/ antioxidant status of the investigated animals. It was found that MDA and NO values were significantly high ( $P < 0.01$ ) while, ASC, GSH-R, Zn, Cu, Fe and Se values were significantly( $P < 0.01$ ) low in repeat breeder buffaloes compared to normal animals.

**Table (1): Oxidant / antioxidants concentrations in repeat breeder buffalo- cows (Mean ± SE).**

Oxidant/antioxidant	Normal cows (N=10)	Repeat breeder buffalo-cows ( N=10)
MDA (mmol/ml)	1.98 ± 0.09	3.70 ± 0.48 **
NO (mmol/L)	15.55 ± 1.58	25.17 ± 0.85**
CAT ( U/ml)	2.28 ± 0.4	1.99 ± 0.10
ASC (µg/dl)	132.17 ± 5.12	95.16 ± 2.37**
SOD (U/ml)	338.16 ± 7.11	332.12 ± 16.14
GSH-R (mmol/L)	6.38 ± 0.11	2.66 ± 0.09**
TAC (mmol/L)	1.43 ± 0.08	0.46 ± 0.50
Zinc (µg/dl)	139.11 ± 2.17	120.21 ± 5.20**
Copper ( µg/dl)	78.65 ± 0.13	68.33 ± 2.05**
Iron (µg/dl)	168.40 ± 4.11	152.13 ± 2.05**
Selenium ( µg/L)	144.85 ± 0.34	130.12 ± 2.01**

\*\* P&lt; 0.01

### 3.4. Treatment trials:

Field trials to treat the typical repeat breeding syndrome using Lugol's solution, mineral mixture and GnRH indicated that 60 – 63 % of the treated animals get conceived as indicated by

gynecological examination in 40 – 60 days later, while no animal from the untreated group get conceived. It was found that mineral mixture- treated group gives the highest response.

**Table (2): Treatment trials for repeat breeder buffalo-cows (Mean ± SE).**

Treatment	Repeat breeder-cows	Recovered animals	Recovery (%)
No treatment	5	0	00
Lugol's solution	5	3	60
Mineral mixture	11	7	63.64
GnRH	13	8	61.54

### 3.5. Economic evaluation

In the present study, economic losses were estimated as 1588 LE =288\$ for every unsuccessful service. Moreover, such losses become greater if the animal did not get pregnant before the end of breeding season.

### 4- Discussion:

Repeat breeding is among reproductive disorders which hinder favorable productivity in buffaloes [15].

In the present study, the incidence of typical repeat breeders was 4.34% of the total examined animals and 7.25 % of all cases having reproductive disorders (813). The same result was found by Ahmed et al. [3]. Meanwhile, Cebra et al. and Bage et al [16 and 17] reported a range of 8.33 - 28% for this syndrome in bovines. Variations in incidences may be attributed to the heterogeneity or multifactorial causes of the repeat-breeder syndrome as well as the effect of locality and season [18].

The low progesterone level that was recorded in the current study in repeat breeder buffaloes during the luteal phase was similar to the result of Rizzo et al[19] that attributed the failure of conception in these

animals to their low progesterone level. Moreover, Sigh et al. [20] indicated that RB heifers revealed higher P4 concentrations during estrus and early metoestrus, and lower P4 concentrations during late metoestrus and onwards. In this respect, Binelli et al.[21] suggested that the supra basal level of P4 during estrus reduced tubal contractility and delayed sperm transport to the site of fertilization and early embryonic mortality. Also, Bage et al.[17] mentioned that the disturbed hormone level which prolonged standing estrus and delayed ovulation causes changes in the microenvironment of the preovulatory follicle, negatively affecting the final maturation of the oocyte leading to fertilization failure in those repeat-breeder heifers. In another study, he reported a negative correlation between conception rate and skim milk progesterone level in cows artificially inseminated [22].

It is well known that in a healthy body, reactive oxygen species (ROS) and antioxidants remain in balance. When the balance is disrupted towards an overabundance of ROS, oxidative stress (OS) occurs. Also, ROS have a role in pathological processes involving the female reproductive tract, whereas, it affect multiple physiological processes

from oocyte maturation to fertilization, embryo development and pregnancy [23]. This theory was confirmed in the current study where RB buffalocows showed increased MDA and NO and decreased CAT, SOD , ASCA, GSH-R and TAC. An endogenous NO system exists in the fallopian tubes [24].

NO has a relaxing effect on smooth muscles and it has similar effects on tubular contractility. Abnormal concentration of NO may lead to tubal motility dysfunction, resulting in retention of the ovum, delayed sperm transport and infertility. On the other hand, it was reported that increased NO levels in the fallopian tubes are cytotoxic to the invading microbes and also may be toxic to spermatozoa [24]. Moreover, Seino et al.[25] found that NO inhibits ovarian steroidogenesis. The presence of endothelial NO synthase in corpora lutea and its expression has been reported in the mid and early luteal phase and to a lesser extent in the late luteal phase Moreover, [26] and [27] added that NO inhibits steroidogenesis in the corpus luteum and has luteolytic action mediated through increased prostaglandins and by apoptosis.

SOD is present in the ovarian tissue and it was found that there is a correlation between SOD and Ad4BP which is a steroidogenic transcription factor that induces transcription of the steroidogenic P450 enzyme. Thus, it controls steroidogenesis in the ovaries. The correlation between Ad4BP and SOD expression suggests an association between OS and ovarian steroidogenesis [28]. The preovulatory follicle has a potent antioxidant defense, which is depleted by the intense peroxidation [29].

Glutathione peroxidase may also maintain low levels of hydroperoxides inside the follicle and thus play an important role in gametogenesis and fertilization [30]. Meanwhile, De Matos and Furnus [31] reported that glutathione is present in the oocyte and tubal fluid and has a role in improving the development of the zygote beyond the 2-cell block to the morula or the blastocyst stage

Vitamin C is a chain breaking antioxidant that stops the propagation of the peroxidative process and helps to recycle oxidized vitamin E and glutathione [32].

Increase in TAC was seen in follicular fluid of oocytes that later were successfully fertilized. Therefore, lower TAC is predictive of decreased fertilization potential [33].

The low concentrations of zinc, copper, iron and selenium traced in this study coincide with Das et al. and Ceylan et al. [34 and 35]who recorded that serum zinc and copper were significantly low in repeat breeders if compared to normal buffalo cows and added that when these animals were supplemented with 500 ppm zinc acetate in the

drinking water and sodium phosphate 40 g/head/day in the diet for 1 month, respectively , the conception rate improved by 80%.and this explains our findings where the treatment with mineral supplementation gave the best results for conception (63.64 %) . This is in agreement with Sah and Nakao [15] who reported that 64.6 % of repeat breeder buffaloes came to estrus and 58.4 % conceived within one month after supplementation with vitamin/mineral mixture for 3 weeks. Then, he added that the hormone treatment is more effective than 3 weeks supplementation with vitamin/ mineral mixture.

Use of hormonal treatments such as GnRH or hCG, have been used by many investigators to increase the rate of pregnancy for repeat breeder cows [36, 37, 38]. It is suggested that it has a role in the expression of SOD as it is found that the Cu-Zn SOD expression in the corpora lutea paralleled with levels of progesterone and these levels rose from early to the mid luteal phase and decreased during the regression of the corpus luteum. However, in the corpus luteum from pregnant cases, the mRNA expression for Cu-Zn superoxide dismutase was significantly higher than that in midcycle corpora lutea [28]. Other investigators reported that when 36.4- 50.0 % repeat breeder buffaloes washed by 1 liter of 1% Lugol's solution conceived within one month after treatment [15].

From the economic point of view, the repeat breeding syndrome impacts the buffalo industry as it causes increased culling, reduced milk production, and reduced value of breeding stock. On the other side, the indirect costs of sound diagnosis, treatment trials, repeated artificial insemination should also be considered. RBS return the animal to service, increased time to conception and thus increased calving interval in the long-term reduced milk production or permanent infertility. The profitability of milking buffalo-cow increases with age, and culling earlier than the fourth lactation may result in net cost.Also reduced fertility is the commonest reason for culling in the UK [39], so any disease or syndrome affects fertility will have an economic impact. RBS may negatively affect milk production. Whilst an increased calving interval would reduce the number of lactations within a period of years, an RB may increase or prolong the lactation in which it happens. Thus, the impact of RB on milk production is complex and has not been fully quantified. In twenty-two Michigan dairy herds, repeat-breeder syndrome was observed in 24% of 3,309 lactations. Cost components associated with unsuccessful inseminations included costs of delayed conception, extra inseminations, extra veterinary service and losses due to culling. Lactations with repeat-breeder syndrome were associated with a loss of

approximately \$385. An estimated extra cost of \$140 was associated with a second insemination, \$279 with three inseminations, \$429 with four inseminations and \$612 with five inseminations [40].

It was concluded that RB has economic impact on buffalo production and consequently, local meat and milk production in Egypt. Veterinary supervision should provide better animal health care and education to farmers regarding the risk factors that may lead to RB. Also, use of ultrasonography may help to get rapid and sound diagnosis. Great efforts should be done to catch up the breeding season not to lose the proposed new individual and lactation season. Also special care should be paid for minerals and food additive in animal's food stuff for animal welfare and breeder income.

#### **Corresponding author**

Ahmed W.M

Department of Animal Reproduction and Artificial Insemination, National Research Centre Dokki, Cairo, Egypt.

[wahidmma@hotmail.com](mailto:wahidmma@hotmail.com)

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9/20/2010

## Biologically evaluation of pan bread supplemented with vital gluten, lupin, fenugreek and turmeric flour blends

Aly, M. H\*; El Nikeety, M. M\*; Saleh, M. A. M\*\*. and Abd El-Hak, N. A. M.\* \*\*

\*Cairo University, Faculty of Agric., Food Science & Technology Dept.

\*\* Food Technology Research Institute, Special Food & Nutrition Dept.

\*\*\*Food Technology Research Institute, Experimental Kitchen Unit.

**ABSTRACT:** The current study was carried out to utilize each of whole meal wheat flour (control), some legumes (lupin and fenugreek), turmeric and vital gluten flour in blends for preparation of pan bread more nutrients and healthy in order to enhance the dietary fiber and amino acids contents. The biological parameters of rats (non and induced diabetic) fed on such pan bread was also estimated. A significant higher amount of soluble, insoluble and total dietary fiber contents was found in the turmeric, fenugreek and lupin, pan bread compared to that found in control once( whole wheat flour). Normal rats (nondiabetic and fed on basal diet) exhibited an insignificant decrement in blood glucose. However, in the diabetic rats a significantly lowered blood glucose trend was found. The tested pan bread samples were more slightly effective in lowering liver and kidney function in the diabetic rats in a relation to diabetic rats, when compared with the positive control. Finally, it is recommended to utilize whole meal flour to prepare healthy diets to deal with diabetic status and control of some biological parameters.

[Aly, M. H; El Nikeety, M. M; Saleh, M. A. M. and Abd El-Hak, N. A. M. Biologically evaluation of pan bread supplemented with vital gluten, lupin, fenugreek and turmeric flour blends. Journal of American Science 2010;6(11):667-79]. (ISSN: 1545-1003).

**Key words:** Whole meal wheat, Vital gluten, Fenugreek seeds, Legumes, Turmeric, Diabetes.

### **INTRODUCTION**

Diabetes mellitus (DM), one of the major metabolic disorders, is characterized by high blood glucose levels due to the inability of body cells to utilize glucose properly. By the 2010 year, the total number of people worldwide with DM will be as high as 239 millions. Regions with greatest potential are Asia and Africa, where DM incidence could rise to 2-3-folds of the present incidence (Xue *et al.*, 2007). Recently, Vijayalakshmi *et al.* (2009) reported that diabetes is a disease of great concern to many all over the world and is known for its complications that include diabetic nephropathy, neuropathy, and retinopathy. In any form of management of diabetes, be it with insulin or drug, diet is a common factor. Some of the foods and their derivatives are recommended for better management of diabetes. In this direction, bitter gourd is one of the vegetables, which is advocated and well practiced in the control of diabetes.

Marques *et al.* (2007) reported that, more wheat -based products, including flour, bread, breakfast cereals, pasta and crackers, are available. It seems that such cereals products possess valuable nutritional and/or physiological properties, which

could help promoting the consumption of these products.

Wheat gluten is a readily available protein source that has been extensively used in baked products (Barber and Warthesen, 1982). Hemstad (2005) reported that vital gluten is a unique water-insoluble protein and carbohydrate complex that is extracted from wheat by wet processing.

Legumes play an important role in human nutrition since they are rich sources of protein, calories, certain minerals and vitamins. In African diets legumes are also, the major contributors of protein and calories for economic and cultural reasons (ELMaki *et al.*, 2007). Madhusudhan and Tharanathan (1995) found that, legumes have been shown to decrease blood glucose responses compared to other cereal based foods such as whole meal bread and are of very vital benefit in the diets of diabetes and hyperlipidemia patients. Moreover, Wolever *et al.* (2003) reported that the low-fat, high-carbohydrate diets are known to stimulate hepatic triglyceride production in diabetic subjects.

The turmeric is rich in dietary fibers and contains both soluble and insoluble dietary fibers. Dietary fibers are well established to play a beneficial role against various diseases like diabetes, colon cancer, heart disease.... etc, as reported by

Vijayalakshmi *et al.* (2009). Turmeric (*Curcuma longa L.*) is a medicinal plant extensively used in Ayurveda, Unani and Siddha medicine as a home remedy for various diseases (Ammon and Wahl, 1991). Turmeric is used as a food additive (spice), preservative and coloring agent in Asian countries, including China and South East Asia. It is also considered as an auspicious and is a part of religious rituals. In old Hindu medicine, it is extensively used for the treatment of sprains and swelling caused by injury (Kamal-Eldin *et al.*, 2000). Arun and Nalini (2002) reported that, both turmeric and curcumin decrease blood sugar level in alloxan-induced diabetes rats. Curcumin also decreases advanced glycation end products induced complications in diabetes mellitus.

On the other hand, Patil *et al.* (2009) reported that, during diabetes, a profound alteration in the concentration and composition of lipids occurs. Liver and kidney organs are important for glucose and lipid homeostasis, they participates in the uptake, oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol, phospholipids and triglycerides. Basch *et al.* (2003) reported that, in human studies, fenugreek seeds reduced the area under the plasma glucose curve and increased the number of insulin receptors. Also, fenugreek seeds exert hypoglycemic effects by stimulating glucose-dependent insulin secretion from pancreatic beta cells as well as by inhibiting the activities of alpha-amylase and sucrase, two intestinal enzymes involved in carbohydrate metabolism.

Therefore, the objectives of the current study are to incorporate and utilization of whole wheat flour, vital gluten, fenugreek, lupin and Turmeric rhizome to prepare an edible and healthy. The objectives are extended also to *invitro* and *invivo* estimation of such products.

## MATERIALS AND METHODS

### Materials:-

Wheat grains (*Triticum aestivum*, Skha 69 variety) were obtained from the Wheat Research Department, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt. The tested legumes lupin, *lupinus albus*, Giza 1variety and fenugreek seeds, *Trigonella foenung raecum*, Giza 30 variety were obtained from Legumes Research Department, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt. Turmeric rhizome (*Curcuma longa*) was obtained from the local market at Giza, Egypt. Commercials wheat gluten which was purchased from Crestar Co. 7 Rue Du Marechal, Jaffre, BP 109, France, was obtained from Cairo Univ., Faculty of Agricultural, Special Baking Technology

Unit. Bread improver was obtained from Saint Paul Company Milk and Chocolate, Badr Industrial City, Cairo, Egypt. Commercial compressed yeast: The compressed Bakers yeast was purchased from the local market at Giza, Egypt. Alloxan (the diabetes mellitus inducer drug for rats) was obtained from Sigma Company, USA. Blood glucose, albumin, total protein, alkaline phosphatase, glutamic-oxalacetic transaminase(GOT), glutamic-pyruvic transaminase (GPT), creatinine, urea and uric acid kits were obtained from Biodagnostic Company, 29 El Tahrer street, Dokki, Giza, Egypt.

### **Methods:-**

#### **-Preparation of raw materials**

##### **-Germination and cooking of legumes**

Germination of fenugreek seeds were carried out according to the method of Marero *et al.* (1988). fenugreek seeds were cooked individually by boiling with sufficient amounts of water, till they became tender and well cooked. Lupin debittering processes, were carried out in the laboratory by washing and soaking the lupin in tap water for 24 h at room temperature, followed by germinating of whole seeds at 30 °C in the dark for 3 days, followed by warming using boiled water for 30 min and submerging the lupin seeds in a running tap water at room temperature for 4 days (Trugo *et al.*, 1993).

#### **Drying and milling of materials**

All such materials (legumes) were dried at 55° C for 12 h, in an air forced oven. Wheat grains, turmeric, dried germinated fenugreek and lupin seeds were milled with a laboratory mill (MLW, Type: Sk1, watt100, West Germany).

#### **Preparation of blends**

The optimum gluten scheme amount was 30%. Exactly 5, 5 and 1% of fenugreek, lupin and turmeric flour, respectively, were substituted (except the control blend) instead of a resemble amount of whole meal wheat flour to achieve the healthy impact.

#### **Baking procedures:**

A straight dough bread making process was performed according to Wang *et al.*, (2002). Basic dough formula of 500g flour basis was consisted of salt (5g), compressed yeast (10g), sugar (5g), bread improver (0.2g), oil (5g) and the required amount of water to reach 500 BU of consistency. The doughs were optimally mixed, fermented for 10min, and then dough pieces (450g) were divided, hand -moulded and sheeted. The dough was proofed for 55 min in a fermentation cabinet under controlled temperatures (30°C) and a relative humidity (78%) for 50 min and

then baked for 40 min at 180 °C in a baking oven. The pan bread attributes were evaluated after cooling for 1hr at room temperature.

### **Methods of analysis**

#### **Determination of amino acids**

All amino acids content, except tryptophan, of the cooked flour of each of lupin, fenugreek, as well as the flour of whole wheat meal, gluten and turmeric were determined using HPLC- PICO- TAG method according to the method described by Cohen *et al.* (1989). Tryptophan was calorimetrically determined in A.O.AC. (2000).

#### **Determination of total dietary fiber (TDF)**

Total dietary fiber (TDF) was determined in according to the method described by Prosky *et al.* (1984) and the modification by Vadivel and Janardhanan (2001).

#### **Determination of soluble and insoluble dietary fiber**

Soluble and insoluble dietary were determined according to the method described by Asp *et al.* (1983).

#### **Computation of protein efficiency ratio (PER).**

Protein efficiency ratio of pan bread was calculated using the equation suggested by Alsmeyer *et al.* (1974) as follows:

$$\text{PER} = -1.816 + 0.435 \text{ (methionine)} + 0.78 \text{ (leucine)} + 0.211 \text{ (histidine)} - 0.944 \text{ (tyrosine).}$$

#### **Biological assay:**

The experimental study was conducted on 45 adult male albino rats, 180-200 g weighed. Animals were housed at the animal house, Crops Technology Department, Food Technology Research Institute (FTRI). Before and during the experiment rats were fed on a basal diet containing 20% casein, 10% corn oil, 5% cellulose, 4% salt mixture and 1% vitamin and completed to 100% with corn starch (AOAC, 2000). After randomization to various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under standard environmental condition of temperature, relative humidity (55%), and dark/light cycle.

#### **The experimental design:**

All the animals were randomly divided in nine groups (five rats for each one group) and namely negative control (the normal group which fed on a

basal diet), positive (the diabetic group fed on basal diet), WWF (the diabetic group fed on whole meal wheat pan bread diet), WWG (the diabetic group fed on whole meal and gluten pan bread), WWGT (the diabetic fed on whole meal, gluten and turmeric pan bread), WWGL (the diabetic fed on whole meal, gluten and lupin pan bread), WWGF (the diabetic fed on whole meal, gluten and fenugreek pan bread), WWGLT (the diabetic fed on whole meal, gluten, lupin and turmeric pan bread), WWGFT (the diabetic fed on whole meal, gluten, fenugreek and turmeric pan bread) and WWGFLT (the diabetic fed on whole meal, gluten, fenugreek, lupin and turmeric pan bread). During the experiment, rats were separately kept in well aerated cages and the diet as well as water were *ad libitum* supplied.

#### **Induced diabetic animals:**

Rats were diabetic induced by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg rat). Alloxan was first individually amount calculated for each animal according to the weight and the proper amount was solubilized with saline just prior to injection (Ahmed *et al.*, 2005) and the control group was saline injection only. Three days after alloxan injection, rats with plasma glucose levels of >140mg/dl were included in the study. Fasting blood glucose estimation was done at zero, 15, 30 and 45 day of the study. Urea, uric acid, creatinine, GOT, GPT, total protein, albumin and alkaline phosphatase estimation, as well as measurements were immediately carried out after the successful injection and at 45 day of the study start. At the end of the feeding period (after 45 days) rats were anaesthetized using diethyl ether and sacrificed. Insulin in plasma was also measured at the experiment end.

#### **Biochemical analysis:**

The blood samples were collected in tubes and centrifuged at 500 xg to obtain serum. It was kept in a deep-freezer until biological analysis was performed and subjected to the following biochemical analysis: fasting blood sugar (according to Trinder, 1969) in the separated serum samples. Serum insulin level (Temple *et al.*, 1992, at National Institute of the Diabetic and Endocrine Discos). Serum glutamic oxaloacetic transaminase (GOT) and glutamic- pyruvic transaminase (GPT) activities were calorimetrically measured according to the method described by Reitman and Frankel (1957). The protein content was determined using the method of Gornall *et al.* (1949). The alkaline phosphatase was determined using the method of Belfield and

Goldbery (1971). The albumin content was determined using the method of Doumes (1971).

### **Statistical analysis:**

Data analysis was performed using SAS (1987), software. All data were expressed as mean of three replicates. Analysis of variance was used to test for differences between the groups. Least Significant Differences (LSD) test was used to determine significant differences ranking among the mean values at  $P < 0.05$ .

## **RESULTS AND DISCUSSION**

### **Dietary fiber content of the tested materials**

Whole meal wheat, cooked lupin, cooked fenugreek, turmeric and vital gluten flour were analyzed for their total dietary fiber (TDF) contents and their soluble (SDF) as well as insoluble (IDF) fractions, the results are given in Table (1).

It is clear from data showed in Table (1) that fenugreek, lupin and turmeric flour were rich sources of dietary fibers and possessed the significant differences for TDF content (37.210, 23.150 and 20.20% on dry weight basis, respectively), of which the IDF represented the major fraction except turmeric flour (20.47 and 21.50 of TDF, respectively). While the highest amount of soluble form of dietary fiber (SDF) was found in turmeric

flour (19.80 %). Such results suggested that fenugreek, lupin and turmeric could be considered a good and inexpensive source of fiber content to be a suitable tool for flour and baked products enrichment. The fenugreek powder addition to the experimental diet in the form of dietary fiber, resulting in a higher fiber content in the experimental diet than that found in the control diet. The dietary fiber of fenugreek seeds is dispersed throughout the seed coat and is also found in the endosperm as reported by Madar and Stark (2002). The results of dietary fiber content of lupin flour (21.5 % and 2.45 % IDF and SDF, respectively) are in agreement with the results of Mohamed and Rayas-Durate (1995) who pointed out that lupin cotyledons contained 21.5 and 2.2% insoluble and soluble fiber, respectively. Therefore, while the hull contained 86.2 and 1% insoluble and soluble fiber, respectively, lupin could be used in existing or new products. Consequently, lupin can also be used in bread making, biscuits, pasta products, and a variety of other food products.

On the other hand, data presented in Table (1) showed that vital gluten contained the lowest amounts of total, soluble and insoluble dietary fiber compared to that found in whole meal wheat flour. Kahlon and Woodruff (2002) reported that the gluten is contained 1.90% of total dietary fiber 1.4 % insoluble dietary fiber and 0.5% of soluble dietary fiber (as dry matter basis).

**Table 1: Dietary fiber content of the tested materials (on dry weight basis).**

<b>Sample</b>	<b>Dietary fiber %</b>		
	<b>Insoluble(IDF)</b>	<b>Soluble(SDF)</b>	<b>Total(TDF)</b>
<b>Whole cooked lupin flour</b>	21.500 <sup>a</sup>	2.450 <sup>c</sup>	23.150 <sup>b</sup>
<b>Whole cooked fenugreek flour</b>	20.475 <sup>b</sup>	17.035 <sup>b</sup>	37.210 <sup>a</sup>
<b>Whole wheat flour</b>	12.172 <sup>c</sup>	1.337 <sup>e</sup>	13.109 <sup>d</sup>
<b>Turmeric flour</b>	0.900 <sup>d</sup>	19.800 <sup>a</sup>	20.200 <sup>c</sup>
<b>Vital gluten</b>	0.515 <sup>e</sup>	1.440 <sup>d</sup>	1.755 <sup>e</sup>

-Each value (an average of three replicates) within the same column, followed by the same letter is not significantly different at  $< 0.05$ .

### **Amino acids content of the tested materials and pan bread.**

The nutritive value of dietary protein is determined by the pattern and quantity of essential amino acids present of the tested materials. The presence of one or more specified of the essential amino acids in adequate amounts would increase the nutritive value of protein. The amino acid contents (g /100g protein) of the tested materials were determined and the results are shown in Table (2).

From the data in Table (2) it could be noticed that turmeric flour had lower quantities of isoleucine and lysine. Moreover, cooked lupin flour contained lower amounts of total sulfur amino acids while cooked fenugreek flour contained lower amounts of valine. On the other hand, gluten had lower quantities of lysine and threonine in relative

to the other tested materials. Whole meal wheat flour also, had lower amounts of lysine compared to the other tested materials. However, a significant variation was existed in the contents of some amino acids, particularly for valine and threonine.

The valine contents was varied from 2.72 gm / 100gm protein in turmeric flour to 4.50 gm /100 gm protein in gluten. Among the tested legumes, lysine, sulfur amino acids, serine, proline, glycine, arginine and glutamic acid were found to be rich in fenugreek, while isoleucine and lysine were found in appreciable amounts in turmeric among the different tested materials. Whole meal wheat and gluten were higher in total sulfur amino acids contents (methionine and cystine) than the other tested materials.

Glutamic acid was found to be the major non-essential amino acids in the tested samples, while maximum amount of proline was found in gluten. Total essential amino acids contents were the highest in gluten. On the other hand, legumes have been reported to contain adequate amounts of lysine, but are deficient in S-containing amino acids (methionine and cystine). However, Roccia *et al.* (2009) reported that the wheat flour is deficient in lysine. A common practice in breadmaking is to incorporate proteins in the product formulation to increase their nutritional value. Legume proteins are the major component of the diet of food producing animals and are increasingly important in human nutrition. Finally, the same data presented in Table (2) showed that the fenugreek flour contained highest amounts of tryptophan when compared to turmeric flour.

Data presented in Table (3) showed the mathematically amino acids content of 100gm of the tested pan bread. It could be concluded from such data that each mixture seemed to contain the same amount, with a slightly changes, of all the estimated amino acids. It was due to the variation of the ingredients in each mixture to prepare a complementary mixture containing the required amino acids with an adequate amount.

Data presented in Table (3) showed the amino acids content of manufactured pan bread of the suggested blends. The highest essential amino acids content was noticed in the tested blends pan bread, than that found in the control pan bread (prepared from whole meal wheat flour). Results in Table (3) showed that the amino acid contents of isoleucine, leucine, lysine and valine in WWG, WWGL, WWGT, WWGF, WWGFT, WWGLT and WWGFLT pan bread blends were higher than that found in pan bread from whole meal wheat flour. On the other hand, tryptophan content was higher in pan bread originated from whole meal wheat flour, vital gluten, turmeric and fenugreek flour than that found in all the other pan bread blends.

**Table 2: Amino acids content of the tested materials (calculated as g/100g protein).**

Amino acid	Whole meal wheat	Gluten	Lupin	Fenugreek	Turmeric
<b>Isoleucine</b>	3.28	3.40	5.15	3.64	1.12
<b>Leucine</b>	6.35	6.20	2.01	1.93	3.23
<b>Lysine</b>	0.44	0.31	3.4	4.90	1.35
<b>Methionine</b>	0.42	0.15	0.82	0.77	1.72
<b>Cystine</b>	4.06	3.51	0.77	0.83	0.038
<b>Phenylalanine</b>	1.58	4.07	2.91	0.389	3.070
<b>Tyrosine</b>	2.85	3.49	3.95	5.280	6.340
<b>Threonine</b>	2.78	0.00	2.78	3.57	4.67
<b>Valine</b>	4.14	4.50	3.01	1.27	2.72
<b>Tryptophan</b>	0.72	0.75	0.60	1.25	0.35
<b>Total essential amino acids</b>	26.62	27.38	25.40	23.82	24.61
<b>Aspartic acid</b>	4.83	2.04	14.50	4.00	12.99
<b>Serine</b>	2.60	5.58	1.91	8.00	3.03
<b>Glutamic</b>	26.78	25.75	29.80	37.55	21.85
<b>Proline</b>	1.48	15.22	1.01	1.58	1.90
<b>Glycine</b>	4.88	3.32	4.02	6.12	3.67
<b>Alanine</b>	18.66	8.71	3.80	2.71	8.52
<b>Arginine</b>	3.09	9.44	6.095	9.66	7.66
<b>Histidine</b>	6.12	1.42	10.80	1.54	5.89
<b>Total amino acids</b>	95.06	97.86	97.33	94.98	90.12

Data presented in Table (3) showed also, that the calculated PER of manufactured pan bread could be divided into two groups. The first group included WWG, WWGT, WWGF and WWGL mixtures which had PER values less than 1.10. The second group included WWF, WWGFT, WWGLT and WWGFLT mixtures which had PER values more than 1.10. This variation in PER could be attributed to the variation of essential amino acids in the tested pan bread. However, WWGLT pan bread had the highest PER value when compared with other manufactured pan bread. Such results are due to its high amounts of lysine, phenylalanine and threonine. These results agreed with those of Doxastakis *et al.*, (2002) who reported that the lupin flours can be considered as an excellent choice for improving the nutritional value of bread.

**Table 3: Amino acids content of the pan bread (calculated as g/100g sample).**

Amino acid	WWF	WWG	WWGT	WWGF	WWGL	WWGFT	WWGLT	WWGFLT
<b>Isoleucine</b>	0.434	1.068	1.064	1.105	1.144	1.101	1.139	1.176
<b>Leucine</b>	0.841	1.984	1.978	1.972	1.980	1.967	1.974	1.963
<b>Lysine</b>	0.058	0.109	0.110	0.184	0.170	0.185	0.171	0.273
<b>Methionine</b>	0.055	0.071	0.0722	0.081	0.083	0.0818	0.084	0.093
<b>Cystine</b>	0.537	1.165	1.160	1.152	0.839	1.146	1.147	1.133
<b>Phenylalanine</b>	0.209	1.062	1.062	1.057	1.106	1.057	1.106	1.102
<b>Tyrosine</b>	0.377	1.049	1.050	1.089	1.080	1.115	1.106	1.171
<b>Threonine</b>	0.336	0.235	0.234	0.275	0.270	0.275	0.270	0.310
<b>Valine</b>	0.548	1.396	1.385	1.389	1.426	1.385	1.422	1.415
<b>Tryptophan</b>	0.095	0.234	0.233	0.544	0.240	0.248	0.241	0.257
<b>Total essential amino acids</b>	3.527	8.578	8.639	6.611	8.942	8.818	8.918	9.123
<b>Aspartic acid</b>	0.639	0.906	0.909	2.810	1.149	0.941	1.152	1.185
<b>Serine</b>	0.344	1.497	1.496	1.800	1.516	1.607	1.515	1.625
<b>Glutamic</b>	3.548	8.284	8.250	6.620	8.673	8.690	8.653	9.079
<b>Proline</b>	0.196	3.566	3.566	5.760	3.575	3.580	3.574	3.589
<b>Glycine</b>	0.646	1.199	1.198	1.264	0.950	1.260	1.239	1.305
<b>Alanine</b>	2.472	3.692	3.672	3.611	2.453	3.593	3.622	3.541
<b>Arginine</b>	0.409	2.412	2.407	2.546	3.847	2.547	2.507	2.642
<b>Histidine</b>	0.810	0.886	0.881	0.869	1.050	0.866	1.046	1.030
<b>Total amino acids</b>	12.59	30.853	30.796	31.745	32.07	31.691	32.016	32.91
<b>PER</b>	1.321	1.043	1.049	1.088	1.049	1.116	1.954	1.134

#### Biological estimation of different tested blends.

It was of importance to estimate the impact of different tested diet types on some specific biological parameters (blood glucose, insulin levels, liver and kidney function). The values of blood glucose, GPT and GOT, alkaline phosphatase (ALP), total protein, albumin, serum uric acid, urea and creatinine of each rat group for zero time (initial period) and after adaptation period (where the rats fed on basal diet were nearly the same level).

#### - Effect of the different pan breads on blood glucose and insulin levels of nondiabetic and diabetic rats.

Blood glucose and insulin levels of the rat groups fed on the tested pan bread are presented in Table (4). The diabetic rats showed a range of 2.24-2.89 folds increment in the blood glucose after injection with alloxan. This increased of blood glucose after injection with alloxan may be due to that the alloxan may either increase the entrance rate of glucose into the blood stream from the liver (increased hepatic glycogenolysis or gluconeogenesis) or decrease the rate of glucose removal from the blood by tissues (decrease the storage and utilization). These influences might be due to the absence of an adequate amount of insulin. The blood glucose concentration was significantly higher in the diabetic rats fed on the basal diet (positive control) than of those diabetic rats fed on the tested pan bread (diabetic) as well as the nondiabetic rats fed on basal diet (negative control).

**Table4: Blood glucose level (mg/dl) and insulin level (mu/l) in serum of rat groups, fed on the tested diets.**

Groups*	After injection (3days)	Feeding period			Insulin level**
		15 day	30 day	45 day	
<b>Negative control</b>	95.0 <sup>h</sup>	97.0 <sup>j</sup>	95.0 <sup>j</sup>	93.0 <sup>l</sup>	0.525 <sup>b</sup>
<b>Positive control</b>	264.0 <sup>c</sup>	258.0 <sup>a</sup>	252.0 <sup>a</sup>	246.0 <sup>a</sup>	0.220 <sup>k</sup>
<b>WWF</b>	213.0 <sup>f</sup>	200.0 <sup>k</sup>	195.0 <sup>c</sup>	183.0 <sup>b</sup>	0.315 <sup>j</sup>
<b>WWG</b>	262.0 <sup>d</sup>	240.5 <sup>b</sup>	202.0 <sup>b</sup>	161.0 <sup>c</sup>	0.365 <sup>h</sup>
<b>WWGL</b>	268.0 <sup>a</sup>	235.0 <sup>c</sup>	191.7 <sup>d</sup>	154.5 <sup>e</sup>	0.390 <sup>g</sup>
<b>WWGF</b>	264.5 <sup>c</sup>	231.0 <sup>d</sup>	184.0 <sup>f</sup>	150.0 <sup>f</sup>	0.420 <sup>f</sup>
<b>WWGT</b>	261.0 <sup>d</sup>	235.0 <sup>c</sup>	189.0 <sup>e</sup>	156.0 <sup>d</sup>	0.430 <sup>e</sup>
<b>WWGLT</b>	253.5 <sup>e</sup>	228.0 <sup>e</sup>	189.0 <sup>e</sup>	148.0 <sup>g</sup>	0.480 <sup>d</sup>
<b>WWGFT</b>	261.0 <sup>d</sup>	225.0 <sup>g</sup>	178.0 <sup>g</sup>	143.0 <sup>h</sup>	0.490 <sup>c</sup>
<b>WWGFLT</b>	267.0 <sup>b</sup>	211.0 <sup>h</sup>	160.0 <sup>h</sup>	129.0 <sup>j</sup>	0.530 <sup>a</sup>

\* Previously identified and listed in Materials and Methods of the current study.

\*\*At the end of experiment.

-Each value (an average of three replicate) within the same column, followed by the same letter are not significantly different at <0.05.

-Value of blood glucose was 84.0 and 95.0 at zero time and after adaptation period, respectively.

There were significant differences in serum blood glucose level in both negative control groups compared with the other diabetic rats groups. The decrease in blood glucose is due to the effect of dietary fiber from the tested materials. These results are agreed with Adam *et al.*, (2003) who reported that the dietary fiber intake, especially from whole grain sources, reduced the serum glucose level and lead to reduce the risk of coronary heart disease and diabetic. Such treatments containing fenugreek and turmeric flour reduced the serum blood glucose. These results agreed with Patil *et al.* (2009) who found that the mean fasting blood glucose in the diabetic untreated group (control positive) was  $280 \pm 8.33$  mg/dl after 21 days of diabetes induction. In the normal health group this value was  $76 \pm 2.59$  mg/dl. In comparison with the positive control group, the group which consumed fenugreek extract showed significantly lower mean fasting blood glucose  $141.83 \pm 9.04$  mg/dl ( $P < 0.05$ ) after 21 day of induced of diabetes. These results confirmed with Kumar *et al.* (2005) who reported that the beneficial effect of fenugreek seed mucilage is due to some of the bioactive compounds present in the mucilage, including 4-hydroxy isoleucine. 4-Hydroxy isoleucine is a novel amino acid known to facilitate insulin secretion. The effect of spent turmeric in rats may be mainly due to the high amount of dietary fiber, which would facilitate a slower absorption of glucose in the gastrointestinal tract. Results in Table (4) revealed that, within the diabetic rat groups, the whole meal possessed lower serum glucose content than that of positive control groups, respectively.

Kim *et al.*, (2006) reported that alloxan induces “chemical diabetes” in a wide variety of animal species by damaging the insulin secreting pancreatic  $\beta$ -cell, resulting in a decrease in endogenous insulin release. Therefore, Table (4) concerned the plasma insulin of the different groups at the end of the present study. It was found that the insulin level was significantly higher in diabetic rats fed on the WWGFLT and the negative control, while it was significantly lower in the positive control. The results showed that serum glucose level was increased, whereas serum insulin was decreased in the diabetic rats groups. It could finally concluded that the whole meal diet and the other blends containing fenugreek and turmeric flour could be used as a serum glucose controller via the lower sugar content either be decrease the glucose level or increase the insulin level in the serum. Such treatments contained fenugreek and turmeric flour may potentiate the insulin secretion by 4- hydroxyisocucine in fenugreek seed and this effect of turmeric could be done by antioxidant and dietary fiber influence. Also, demonstrated that the in vitro amino acid 4-hydroxyisocucine in fenugreek seeds, increased glucose-induced insulin release in human and rat pancreatic islet cells. This amino acid appeared to act only on pancreatic beta cells, since the levels of somatostatin and glucagon were not altered. Chattopadhyay *et al.*, (2004) reported that, both turmeric and curcumin decrease blood sugar level in alloxan-induced diabetes in rat. Curcumin also decreases advanced glycation end products

induced complications in diabetes mellitus. Srinivasan (2005) mentioned that, daily intake of curcumin (coloring principle of turmeric) not only reduced the fasting sugar level, but also lowered the dosage of insulin needed for normoglycemia. It was observed that the rhizome of turmeric showed blood glucose lowering activity in alloxan diabetic rats.

Vijayalakshmi *et al.*, (2009) reported that the role of spent turmeric in the management of diabetes could be mainly due to the presence of dietary fibers. Both soluble and insoluble dietary fibers contribute to the management of diabetes. Both of them not only serve in the slow absorption of glucose (in the gastrointestinal tract) but also are fermented by the microflora present in the colon, which release short chain fatty acids. In recent years, beneficial effect of short-chain fatty acids and that of butyric acid are receiving much attention in the improvement of disease conditions like cancer and diabetes.

**Table 5. GOT, GPT (U/L) and alkaline phosphatase (IU/L) in rat groups fed on the tested diets.**

Groups*	GOT (U/L)		GPT (U/L)		Alkaline phosphatase (IU/L)	
	After injection (3days)	45 day	After injection (3days)	45 day	After injection (3days)	45 day
<b>Negative control</b>	29.75 <sup>h</sup>	25.00 <sup>i</sup>	38.75 <sup>k</sup>	30.00 <sup>h</sup>	25.50 <sup>h</sup>	20.50 <sup>h</sup>
<b>Positive control</b>	78.25 <sup>a</sup>	57.75 <sup>a</sup>	48.50 <sup>g</sup>	46.95 <sup>a</sup>	48.75 <sup>a</sup>	40.45 <sup>a</sup>
<b>WWF</b>	70.25 <sup>f</sup>	56.25 <sup>b</sup>	44.75 <sup>i</sup>	32.75 <sup>b</sup>	39.50 <sup>g</sup>	31.50 <sup>b</sup>
<b>WWG</b>	65.50 <sup>g</sup>	41.25 <sup>c</sup>	47.50 <sup>h</sup>	31.25 <sup>g</sup>	39.50 <sup>g</sup>	31.25 <sup>c</sup>
<b>WWGL</b>	70.75 <sup>e</sup>	41.00 <sup>d</sup>	51.50 <sup>e</sup>	32.50 <sup>c</sup>	41.53 <sup>f</sup>	30.95 <sup>d</sup>
<b>WWGF</b>	73.25 <sup>d</sup>	39.75 <sup>f</sup>	54.50 <sup>d</sup>	31.75 <sup>d</sup>	45.58 <sup>d</sup>	31.25 <sup>c</sup>
<b>WWGT</b>	65.50 <sup>g</sup>	41.00 <sup>d</sup>	49.50 <sup>f</sup>	31.75 <sup>d</sup>	47.50 <sup>b</sup>	31.25 <sup>c</sup>
<b>WWGLT</b>	75.25 <sup>c</sup>	40.00 <sup>e</sup>	56.0 <sup>b</sup>	31.50 <sup>e</sup>	45.50 <sup>d</sup>	30.65 <sup>f</sup>
<b>WWGFT</b>	76.50 <sup>b</sup>	39.25 <sup>g</sup>	55.50 <sup>c</sup>	32.50 <sup>e</sup>	44.50 <sup>e</sup>	30.75 <sup>e</sup>
<b>WWGFLT</b>	78.25 <sup>a</sup>	38.50 <sup>h</sup>	58.25 <sup>a</sup>	32.50 <sup>e</sup>	46.25 <sup>c</sup>	29.50 <sup>g</sup>

\* Previously identified and listed in Materials and Methods of the current study.

-Each value (an average of three replicates) within the same column, followed by the same letter are not significantly different at <0.05.

Value of GOT was 25.0 and 30.0 at zero time and after adaptation, respectively.

Value of GPT was 40.0 and 39.0 at zero time and after adaptation, respectively.

Value of Alkaline phosphatase was 25.0 and 23.0 at zero time and after adaptation, respectively.

#### - Effect of the tested pan bread on liver functions of nondiabetic and diabetic rats

##### - Effect of the tested pan bread on GOT, GPT and ALP

The results in Table (5) showed that serum GOT, GPT and ALP activities were significantly increased in diabetic rats after injection with alloxan when compared with the nondiabetic rat. These results agreement with Eidi *et al.*, (2007) who reported that the serum GOT and GPT levels increased in diabetic control rats when compared with nondiabetic control rats. There was a decrement of liver function, serum GOT, GPT and ALP as a result of feeding the rats on the tested pan bread in the 45 days *in vivo* experimental assay, as shown in Table (5). Data presented in Table shows that there were significant changes in impact of all the tested diets under investigation on both of serum GOT, GPT and ALP tests either in diabetic control or healthy (normal rats). Serum GOT was the lowest in all diabetic rats fed on pan bread prepared from fenugreek, lupin and turmeric and pan bread from fenugreek and turmeric. GPT was lower in diabetic rats fed on WWG, WWGLT, WWGF and WWGT pan

bread blends compared with values of the other groups. There was an increment in liver function, serum GOT and GPT, as a result of feeding the rats on the basal diet (positive control). The decrement in GOT and GPT may be due to the decreased in serum glucose level which inhibited the glycogenesis (conversion of amino acids into sugars). These results are in agreement with Abbas (2008).

These findings confirmed, also, the healthy roles of legumes and whole meal in lowering the GOT and GPT activities as reported by Eidi *et al.*, (2007). It was found that, an increase in these enzyme activities reflects active liver damage. Inflammatory hepatocellular disorders result in extremely elevated transaminase levels. Administration of the fenugreek seeds extract (0.25 and 0.5 g/kg body wt) and glibenclamide (600 $\mu$ g/kg) similarly decreased GOT and GPT levels when compared with control diabetic rats. Data presented in (Table 5) showed that alkaline phosphatase activity in nondiabetic rats fed on basal diet and diabetic rats fed on WWGFLT pan bread blend was significantly lower than that found in the other rats groups fed on the suggested blends. The significant highest activity in ALP was noticed in case of diabetic rats fed on basal diet (positive control). There was, also, an improvement in ALP of in all groups relative to diabetic control. These results agreed with Mahmoud *et al.*, (2007) who reported that the feeding with legumes to alloxanized diabetic rats was characterized by a significant inhibition in ALP activity.

**Table 6. Albumin and total protein concentration and(g/dl) in rat groups fed on the tested diets.**

Groups*	Albumin concentration (g/dl)		Total protein (g/dl)	
	After Injection (3days)	45 days	After Injection (3days)	45 day
<b>Negative control</b>	2.31 <sup>a</sup>	4.93 <sup>a</sup>	4.93 <sup>a</sup>	7.53 <sup>f</sup>
<b>Positive control</b>	1.71 <sup>d</sup>	3.52 <sup>g</sup>	3.14 <sup>g</sup>	5.94 <sup>i</sup>
<b>WWF</b>	1.92 <sup>b</sup>	3.54 <sup>g</sup>	3.34 <sup>f</sup>	6.53 <sup>h</sup>
<b>WWG</b>	1.82 <sup>c</sup>	4.23 <sup>d</sup>	3.35 <sup>f</sup>	7.10 <sup>g</sup>
<b>WWGL</b>	1.96 <sup>b</sup>	4.52 <sup>c</sup>	3.47 <sup>e</sup>	8.52 <sup>c</sup>
<b>WWGF</b>	1.86 <sup>c</sup>	4.12 <sup>e</sup>	3.67 <sup>d</sup>	8.13 <sup>e</sup>
<b>WWGT</b>	1.72 <sup>d</sup>	4.08 <sup>f</sup>	3.85 <sup>c</sup>	7.15 <sup>g</sup>
<b>WWGLT</b>	1.81 <sup>c</sup>	4.73 <sup>b</sup>	3.43 <sup>e</sup>	8.61 <sup>b</sup>
<b>WWGFT</b>	1.93 <sup>b</sup>	4.22 <sup>d</sup>	3.97 <sup>b</sup>	8.22 <sup>d</sup>
<b>WWGFLT</b>	1.85 <sup>c</sup>	4.97 <sup>a</sup>	3.65 <sup>d</sup>	8.75 <sup>a</sup>

\* Previously identified and listed in Materials and Methods of the current study.

-Each value (an average of three replicates) within the same column, followed by the same letter are not significantly different at <0.05.

Value of albumin concentration (g/dl) was 1.50 and 2.10 at zero time and after adaptation, respectively.

Value of total protein (g/dl) was 4.52 and 4.90 at zero time and after adaptation, respectively.

#### **- Effect of the tested pan bread on albumin and total protein concentration of nondiabetic and diabetic rats**

Data presented in Table (6) showed that a significant lower in total protein and albumin content was found among the rat groups serum after injection with alloxan. In this study, the decrease in serum protein may be due to increasing the excretion of nitrogen in urine and protein synthesis. The present results are in a good agreement with Venkateswamy *et al.*, (1993) who proved that there was a marked decrease in the serum protein content of alloxanized diabetic rats when compared with that of the normal control animals. These effect may be due to the increasing in the excretion of nitrogen in urine alloxanized diabetic rats and increased the activity of transaminases. After 45 days feeding, the protein level was significantly lower in the diabetic rats fed on the basal diet (positive

control) than those diabetic rats fed on the tested pan bread, nondiabetic fed on basal diet (negative control). There were significant differences in serum total protein among the diabetic rats fed on the WWGFLT, WWG, WWGT and WWF pan bread.

Table (6) showed that, after feeding the rats on the tested pan bread, it was found that there was a significant increment in serum albumin content in diabetic rats at the experimental feeding period end (45 days). It was also found that there were significant differences in serum albumin in normal rats and diabetic rats at each feeding stage(after and after adaptation) and within the tested period. The present results showed that serum albumin increased in diabetic rats fed on the tested pan bread when compared with diabetic control (positive control) rats. These results agreed with that found by Mahmoud *et al.*, (2007) who found that the feeding with legumes to alloxanized diabetic rats was characterized by improvement in protein and albumin.

#### - Effect of the tested pan bread on kidney function of nondiabeti and diabetic rats

It is of great importance to estimate the renal function which includes the determination of serum uric acid, urea and creatinine to evaluate disorders which may be occurred as a result of alloxan injection (El-Abd *et al.*, 2007).

The results in Table (7) indicated that alloxan injection caused a highly increase in serum uric acid, urea and creatinine values. The present results showed that serum urea, uric acid and creatinine increased in diabetic control rats when compared with nondiabetic control rats. This may be due to the protein glycation in diabetes may lead to muscle wasting and increased release of purine, the main source of uric acid, as well as increased activity of xanthine oxidase. These results confirmed by Eidi *et al.*, (2007) who showed that serum uric acid, urea, and creatinine levels were increased in diabetic rats. This may due to metabolic disturbance in diabetes reflected in high activities of xanthine oxidase, lipid peroxidation, and increased triacylglycerol and cholesterol levels. Data presented in Table (7) shows that there were significant changes in impact of all the tested diets under investigation serum uric acid, urea and creatinine amounts either in diabetic or healthy (normal rats). Serum uric acid was significantly decreased in diabetic rats fed on WWG, WWGF, WWGFT, WWGFLT and WWF followed by WWGLT and WWGT at the experimental period (45 days) end compared with that of diabetic (whole meal wheat and positive control) rat groups. Also, results in the same Table showed that, feeding on pan bread samples prepared from the tested materials flour led to a serum significantly decrement in urea amount in the diabetic rats group fed on the WWGFLT and nondiabetic rats group fed on the basal diet or whole meal wheat bread for 45 days compared with that of the positive control. These results agreed with Rubio *et al.*, (1998) who mentioned that, there is an inverse correlation between the biological value of foodstuff and blood urea concentration in rats. Because the urea is one of the main end products of protein catabolism in mammals, high plasma values are associated with disturbances in protein metabolism and increased protein degradation, which can finally result in a high loss of N through the urine.

However, data in Table (7) showed that the serum creatinine was significantly decreased in diabetic rats fed on WWGFLT and WWGFT followed by WWGLT for the experimental period (45 days) compared with that of diabetic (whole meal wheat, positive control) rat groups. These results agreed with Yadav *et al.*, (2004) who showed the presence of lipid deposits in the kidney of diabetic human and experimental animals and they have proposed that these deposits may play an important role in the pathogenesis of diabetic kidney disease.

From aforementioned results in Table (7) it could be concluded that fenugreek flour decreased the serum uric acid, urea, and creatinine levels in diabetic rats. These results are concurrent with Eidi *et al.*, (2007) who reported that the fenugreek seeds significantly decreased serum urea, uric acid, and creatinine when compared with control diabetic rats. Elevation of the serum urea and creatinine, as significant renal function markers, are related to renal dysfunction in diabetic hyperglycemia. However, Tharanathan and Mahadevamma (2003) showed that a high fiber diet seemed to lower the urinary phenol and cresol concentrations in humans. It has been suggested that the presence of undigested carbohydrates stimulates rapid growth of colonic bacteria, which can act as 'nitrogen sinks' for using remaining protein and protein metabolites for their metabolism and growth. Kumar *et al.*, (2005) also, mentioned that the water extract of fenugreek seeds is used in the management of diabetes and is known to improve kidney function during diabetes.

Finally, it is recommended to utilize whole meal flour, fenugreek, lupin and turmeric flour to prepare healthy diets to deal with diabetic status and control of some biological parameters.

**Table 7. Uric acid, urea and creatinine (mg/dl) in rats groups fed on the tested diets.**

Groups*	Uric acid (mg/dl)		Urea (mg/dl)		Creatinine level (mg/dl)	
	After injection (3days)	45 day	After injection (3days)	45 day	After injection (3days)	45 day
Negative control	3.47h	3.01h	35.23j	20.25j	0.76e	0.62g
Positive control	6.13a	5.70a	54.29a	40.14a	1.52b	1.4a
WWF	5.18d	3.91d	45.16i	34.18c	1.46c	1.38b
WWG	4.27g	3.14g	50.30f	32.51d	1.53b	1.37b
WWGL	5.87c	4.80b	51.32c	32.32e	1.51b	1.32d
WWGF	4.56f	3.16g	50.22h	31.48f	1.55a	1.30d
WWGT	5.19d	4.09c	50.28g	35.51b	1.50b	1.35c
WWGLT	4.83e	4.09c	50.78d	31.22g	1.42d	1.25e
WWGFT	5.92b	3.29f	50.63e	30.49h	1.48c	1.22e
WWGFLT	4.58f	3.82e	53.45b	26.21i	1.44d	1.19f

\* Previously identified and listed in Materials and Methods of the current study.

-Each value (an average of three replicates) within the same column, followed by the same letter are not significantly different at <0.05.

Value of uric acid (mg/dl) was 3.90 and 3.70 at zero time and after adaptation, respectively.

Value of urea (mg/dl) was 37.10 and 36.3 at zero time and after adaptation, respectively.

Value of creatinine(mg/dl) was 0.85 and 0.80 at zero time and after adaptation, respectively.

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### المستخلص العربي

#### التقييم البيولوجي لخبز القالب المدعم بالجلوتين ودقيق الحبة والترمس والكركم

محمد حسن على\*, محمد محمد أحمد النقطي\*, محمود عبدالله محمد صالح\*\* نصرة أحمد محمد عبدالحق\*\*\*

\* جامعة القاهرة- كلية الزراعة- قسم الصناعات الغذائية ،\*\* معهد بحوث تكنولوجيا الأغذية- قسم الأغذية الخاصة والتغذية،\*\*\* معهد بحوث تكنولوجيا الأغذية- وحدة المطبخ التجاري

الهدف من الدراسة هو الاستفادة من حبة القمح الكاملة والجلوتين وبعض البقوليات والكركم لتجهيز خبز القالب وتقييمه من حيث احتواه على الألياف الغذائية والأحماض الامينية الأساسية وبعض صفاتي الباليولوجية. وجد ارتفاع معنوي في الألياف الغذائية الذائبة وغير ذاتية والكلية في كلا من الحبة والترمس والكركم بالمقارنة بقيق القمح الحبة الكاملة. قد ارتفعت بدرجة معنوية نتيجة عملية اصابة الفران بالسكر. وكانت الفران الطبيعية ( التي تم تغذيتها على الوجبة الاساسية دون احداث اصابة بمرض السكري لها) قد أعطت انخفاض غير معنوي في مستوى السكر الدم بينما حدث انخفاض معنوي في حالة الفران المصابة بمرض السكر في وظائف الكبد والكلى بعد تغذيتها بالخبز المختبر . ولذلك فإنه ينصح باستخدام دقيق حبة القمح الكاملة مع الجلوتين و البقوليات والكركم في تجهيز وجبات صحية تعمل على تنظيم بعض الاختبارات الحيوية في الحالات المصابة بمرض السكري او العادية .

**الكلمات الدالة:** حبة القمح الكامل - الجلوتين- بذور الحبة- البقوليات- الكركم-السكري .

8/31/2010

## Scleral Fixation Intraocular lenses

<sup>1</sup>Ayman Shouman, <sup>1</sup>Mohamed Marzouk, <sup>1</sup>Hesham Ali and <sup>1</sup>Ehab Zakzook

<sup>1</sup>Ophthalmology Department, Research Institute of Ophthalmology, Giza

[shoumannaes@yahoo.co.uk](mailto:shoumannaes@yahoo.co.uk)

**Abstract:** Background: The 1ry indication for scleral fixation of intraocular lenses (IOL) is dislocation as a principal complication of cataract surgery. Inadequate capsular support is the most common cause of IOL dislocation. Other indications include traumatic phakic lens dislocation (cataractous or clear), surgically aphakic eyes or anterior chamber IOL with complications (persistent hyphema, uveitis). Methods: 20 eyes of 20 patients were done, surgery was done only when the IOL was dislocated peripheral to the visual axis and was causing symptoms of visual loss sufficient to interfere with the patient's activities of daily living, or patients who were left aphakic for a 2ry implantation procedure. A modification of the technique was done which made the procedure faster and preserved the surrounding conjunctiva. Results: Best corrected visual acuity (BCVA) preoperatively ranged from 1/60 -6/60 and postoperatively between 6/60 – 6/6. Statistical analysis of the logarithm of the minimum angle of resolution (LogMAR) between the preoperative and postoperative visual acuity revealed significant improvement ( $p \leq 0.05$ ). Intraoperative complications included one case of accidental iris injury, two cases of mild vitreous hemorrhage, two cases of moderate vitreous hemorrhage. Early postoperative complications included pupillary block. Midterm post operative complications occurred in one case with the occurrence of cystoid macular edema. Conclusion: Scleral fixation of IOL is a safe procedure with minimal complications, but needs surgical skills to be managed optimally.

[Ayman Shouman, Mohamed Marzouk, Hesham Ali and Ehab Zakzook. **Scleral Fixation Intraocular lenses.** Journal of American Science 2010;6(11):680-687]. (ISSN: 1545-1003).

**Key words:** Scleral fixation, Intraocular lenses, Aphakia, IOL dislocation.

### Introduction

The indication for intraocular lens (IOL) scleral fixation is widely variable. The 1ry indication is IOL dislocation as a principal complication of cataract surgery, with early reports of 13% rate of IOL dislocation<sup>(1)</sup>. Inadequate capsular support is the most common cause of IOL dislocation and most commonly manifests in the early postoperative period<sup>(2)</sup>. Fixation of intraocular lenses in cases of insufficient or no capsular support is challenging and requires a large armamentarium of techniques to resolve different situations<sup>(3-24)</sup>. Other indications include traumatic phakic lens dislocation (cataractous or clear), surgically aphakic eyes or anterior chamber IOL with complications (persistent hyphema, uveitis). The reported incidence of dislocation of rigid posterior chamber IOLs (PCIOLs) is from 0.2% to 2%<sup>(25,26)</sup>. Management decisions are based on the clinical features of every individual case and surgeon preference. Management options described for dislocated PCIOLs include IOL repositioning with or without scleral fixation sutures (depending on residual capsular support) and IOL removal (with or without reimplantation of the same or alternative

IOL)<sup>(19,27,28)</sup>. IOL repositioning without scleral fixation suture is generally preferred technique if there is adequate capsular support. In the absence of adequate capsular support, the dislocated PCIOL may be sutured to the sclera or exchanged. The scleral suture fixation technique requires undamaged open-loop haptics. A PCIOL may be exchanged for a sutured PCIOL or an anterior chamber IOL (ACIOL)<sup>(29,30)</sup>. Dislocation of the phakic human lens is uncommon but serious complication, as it requires sophisticated techniques to remove safely, especially if it is dislocated in the vitreous cavity. Dislocation of a PCIOL into the vitreous cavity is another uncommon but serious complication with numerous techniques for its management<sup>(3,5,6,13,14,17,18,31-41)</sup>. During surgical intervention, an important consideration is whether to remove, reposition, or exchange the dislocated PCIOL after performing a pars plana vitrectomy. If adequate capsular support is found to be present, the same intraocular lens may be positioned into the ciliary sulcus. Alternatively, the dislocated PCIOL may be sutured to the sclera if capsular support seems inadequate. If the PCIOL is removed, the patient may be left aphakic if intraocular lens implantation is contraindicated for

any reason. However, when there is no contraindication to lens insertion, the patient may receive either a sutured PCIOL or an anterior chamber intraocular lens (ACIOL). Historically, ACIOLs, especially rigid and closed-loop lenses, have been associated with a higher rate of complications compared with PCIOLs<sup>(42)</sup>. These complications can lead to poor visual results and include bullous keratopathy, glaucoma, uveitis, hyphema, and cystoid macular edema. Newer, open-loop ACIOL designs, however, seem to have a lower rate of complications<sup>(43)</sup>. In case of ACIOL complications, exchange of the anterior chamber IOL to a sclerally fixated PCIOL is done. Previous studies describing the visual results in subjects who underwent intraocular lens exchange have reached conflicting conclusions. Some studies have found similar final visual results between eyes that received an ACIOL and those that received a PCIOL<sup>(44)</sup>, whereas other studies have found better postoperative visual results in eyes that received a PCIOL<sup>(45)</sup>.

### **Patients and Methods**

The study cohort included 20 eyes of 20 patients. Preoperative data included age, gender, type & duration of dislocation (surgery, trauma) or aphakia, coexisting ocular diseases, preoperative best-corrected visual acuity (BCVA), preoperative type of IOL, category of IOL dislocation (in the bag), and previous surgery including pars plana vitrectomy, sclera buckling procedure, or neodymium:yttrium-aluminium-garnet (Nd:YAG) capsulotomy. Surgery is recommended only when the IOL was dislocated peripheral to visual axis and was causing symptoms of visual loss sufficient to interfere with the patient's activities of daily living, or patients who were left aphakic for a 2ry implantation procedure. The scleral suture fixation technique for acrylic IOLs was previously reported<sup>(18, 46)</sup>. A modification of the technique was done which made the procedure faster and preserved surrounding conjunctiva (fig. 1). All surgeries were done by one surgeon (1<sup>st</sup> author A.S.).

### **Scleral fixation technique:**

We followed the technique reported by Richard Hoffmann (fig 1) where two limbal vertical incisions half corneal thickness 2.5 mm in length were done at 1:30 and 7:30 meridians. A 2.6 mm crescent knife was used to create a reversed sclera pocket 2.5mm backwards across the limbus into the sclera subconjunctivally. Polypropylene sutures (10-0) on a straight needle were introduced 1 mm

posterior to the limbus through the wall of the scleral pocket traversing the conjunctiva, sclera pocket and into the eye. The needle is passed across the eye behind the iris and delivered from the opposite side 1 mm behind the limbus through guidance by a 25 G needle introduced through the sclera pocket of the other side 180 degrees away. This needle is again introduced into the eye 1mm adjacent to its exit to pass to the opposite side in the same manner previously described. Finally, we have two 10/0 threads passing across the eye and 2 separate ends on either side of the limbus. A two step corneal incision 7mm is done. The two 10/0 threads are retrieved through the wound by a sinsky hook. Every two ends are tied to the hole on the haptic of sclera fixation PMMA 6.50 optic IOL, whenever the diopteric power was available or on the haptics if not. The two ends were pulled to centralize the IOL. The corneal wound is closed with 3 interrupted 10/0 nylon stitches. The 2 10/0 prolene threads on either side were retrieved from the corneal side of the scleral pocket and tied to one another on either side separately and buried in the sclera pocket.

In vitreous dislocated IOLs; 3 port 20 Gauge pars plana vitrectomy was done in the usual manner with perflurodecalin floatation of the dislocated IOL and retrieval with serrated vitrectomy forceps and into the anterior chamber above the iris, where it is removed through corneal incision. In cases where there was traumatic dislocation of the cataractous lens, the same former technique was done with removal of the lens by phacofragmatome. In cases where total vitrectomy was performed, peripheral retinal indentation was done with intraocular laser treatment to any suspicious peripheral tears and fluid air exchange was done with the eye left with air tamponade with no traction on the retina. In case of surgical aphakia, conjunctiva was not opened and anterior chamber vitrectomy was done either through two corneal side ports or through the main corneal wound. In cases of anterior chamber IOL subluxation, anterior vitrectomy was done through 2 corneal side ports and IOL was fixated with an anteriorly opened needle introducing the 10/0 prolene through the sclera pocket and retrieving it in the manner described earlier. The anterior chamber iris captured IOL was removed through corneal incision with anterior vitrectomy. All were followed by sclera fixation of a 6.5 optic Polymethylmethacrylate (PMMA) IOL. Visual acuity was converted to the logarithm of minimum angle of resolution for statistical analysis.

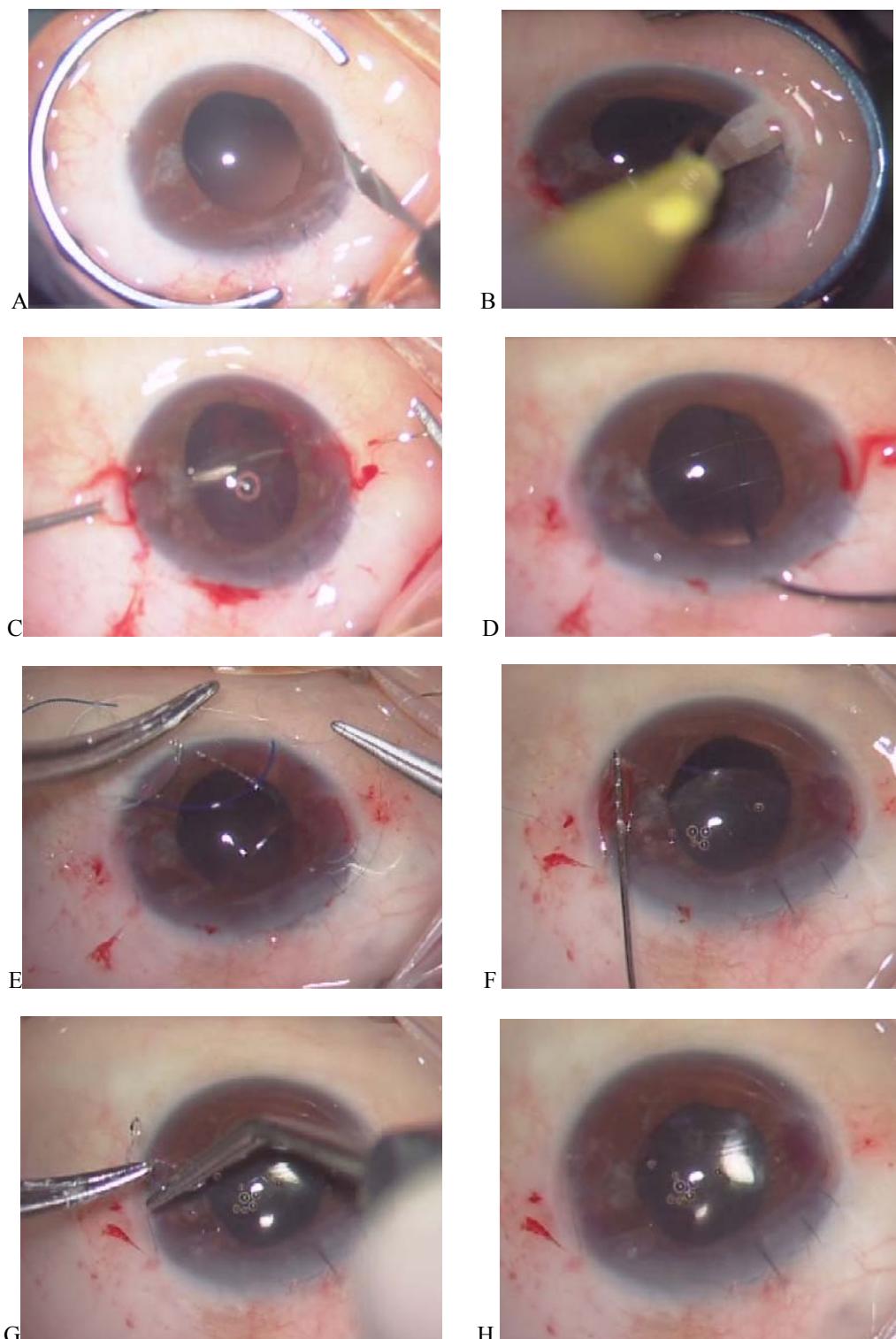


Figure1: Steps of scleral fixation: A- Limbal incision. B- Tunnel dissection. C- 10/0 prolene introduction and retrieval. D- 10/0 prolene thread retrieval. E-Haptic tying. F- 10/0 prolene pocket retrieval. G- 10/0 prolene tying and embedding in the sclera pocket. H- Final picture with no conjunctival opening.

### Statistical analysis:

The mean differences in paired designs were applied using paired t-test to analyze the differences in logMAR of visual acuity in the collected sample of this study at a confidence interval 95% and significance level 0.05.

### Results:

#### Preoperative Characteristics

The study cohort included 20 eyes of 20 patients (8 males, 12 females), with mean age of the patients was 52.9 years (range, 25-70 years; (Table 1). There were 11 (55%) right eyes. The median interval from 1<sup>ry</sup> procedure to the present procedure is 66 days (range, 14–120 days). The indications for scleral fixation were vitreous IOL dislocation during the 1<sup>ry</sup> procedure in 3(15%) eyes; surgical aphakia without enough capsular support for 1<sup>ry</sup> IOL implantation in 9 (45%) eyes; Traumatic cataractous phakic lens vitreal dislocation in 5 (25%) eyes; IOL subluxation with the optic bisecting the pupil and peripheral anterior synechiae and residual cortical matter in 2 (10%) eyes; Anterior chamber captured IOL suffering from chronic iritis in one (5%) eye. The preoperative mean BCVA logarithm of minimum angle of resolution was 1.67 (range of Snellen visual acuity, 1/60 – 6/60). There were 2 (10%) polymethyl methacrylate (PMMA) IOLs, 3 (15%) one piece acrylic IOLs, and 1 (5%) ACIOLs (Table 1).

Table 1. Baseline Characteristics

No. of patients	20
Eyes	20
Gender (%)	Female 12 eyes 60 %
Age (yrs)	Mean 52.9 Range 25–70
Right	11eyes 55 %
Types of dislocated IOL (n = 6)	
PMMA 1-piece	2 (10 %)
1-Piece acrylic IOL	3 (15 %)
ACIOL	1 (5 %)
Preoperative BCVA	
Mean logMAR	1.67
Range (Snellen)	1/60 – 6/60
Interval from IOL implantation to dislocation	Range 14 day to 120 days

ACIOL \_ anterior chamber intraocular lens; BCVA \_ best corrected visual acuity; IOL \_ intraocular lens; logMAR \_ logarithm of minimal angle resolution; PMMA \_ polymethylmethacrylate.

The median follow-up after dislocated IOL management or implantation was 18 months (range, 11months-24months). The postoperative mean BCVA logarithm of minimum angle of resolution was 0.24 (range of Snellen visual acuity, 6/60 – 6/6). Intraoperative complications included one case of accidental iris injury during anterior vitrectomy (Table 2). Two cases of mild vitreous hg which resolved after 2 weeks of bed rest (Fowler position). Two cases of moderate vitreous hg which mandated doing total vitrectomy during the procedure. Early postoperative complications included pupillary block with elevation of the IOP which resolved completely after a course of mydriatics. Mid term post operative complications occurred in one case with the occurrence of cystoid macular oedema (Irvine gass syndrome).

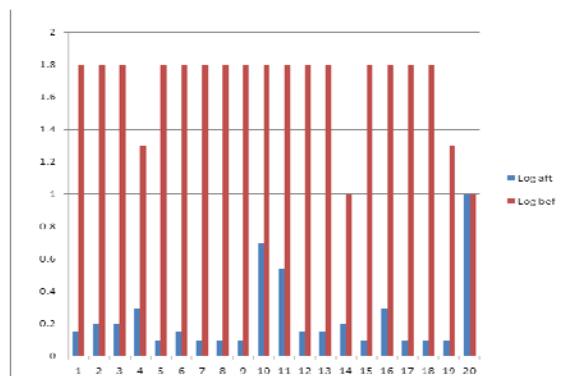
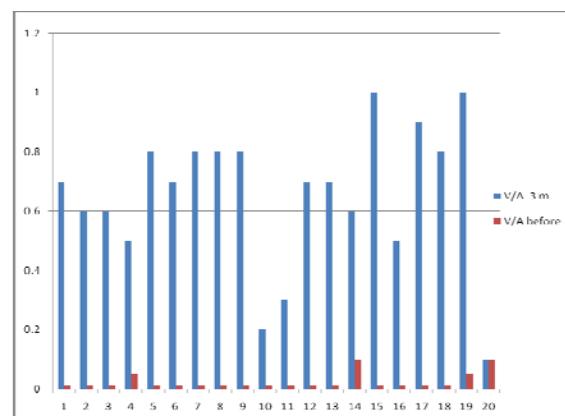


Figure2: graph of visual acuity pre and postoperative by 3 months, Figure3: graph of visual acuity LogMar conversion pre and postoperative by 3 months.

Best corrected visual acuity (BCVA) preoperatively ranged from 1/60 -6/60 and postoperatively between 6/60 – 6/6 (Figure 2, 3). Statistical analysis of the logarithm of the minimum angle of resolution (LogMAR) between the

preoperative and postoperative visual acuity revealed significant improvement ( $p \leq 0.05$ ).

Table 2: complications.

Complications	
Intraoperative	accidental iris injury (n:1)
	Mild vitreous hemorrhage (n:2)
	Moderate vitreous hemorrhage (n:2)
	Retinal tears (n:2)
Postoperative	
Early	Papillary block (n:1)
Midterm	Cystoid macular oedema (n:1)

### **Discussion:**

Previous studies have not definitively set surgical indications and optimal timing for management of dislocated IOLs; neither did this study. The management approach depends on surgeon preferences and individual case specifics, including integrity of capsule remnants, type of IOLs, and coexisting ocular pathology<sup>(47-50)</sup>. The surgical approach in this series was influenced by the specialty (vitreoretinal) and the preferences of the operating surgeon (AS), but standardization was established regarding the scleral fixation itself, while the remainder of the procedure was determined by the case pathology and operative circumstances. The stability and centralization of the sclerally fixated IOLs in this study were excellent during the follow up period with no rotation or subluxation. The visual acuity significantly improved postoperatively. Postoperative retinal detachment has been reported in 3%<sup>21</sup> to 14%<sup>10</sup> of cases. In the current study, postoperative retinal detachment didn't develop in any case postoperatively. In cases where total vitrectomy was performed, peripheral retinal indentation was done with intraocular laser treatment to any suspicious peripheral tears and fluid air exchange was done with the eye left with air tamponade with no traction on the retina. Knot exposure, intraocular hemorrhage, trans-scleral suture fistulas, and endophthalmitis have been reported with transscleral sutures, but none with the present technique. The scleral pocket technique done minimized this incidence. Intraoperative and postoperative intraocular hemorrhage is usually self-limited, with two cases resolved after 2 weeks but 2 (10%) patients received vitrectomy during the scleral fixation owing to moderate nature of the hemorrhage, which was related to the transscleral suture passes. Cystoid macular edema occurred in one case and was treated accordingly. Recent case studies of in-the-bag IOL dislocation have suggested predisposing factors

of pseudoexfoliation, uveitis, trauma, status after vitrectomy, and long axial length<sup>(27,33,48,51-59)</sup>. Postoperative retinal detachment occurred more commonly with in-the-bag IOL dislocation compared with extracapsular dislocation, but none in this study. Intraoperative retinal breaks were encountered in 2 eyes; 10% in this study during the course of vitrectomy and were treated with intraocular laser. Suturing of a dislocated PCIOL to the sclera could be technically demanding, potentially resulting in an increased number of intraoperative and postoperative complications such as intraocular hemorrhage, retinal tears, and retinal detachment, with a long-term risk of lens rotation, as well as endophthalmitis<sup>(36,48,60,61)</sup>. Repositioning a dislocated PCIOL into the ciliary sulcus is easier and less traumatic than suturing a lens into the sclera, however, there is the possibility of postoperative lens dislocation because of inadequate capsular support<sup>(35)</sup>. Finally, although technically easier to place, ACIOLs may be associated with a higher long term risk of corneal decompensation, glaucoma, and uveitis<sup>(42,43,62)</sup>. In contrast, PCIOLs have a long-term safety record in eyes with other ocular diseases such as glaucoma, uveitis, and diabetic retinopathy<sup>(63,64)</sup>. This series is rather few, but it adds to the belief of the safety and stability of sclerally fixated IOLs.

### **Corresponding Author:**

Ayman Shouman, MD, FRCS, Ophthalmology Department, Research Institute of Ophthalmology, Giza, Egypt. E-mail: [shoumanaes@yahoo.co.uk](mailto:shoumanaes@yahoo.co.uk)

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8/31/2010

# Technological Properties of some Egyptian New Wheat Varieties

Ahmed M. S. Hussein, Mohie M. Kamil and Gamal H. Ragab

National Research Centre, Food Technology Department, Dokki, Cairo, Egypt  
email: [ResearchTeamMMK@yahoo.com](mailto:ResearchTeamMMK@yahoo.com)

**Abstract:** Whole meal and flour 72% of Gemmeiza 7, Gize 168, Sohage 3 and Sakha 93 wheat varieties were evaluated to produce pan bread, pasta and biscuits. Pan bread of whole meal wheat varieties had higher contents of moisture, protein, fat, ash and fiber than wheat flour 72% of the same varieties. Pan bread of Sakha 93 characterized with its higher baking quality (weight, volume and specific volume) than pan breads of other varieties. Crust color of pan bread slightly affected with whole-meal wheat varieties, where its color score maximized in Sakha 93 (7.7) and Sohage 3 (6.7). This result agreed with the obtained color parameter of Hunter, where lightness (L) maximized to 55.95 and 49.79 in pan bread crust of Sohage 3 and Sakha 93, respectively. Pasta characterized with its higher protein (13.12%), fat (2.59%) and crude fiber (2.82%) in case of using whole meal of Sohage 3, Giza 168 and Gemmeiza 7 varieties, respectively. Pasta cooking quality ranked first in case of using Sohage 3 whole meal, where its weight increase, volume increase and cooking loss reached to 265%, 305.3% and 8.3%, respectively. Pasta color parameter showed that, wheat flour 72% and whole meal of Sakha 93 characterized with its higher lightness (L). Sensory evaluation showed that, pasta of wheat flour 72% accepted slightly in appearance and color if compared with whole meal pasta of the same variety. In addition, there were no significant difference between pasta of wheat flour 72% and whole meal in flavor, tenderness and stickiness. Biscuit of whole meal characterized with its higher content of protein, fat, ash and crude fiber than wheat flour 72%. Whole meal biscuit of Sohage 3 characterized with its higher protein (12.13%), fat (31.0%) and ash (2.51%) contents; and lowest carbohydrate content (52.18%). Biscuit of Sakha 93 variety (whole meal or flour 72%) was higher in baking quality. Hunter color parameter and sensory evaluation showed that, biscuit of whole meal varieties was slightly darker than biscuit of wheat flour 72% varieties. In addition, biscuits flavor, taste, texture, appearance and overall acceptability of wheat flour 72% not affected significantly in case of using whole meal flour of the same variety.

[Ahmed M. S. Hussein, Mohie M. Kamil and Gamal H. Ragab. Technological Properties of some Egyptian New Wheat Varieties. Journal of American Science 2010;6(11):688-699]. (ISSN: 1545-1003).

**Keywords:** Egyptian wheat varieties – technological properties - Pan bread – Pasta – Biscuit - whole meal – wheat flour 72%.

## INTRODUCTION

In Egypt, there is a big gap between wheat production and consumption. Therefore, National Program for Wheat Research developed new wheat varieties characterized with its higher yield and persist pests, i.e. Gemmiza 7, Giza 168, Sohag 3 and Sakha 93 (**Anon., 2005**). The importance of wheat is mainly due to its nutritive value and the fact that its seed can be ground into flour, semolina, etc., which form the basic ingredients of bread and other bakery products, as well as pastas.

Milling of wheat has two important purposes, one is to remove the bran and the other is to reduce the endosperm to small and fine particles. Such process decrease the nutritive value of wheat, so several researchers (**Collins et al., 1983; Finney et al., 1985; Jenkins et al., 1988; Lai et al., 1989a; Mugford, 1993; Shouk, 1996 and Czerny and Schieberle, 2002**) studied the possibility of using whole meal grains (100%) in several products to

utilize from its bran and germ that contain several healthy and nutrient components, furthermore, maximize the yield of flour to reduce the big gape between wheat production and consumption in Egypt.

The differences usually occurred in the wheat flour products depend on the wheat variety (hard or soft wheat), rate of flour milling and particle size. On the other hand, whole meal flour contains the whole of the product derived from the milling of cleaned wheat. Bread can only legally be described as whole meal bread if the flour used in its manufacture is whole meal flour, and no other flour is added. Production of whole meal bread, which is produced from whole meal grains (100%) should reduce bread making cost.

This research aimed to evaluate processing quality of such new varieties to produce some important wheat products, i.e. bread, pasta and biscuit.

## Material and Methods

### Materials

Wheat grains varieties of Gemmeza 7, Giza168, Sohage 3and Sakha93 were obtained from Field Crops Department, Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt. Shortening, sugar, salt and sodium bicarbonate were obtained from Egyptian local market.

### 1. Analytical methods

Moisture, ash, crude protein, fat and crude fiber contents of wheat products were determined according to the methods outlined in AOAC (2000), while carbohydrates were calculated by difference as mentioned by Tadrus (1989).

### 2. Statistical analysis

All results were evaluated statistically using analysis of variance as reported by McClave and Benson (1991).

### 3. Methods of Processing:

Wheat grains varieties were manually cleaned, tempered to 14% moisture content, then milled using Quadrumat Junior flour mill. The obtained flour represent whole flour mill (100% extraction), then sieved to obtain flours of 72% extraction.

#### 3.1 Preparation of Pan Bread

Pan bread baking carried out as described by Lazaridou *et al.* (2007) as follows: Firstly, yeast dissolved in warm water (35°C), then mixed and kneaded with flour, salt (1.5%) and sugar (1%). Then dough fermented at 30°C/30 min in a fermentation cabinet under 80-85% relative humidity. after which, dough was placed in the pan (150 g/piece) and kept under the same fermentation condition for 45 min. Bread dough loaves were baked at 240 °C for 20–25 min in an electric oven (Mondial Formi, 4T 40/60, Italy), then subjected to steam for 10 sec to enhance the browning process of bread. The obtained pan bread packed in a polyethylene bags and stored at room temperature for evaluation and analysis.

#### 3.2 Preparation of Biscuits

Tow type of biscuits were prepared, i.e. biscuit of whole meal flour (100%) and traditional biscuit of wheat flour 72% extraction. Biscuit were prepared according to the method of AACC (2000) by using the following formula: wheat flour 100% or 72% extraction (100 g), sugar (50 g), shortening (28 g), salt (0.93 g), sodium bicarbonate (1.11g), 14.66 ml of dextrose solution (5.93%).

#### 3.3 Preparation of Pasta:

Pasta samples were prepared in Food Technology Department, National Research Centre,

Cairo, Egypt, according to the method of AACC (2000) by using Pasta Matic 1000 Simac Machine Corporation, Milano, Italy. Pasta hydrated for 15 min under atmospheric air, dried in a cabinet dryer at 40°C for 14 hrs, then cooled at room temperature, packed in polyethylene bags and kept at room temperature for analysis.

### 4. Baking quality of Pan Bread and Biscuit:

Weight, volume and specific volume of manufactured pan bread were determined as described by AACC (1983).

Biscuits Diameter, volume and thickness were determined according to the standard method of AACC (2000). The spread ratio (Diameter/thickness) was calculated according to standard methods of AACC (2000).

### 5. Cooking quality of pasta

Cooking quality of pasta carried out by measuring the increases in weight, volume and cooking loss after cooking according the methods of AACC (2000).

### 6. Color quality of Processed Products:

The color of pan bread (crust and crumb), biscuit and pasta samples were evaluated using Hunter, Lab Scan XE, Reston VA., calibrated with a white standard tile of Hunter Lab color standard (LX No. 16379) x = 77.26, y = 81.94 and z = 88.14 (L\* = 92.43, a\* = -0.88, b\* = 0.21).

### 7. Freshness of pan Bread

Pane bread was packed in polyethylene bags and stored at room temperature. Loaves freshness of pan bread was tested at interval time (1, 3 and 5 days) using alkaline water retention capacity (AWRC) according to Kitterman and Rubenthaler (1971).

### 8. Sensory Evaluation of Processed Products:

Sensory evaluation of pan bread was performed by 10 trained panelists as described by Kulp *et al.* (1985) for symmetry of shape (5), crust color (10), break & shred (10), crumb texture (15), crumb color (10), aroma (20), taste (20) and mouth feel (10).

Cooked pasta organoleptically evaluated by ten panelists for its appearance (10), color (10), flavor (10), tenderness (10) and stickiness (10) as described by Hallabo *et al.* (1985).

Sensory characteristics of biscuits evaluated by ten trained panelist for its color (10), flavor (10), taste (10), texture (10), appearance (10) and overall acceptability (10) as mentioned by Zabic and Hoojjat (1984).

## Results and Discussion

### Technological properties of wheat varieties:

#### 1 Pan Bread

##### 1.1 Chemical composition:

Pan bread of whole meal or wheat flour 72% varieties were evaluated chemically and presented in Table (1). The obtained results showed that, pan bread of whole meal characterized with its higher moisture content if compared with wheat flour 72% of all the studied varieties. In addition, moisture content of whole meal pan bread increased significantly to the highest level (37.02%) in Sohage 3 variety and decreased to the lowest level (33.09%) in gemmiza 7. Sohage 3 also characterized with is higher protein content where it reached to 12.43% and 12.22% in pan bread of whole meal and wheat flour 72%, respectively. Fat content of whole meal ban bread decreased slightly in Giza 168, Sohage 3, Gemmiza 7 and Sakha 93 to reach 3.73, 3.42, 3.43 and 3.22%, respectively. The same trend also observed in ash content of pan bread for the same varieties. Crude fibre of whole meal pan bread reached to the highest level in Sakha 93 (3.07%) and lowest level in Sohage 3 (1.39%). Table (1) showed also that, all pan bread of wheat flour 72% varieties characterized only by its higher content of carbohydrate than whole meal, while whole meal had higher contents of moisture, protein, fat, ash and

fiber. This result agreed with those found by Kent-Jones and Amos (1967) and Shouk (1996).

##### 1.2 Baking quality:

The quality of pan bread depends on flour type, so baking quality of whole meal flour and wheat flour 72% of the studied wheat varieties evaluated. Data presented in Table (2) showed that, pan bread of whole meal varieties characterized with its higher weight (140-133.3 g) if compared with its wheat flour 72% varieties (134.8-131.0 g). In addition, the volume of pan bread whole meal increased and ranged between 312.5-265 cm<sup>3</sup>, while its wheat flour 72% decreased between 290-255 cm<sup>3</sup>. On other hand, pan bread of wheat flour 72% varieties characterized with its higher specific volume (2.16-1.91) if compared with its whole meal (2.25-1.94). The obtained results well agree with those found by He and Hoseny (1992) and shouk (1996). Also, Pommeranz et al (1977), stated that, as fiber content increase loaf volume and weight increase, also, Chen et al (1988) stated that, the effect could result from the interaction between gluten and fiber. It could concluded from Table (2) that, whole wheat meal and its flour 72% of Sakha 93 characterized with higher baking quality (weight, volume and specific volume) for pan bread than Gemmeiza 7, Giza 168 and Sohage 3 varieties.

**Table 1:** Effect of wheat varieties and extraction rate on chemical composition of pan bread.

Sample	Moisture	Protein	Fat	Ash	Crude fiber	Total Carb.
<b>Gemmiza 7:</b>						
Whole meal	33.09 <sup>c</sup> ± 0.56	11.26 <sup>bc</sup> ± 0.25	3.43 <sup>b</sup> ± 0.07	1.94 <sup>b</sup> ± 0.09	2.86 <sup>b</sup> ± 0.03	80.48 <sup>de</sup> ± 0.16
Wheat flour 72%	29.37 <sup>e</sup> ± 1.35	10.88 <sup>c</sup> ± 0.08	2.50 <sup>e</sup> ± 0.06	1.06 <sup>c</sup> ± 0.13	0.69 <sup>b</sup> ± 0.14	84.91 <sup>ab</sup> ± 0.28
<b>Gize 168:</b>						
Whole meal	35.50 <sup>b</sup> ± 0.62	11.25 <sup>bc</sup> ± 0.25	3.73 <sup>a</sup> ± 0.15	2.18 <sup>a</sup> ± 2.89	3.00 <sup>ab</sup> ± 0.05	79.72 <sup>e</sup> ± 0.34
Extraction: 72%	31.26 <sup>d</sup> ± 0.91	10.95 <sup>c</sup> ± 0.05	2.57 <sup>de</sup> ± 0.06	1.07 <sup>c</sup> ± 0.06	0.88 <sup>d</sup> ± 0.04	84.61 <sup>b</sup> ± 0.18
<b>Sohage 3:</b>						
Whole meal	37.02 <sup>a</sup> ± 0.58	12.43 <sup>a</sup> ± 0.69	3.48 <sup>b</sup> ± 0.03	1.94 <sup>b</sup> ± 0.09	1.39 <sup>c</sup> ± 0.03	80.82 <sup>d</sup> ± 0.59
Wheat flour: 72%	30.15 <sup>de</sup> ± 0.45	12.22 <sup>a</sup> ± 0.26	2.64 <sup>d</sup> ± 4.20	0.93 <sup>cd</sup> ± 0.07	0.70 <sup>d</sup> ± 0.09	83.51 <sup>c</sup> ± 0.26
<b>Sakha 93:</b>						
Whole meal	35.29 <sup>b</sup> ± 0.49	11.53 <sup>b</sup> ± 0.30	3.22 <sup>c</sup> ± 0.08	1.82 <sup>b</sup> ± 0.12	3.07 <sup>a</sup> ± 0.16	80.35 <sup>de</sup> ± 0.43
Wheat flour 72%	31.19 <sup>d</sup> ± 0.42	10.95 <sup>c</sup> ± 0.13	2.32 <sup>f</sup> ± 0.03	0.85 <sup>d</sup> ± 0.10	0.77 <sup>d</sup> ± 0.12	85.24 <sup>a</sup> ± 0.07
<b>LSD at 0.05</b>	1.27	0.54	0.13	0.16	0.17	0.57

Total Carb. = Total carbohydrate.

**Table (2):** Effect of wheat varieties and extraction rate on baking quality of pan bread.

Sample	Weight (g)	Volume (cc)	Specific volume (cc/g)
<b>Gemmiza 7:</b>			
Whole meal	133.30 <sup>c</sup> ± 1.50	274 <sup>c</sup> ± 8.4	2.06 <sup>bc</sup> ± 5.51
Wheat flour 72%	131.00 <sup>d</sup> ± 0.50	255 <sup>e</sup> ± 5.0	1.95 <sup>d</sup> ± 6.81
<b>Gize 168:</b>			
Whole meal	135.30 <sup>bc</sup> ± 1.52	305.0 <sup>a</sup> ± 5.0	2.25 <sup>a</sup> ± 4.93
Wheat flour 72%	133.80 <sup>c</sup> ± 1.75	267.5 <sup>cd</sup> ± 2.5	1.99 <sup>cd</sup> ± 4.43
<b>Sohage 3:</b>			
Whole meal	136.30 <sup>b</sup> ± 0.763	265.0 <sup>cde</sup> ± 5.0	1.94 <sup>d</sup> ± 0.025
Wheat flour 72%	134.80 <sup>bc</sup> ± 0.289	257.5 <sup>de</sup> ± 7.5	1.91 <sup>d</sup> ± 0.049
<b>Sakha 93:</b>			
Whole meal	140.00 <sup>a</sup> ± 1.50	312.5 <sup>a</sup> ± 10.5	2.23 <sup>a</sup> ± 0.115
Wheat flour 72%	134.20 <sup>bc</sup> ± 1.52	290.0 <sup>b</sup> ± 5.0	2.16 <sup>ab</sup> ± 0.065
<b>LSD at .05</b>	2.227	11.983	0.111

**Table (3):** Color quality of pan bread as affected by wheat varieties and extraction rate.

Sample	Lightness "L"		Redness "a"		Yellowness "b"	
	Crust	Crumb	Crust	Crumb	Crust	Crumb
<b>Gemmiza 7:</b>						
Whole meal	51.32 <sup>c</sup> ± 0.50	53.80 <sup>c</sup> ± 3.31	9.48 <sup>f</sup> ± 0.11	6.48 <sup>a</sup> ± 0.16	27.50 <sup>d</sup> ± 0.50	25.46 <sup>a</sup> ± 2.02
Wheat flour (72%)	56.96 <sup>b</sup> ± 0.72	64.46 <sup>a</sup> ± 3.41	6.44 <sup>f</sup> ± 0.23	3.41 <sup>c</sup> ± 1.19	26.94 <sup>d</sup> ± 0.31	25.73 <sup>e</sup> ± 3.35
<b>Gize 168:</b>						
Whole meal	44.18 <sup>d</sup> ± 0.55	60.03 <sup>ab</sup> ± 2.92	14.21 <sup>b</sup> ± 0.18	5.93 <sup>ab</sup> ± 0.83	24.80 <sup>e</sup> ± 1.79	25.79 <sup>a</sup> ± 1.34
Wheat flour (72%)	57.01 <sup>b</sup> ± 2.92	63.35 <sup>a</sup> ± 3.28	10.05 <sup>a</sup> ± 0.46	4.04 <sup>c</sup> ± 0.64	30.79 <sup>bc</sup> ± 0.80	25.26 <sup>a</sup> ± 1.26
<b>Sohage 3:</b>						
Whole meal	55.95 <sup>b</sup> ± 0.78	59.12 <sup>abc</sup> ± 3.22	11.72 <sup>d</sup> ± 1.01	6.09 <sup>ab</sup> ± 0.60	31.11 <sup>b</sup> ± 0.56	25.28 <sup>a</sup> ± 0.97
Wheat flour (72%)	60.06 <sup>a</sup> ± 1.25	64.96 <sup>a</sup> ± 4.16	12.71 <sup>c</sup> ± 0.38	4.83 <sup>bc</sup> ± 0.61	33.91 <sup>a</sup> ± 0.23	26.01 <sup>a</sup> ± 0.91
<b>Sakha 93:</b>						
Whole meal	49.79 <sup>c</sup> ± 0.34	56.19 <sup>bc</sup> ± 3.49	14.88 <sup>ab</sup> ± 0.30	6.45 <sup>a</sup> ± 0.95	29.35 <sup>c</sup> ± 0.39	23.98 <sup>a</sup> ± 1.42
Wheat flour (72%)	50.98 <sup>c</sup> ± 1.59	63.63 <sup>a</sup> ± 4.49	15.37 <sup>a</sup> ± 0.53	3.92 <sup>c</sup> ± 0.71	32.03 <sup>b</sup> ± 1.61	23.55 <sup>a</sup> ± 1.62
<b>LSD at 0.05</b>	2.33	6.15	0.83	1.32	1.66	2.70

### 1.3 Color quality:

Data presented in Table (3) represent color attributes of pan bread as affected by wheat varieties and its extraction rate (72%). The obtained results indicated that, the lightness color (L) of crust whole-wheat meal affected with wheat varieties, where it maximized in Sohage 3 to reach 55.95 and minimized to 44.18 in Giza 168. In addition, extraction rate (72%) and wheat variety increased lightness to 60.06, 57.01, 56.96 and 50.98 in Sohage, Giza 168, Gemmiza 7 and Sakha93, respectively. The obtained

results showed also that, the color parameter (a & b) of crust pan bread were affected with wheat varieties, but crumb not affected.

As expected, crumb color layer characterized with its higher whiteness than crust layer where lightness (L) score increased, and both redness and yellowness scores decreased. This result could explain as follows: the parameter L, a and b of crust color (wheat flour 72%) in Sohage 3 was 55.95, 11.72 and 31.11, respectively, while the same parameter color in crumb was 60.06, 6.09 and 25.28, respectively. This result could explained as stated by

**Kordonowy & Young (1985), Kim et al.(1997) and Ramy et al. (2002)** that darkness increased in whole-meal pan bread as a result of bran and germ that present in whole-meal.

#### 1.4 Sensory Properties:

Effect of wheat varieties on sensory properties of pan bread evaluated and presented in Table (4). The obtained results showed that, the symmetric shape of whole meal pan bread or its 72% extracted flour not affected significantly in case of using any one of the studied wheat varieties, where it ranged between 3.35 – 4.05. Crust color slightly affected with whole-meal wheat varieties, where its color score maximized in Sakha 93 (7.7) and Sohage 3 (6.7). This result agreed with the obtained color parameter of Hunter (Table 6), where lightness (L) maximized to 55.95 and 49.79 in pan bread crust of Sohage 3 and Sakha 93, respectively. In addition, sensory property and Hunter color parameter not affected significantly with wheat varieties flour (72%) of pan-bread crumb.

In case of comparing crust color pan bread of wheat flour (72%) with whole meal, it could state that, the crust color quality related to maillard reaction that affected by sugars content of flour. Therefore, wheat flour 72% had higher content of total and reducing sugars than whole meal, consequently crust color of wheat flour pan-bread was better than that of whole meal pan bread. This effect observed in all samples of the prepared pan-bread regardless of wheat variety. These findings are in agreement with those obtained by **Lai et al (1989b) and Shouk (1996)**. They proposed that, bran binds relatively large amount of water, changes the appearance and the handling properties of the dough. Therefore, the gluten not properly hydrated and developed at normal absorption levels.

Concerning break and shred, it's clear that pan bread of wheat flour 72% had better break and shred than that of whole meal pan bread. These findings observed in all tested samples regardless of wheat variety.

The same table showed that, crumb-texture, aroma, taste and mouth feel of pan bread wheat flour (72%) were better than whole meal pan bread regardless of wheat variety.

#### 1.5 Freshness:

Data in Table (5) show and compare the effect of whole meal, wheat flour (72%) and wheat varieties on the pan bread freshness that stored at room temperature for 1, 3 and 5 days. The obtained results showed that, pan bread wheat flour 72% had the lowest values of alkaline water retention capacity (AWRC) than that of whole meal pan-bread. Furthermore, all tested samples caused a noticeable

decrease in AWRC values at 1, 3 and 5 days storage. This could relate to whole meal pan-bread that contained more fiber and protein than wheat flour 72% bread, consequently could retain more water. **Mohamed et al. (2006)** reported that bread samples with higher wheat flour (higher amylase) and lower protein content showed higher firmness values. In addition, **Parker and Ring (2001)** stated that, bread staling is caused by amylose and to a lesser extent, amylopectin retrogradation. The high protein content altered the macromolecular content of the bread and thus the overall glass transition of the system. The change in the glass transition directly related to the molecular relaxation of the bread, which in turn affected the staling process as explained.

#### 2 Pasta:

Gemmeiza 7, Gize 168, Sohage 3 and Sakha 93 wheat varieties were evaluated also to produce pasta from its whole meal and flour 72%.

##### 2.1 Chemical composition of pasta:

Data presented in Table (6) showed that, moisture content of whole meal pasta was higher than pasta of wheat flour 72% in all studied varieties. This could attribute to higher content of crude fiber (ranged between 2.82 to 1.65) in pasta of whole meal than pasta of wheat flour 72% (ranged between 0.78-0.5). In addition, whole meal pasta of all wheat varieties characterized with its higher protein, fat and ash contents than wheat flour 72% pasta. In contrast, whole meal pasta was lower in carbohydrate than flour 72% pasta. This result agreed with those found by **Kent-Jones and Amos (1967) and Shouk (1996)** where they stated that, whole meal wheat bread was higher than wheat flour 72% bread in protein, fat, ash and fiber. Furthermore, whole meal pasta of Sohage 3, Giza 168 and Gemmeiza 7 characterized with its higher protein (13.12%), fat (2.59%) and crude fiber (2.82%) contents than other studied varieties, respectively.

##### 2.2 Cooking quality:

Table (7) showed that, cooked pasta of whole meal characterized with its higher increase in weight (ranged between 265-219.6%) and volume (ranged between 305.3-247.4%), while weight and volume of wheat flour 72% cooked pasta ranged between (230-175.8%) and (188-183.3%), respectively. Cooking loss (solids loss into cooking water) of wheat flour 72% pasta characterized with its lower cooking loss (ranged between 8.8-6%), while whole meal pasta ranged between 12.8-8.3%. Regarding to pasta cooking quality of four studied varieties, Sohage 3 ranked first in both whole meal and wheat flour 72% pasta, followed by Giza 168, Sakha 93, and Gemmeiza 7.

Weight increasing of whole meal pasta could due to its high fiber content (table 6), where fiber have a lower bulk density, more surface area, polar groups, and uronic acid groups leading to surrounding water, and increase in its swelling volume (**Lo et al, 1991 and Bao & Chang, 1991**).

The undesirable effect on cooking loss may be due to dilution of gluten, or the interaction between gluten and fiber that allows high starch to leach out from the pasta, consequently, resulting an increase in cooking losses.

**Table 4:** Effect of wheat varieties and extraction rate on sensory properties of pan bread.

L.S.D 5%	Sakha 93		Sohage 3		Gize 168		Gemmeiza 7		Characterisctics
	Flour 72 %	Whole meal	Flour 72 %	Whole meal	Flour 72 %	Whole meal	Flour 72 %	Whole meal	
---	4.05 ± 0.726	4.00 ± 0.707	3.6 ± 0.768	3.50 ± 0.726	3.95 ± 0.79	3.35 ± 5.27	3.50 ± 1.01	3.45 ± 2.90	Symmetrical Shape (5)
1.222	7.8 <sup>abc</sup> ± 1.51	7.7 <sup>abc</sup> ± 0.66	7.2 <sup>bc</sup> ± 1.50	6.70 <sup>c</sup> ± 0.66	8.60 <sup>a</sup> ± 1.0	7.65 <sup>ab</sup> ± 1.43	8.20 <sup>ab</sup> ± 1.48	7.6 <sup>abc</sup> ± 1.5	Crust color (10)
1.48	7.8 <sup>a</sup> ± 0.86	7.33 <sup>a</sup> ± 1.16	7.7 <sup>a</sup> 1.30	6.88 <sup>a</sup> ± 1.17	7.88 <sup>a</sup> ± 0.78	7.11 <sup>a</sup> ± 1.16	8.00 <sup>a</sup> ± 1.73	7.6 <sup>a</sup> ± 1.11	Break & shred (10)
2.268	11.8 <sup>a</sup> ± 2.06	11.50 <sup>a</sup> ± 2.69	11.1 <sup>a</sup> ± 2.00	10.80 <sup>a</sup> ± 2.04	12.10 <sup>a</sup> ± 2.65	11.60 <sup>a</sup> ± 2.65	12.13 <sup>a</sup> ± 2.28	11.3 <sup>a</sup> ± 2.1	Crumb texture (15)
1.302	7.8 <sup>a</sup> ± 1.26	7.40 ± 1.73	7.9 <sup>a</sup> ± 1.41	7.50 <sup>a</sup> ± 0.88	8.20 <sup>a</sup> ± 1.2	7.30 <sup>a</sup> ± 1.36	8.00 <sup>a</sup> ± 1.11	7.2 <sup>a</sup> ± 1.64	Crumb color (10)
2.295	16.5 <sup>ab</sup> ± 2.60	16.33 <sup>ab</sup> ± 2.29	15.66 <sup>ab</sup> ± 2.70	15.22 <sup>b</sup> ± 2.73	16.66 <sup>ab</sup> ± 2.73	16.33 <sup>ab</sup> ± 1.66	17.55 <sup>a</sup> ± 1.66	16.0 <sup>ab</sup> ± 2.39	Aroma (20)
1.735	16.0 <sup>ab</sup> ± 1.66	16.55 <sup>ab</sup> ± 1.24	16.33 <sup>ab</sup> ± 2.29	15.44 <sup>b</sup> ± 1.66	17.33 <sup>a</sup> ± 2.12	16.22 <sup>ab</sup> ± 1.20	17.33 <sup>a</sup> ± 1.80	17.0 <sup>ab</sup> ± 2.12	Taste (20)
1.056	7.8 <sup>a</sup> ± 0.87	7.77 <sup>a</sup> ± 1.09	7.52 <sup>a</sup> ± 53	7.44 <sup>a</sup> ± 93	7.7 <sup>a</sup> ± 0.08	7.33 <sup>a</sup> ± 1.22	8.1 <sup>a</sup> ± 1.52	8.0 <sup>a</sup> ± 1.32	Mouth feel (10)

- Significant at 0.05 probability level.

- There is no significant difference between two means (within the same property) designed by the same letter.

\*NS=Not Significant

**Table 5:** Effect of wheat varieties and extraction rate on Freshness properties of stored pan bread.

Storage period (days)						Sample	
5		3		1			
Freshness	Moisture	Freshness	Moisture	Freshness	Moisture		
<b>Gemmeiza 7 :</b>							
268.90 <sup>a</sup> ± 3.12	27.45 <sup>c</sup> ± 0.57	293.50 <sup>a</sup> ± 3.04	31.27 <sup>c</sup> ± 0.48	303.70 <sup>d</sup> ± 5.98	33.09 <sup>c</sup> ± 0.56	Whole meal	
215.50 <sup>e</sup> ± 5.20	25.60 <sup>e</sup> ± 0.79	231.96 <sup>d</sup> ± 0.83	27.31 <sup>e</sup> ± 0.79	252.47 <sup>e</sup> ± 7.29	29.37 <sup>c</sup> ± 1.35	Extraction: (72%)	
<b>Gize 168:</b>							
262.10 <sup>b</sup> ± 3.05	30.03 <sup>b</sup> ± 0.39	286.20 <sup>a</sup> ± 8.43	32.95 <sup>d</sup> ± 0.43	305.33 <sup>b</sup> ± 8.38	35.50 <sup>b</sup> ± 0.62	Whole meal	
235.46 <sup>d</sup> ± 4.70	26.71 <sup>cd</sup> ± 0.61	255.27 <sup>c</sup> ± 5.20	29.23 <sup>d</sup> ± 1.03	275.00 <sup>d</sup> ± 5.00	31.26 <sup>d</sup> ± 0.92	Extraction: (72%)	
<b>Sohage 3:</b>							

271.27 <sup>a</sup> ± 1.16 238.60 <sup>cd</sup> ± 3.13	31.97 <sup>a</sup> ± 0.79 25.75 <sup>de</sup> ± 0.32	292.90 <sup>a</sup> ± 2.56 258.33 <sup>bc</sup> ± 2.88	34.99 <sup>a</sup> ± 0.54 28.18 <sup>de</sup> ± 0.49	315.37 <sup>a</sup> ± 4.95 278.33 <sup>cd</sup> ± 2.88	37.02 <sup>a</sup> ± 0.58 30.15 <sup>de</sup> ± 0.45	Whole meal Extraction: (72%)
<b>Sakha 93:</b>						
244.70 <sup>c</sup> ± 4.09 212.27 <sup>e</sup> ± 2.97	29.84 <sup>b</sup> ± 0.67 26.36 <sup>de</sup> ± 0.66	265.40 <sup>b</sup> ± 5.00 232.13 <sup>d</sup> ± 2.92	32.89 <sup>b</sup> ± 0.56 29.09 <sup>d</sup> ± 0.81	285.60 <sup>c</sup> ± 4.76 251.83 <sup>e</sup> ± 2.75	35.29 <sup>d</sup> ± 0.48 31.23 <sup>d</sup> ± 0.42	Whole meal Extraction: (72%)
6.27	1.07	7.66	1.16	9.63	1.27	<b>LSD at 5%</b>

**Table 6:** Effect of wheat varieties and extraction rate on chemical composition of pasta.

Total Carb.	Crude fiber	Ash	Fat	Protein	Moisture	Sample
<b>Gemmeiza 7 :</b>						
81.42 <sup>ab</sup> ± 0.06	2.82 <sup>a</sup> ± 0.03	2.52 <sup>a</sup> ± 0.03	2.19 <sup>d</sup> ± 0.10	11.05 <sup>de</sup> ± 0.06	8.07 <sup>a</sup> ± 0.03	Whole meal
86.3 <sup>a</sup> ± 0.9	0.5 <sup>d</sup> ± 0.05	0.99 <sup>e</sup> ± 0.03	1.52 <sup>g</sup> ± 0.03	10.69 <sup>f</sup> ± 0.45	7.71 <sup>c</sup> ± 0.11	Wheat flour 72%
<b>Gize 168:</b>						
82.12 <sup>ab</sup> ± 0.03	2.17 <sup>b</sup> ± 0.17	2.03 <sup>b</sup> ± 0.03	2.59 <sup>a</sup> ± 0.04	11.09 <sup>d</sup> ± 0.03	7.82 <sup>b</sup> ± 0.03	Whole meal
85.73 <sup>a</sup> ± 0.04	0.78 <sup>d</sup> ± 0.05	0.98 <sup>e</sup> ± 0.03	1.64 <sup>f</sup> ± 0.04	10.87 <sup>ef</sup> ± 0.03	7.23 <sup>e</sup> ± 0.06	Wheat flour 72%
<b>Sohage 3:</b>						
80.90 <sup>ab</sup> ± 0.26	1.65 <sup>d</sup> ± 0.06	1.91 <sup>c</sup> ± 0.10	2.42 <sup>c</sup> ± 0.05	13.12 <sup>a</sup> ± 0.11	8.03 <sup>a</sup> ± 0.02	Whole meal
84.58 <sup>a</sup> ± 0.12	0.64 <sup>d</sup> ± 0.05	0.97 <sup>e</sup> ± 0.08	1.66 <sup>f</sup> ± 0.03	12.05 <sup>b</sup> ± 0.06	7.52 <sup>d</sup> ± 0.03	Wheat flour 72%
<b>Sakha 93:</b>						
81.88 <sup>ab</sup> ± 0.46	2.45 <sup>b</sup> ± 0.40	1.70 <sup>d</sup> ± 0.02	2.52 <sup>b</sup> ± 0.05	11.45 <sup>c</sup> ± 0.29	7.70 <sup>c</sup> ± 0.09	Whole meal
85.87 <sup>a</sup> ± 0.19	0.73 <sup>d</sup> ± 0.12	0.84 <sup>f</sup> ± 0.09	1.74 <sup>e</sup> ± 0.04	10.82 <sup>f</sup> ± 0.06	7.18 <sup>e</sup> ± 0.03	Wheat flour 72%
18.79	0.29	0.10	0.21	0.6	0.9	<b>LSD at .05</b>

Total Carb. = Total carbohydrate.

**Table 7:** Effect of wheat varieties and extraction rate on cooking quality of pasta.

Cooking loss (%)	Volume increase (%)	Weight increase (%)	Sample
<b>Gemmeiza 7:</b>			
11.9 <sup>b</sup> ±0.09	247.4 <sup>d</sup> ±1.72	219.6 <sup>c</sup> ±1.65	Whole meal
8.8 <sup>c</sup> ±0.25	184.4 <sup>e</sup> ±1.25	177.2 <sup>d</sup> ±1.1	Wheat flour 72%
<b>Gize 168:</b>			
11.63 <sup>c</sup> ±0.15	285.0 <sup>b</sup> ±5.00	247.6 <sup>b</sup> ±2.51	Whole meal
8.6 <sup>c</sup> ±0.09	183.3 <sup>e</sup> ±1.71	175.8 <sup>b</sup> ±1.40	Wheat flour 72%
<b>Sohage 3:</b>			
8.3 <sup>d</sup> ±0.25	305.3 <sup>a</sup> ±4.50	265 <sup>a</sup> ±5.0	Whole meal
6.0 <sup>e</sup> ±0.21	265.7 <sup>c</sup> ±4.51	230.0 <sup>c</sup> ±5.0	Wheat flour 72%
<b>Sakha 93:</b>			
12.8 <sup>a</sup> ±0.25	263.8 <sup>c</sup> ±8.14	248.3 <sup>b</sup> ±7.63	Whole meal
8.5 <sup>c</sup> ±0.25	188.0 <sup>e</sup> ±2.00	178.0 <sup>de</sup> ±1.0	Wheat flour 72%
0.30	7.33	16.51	LSD at .05

\*Average of three determinations

### 2.3 Color quality:

Data presented in Table (8) showed the effect of wheat variety and extraction rate (whole meal and flour 72%) on the color quality of pasta. Regarding to pasta color parameter of four wheat varieties, Sakha 93 ranked first in lightness (L) in its flour 72% and whole meal, where L reached to 63.37 and 54.39, respectively. While, L value of pasta of other variety ranged between 59.05-57.76 and 52.12-49.90 in flour 72% and whole meal, respectively. Pasta of whole meal Gemmeiza 7 characterized with higher redness (a = 5.46) and yellowness (b = 20.62). In comparing pasta color of whole meal with flour 72%, pasta whole meal of studied varieties were darker, where its color parameter was lower in lightness (L) and higher in redness (a) and yellowness (b). This result could due to pasta of whole meal containing higher level of fiber if compared with pasta of wheat flour 72%.

**Table (8):** Effect of wheat varieties and extraction rate on color quality of pasta.

Yellowness "b"	Redness "a"	Lightness "L"	Sample
<b>Gemmeiza 7</b>			
18.85 <sup>c</sup> ±1.21	3.03 <sup>d</sup> ±0.27	59.05 <sup>b</sup> ±2.64	Wheat flour 72%
20.62 <sup>a</sup> ±0.17	5.46 <sup>a</sup> ±0.05	49.98 <sup>d</sup> ±0.71	Whole meal
<b>Gize 168:</b>			
17.17 <sup>ab</sup> ±0.25	3.60 <sup>c</sup> ±0.21	58.86 <sup>b</sup> ±1.03	Wheat flour 72%
20.1 <sup>ab</sup> ±0.52	5.32 <sup>a</sup> ±0.24	49.90 <sup>d</sup> ±1.87	Whole meal
<b>Sohage 3</b>			
18.49 <sup>c</sup> ±0.36	3.02 <sup>d</sup> ±0.12	57.76 <sup>b</sup> ±2.64	Wheat flour 72%
19.49 <sup>bc</sup> ±0.67	4.19 <sup>b</sup> ±0.30	52.12 <sup>cd</sup> ±0.98	Whole meal
<b>Sakha 93</b>			
14.52 <sup>e</sup> ±0.74	2.17 <sup>c</sup> ±0.02	63.37 <sup>a</sup> ±2.58	Wheat flour 72%
16.64 <sup>d</sup> ±0.45	3.83 <sup>c</sup> ±0.18	54.39 <sup>b</sup> ±1.69	Whole meal
1.08	0.34	2.97	<b>LSD at .05</b>

### 2.4 Sensory evaluation:

Pasta of studied wheat varieties (whole meal and wheat flour 72%) evaluated sensorially and presented in Table (9). The obtained results showed that, pasta of wheat flour 72% increased slightly in appearance and color if compared with whole meal pasta of the same variety. The obtained color score agreed with the obtained color parameter of Hunter (Table 8). In addition, **Kordonowy & Young (1985)** reported that flavor, texture and color of no bran spaghetti were rated significantly higher than those of other samples containing bran. Table (9) indicated also that, there were no significant difference between wheat flour 72% and whole meal in flavor, tenderness and stickiness.

**Table 9:** Effect of wheat varieties and extraction rate on sensory properties of pasta.

Stickiness (10)	Tenderness (10)	Flavor (10)	Color (10)	Appearance (10)	Sample
<b>Gemmeiza 7:</b>					
7.2 <sup>ab</sup> ±0.78	7.2±1.39	7.4±1.6	7.2 <sup>ab</sup> ±1.2	7.8 <sup>a</sup> ±1.03	Wheat flour 72%
7.7 <sup>a</sup> ±0.91	7.0±1.33	7.4±1.4	6.5 <sup>ab</sup> ±1.4	6.4 <sup>b</sup> ±1.64	Whole meal
<b>Gize 168:</b>					
6.8 <sup>ab</sup> ±1.13	6.5±1.27	6.5±1.7	7.3 <sup>a</sup> ±1.4	6.8 <sup>ab</sup> ±1.62	Wheat flour 72%
7.0 <sup>ab</sup> ±1.33	6.7±1.34	6.6±1.3	6.7 <sup>ab</sup> ±1.3	6.9 <sup>ab</sup> ±1.85	Whole meal
<b>Sohage 3:</b>					
6.8 <sup>ab</sup> ±0.92	6.5±1.35	6.7±1.2	6.0 <sup>b</sup> ±1.8	6.4 <sup>ab</sup> ±1.43	Wheat flour 72%
6.3 <sup>b</sup> ±1.15	7.3±1.30	6.5±1.1	6.1 <sup>ab</sup> ±1.5	5.8 <sup>b</sup> ±1.37	Whole meal
<b>Sakha 93:</b>					
6.4 <sup>b</sup> ±1.07	7.3±1.05	6.7±1.2	6.7 <sup>ab</sup> ±1.5	6.7 <sup>ab</sup> ±1.34	Wheat flour 72%
6.7 <sup>b</sup> ±1.33	7.4±1.05	7.1±1.3	6.4 <sup>ab</sup> ±1.1	6.3 <sup>b</sup> ±1.25	Whole meal
1.00	---	---	1.29	1.34	<b>LSD at 0.05</b>

### 3 Biscuit

#### 3.1 Chemical composition of biscuit samples:

The effect of using some Egyptian new wheat variety (whole meal, flour 72%) on gross chemical composition of biscuit studied. Table (10) showed that, whole meal biscuit characterized with its higher content of protein, fat, ash and crude fiber than wheat flour 72%. While wheat flour 72% was higher than whole meal in total carbohydrate (TC). This result agreed with those found by **Kent-Jones and Amos (1967)** and **Shouk (1996)**, they stated that, whole meal wheat bread was higher than wheat flour 72% bread in protein, fat, ash and fiber. In addition, whole meal biscuit of Sohage 3 characterized with its higher protein (12.13%), fat (31.0%) and ash (2.51%) contents; and lowest carbohydrate content (52.18%), while whole meal biscuit of Sakha 93 characterized with its higher moisture (4.5%) and crude fiber contents (3.0%).

**Table 10:** Effect of wheat varieties and extraction rate on chemical composition of biscuit.

Total Carb.	Crude fiber	Ash	Fat	Protein	Moisture	Sample
<b>Gemmeiza 7:</b>						
58.61 <sup>a</sup> ± 0.23	1.10 <sup>e</sup> ± 0.09	1.65 <sup>d</sup> ± 0.05	29.08 <sup>f</sup> ± 0.10	9.50 <sup>f</sup> ± 0.06	3.60 <sup>ef</sup> ± .005	Wheat flour 72%
54.91 <sup>d</sup> ± 0.62	2.28 <sup>b</sup> ± 0.26	2.12 <sup>b</sup> ± 0.10	30.35c ± 0.15	10.34 <sup>d</sup> ± 0.14	4.20 <sup>c</sup> ± 0.02	Whole meal
<b>Gize 168:</b>						
57.65 <sup>b</sup> ± 0.09	1.48 <sup>de</sup> ± 0.08	1.83 <sup>c</sup> ± 0.05	29.29 <sup>e</sup> ± 0.04	9.75 <sup>e</sup> ± 0.05	3.64 <sup>de</sup> ± 0.04	Wheat flour 72%
54.18 <sup>c</sup> ± 0.11	2.24 <sup>bc</sup> ± 0.06	2.40 <sup>a</sup> ± 0.10	30.51b ± 0.04	10.76 <sup>c</sup> ± 0.21	4.31 <sup>b</sup> ± 0.08	Whole meal
<b>Sohage 3:</b>						
56.37 <sup>c</sup> ± 0.20	1.85 <sup>cd</sup> ± 0.61	1.75 <sup>cd</sup> ± 0.03	29.36 <sup>e</sup> ± 0.04	11.34 <sup>b</sup> ± 0.14	3.72 <sup>d</sup> ± 0.03	Wheat flour 72%
52.18 <sup>f</sup> ± 0.15	1.22 <sup>e</sup> ± 0.61	2.51 <sup>a</sup> ± 0.1e	31.00 <sup>a</sup> ± 0.06	12.13 <sup>a</sup> ± 0.08	4.55 <sup>a</sup> ± 0.05	Whole meal
<b>Sakha 93:</b>						
57.89 <sup>b</sup> ± 0.29	1.66 <sup>d</sup> ± 0.14	1.42 <sup>e</sup> ± 0.09	29.16 <sup>f</sup> ± 0.05	9.86 <sup>e</sup> ± 0.05	3.54 <sup>f</sup> ± 0.03	Wheat flour 72%
53.71 <sup>e</sup> ± 0.16	3.00 <sup>a</sup> ± 0.15	2.21 <sup>b</sup> ± 0.04	30.22 <sup>d</sup> ± 0.03	10.86 <sup>c</sup> ± 0.05	4.55 <sup>a</sup> ± 0.05	Whole meal
0.485	0.432	0.133	0.128	0.196	0.078	<b>LSD at 0.05</b>

Total Carb. = Total carbohydrate.

#### 2.3.2 Baking quality:

Table (11) showed that, whole-meal biscuits were higher in weight and volume than biscuit of wheat flour 72%, where biscuit weight of whole meal and wheat flour 72% varieties ranged between (87.47-81.49g) and (81.51-74.38g), respectively; while biscuit volume of whole meal and wheat flour 72% varieties ranged between (160.0-135.0cc) and (143.30-123.3cc) respectively. Whole meal biscuits were also higher in height than wheat flour 72% biscuits, where biscuits height of whole meal varieties ranged between (1.15-0.95cm), while it decreased to be ranged between (1.01-0.87cm) in wheat flour 72% varieties. These results could due to increasing level of crude fiber in biscuit of whole meal (Table 10). In contrast, biscuits diameter and spread ratio of whole meal varieties were lower than biscuit of wheat flour 72% varieties, where diameter and spread ratio of biscuit of whole meal varieties ranged between (7.28-6.78cm) and (6.86-6.38diam/ht), while they increased to (7.62-7.13cm) and (8.73-7.08diam/ht) in wheat flour 72% varieties, respectively.

It could conclude from Table (11) that, the higher baking quality of whole meal biscuits or wheat flour 72% was obtained from Sakha 93 variety.

**Table 11:** Effect of wheat varieties and extraction rate on biscuits baking quality.

Spread ratio (diam./ht.)	Height (cm)	Diameter (cm)	Specific volume (cc/g)	Volume (cc)	Weight (g)	Samples
<b>Gemmeiza 7:</b>						
8.73 <sup>a</sup> ± 0.02	0.87 <sup>d</sup> ± 0.01	7.62 <sup>a</sup> ± 0.16	1.63 <sup>b</sup> ± 0.03	135.0 <sup>c</sup> ± 5.00	81.51 <sup>bc</sup> ± 2.19	Wheat flour (72%)
6.86 <sup>e</sup> ± 0.08	0.95 <sup>bc</sup> ± 0.09	6.92 <sup>e</sup> ± 0.34	1.54 <sup>d</sup> ± 0.02	135.0 <sup>c</sup> ± 5.00	87.47 <sup>a</sup> ± 3.40	Whole meal
<b>Giza 168:</b>						
8.49 <sup>b</sup> ± 0.13	0.93 <sup>cd</sup> ± 0.09	7.6 <sup>ab</sup> ± 0.18	1.81 <sup>a</sup> ± 0.02	123.3 <sup>d</sup> ± 5.77	74.38 <sup>d</sup> ± 1.73	Wheat flour (72%)
6.73 <sup>f</sup> ± 0.02	1.01 <sup>b</sup> ± 0.04	6.78 <sup>e</sup> ± 0.09	1.82 <sup>a</sup> ± 0.02	138.5 <sup>c</sup> ± 2.89	83.95 <sup>abc</sup> ± 1.59	Whole meal
<b>Sohage 3:</b>						
7.55 <sup>c</sup> ± 0.04	0.99 <sup>bc</sup> ± 0.08	7.46 <sup>b</sup> ± 0.13	1.52 <sup>c</sup> ± 0.02	143.00 <sup>bc</sup> ± 5.77	79.28 <sup>c</sup> ± 2.67	Wheat flour (72%)
6.63 <sup>g</sup> ± 0.09	1.1 <sup>a</sup> ± 0.05	7.1 <sup>d</sup> ± 0.12	1.62 <sup>b</sup> ± 0.03	152.33 <sup>ab</sup> ± 2.51	81.49 <sup>ab</sup> ± 1.61	Whole meal
<b>Sakha 93:</b>						
7.08 <sup>d</sup> ± 0.07	1.01 <sup>b</sup> ± 0.09	7.13 <sup>cd</sup> ± 0.12	1.83 <sup>a</sup> ± 0.03	143.30 <sup>bc</sup> ± 5.70	79.28 <sup>c</sup> ± 2.81	Wheat flour (72%)
6.38 <sup>h</sup> ± 0.05	1.15 <sup>b</sup> ± 0.09	7.28 <sup>e</sup> ± 0.08	1.85 <sup>a</sup> ± 0.03	160.00 <sup>a</sup> ± 10.00	85.33 <sup>ab</sup> ± 0.84	Whole meal
0.07	0.08	0.16	0.07	6.96	4.85	<b>LSD at .05</b>

**2.3.3 Color quality:**

Biscuits color quality of whole meal and flour 72% for Gemmeiza 7, Giza 168, Sohage 3 and Sakha 93 varieties studied and presented in Table (12). As expected biscuits face or back of flour 72% varieties characterized with its higher lightness (L) than whole meal varieties. Where, (L) value of biscuits face and back of flour 72% varieties ranged between (75.16-68.58) and (63.46-45.12), respectively, while its whole meal ranged between (72.69-64.28) and (51.56-41.28), respectively. In addition, biscuits face or back of flour 72% varieties characterized with its higher redness (a) than whole meal varieties. In contrast, yellowness (b) of biscuits face or back of flour 72% varieties decreased if compared with biscuit of whole meal varieties. From the obtained result of Hunter color parameter (Table 12), it was found that biscuit of whole meal varieties was slightly darker than biscuit of wheat flour 72% varieties, where whole meal contain higher level of dietary fiber. This result agreed with those found by **Kim et al (1997)** who stated that adding dietary fiber to bread dough increase darkness.

**Table 12:** Effect of wheat varieties and extraction rate on biscuits color quality.

Yellowness "b"		Redness "a"		Lightness "L"		Sample
Back	Face	Back	Face	Back	Face	
<b>Gemmeiza 7:</b>						
34.71 <sup>abc</sup> ± 0.83	27.79 <sup>b</sup> ± 0.50	15.61 <sup>c</sup> ± 0.29	6.44 <sup>a</sup> ± 0.52	63.46 <sup>a</sup> ± 1.20	68.58 <sup>de</sup> ± 0.51	Wheat flour (72%)
36.62 <sup>a</sup> ± 0.57	29.72 <sup>a</sup> ± 0.75	12.08 <sup>d</sup> ± 1.0	3.89 <sup>c</sup> ± 0.53	51.56 <sup>b</sup> ± 1.24	64.28 <sup>f</sup> ± 0.46	Whole meal
<b>Giza 168:</b>						
32.1 <sup>cd</sup> ± 3.02	29.67 <sup>a</sup> ± 0.68	17.34 <sup>ab</sup> ± 0.47	6.75 <sup>a</sup> ± 0.50	45.12 <sup>bcd</sup> ± 6.22	68.72 <sup>d</sup> ± 0.59	Wheat flour (72%)
33.80 <sup>abcd</sup> ± 3.99	29.27 <sup>a</sup> ± 0.67	16.7 <sup>b</sup> ± 0.43	6.7 <sup>a</sup> ± 0.48	41.28 <sup>d</sup> ± 3.13	68.09 <sup>de</sup> ± 0.37	Whole meal
<b>Sohage 3:</b>						
36.25 <sup>ab</sup>	29.27 <sup>a</sup>	17.92 <sup>a</sup>	3.59 <sup>c</sup>	48.81 <sup>bc</sup>	75.16 <sup>a</sup>	Wheat flour

$\pm 3.36$	$\pm 0.66$	$\pm 0.60$	$\pm 0.34$	$\pm 7.01$	$\pm 0.46$	(72%)
35.46 <sup>abc</sup> $\pm 1.25$	30.11 <sup>a</sup> $\pm 1.14$	17.50 <sup>ab</sup> $\pm 0.26$	5.34 <sup>b</sup> $\pm 0.06$	45.64 <sup>bcd</sup> $\pm 3.71$	72.69 <sup>b</sup> $\pm 0.73$	Whole meal
<b>Sakha 93:</b>						
32.66 <sup>bcd</sup> $\pm 0.77$	29.43 <sup>a</sup> $\pm 0.9$	17.71 <sup>a</sup> $\pm 0.12$	7.26 <sup>a</sup> $\pm 0.89$	46.12 <sup>b</sup> $\pm 1.52$	70.37 <sup>c</sup> $\pm 1.31$	Wheat flour (72%)
30.25 <sup>d</sup> $\pm 0.85$	26.28 <sup>c</sup> $\pm 0.57$	16.72 <sup>b</sup> $\pm 0.06$	6.73 <sup>a</sup> $\pm 0.21$	42.40 <sup>cd</sup> $\pm 1.75$	67.55 <sup>e</sup> $\pm 0.29$	Whole meal
2.70	1.66	0.81	0.86	6.69	1.15	<b>LSD at 0.05</b>

### 3.4 Sensory properties:

Biscuits quality of whole meal and flour 72% of studied wheat varieties evaluated sensorially and presented in Table (13). Biscuit color of wheat flour 72% varieties ranged between (8.17-7.7), while in case of whole meal varieties showed non-significant decrease (ranged between 8.16-6.66). This result agreed with the obtained Hunter color parameter (Table 12), where biscuit of whole meal varieties was slightly darker than biscuit of wheat flour 72% varieties. In addition, biscuits flavor, taste, texture, appearance and overall acceptability of wheat flour 72% showed non-significant increase if compared with whole meal of the same variety.

**Table 13:** Effect of wheat varieties and extraction rate on sensory properties of biscuits.

Overall acceptability (10)	Appearance (10)	Texture (10)	Taste (10)	Flavor (10)	Color (10)	Samples
<b>Gemmeiza 7:</b>						
5.8 <sup>a</sup> $\pm 1.08$	8.25 <sup>a</sup> $\pm 1.14$	8.25 <sup>a</sup> $\pm 1.3$	8.5 <sup>a</sup> $\pm 1.17$	8.75 <sup>a</sup> $\pm 1.08$	8.17 <sup>a</sup> $\pm 1.64$	Wheat flour (72%)
7.83 <sup>ab</sup> $\pm 1.61$	7.58 <sup>ab</sup> $\pm 1.73$	7.66 <sup>ab</sup> $\pm 1.1$	7.5 <sup>ab</sup> $\pm 1.83$	7.75 <sup>ab</sup> $\pm 1.35$	7.9 <sup>a</sup> $\pm 1.08$	Whole meal
<b>Giza 168:</b>						
7.58 <sup>ab</sup> $\pm 1.86$	7.58 <sup>ab</sup> $\pm 1.73$	7.5 <sup>ab</sup> $\pm 1.83$	7.3 <sup>ab</sup> $\pm 2.03$	7.75 <sup>ab</sup> $\pm 1.46$	7.7 <sup>a</sup> $\pm 1.16$	Wheat flour (72%)
7.42 <sup>ab</sup> $\pm 1.16$	7.75 <sup>ab</sup> $\pm 0.96$	7.2 <sup>ab</sup> $\pm 1.4$	7.2 <sup>b</sup> $\pm 1.69$	7.08 <sup>bc</sup> $\pm 1.24$	8.16 <sup>a</sup> $\pm 0.75$	Whole meal
<b>Sohage 3:</b>						
7.58 <sup>bc</sup> $\pm 1.62$	7.92 <sup>ab</sup> $\pm 0.9$	7.7 <sup>ab</sup> $\pm 1.32$	7.6 <sup>ab</sup> $\pm 1.55$	7.5 <sup>bc</sup> $\pm 1.0$	8.0 <sup>a</sup> $\pm 1.21$	Wheat flour (72%)
7.17 <sup>bc</sup> $\pm .40$	7.58 <sup>ab</sup> $\pm 1.16$	7.6 <sup>ab</sup> $\pm 1.08$	7.1 <sup>b</sup> $\pm 1.24$	6.83 <sup>bc</sup> $\pm 0.86$	7.92 <sup>a</sup> $\pm 0.99$	Whole meal
<b>Sakha 93:</b>						
7.33 <sup>bc</sup> $\pm 0.51$	7.08 <sup>bc</sup> $\pm 0.79$	7.0 <sup>b</sup> $\pm 1.52$	7.5 <sup>ab</sup> $\pm 0.80$	7.08 <sup>bc</sup> $\pm 1.38$	7.7 <sup>a</sup> $\pm 0.98$	Wheat flour (72%)
6.29 <sup>c</sup> $\pm 1.32$	6.25 <sup>c</sup> $\pm 0.62$	6.6 <sup>b</sup> $\pm 1.24$	6.5 <sup>b</sup> $\pm 2.02$	6.58 <sup>c</sup> $\pm 1.24$	6.66 <sup>b</sup> $\pm 0.96$	Whole meal
1.12	0.88	1.15	1.33	1.01	0.99	<b>LSD at 0.05</b>

### Correspondence author:

Dr. Mohie M. Kamil

email: ResearchTeamMMK@yahoo.com

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# Circulating Endothelial Cells And Cardiovascular Risk In Systemic Lupus Erythematosus

Elham Kassem<sup>1</sup>, Mervat El-Sergany<sup>2</sup>, Hanan El-Saadany<sup>3</sup> Abeer Shahba<sup>4</sup> and Wesam Salah<sup>5</sup>

Departments of Rheumatology & Rehabilitation<sup>1,2,3</sup>, Internal Medicine<sup>4</sup> and Clinical Pathology<sup>5</sup>

Faculty of Medicine, Tanta University, Egypt

[Elahm77@hotmail.com](mailto:Elahm77@hotmail.com)

**ABSTRACT:** Premature atherosclerosis seen in systemic lupus erythematosus (SLE) patients is not explained by traditional risk factors. Circulating endothelial cells (CECs) have been shown to be a surrogate marker of endothelial dysfunction. The aim of this study was to assess the number of CECs in SLE patients and to determine any potential correlation between CEC count and endothelial function (FMD%), disease activity, organ involvement and therapy used. Also, to investigate VCAM-1 and ICAM-1 levels as markers of vascular inflammation and injury. This study was performed on 30 premenopausal female SLE patients and 20 age and sex matched healthy controls (HC). Patients were subjected to full history taking, complete clinical examination and assessment of disease activity using (SLAM) score. For both patients and controls, endothelial function (FMD%), laboratory estimation of CEC count, and serum level of VCAM-1 and ICAM-1 were performed. CEC count was significantly elevated in SLE patients comparing to HC ( $P<0.001$ ). CEC count was positively correlated with SLAM score, while negatively correlated with FMD%. Serum levels of VCAM-1 and ICAM-1 were significantly increased in SLE patients than controls. Moreover, VCAM-1 correlated significantly with disease activity and CEC count while ICAM-1 did not correlate with any of them. There was significant correlation between CEC count and skin vasculitis, renal involvement and anti-malarial medications. In conclusion, increased number of CEC may be a biomarker of disease activity and disseminated vasculopathy occurring in the course of SLE and may represent one of the first specific cellular markers to provide a direct link with the pathophysiology of cardiovascular disease (CVD). VCAM-1 is considered a marker of activation of endothelial cells. Taking together, this may predict patients at increased risk of CVD complications, lupus nephritis or vasculitic skin affection.

[Elham Kassem, Mervat El-Sergany, Hanan El-Saadany Abeer Shahba and Wesam Salah. Circulating Endothelial Cells And Cardiovascular Risk In Systemic Lupus Erythematosus. Journal of American Science 2010;6(11):700-707]. (ISSN: 1545-1003).

**Key words:** Circulating endothelial cells (CEC), SLE, adhesion molecules

## 1-INTRODUCTION:

It has been established that patients with systemic lupus erythematosus (SLE) are at increased risk of cardiovascular (CV) mortality and morbidity (Roman et al., 2003). However, the premature atherosclerosis and endothelial dysfunction in SLE are not solely attributed to traditional risk factors (Edile et al., 2001).

The risk of developing coronary heart disease remains increased 8-10 fold even after adjustment of risk factors identified in the Framingham Heart Study (Edile et al., 2001). This prompted us to investigate additional factors that might be related to disease process itself.

As we all know, the primary pathological findings in SLE patients are those of inflammation, immune complex deposition, altered angiogenesis and vacuities (Robak et al., 2009). Furthermore, the vascular endothelium in general is "primed" for injury by activated leucocytes (Belmont et al., 1997).

Circulating endothelial cells (CEC) are thought to be mature cells that have detached from the intimal

mono layer in response to endothelial injury (Boos et al., 2006). Several possibilities can be considered for the mechanism responsible for endothelial detachment. It might be due to apoptosis, mechanical dislodgment of cells, proteolysis of subendothelial matrix proteins, or a consequence of complement dependent injury (Hunting et al., 2005).

Endothelial cells (EC) are potential participant in the inflammatory processes that contribute to tissue damage. Furthermore, the activated phenotype of circulating endothelial cells suggests that they may be capable of vascular injury by producing prothrombotic mediators (Robak et al., 2009).

Although endothelial damage due to deposition of immune complexes is considered to be one of the main pathogenetic traits of SLE, other alternative mechanisms should also be taken into account when pondering the etiology of SLE microangiopathy - first and foremost inflammatory immune lesions of endothelial cells (Kluz et al., 2009).

Endothelial damage and dysfunction as well as increased leukocyte migration to loci of inflammation, mediated by adhesion molecules, are

believed to be key factors in the induction of vasculitis (Guillevin et al., 2007). Growing evidence, including increase in the expression of vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1) on the endothelial cell surface, speaks for endothelial activation in SLE (Robak et al., 2007 and Constans et al., 2003).

The aim of this study was to assess the number of CECs in SLE patients and to determine any potential correlation between CEC count and endothelial function (FMD%), disease activity, organ involvement and therapy used. Also, to investigate VCAM-1 and ICAM-1 levels as markers of vascular inflammation and injury.

## 2-SUBJECTS AND METHODS

This study was conducted on 30 premenopausal SLE female patients and 20 age and sex-matched healthy controls (HC). They were recruited from the Internal Medicine and Rheumatology & Rehabilitation Departments, Tanta University Hospital, Egypt. All patients met the American College of Rheumatology (ACR) criteria for SLE (Tan et al., 1982). Assessment of disease activity was achieved by use of (SLAM) score (Liang et al., 1989). Clinical assessment included physical examination and laboratory investigations; also a complete medication history was obtained.

A written consent prior to participation in the study was taken from all patients and controls.

### Exclusion criteria:

- 1- Coronary artery disease, myocardial infarction and cardiac insufficiency which affect CEC count (Robak et al., 2009).
- 2- Patients received hemoperitoneal dialysis or had undergone kidney transplantation(de Groot et al., 2005)
- 3- Patients with clinical signs of infection or neoplastic disease(3 Robak et al., 2009 )
- 4- Diabetic patients(Deng et al., 2009)
- 5- Patients with evidence with other disease known to cause endothelial dysfunction.
- 6- Patients received other medications for at least 4 weeks before blood donation (Robak et al., 2009).

### Assessment of endothelial function by flow mediated dilatation (FMD %):

Endothelial function was assessed with high-resolution B-mode Doppler (ATL HDI 5000 with a 7.4 – MHz linear –array transducer) examination of the brachial artery using the protocol described by Rajagopalan et al. (2002). Briefly, the test was reformed in the morning in quiet, low light room; subjects had fasted and not smoked for at least the

preceding 12hs. The brachial artery was scanned 5–15 cm above cubital fossa. Resting diameter was measured. Then blood pressure cuff inflated to 300 mmHg around forearm and further scan was done 1 minute during occlusion then after occlusion (cuff release) by 1 minute.

FMD was calculated as follows:

$$\frac{[(\text{post deflation diameter} - \text{resting diameter}) / \text{resting diameter}]}{100}$$

### Sampling:

Venous blood samples (10 ml) were taken from each patient and controls and separated into two tubes: one tube (5ml) was collected into ethylene diaminetetra acetic acid (EDTA) and immediately transferred into citrate-theophylline-adenosine-dipyridamole (CTAD) anticoagulant, which recently has been shown to maximize antigen stabilization on leucocytes. Anticoagulated blood samples were kept at 4°C and analyzed by flow cytometry within 4 hr of venesection (Macey et al., 2003).

The remaining 5 ml were left to clot at room temperature for 30 min in the second tube then centrifuged at 1500 rpm for 15 min and serum was separated stored at -80°C till time of assay of serum ICAM-1, VCAM-1, anti-phospholipid antibodies, C3, C4, ANA, anti-dsDNA, urea, creatinine, lipid profile and blood glucose

24 hours urine was collected from patients for creatinine clearance, urinary protein and complete urinalysis.

### Study measurements:

- Complete blood count using Advia 60 Cell Counter (Bayer),
- Erythrocyte sedimentation rate by Westergren method.
- Serum ANA was assessed by indirect immunofluorescence using Hep-2 cells and anti-double-stranded DNA by indirect immunofluorescence on Crithidia luciliae (Sanofi Diagnostics Pasteur Inc, Minnesota, USA) (Fritzler 1992).
- Complement 3 (C3) and complement 4 (C4) were assayed by nephelometry (Behringwerke, Marburg, Germany)(Virella 1980). Antiphospholipid (APL) antibodies were measured by ELISA technique (HM007, Technoclone Diagnostics Ltd., UK).
- Quantitative sandwich ELISA technique was used to measure serum concentration of ICAM-1 (BMS201CE human sICAM-1, Bender Biosystem, Vienna, Austria, Europe) and VCAM-1 (BMS232CE human sVCAM-1, Bender Biosystem, Vienna, Austria, Europe). The standard range of sICAM-1

was 6.25 - 100 ng/ml and sVCAM-1 was 3.2 - 100 ng/ml (Robak et al., 2007 ).

- Serum creatinine, lipid profile, blood urea and glucose concentration were measured by using standard laboratory techniques on Synchron CX7 autoanalyzer (Beckman Instruments, CA, USA).
- Complete microscopic urine analysis for WBCs, RBCs, and casts, 24-hour urinary protein excretion (UPE) by the turbidimetric method using TP Kit supplied by Stanbio (Stanbio Laboratory Inc., San Antonio, USA) and creatinine clearance were measured for renal assessment.

#### **Immunophenotyping of CECs Flow cytometry (Goon et al., 2006):**

Freshly isolated peripheral blood mononuclear cells (PBMCs) were washed and separated from blood of patients and healthy control using 1X FACS lysis solution (BD) for erythrocytes lysis then PBMCs were resuspended in phosphate buffered saline (PBS, pH 7.4) containing 20 uL of the appropriate antibody and cells were double stained with mouse anti-human fluorescein isothiocyanate (FITC) conjugated CD45 antibody and mouse anti-human phycoerythrin conjugated CD 146 antibody (BD Biosciences) to identify CD45- and CD146+ respectively. The isotype control was used to determine nonspecific binding of the lymphocyte subset-specific antibodies and to set the cut-off between fluorescence-negative and fluorescence-positive staining. Stained cells were washed three times with 1% bovine serum albumin BSA-PBS, pH 7.2, and then 7AAD was added to stain dead cells. The cells were analyzed within 15 minutes after addition of 7AAD using a fluorescence-activated cell scanner and Cell Quest software [FACS Caliber, Becton-Dickinson (BD)]. Cells were plotted according to forward scatter (FSC) and side scatter profiles (SSC) and a region was drawn around the small, live cell population containing the lymphocyte. The cell population data obtained from the quadrant statistics (2- color staining) was standardized for the number of mature CEC using the sum of CD45-, CD146+ and 7-AAD negative (Live) cells within this region (i.e., CD45-, CD146+ and 7-AAD+ cells were not accounted). Normal CEC count by flow cytometry was < 20 cells/ml (Woywodt et al., 2006).

#### **Statistical analysis:**

Data were analyzed using SPSS version 11.5. Descriptive statistics were done by number and percent as well as mean, median and range. Unpaired student's t-test was used for comparison between groups. Correlation between variables was calculated

using Spearman's correlation coefficient.  $P < 0.05$  was considered statistically significant.

### **RESULTS:**

The cardiovascular (CV) risk profile regard to obesity, smoking, hypertension, hyper lipidaemia and diabetes mellitus did not differ significantly between patients and controls (Table 1).

#### **Circulating endothelial cells are elevated in SLE patients:**

Circulating endothelial cells (CEC) count in peripheral blood was significantly elevated in SLE patients than HC  $p<0.001\{39.1 (22.5 - 55.7) \text{ vs. } 7.8 (0.9 - 14.7)\}$  and in active than in non active disease  $p<0.001\{42.6 (29.5 - 55.7) \text{ vs. } 25.7 (22.5 - 28.9)\}$  (table 2, figure 1). CEC count from patients with vasculitic skin lesion and renal manifestations was significantly higher than patients without these manifestations ( $p<0.01$ ).

#### **Impaired endothelial function (FMD %) is linked to CEC count:**

FMD% was significantly reduced in SLE patients than HC [3.8(0.5-7.25)] versus [8.45(4.50-12.40)] respectively ( $P<0.05$ ), and in the active than in non active disease [2.55 (0.60 - 4.50)] versus [4.2 (1.20 - 7.20)] respectively ( $P<0.05$ ) (Table2).

There was a significant negative correlation between CEC count and FMD% ( $r=-0.942$ ,  $P<0.001$ ) (Table 3).

**VCAM-1 and ICAM-1**were significantly increased in SLE patients than HC [276.5(103-450)] and [149.5 (103-196)] versus [66 (37- 95)] and [69.5 (57- 82)]  $P<0.05$  respectively.

Moreover, VCAM-1 was significantly increased in the active than in non active disease, with a significant correlation with CEC count [ $r=0.917$ ,  $P<0.001$ ] (Table 2, 3), while there was no significant variation in ICAM-1 during SLE flare or any correlation with CEC count.

Analyzing the relationship between CEC count and the presence of particular clinical and laboratory parameters of the disease, organ involvement and therapy used, revealed significant positive correlation between CEC count and SLAM score, vasculitic skin lesion and renal involvement, but there was significant negative correlation between CEC count and low complement and antimalarial medications. On the other hand, we did not find any correlation between CEC count and joint involvement, CNS involvement or steroid medication.

**Table (1): Characteristics of SLE patients and controls**

	SLE (n=30)	HC(n=20)	P
<b>Demographics</b>			
Age (years mean ±SD)	25.68±7.78	23 ± 5.8	NS
Disease duration (years mean ±SD)	9 ± 3.7	-	
<b>Cardiovascular risk factors</b>			
BMI (kg/m <sup>2</sup> )	7%	8%	NS
Smoking (%)	0%	0%	NS
Hyperlipidaemia (%)	6%	4%	NS
Hypertension (%)	19%	15%	NS
Diabetes mellitus (%)	2%	0%	NS
<b>Clinical &amp; laboratory features of patients (%)</b>			
Active (%)	57%		
Inactive (%)	43%		
Low C3&C4 (%)	45%		
ANA +ve (%)	100%		
Anti-ds DNA +ve (%)	60%		
Anti-phospholipids antibody positive (%)	50%		
<b>Medication usage (%)</b>			
Antimalarials (%)	69%		
Steroids (%)	45%		
Cyclophosphamide (%)	13%		
Steroids + immunosuppressant (%)	34%		
<b>Organ involvement (%)</b>			
Joint involvement (%)	56%		
Renal involvement (%)	48%		
Cerebral involvement (%)	11%		
Skin involvement (%)	28%		

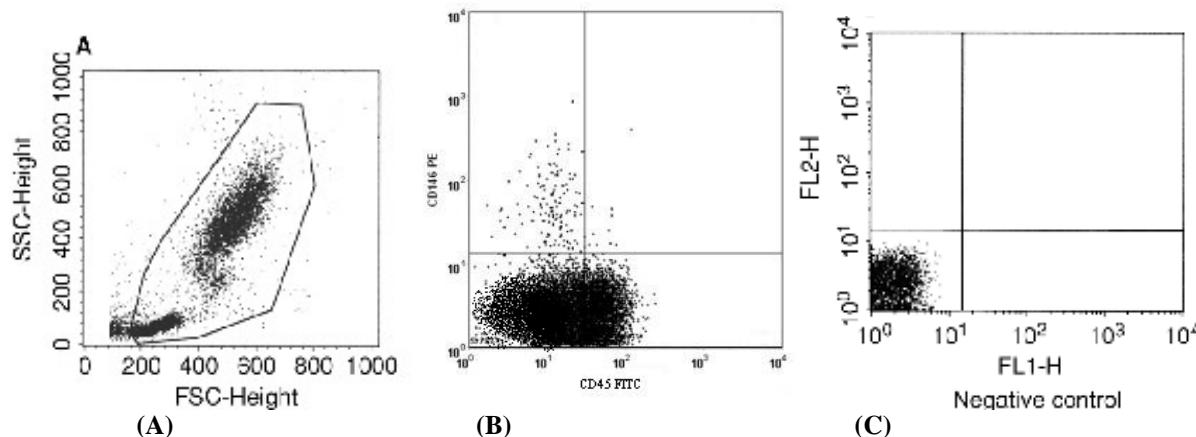
**Table (2): CEC count and other parameters in SLE patients and controls.**

		CEC count (cells/ml)	FMD%	VCAM-1 (ng/ml)	ICAM-1 (ng/ml)
<b>(a)</b>	<b>SLE=30 Median (Range)</b>	39.1 (22.5-55.7)	3.8(0.5-7.25)	276.5(103-450)	149.5(103-196)
<b>(b)</b>	<b>Active =17 Median (Range)</b>	42.6(29.5-55.7)	2.55(0.60-4.50)	288(126-450)	156(116-196)
<b>(c)</b>	<b>Inactive=13 Median (Range)</b>	25.7(22.5-28.9)	4.2(1.20-7.20)	149.5(103-196)	123(103-143)
<b>(d)</b>	<b>Control =20 Median (Range)</b>	7.8 (0.9-14.7)	8.45(4.50-12.40)	66 (37-95)	69.5(57-82)
<b>P</b>		(a)vs(d)P<0.001 (b)vs(c)P<0.001	(a)vs(d)P<0.05 (b)vs(c)P<0.05	(a)vs(d)P<0.05 (b)vs(c)P<0.05	(a)vs(d)P<0.05 (b)vs(c)P>0.05

CEC: circulating endothelial cells; FMD: flow mediated dilatation; VCAM-1: vascular cell adhesion molecule; ICAM-1 intracellular adhesion molecule

**Table (3): Correlation between CEC count and different parameters**

	CEC count	
	r	p
FMD%	-0.942	<0.001
SLAM score	0.966	<0.001
Low complement	-0.384	<0.05
VCAM-1 ng/ml	0.917	<0.001
ICAM-1 ng/ml	0.201	>0.05
Steroid therapy	0.101	>0.05
Antimalarial	-0.451	<0.05
Vasculitic skin lesion	0.662	<0.01
Renal involvement	0.541	<0.01
Joint involvement	0.132	>0.05
CNS involvement	0.168	>0.05



**Figure (1):** Flow cytometry evaluation of circulating endothelial cells (CECs). (A) Representative panel showing the analysis gate used to exclude platelets and debris. (B) Representative histogram of CECs cells (CD45- and CD146+). (C) Representative panels showing the negative control. PE, phycoerythrin; FITC, fluorescein isothiocyanate.

#### 4-DISCUSSION

Although premature atherosclerosis and endothelial dysfunction are well known comorbidities associated with SLE, their underlying cause is not fully explained by traditional risk factors (Lee et al., 2007).

Circulating endothelial cells (CECs) predict vascular function and serve as a surrogate marker of endothelial dysfunction and cumulative CV risk (Lee et al., 2007). CEC activation, described in patients with SLE, was suggested to be a potential inflammatory process mediator, able to induce progressive vascular damage on the vicious circle principle (Clancy et al., 2001).

Our results showed significant elevation in CEC count in SLE patients than healthy controls. This result was in agreement with those reported by Clancy et al. (2001) Woywodt et al.(2003) Robak et al.(2009) and Kluz et al. (2009). This suggests that increased number of CEC may be a marker of

disseminated vasculopathy occurring in the course of SLE (Robak et al.2009).

In the contrary to our data, however, two other reports showed significant deficiency of CEC count in their SLE patients (Lee et al., 2007 and Westerweel et al., 2007 ). The explanation of these discrepancies may be due to the fact that the population studied here comprised totally Egyptian, whereas the study of Lee et al. (2007) involved Africans, Americans, whites and others to nearly equal parts. In their study the ethnic distribution among controls was not matched to that among patients. Also those involved in Westerweel et al. (2007) study were in clinical remission but our patients include active and inactive disease.

Although elevated CEC count are observed mostly in conditions linked with endothelial damage, It seems that the dissociation of mature endothelial cells (ECs) from the vascular wall due to its damage is not the sole reason for increasing CEC numbers noted in those patients. It seems more probable that

extensive vascular involvement, resulting in the release of "desquamated" EC into peripheral blood, also mobilizes medullary endothelial progenitor cells (EPCs) reserves, as well as stimulates their differentiation into mature endothelial cells as a compensatory response to its damage (Kluz et al., 2009).

In our study, there was strong positive correlation between CEC count and disease activity. This observation matches with those reported by Elshal et al.(2009), Kluz et al.(2009), Sesin et al.,(2005) and Clancy et al.(2001), but, contrasting that reported by Robak et al. (2009).

Many previous studies concluded that endothelial function (estimated by FMD) serves as a better marker of vascular reactivity than traditional risk factors (Lee et al., 2007).Also, Moreover, endothelial dysfunction is the key point in both the development of vascular inflammation and atherosclerosis (Deng et al., 2009). In our study FMD% was significantly reduced in SLE patients than HC with significant negative correlation between CEC count and FMD. Soltecz et al. (2010) found that the endothelium dependent vasodilation (FMD) was significantly impaired in patients with MCTD, as compared to controls and concluded that FMD is a reliable sensitive marker of endothelial cell dysfunction in MCTD.

In analyzing the relationship between CEC count and organ involvement, we found that CEC count from patients with vasculitic skin lesion and renal manifestations was significantly higher than patients without these manifestations, a result which matches with that of Elshal et al. (Elshal et al., 2009). Also, we found a significant positive correlation between CEC count and both of skin vasculitis and renal involvement. This indicates that endothelial injury, as a part of immune mediated vascular damage, could play a crucial role in the pathogenesis of these manifestations. Sesin et al. (2005) showed that decreased expression of endothelial protein c receptor in CEC of SLE patients may predict and/or reflect vasculopathy and renal injury in SLE. Moreover, we found a significant correlation between CEC count and low complement, a finding which is in consistent with the previous study of Clancy et al. (2001).

Regarding the relationship between CEC and therapy used, we found a significant negative correlation between CEC count and antimarial medications, but no correlation with steroids. Jung et al. (2010) demonstrated that antimarial drugs are thromboprotective in SLE with a risk reduction of thrombovascular events of 68%. However, Lee et al.(2007) and Robak et al.(2009) concluded that

there was no correlation between CEC count and steroids, anti- malarials or cytotoxic agents.

It has been established that leukocyte stimulation due to complement activation (C3 and C4) is an important step in the development of endothelial dysfunction. Simultaneously, through the influence of numerous immune stimuli such as cytokines, immune complexes, antiendothelial or antiphospholipid antibodies, surface expression of adhesion molecules in endothelial cells is enhanced (Clancy et al., 2001 ).

In glomerulonephritis murine models, increased expression of VCAM-1 and ICAM-1 was found in renal tissue (McHal et al., 1999). Moreover, greater survival rates were observed in mice deprived of ICAM-1, suggesting that their enhanced expression may play a considerable role in SLE pathogenesis (Kevil et al., 2004). Furthermore, in numerous patients with active vasculitis, no circulating or deposited immune complexes are found. Similarly, in many instances, such a deposition within vessel wall does not lead to inflammatory infiltration or fibrotic necrosis development. Hence the suggestion that alternative mechanisms might be involved in the pathogenesis of inflammatory vascular lesions, such as interaction between VCAM-1 and very late activation antigen (VLA-4) as well as ICAM-1 and leukocyte function-associated antigen (LFA-1), determining the adhesion of leukocytes to endothelial cells and their subsequent damage linked with cytokines and neutrophils (Guillevin et al., 2007).

In this study, there was significant elevation of VCAM-1 and ICAM-1 in SLE patients than HC, but regarding their relations to disease activity and CEC count, VCAM-1 significantly increased with disease activity and correlate positively with CEC count, which led to the suggestion that increased expression of VCAM-1 plays a leading role in the pathogenesis of SLE and is a direct cause of enhancing activated leukocytes migration, responsible for inflammatory tissue lesions. A similar result was reported by Kluz et al. ( 2009), but opposite result was reported by Robak et al.(2009). Additionally, there was no interrelationship between ICAM-1 and CEC count or disease activity, implying that increased expression of this molecule seems to be primarily an indicator of a generalized EC dysfunction rather than a marker reflecting the degree of endothelial damage. This observation agrees with that reported by Kluz et al. (2009). However, the pathogenesis of SLE is complex, and it is based on several overlapping regulatory loops; so, further studies are needed to determine the relationship between CEC and angiogenic proteins and inflammatory cytokines.

**CONCLUSION:**

- \*Increased circulating endothelial cells number constitute a reliable marker of disease activity in SLE, reflecting endothelial damage and thus enabling the distinction of a patient group running a higher risk of vascular lesion development.
- \* Progressive increase in serum VCAM-1 concentration is linked with progression of SLE activity and development of lupus angiopathy.

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9/6/2010

## Degradation of Polycyclic Aromatic Hydrocarbons As Affected By Some Lactic Acid Bacteria

Abou-Arab, A.A.K\*; Abou-Bakr Salim\*; Maher,R.A. ; El-Hendawy, H.H. \*\* and Awad, A.A. \*\*

\*Food Toxicology and Contaminants, National Research Center.

\*\*Botany and Microbiology Department, Faculty of Science, Helwan University.

[Aak\\_abouarab2007@yahoo.com](mailto:Aak_abouarab2007@yahoo.com)

**ABSTRACT:** Polycyclic aromatic hydrocarbons (PAHs) are a group of chemicals composed of two or more fused aromatic rings that are formed from the incomplete combustion or high-temperature pyrolysis of coal, oil, gas, wood, fossil fuel, garbage or other organic substances, such as tobacco, charbroiled meat and exhaust from automobile and trucks. They enter the environment and release to air, soil, water and food. Some PAHs have shown to have toxicological, carcinogenic and mutagenic effects on animals and humans. Biodegradation of PAHs in the presence of the three types of lactic acid bacteria (*Bifidobacterium bifidum*, *Streptococcus thermophilus* and *Lactobacillus bulgaricus*) were studied during the different incubation periods (2, 4, 6,8,10,12,24,48 and 72 h) at 37°C. The reduction of PAHs concentration proved that these compounds were affected by the previous lactic acid bacteria. At the end of incubation period (72 h), the reduction percent were 46.6, 87.7 and 91.5% with *Bifidobacterium bifidum*, *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, respectively. These results could be explained as the bacterial cell is a high proteinous material and so may adsorbs PAHs which could interfere with cellular metabolism. Also, the variation of pH values during the incubation periods may control in the adsorbed PAHs on the cells. The biodegradation of PAHs by yoghurt starter during yoghurt manufacture were studied. Slightly reduction was observed during the incubation periods (1, 2 and 3 h). The reduction percent was 3.46 at the final product. It could be revealing that the persistence of PAHs depend on a number of factors such as the type of microorganism, the interaction between microorganisms, the microbial concentration, the composition of the medium, and the microbial growth conditions of temperature and pH. The foregoing information reveal that extra care must be taken when comparing the results since in-vitro studies are not always relevant to real situation in food products.

[Abou-Arab, A.A.K; Abou-Bakr Salim; Maher,R.A.; El-Hendawy, H.H. and Awad, A.A. Degradation Of Polycyclic Aromatic Hydrocarbons As Affected By Some Lactic Acid Bacteria. Journal of American Science 2010;6(11):708-715]. (ISSN: 1545-1003).

**Key words:** PAHs, Lactic acid bacteria, Degradation, MRS, Milk, Yoghurt.

### INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a group of chemicals composed of two or more fused aromatic rings that are formed during the incomplete combustion or high-temperature pyrolysis of coal, oil, gas, wood, fossil fuels, garbage, or other substances, such as tobacco and charbroiled meat (*Mottier et al.,2000*). The quantity and composition of PAHs produced are closely related to the reaction conditions, temperature and amount of air and, therefore, may vary considerably (*Vaessen et al., 1988*). Over 100 PAHs have been identified and occur as complex mixtures, never as individual components.

PAHs comprise the largest class of known chemical carcinogens and have been detected in the environment especially in air, water, soil and foods. They enter the environment mostly as releases to air from volcanoes, forest fires, and residential wood burning, cigarette smoke, asphalt roads, coal, coal tar, agricultural burning, municipal, industrial waste incineration, hazardous waste sites and exhaust from automobiles and trucks.. They can also enter surface

water through discharges from industrial plants and waste water treatment plants. These compounds can be released to soils at hazardous waste sites if they escape from storage containers (*ATSDR 1995*). The populations may be exposed to PAHs in the soil at or near hazardous waste sites. The movement of PAHs in the environment depends on properties such as how easily they dissolve in water, and how easily they evaporate into the air. PAHs in general do not easily dissolve in water. They are present in air as vapors or stuck to the surfaces of small solid particles. They can travel long distances before they return to earth in rainfall or particle settling (*ATSDR 1995*). The PAHs content of plants and animals living on the land or in water can be many times higher than the content of PAHs in soil or water.

Polycyclic aromatic hydrocarbons (PAHs) are proven animal carcinogens; in humans they are suspected of causing cancer. Clinical studies have shown that exposure a mixture of highly concentrated PAHs may cause various cancers, in skin, lung, stomach and liver. It is generally convinced that PAHs

are responsible for the increasing cancer risks as PAHs are capable of damaging genetic materials and thus initiating the development of cancers (*Schneider et al., 2000*). Some of PAHs compounds such as benzo(a)pyrene and dibenzo (a,h) anthracene were reported to be the most carcinogenic (*Schneider et al., 2000*). So, the presence of these compounds in food has received considerable attention over the past three decades (*Maga, 1988*). Food quality and safety is a pertinent issue, consumers are concerned that their food should be both of high nutritional value and free from chemical residues.

As environment pollution in different countries is becoming a serious problem, it is possible that PAHs may be widely distributed in the environment and thus contaminates food. The occurrence of PAHs in food may result from their sorption from a contaminated environment or from food preparation. The variation in PAHs profile in food products also depends on the source of the contamination (*Vaessen et al., 1988*). PAHs have been detected in fresh vegetables, fruits, and cereals as a result of the deposition of airborne PAHs, particularly near industrial sources or in areas with high traffic (*Dennis, 1991*). They have also been found in mussels, snails, and fish from contaminated waters (*Speer et al., 1990*). *Kan et al.* (2003) reviewed the occurrence of PAHs in animal products. In France, PAHs have been found in milk at total levels of 37 and 27 ng/g fat (*Grova et al., 2001*). Concentrations up to 70 µg/kg were found in meat (SCF, 2002). PAHs are also present at elevated levels in some vegetable oils and margarine (*Thomson et al., 1996*), probably formed during processing. They are also formed during some methods of food preparation, such as charbroiling, grilling, roasting, frying, or baking (*Yabiku et al., 1993*).

PAHs can breakdown to longer-lasting products by reacting with sunlight and other chemicals in the air, generally over a period of days to weeks. Breakdown in soil and water generally takes weeks to months and is caused primarily by microorganisms (ATSDR 1995). Biodegradation of chemicals by living organisms is one of the most important mechanisms for the breakdown of organic compounds and the microorganisms are the most important agents for such degradation. However, degradation is a very specific process and the growth of some microorganisms can even be inhibited by a xenobiotic. If degradation does occur, it is likely to result from enzymatic activity and may either occur immediately or only after a period of adaptation to the chemicals (*Boethling, 1993*).

The study of degradation of such residues in these foodstuffs is very important because of their increasing rate of consumption world-wide. Therefore,

technological procedures in food production should be developed to reduce the content pollutants hazardous to public health in food products. Nowadays, in the food industry it is very common to use starter cultures to improve the characteristics of the food products, and the possibility that these microorganisms would degrade these contaminants is of great interest because the de-chlorinated products are generally less toxic to animals, less likely to bio-accumulate, and more susceptible to further microbial attack (*Bayarri et al., 1997*).

Report on microbial degradation of PAHs appear increasing numbers, but such investigation tend to be focused on soil or aquatic microorganisms (*Luning and Pritchard, 2002 and Story et al., 2004*), while the activity of microorganisms associated with food fermentation has been less will investigated.

With this in view, the present work was conducted to unveil and throw more light on the biodegradation of the target PAHs as affected by some types of lactic acid bacteria (dairy and fermented foods starter) in different media.

## MATERIALS AND METHODS

### 1. Polycyclic aromatic hydrocarbons (PAHs) reference standards

A mixture (16 compounds) of PAHs reference standards containing acetaphthene, acenaphthylene, anthracene, benzo (a) anthracene, benzo (a) pyrene, benzo (b) fluoranthene, benzo (g, h, i) perlylene, chrysene, dibenz (a,h) anthracene, fluoranthene, fluorene, indeno (1,2,3,-cd) pyrene, naphthalene, phenanthrene, pyrene and 2-bromonaphthalene was purchased from Supelco company (Supelco Park, Bellefonte, PA, U.S.A.).

### 2. Bacterial Strains

Strains of *Bifidobacterium bifidum* (*B. bifidum*), *Streptococcus thermophilus* (*S. thermophilus*) and *Lactobacillus bulgaricus* (*L. bulgaricus*) were obtained from Cairo Microbiological Research Center, Cairo MIRCEN, Faculty of Agriculture, Ain-Shams University, Egypt. The strains were stored at -18°C until utilized.

### 3. Degradation of polycyclic aromatic hydrocarbons(PAHs) by lactic acid bacteria

Sterilize liquid medium De Man-Rogosa-Sharpe (MRS) was prepared (300ml) according to *Man et al.* (1960) and spiked by PAHs mixture containing 16 compounds of acetaphthene, acenaphthylene, anthracene, benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(g,h,i)perlylene, chrysene, dibenz(a,h)anthracene, fluoranthene, fluorene, indeno(1,2,3,-cd)pyrene, naphthalene, phenanthrene,

pyrene and 2-bromonaphthalene 0.25 $\mu$ g of each/ml medium). The medium was divided into three portions. The first, second and third portions were inoculated by 1% *B. bifidum*, *S. thermophilus* and *L. bulgaricus*, respectively. All flasks incubated at 37°C for 2, 4, 6, 8, 10, 12, 24, 48 and 72 h .The collected samples (10 ml of each) were extracted according to the method of Hodgeson (1990) for PAHs residues. The extraction method was validated. The limit of detection (LOD) is equal to 3 times the standard deviation (SD) of the lowest standard concentration used for the calibration curve (Chandra and Sangchan, 2009). Minimum detectable concentrations of PAHs in the present investigation were ranged between 0.007 to 0.020  $\mu$ g/ml (Table 1). Recovery results refer to complete method with concentration of 0.25  $\mu$ g/ml of each compound PAHs (total compounds of 16 was 4 $\mu$ g/ml) used in this study ranged from 88 to 96 % (Table 1). The experiment was repeated by injecting a mixture of 16 PAHs standards 6 times.

**Table 1. Validation (detection limits and recovery) of PAHs**

Compound	LOD ( $\mu$ g/ml)	Recovery (%)
1-Naphthalene	0.010	88.0
2-Acenaphthylene	0.020	89.2
3-2.Bromonaphthalene	0.010	94.0
4-Acenaphthene	0.008	94.0
5-Fluorene	0.010	94.0
6-Anthracene	0.009	93.0
7-Phenanthrene	0.008	90.0
8-Pyrene	0.020	92.0
9-Fluoranthene	0.008	90.0
10-Chrysene	0.008	96.0
11-Benzo(a)anthracene	0.007	94.0
12-Benzo(k)fluoranthene	0.007	92.0
13-Benzo(a)pyrene	0.008	96.0
14-Benzo(ghi)perylene	0.009	94.0
15-Dibenz(a,h)anthracene	0.008	94.0
16-Indeno(1,2,3cd)pyrene	0.010	89.0

Measured volumes of the medium were serially extracted with dichloromethane. Sixty ml of dichloromethane was added to the sample in separating funnel with shaking for two minutes with periodic venting to release excess pressure. Then, the organic layer separated from the liquid phase and the dichloromethane extract collected in 250 ml Erlenmeyer flask. The extraction steps repeated by adding another 60 ml dichloromethane. A third extraction in the same manner was performed. The combined extracts of dichloromethane was dried through column containing about 10 cm of anhydrous sodium sulphate and the extracts were collected in Kuderna- Danish (K-D) concentrator and the K-D placed on a hot water bath (60-65°C), so that the concentrator tube is partially immersed in hot water, and the entire lower rounded surface of the flask was bathed with hot vapor. When the apparatus volume of

liquid reaches 0.5 ml, the K-D was removed and allowed it to drain and cool for at least 10 min. Then the synder column was removed. The flask and its lower joint into the concentrator tube were rinsed with 1-2 ml dichloromethane. The extract was evaporated with a gentle steam of N<sub>2</sub> flow to defined volume.

One micro-liter of each sample extract was injected into a Hewlett Packard 5890 gas chromatograph fitted with a HP-5 fused silica capillary column (50m x 0.2mm x 0.33 $\mu$ m film thickness) and connected to Hewlett Packard 5970 series mass selective detector. The carrier gas was helium, maintained at a flow rate of 1.0 ml/min. The injection port temperature was 275°C with electron energy of 70 eV. The quadrupole temperature was 280°C. The oven programmed was as follows: 70°C for 5 min, 3°C/min to 290°C for 30 min. The mass spectrometer is tuned by letting in a small amount of perfluorotributylamine (C<sub>12</sub>F<sub>27</sub>N) gas as a reference. The fragments of peak for m/z, 69, 219 and 502 were observed and tune results were recorded and the masses are calibrated. The mass spectrum for each of the peaks from the resulting chromatogram from analyzed samples was observed by the total ion count (TIC) mode. Calibration was carried out by external standards, mixture of 16 compounds (Fig.1). The mass spectrometer was operated in selective ion monitoring mode using separate ions to identify and confirm compounds. Acquired mass spectrum in samples was compared with the standard and library spectra for identification.

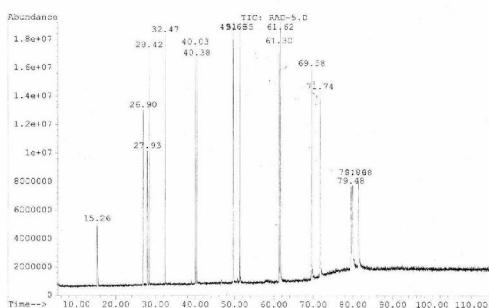
#### Degradation of PAHs by yoghurt starter

Mixture of buffalo's and cow's milk was heated at 80-82°C for 20 min and cooled to 40°C. The milk was polluted by polycyclic aromatic hydrocarbons (PAHs) mixture (16 compounds) to give concentration around 0.02  $\mu$ g/ml of each compound in the mixture and the PAHs concentration of the polluted mixture was determined.

Polluted milk was inoculated with 2% yoghurt starter (mixture of *S. thermophilus* and *L. bulgaricus*) and incubated at 40°C for 3.0 h as described by the Egyptian Organization for Standardization, EOS (1970). The samples were analyzed at zero time and after 1, 2 and 3 h (yoghurt product) intervals. The extracted samples were applied according to the method of Hodgeson (1990) as described before. The residues of PAHs were determined by GC/MS as described before.

#### Statistical analysis

The data were statistically analyzed by analysis of variance and least significant difference (L.S.D) at 0.05 levels according to the method described by Snedecor and Cochran (1980).



**Fig.1. Mixture of polycyclic aromatic hydrocarbons (PAHs) analyzed by GC/MS**

## RESULTS AND DISCUSSION

### RESULTS

#### Degradation of polycyclic aromatic hydrocarbons (PAHs) by lactic acid bacteria:

MRS media broth contaminated by PAHs (16 compounds, 0.25 µg of each/ml media) and inoculated with *B. bifidum*, *S. thermophilus* and *L. bulgaricus* and incubated at 37°C for 72 h, critical and significant role of lactic acid bacteria (LAB) in uptake and/or degrade PAHs was observed. It could be revealing that the persistence of PAHs depends on bacterial species and incubation period.

The obtained results revealed that PAHs was affected by *B. bifidum* strain during the incubation period (Table 2). After 2 to 48 h of incubation, naphthalene, acenaphthylene, 2-bromonaphthalene and acenaphthene weren't detected in the various samples. However, 2-bromonaphthalene and acenaphthene were appeared after 72 h of incubation and the reduction (%) was 74.8 and 87.6, in this order. Regarding to the other compounds in different samples, they were detected at

fluctuation levels and the sum of total mixture compounds was decreased during the incubation periods. The same pattern was detected in case of *S. thermophilus* (Table 3) except, the presence of residues of acenaphthylene, 2-bromonaphthalene and acenaphthene after 2 h incubation, beside presence of 0.017 µg/ml of 2-bromonaphthalene when incubation period was 72 h. Also, fluctuation levels were observed during the different period of incubation. The effect of *L. bulgaricus* (Table 4) on PAHs was similar to that detected with *B. bifidum*. It was observed that naphthalene, acenaphthylene, 2-bromonaphthalene and acenaphthene were disappeared during the incubation for 48 h. However, traces of either naphthalene (0.013 µg/ml) and acenaphthylene (0.003 µg/ml) were detected after 72 h and 48 h incubations, respectively. With the other compounds, the same sequence was detected as in other two strains.

Data presented in Table 5 proved the critical and significant role of LAB in uptake and/or degrade PAHs. During the incubation periods (2, 4, 6, 8, 10, 12, 24, 48 and 72 h), the reduction (%) relative to the initial concentration of PAHs (4 µg/ml) ranged from (46.6 to 92.9), (51.8 to 94.9) and (77.7 to 92.4), by *B. bifidum*, *S. thermophilus* and *L. bulgaricus*, respectively. It is worthy to mention that the highest reduction of PAHs by *B. bifidum* and *S. thermophilus* was observed after incubation for 10 and 12 h, and was found to be 92.6 and 96.0 %, respectively. However, the highest reduction by *L. bulgaricus* was recorded after 48 h and was found to be 92.4%. In a descending order, the strains tested could be arranged according to their ability to assimilate the PAHs at the end of incubation (72 h), to be as follows: *L. bulgaricus* (91.5%), *S. thermophilus* (87.7%) and *B. bifidum* (46.6%) as shown in Table 5.

**Table 2. Persistence of PAHs in MRS media broth during incubation at 37°C as affected by *Bifidobacterium ifidum***

PAHs compounds	Residues of PAHs (µg/ml) during the incubation period (hr)								
	2	4	6	8	10	12	24	48	72
Naphthalene	nd	nd	nd	nd	nd	nd	nd	nd	nd
Acenaphthylene	nd	nd	nd	nd	nd	nd	nd	nd	nd
2.Bromonaphthalene	nd	nd	nd	nd	nd	nd	nd	nd	0.063
Acenaphthene	nd	nd	nd	nd	nd	nd	nd	nd	0.031
Fluorene	0.009	nd	0.017	nd	0.005	0.010	0.017	nd	0.110
Anthracene	0.076	0.248	0.059	0.024	0.038	0.012	0.204	0.071	0.182
Phenanthrene	0.151	0.056	0.037	0.022	0.007	0.099	0.053	0.013	0.023
Pyrene	0.089	0.143	0.068	0.102	0.057	0.121	0.103	0.203	0.220
Fluoranthene	0.080	0.140	0.083	0.106	0.054	0.121	0.097	0.196	0.211
Chrysene	0.008	0.155	0.061	0.085	0.012	0.101	0.059	0.112	0.185
Benzo(a)anthracene	0.083	0.196	0.099	0.118	0.052	0.153	0.109	0.201	0.250
Benzo(k)fluoranthene	0.084	0.163	0.095	0.028	0.045	0.105	0.078	0.127	0.128
Benzo(a)pyrene	nd	0.093	0.015	0.012	0.003	0.009	nd	0.019	0.117
Benzo(ghi)perylene	0.043	0.068	0.143	0.044	0.006	0.042	0.087	0.128	0.111
Dibenz(a,h)anthracene	0.053	0.128	0.144	0.057	0.007	0.094	0.078	0.173	0.229
Indeno(1,2,3cd)pyrene	0.080	0.239	0.186	0.188	0.010	0.090	0.108	0.173	0.250
Total (sum)	0.756	1.629	1.007	0.786	0.296	0.957	0.993	1.428	2.138

-Total of mixture compounds (4.0 µg/ml, 0.25 µg of each 16 compounds). -nd: not detectable.

**Table 3. Persistence of PAHs in MRS media broth during incubation at 37°C as affected by *Streptococcus thermophilus***

PAHs compounds	Residues of PAHs (µg/ml) during the incubation period (hr)								
	2	4	6	8	10	12	24	48	72
Naphthalene	nd	nd	nd	nd	nd	nd	nd	nd	nd
Acenaphthylene	0.023	nd							
2-Bromonaphthalene	0.045	nd	0.017						
Acenaphthene	0.046	nd							
Fluorene	0.051	nd	nd	0.015	0.005	0.003	0.037	0.008	0.017
Anthracene	0.060	0.049	0.202	0.065	0.008	0.004	0.249	0.078	0.072
Phenanthrone	0.046	0.045	0.037	0.017	0.004	0.003	0.056	0.041	0.010
Pyrene	0.066	0.048	0.152	0.071	0.037	0.028	0.241	0.135	0.068
Fluoranthene	0.060	0.047	0.150	0.067	0.031	0.019	0.221	0.135	0.070
Chrysene	0.011	0.024	0.129	0.052	0.008	0.003	0.187	0.071	0.046
Benzo(a)anthracene	0.042	0.047	0.150	0.066	0.035	0.033	0.210	0.103	0.056
Benzo(k)fluoranthene	0.023	0.058	0.167	0.070	0.037	0.002	0.180	0.080	0.015
Benzo(a)pyrene	0.020	0.012	0.056	0.020	0.003	0.003	0.042	0.011	0.034
Benzo(ghi)perylene	nd	0.056	0.147	0.063	0.004	0.005	0.168	0.079	0.025
Dibenz(a,h)anthracene	nd	0.003	0.003	0.034	0.009	0.018	0.088	0.098	0.045
Indeno(1,2,3cd)pyrene	nd	0.071	0.193	0.048	0.019	0.028	0.248	0.150	0.019
Total (sum)	0.493	0.460	1.386	0.588	0.205	0.159	1.927	0.989	0.494

-Total of mixture compounds (4.0 µg/ml, 0.25 µg of each 16 compounds).

-nd : not detectable.

**Table 4. Persistence of PAHs in MRS media broth during incubation at 37°C as affected by *Lactobacillus bulgaricus***

PAHs compounds	Residues of PAHs (µg/ml) during the incubation period (hr)								
	2	4	6	8	10	12	24	48	72
Naphthalene	nd	nd	nd	nd	nd	nd	nd	nd	0.013
Acenaphthylene	nd	nd	nd	nd	nd	nd	nd	nd	nd
2.Bromonaphthalene	nd	nd	nd	nd	nd	nd	nd	nd	nd
Acenaphthene	nd	nd	nd	nd	nd	nd	nd	0.003	nd
Fluorene	nd	nd	0.013	0.027	nd	nd	nd	nd	0.003
Anthracene	0.041	0.068	0.013	0.029	0.025	0.019	0.034	0.016	0.032
Phenanthrone	nd	0.011	nd	0.007	0.016	0.019	0.033	nd	0.009
Pyrene	0.035	0.125	0.115	0.084	0.061	0.073	0.029	0.047	0.037
Fluoranthene	0.024	0.115	0.133	0.080	0.060	0.074	0.010	0.003	0.038
Chrysene	nd	0.047	0.053	0.057	0.051	0.037	0.090	0.072	0.039
Benzo(a)anthracene	0.034	0.111	0.126	0.096	0.073	0.083	0.151	0.002	0.049
Benzo(k)fluoranthene	0.092	0.051	0.094	0.069	0.065	0.087	0.031	0.049	0.033
Benzo(a)pyrene	nd	0.009	0.028	0.019	0.016	0.031	0.035	0.004	0.026
Benzo(ghi)perylene	0.087	0.089	0.029	0.039	0.049	0.032	0.140	0.021	0.006
Dibenz(a,h)anthracene	0.081	0.095	0.045	0.036	0.043	0.073	0.217	0.027	0.024
Indeno(1,2,3cd)pyrene	0.140	0.047	0.172	0.023	0.102	0.071	0.121	0.062	0.032
Total (sum)	0.534	0.768	0.821	0.566	0.561	0.599	0.891	0.306	0.341

-Total of mixture compounds (4.0 µg/ml, 0.25 µg of each 16 compounds).

-nd: not detectable.

**Table 5. Persistence of polycyclic aromatic hydrocarbons (PAHs) in MRS media broth during incubation at 37°C as affected by lactic acid bacteria (LAB).**

Incubation periods/ hr	<i>Bifidobacterium bifidum</i> .		<i>Streptococcus thermophilus</i> .		<i>Lactobacillus bulgaricus</i>	
	Residue (µg/ml)	Reduction (%)	Residue (µg/ml)	Reduction (%)	Residue (µg/ml)	Reduction (%)
2	0.756	81.1	0.493	87.7	0.534	86.7
4	1.629	59.3	0.460	88.5	0.768	80.8
6	1.007	74.8	1.386	65.4	0.821	79.5
8	0.786	80.4	0.588	85.3	0.566	85.9
10	0.296	92.6	0.205	94.9	0.561	86.0
12	0.957	76.1	0.159	96.0	0.599	85.0
24	0.993	75.2	1.927	51.8	0.891	77.7
48	1.428	64.3	0.989	75.3	0.306	92.4
72	2.138	46.6	0.494	87.7	0.341	91.5

-Zero time: 4.0 µg/ml of sum total mixture (16 compounds) of PAHs (0.25 µg of each).

### Degradation of polycyclic aromatic hydrocarbons (PAHs) by yoghurt starter

The purpose of this item, is to determine the role of yoghurt starter (*S. thermophilus* and *L. bulgaricus*) in degradation of PAHs compounds by in milk as complex medium . During the manufacture of yoghurt, data in Table 6 proved slightly significant role of yoghurt starter in degradation of PAHs (0.4044 µg/ml). The mean reduction (%) after 1 h of incubation at 40°C was 1.11%. However, after 2 h and 3 h, the reduction (%) increased to 2.15 and 3.46 % of sum PAHs compounds, respectively. These results indicate that the level of PAHs compounds were variable. The highest reduction were recorded with the compounds of indeno(1,2,3-cd)pyrene (5.81%), benzo (ghi) perylene (5.16%) followed by dibenz(a,h)anthracene (4.17%) at the end of incubation period (3 h). However, these reductions were slightly significant.

**Table 6. Concentrations (µg/g) of PAHs during incubation at 40°C as affected by Yoghurt starter**

PAHs	Zero time concentration µg/g	1 hour Concentration µg/g	Reduction (%)	2 hour Concentration µg/g	Reduction (%)	3 hour Concentration µg/g	Reduction (%)
Naphthalene	0.0261	0.0259	0.77	0.0257	1.53	0.0251	3.83
Acenaphthylene	0.0228	0.0226	0.88	0.0224	1.60	0.0223	2.19
2-Bromonaphthalene	0.0295	0.0293	0.84	0.0291	1.36	0.0288	2.35
Acenaphthene	0.0258	0.0256	0.92	0.0253	1.92	0.025	3.10
Fluorene	0.0299	0.0296	1.00	0.0294	1.67	0.0289	3.34
Anthracene	0.0321	0.0317	1.25	0.0314	2.18	0.0311	3.12
Phenanthrene	0.0291	0.0288	1.03	0.0285	2.06	0.0281	3.44
Pyrene	0.0248	0.0245	1.21	0.0244	1.61	0.0239	3.63
Fluoranthene	0.0268	0.0265	1.12	0.0261	2.61	0.0259	3.36
Chrysene	0.0264	0.0259	1.89	0.0256	3.03	0.0253	4.17
benzo(a)anthracene	0.0212	0.0209	1.42	0.0207	2.36	0.0206	2.83
Benzo(k)fluoranthene	0.0220	0.0217	1.36	0.0215	2.27	0.0212	3.64
Benzo(a)pyrene	0.0212	0.0210	0.94	0.0207	2.36	0.0204	3.77
Benzo(ghi)perylene	0.0252	0.0248	1.59	0.0245	2.78	0.0239	5.16
Dibenz(a,h)anthracene	0.0215	0.0213	0.93	0.0209	2.87	0.0207	3.72
Indeno(1,2,3-d)pyrene	0.0200	0.0198	1.00	0.0195	2.50	0.01920	5.81
Total (sum)	0.4044	0.3999	1.11	0.3957	2.15	0.3904	3.46

-The intial pH of whole milk (O.T) was 6.8; -After 1 hr, pH was 6.1

-After 2 hr, pH was 5.9; -After 3 hr, pH was 4.8

### DISCUSSION

Biodegradation is defined as the biologically catalyzed reduction in complexity of chemical compounds (Wilson and Jones, 1992). It is based on two processes: growth and co- metabolism. In the case of growth, organic pollutants are used as a sole source of carbon and energy. This process results in a complete degradation (mineralization) of organic pollutants. Co-metabolism is defined as the metabolism of an organic compound in the presence of a growth substrate which is used as the primary carbon and energy source.

Enzymatic key reactions of aerobic biodegradation are oxidations catalyzed by oxygenases and peroxidases. Oxygenases are oxidoreductases that used O<sub>2</sub> to incorporate oxygen into the substrate. Degradative organisms need oxygen at two metabolic sites, at the initial attack of the substrate and at the end of the respiratory chain. Although the presence of PAHs in some types of food due to different treatments (FSA, 2002 and Falco *et al.*, 2003), no information available on the degradation of PAHs by LAB or by pure cultures of microorganisms isolated from food and dairy products. Most investigations studied the microbial degradation of PAHs in soil.

Factors that affect biodegradation include pollutant concentration and pure-exposure time. Microbial communities present in contaminated soil can metabolize PAHs at greater rates than soil microbial communities found in uncontaminated soils (Rathbone *et al.*, 1998). Greater population density and diversity of microorganisms often result in increased degradation rates of PAHs in soil (Rathbone *et al.*, 1998). However, organic matter did not appear to increase the population of known PAH-degrading microorganisms as much as general heterotrophic microorganisms (Carmichael and Pfaender, 1997). PAH degradation capabilities are associated with members of certain taxa such as *Pseudomonas*, *Sphingomonas*, and *Burkholderia*, independent of origin of the soil from which bacteria isolated (Mueller *et al.*, 1997). Moreover, genes responsible for PAH degradation are homologous and ordered (Dagher *et al.*, 1997). These genetic characteristics restrict enzymes diversity in microbial communities of pyrene and phenanthrene contaminated soils. Biodegradation of PAHs in the present study by LAB, *B. bifidum*, *S. thermophilus* and *L. bulgaricus* were similarly to that recorded with PAHs degradation in soils. The reduction of PAHs concentration in this investigation proved that the studied microorganisms

degraded the PAHs at different levels. The obtained results could be explained as the bacterial cell is a high proteinous material and so may adsorbs PAHs which could interfere with cellular metabolism. Also, the variations of PAHs levels detected during the incubation periods may be due to the lowering of PAHs values of the medium by the fermentation of their lactose contents. The variations of pH values during the incubation periods may determine whether PAHs could be adsorbed on the cells or became free in the MRS medium. The results of this study indicated that microbial communities exposed to PAHs contaminated media produced distinctive patterns of substrate utilization. The pattern indicated differences in community structure which resulted in a change in decomposition ability by the microorganisms. The PAHs have induced changes in type and amount of enzymes/or composition of the microbial population. The contaminants induced enzyme response from the microorganisms under their influence. The production of aromatic ring deoxygenase one of the PAH-degrading enzymes, was induced by the presence of PAH (*Dagher et al., 1997*). However, organic matter did not appear to increase the population of known PAH-degrading microorganism's as much as general heterotrophic microorganisms (*Carmichael and Pfaender, 1997*).

The slightly reduction of PAHs by yoghurt starter may be related to the pH effects of the culture medium after or during the incubation period. In this sense, several authors labeled the pH as a factor that influences the microbial degradation process (*Furukawa, 1982* and *Fewson, 1988*). On the other hand, the reduction of PAHs may be due to the protein affinity and/or adsorption ability of these compounds on the fat globule. Besides, bacterial cell is high proteinous material and may adsorb PAHs which could interfere with cellular metabolism.

The activity of microorganisms associated with food fermentation on the contaminants especially PAHs has been less will investigated. However, similar finding with pesticides (which represent the same group of PAHs, i.e. persistent organic pollutants) was recorded by *Hantke and Bradley (1972)* who found that adsorption of organochlorine pesticide residues was related to the interference with the cellular metabolism of organisms. Moreover, *Chacko and Lockwood (1967)* reported that bacterial cells can accumulate pesticide molecules. On the other hand, *Kim and Harmon (1970)* observed that amounts of dieldrin as pesticide are adsorbed or incorporated by the cells. In addition *Abou-Arab (1996, 1999 and 2002)* confirmed that the fermentation process in milk to produce dairy products (cheese) and meat products (fermented sausage) reduced pesticide residues and these reductions were due to the activity of milk or meat starter. Besides,

author reported that lactic acid bacteria decreased some types of pesticides (DDT, malathion and fenvalerate) during the incubation periods for 120 h.

Slight reduction of PAHs during yoghurt manufacturing was observed.. This result coincides with those reported by *Montourey and Muldon (1968)*, which explained the reduction in DDT content due to adsorption preferability by the milk protein , likewise, *Hugunin and Bradley (1971)* reported that significant amounts of dieldrin insecticide were associated with serum protein fraction in skimed milk . On the other hand, *Abou-Arab (1987, 1991and 2002)* reported significant role of lactic acid bacteria in degradation of some types of pesticides. The author reported that yoghurt starter reduced lindane, BHC and DDT by 77.3, 9.0 and 2.0 %, respectively. On the other hand, the reduction of DDT and lindane was (24.1-32.5) and (27.9-40.0%), respectively with *Micrococcus varians* as meat starter. Moreover, *Chacko et al. (1966)* reported that bacterial cells can accumulate pesticide molecules. However, *Kim and Harmon (1970)* observed that amount of dieldrin are adsorbed by the cells.

It could be concluded that, LAB may affected by PAHs during the first time of incubation. Nevertheless, the microorganisms rapidly adapted with presence of such PAHs and grow fast. Then critical and significant role of these strains in uptake and/or degrade PAHs was observed. But extra care must be taken when comparing the results since *invitro* studies are not always relevant to real situation in food products. This is due to the fact that the biodegradation process may be affected by a number of factors such as the type of microorganism (even the type of strain), the interaction between microorganisms, the microbial concentration, the composition of the medium, whether the medium is liquid or solid, and the microbial growth conditions of temperature and pH. However, more studies must be done on biodegradation of PAHs in food media.

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## Expression of The Antiapoptotic Gene Survivin in Acute Leukemias

Hoda Sadek,<sup>1</sup> Shadia Ragab,<sup>2</sup> Hanaa Rasmy,<sup>2\*</sup> Nancy M. El Guindy,<sup>1</sup> Wafaa Ezzat,<sup>3</sup> Mona Hamed<sup>2</sup>

<sup>1</sup> Clinical and Chemical Pathology Department, Faculty of Medicine, Cairo University, Egypt

<sup>2</sup> Clinical and Chemical Pathology Department, National Research Center, Egypt

<sup>3</sup> Internal Medicine Department, National Research Center, Egypt

[hanarasmy2000@yahoo.com](mailto:hanarasmy2000@yahoo.com)

**Abstract:** *Objectives:* To assess the level of expression of the antiapoptotic signal "Survivin" in Egyptian patients with acute leukemias and to delineate any significant correlation between the level of Survivin with the clinical and hematological findings in those patients. *Patients and Methods:* Survivin expression was quantitatively determined by a real-time PCR technique in 30 acute leukemia patients; ALL and AML in two age groups; pediatric group (<18 years) and adult group ( $\geq 18$  years) and in age and sex matched control healthy subjects. *Results:* Statistically significant higher expression was noted in both ALL and AML groups when compared to the control group (p-value = 0.0001). A statistically significant negative correlation was detected between Survivin expression and RBCs count, HB level and Platelet count with p-values = 0.01, 0.01 and 0.0001 respectively. Positive correlations were found with T.L.C, peripheral blood blasts, bone marrow malignant cells, LDH, ALP and uric acid levels with p-values = 0.0001, 0.0001, 0.03, 0.0001, 0.006 and 0.001 respectively. During the post-induction phase, Survivin expression showed a statistically insignificant difference between patients achieving complete remission and those showing unfavorable response with a p-value = 0.7. After follow up, the expression change between patients achieving complete remission and those showing unfavorable response was statistically insignificant with a p-value = 0.6. *In summary,* The previous data emphasized important correlations between Survivin expression and established risk factors in acute leukemia patients. Thus Survivin could be used as a marker for assessment of bone marrow infiltration that in future could be used to refine treatment stratification.

[Hoda Sadek, Shadia Ragab, Hanaa Rasmy, Nancy M. El Guindy, Wafaa Ezzat, Mona Hamed. Expression of The Antiapoptotic Gene Survivin in Acute Leukemias. Journal of American Science 2010;6(11):716-725]. (ISSN: 1545-1003).

**Key words:** Survivin - Antiapoptosis function - Hematological malignancies

### Introduction

The balance between cell death and cell viability is important in tissue homeostasis. Abnormalities in the control of programmed cell death (apoptosis) play an important role in tumorigenesis (Tazzari et al. 2008). The evolutionarily conserved multi-step apoptosis cascade is regulated by proteins that promote or inhibit apoptotic cell death (Jakubowska et al. 2007). The inhibitor of apoptosis Survivin is a member of the inhibitor of apoptosis protein (IAP) family that suppresses apoptosis or programmed cell death and regulates cell division and thereby integrates apoptosis and cell division (Badran et al. 2004). Six human IAPs have been identified: XIAP, cIAP1, cIAP2, NAIP, livin, and survivin. Because of their important role in regulating apoptosis, IAPs are being investigated as a potential prognostic factor as well as a treatment target in cancer patients (Wrzesień-Kuś et al. 2004). The survivin protein shuttles between the nucleus and the cytoplasm, where it effectively inhibits apoptosis, most likely by binding to second mitochondrial activator of caspase (Smac) (Song et al. 2003).

One of the clinically significant features of survivin is its differential distribution in many cancers compared with its limited expression in normal terminally differentiated tissues (Johnson and Howerth 2004). Very high levels of survivin have been described in a number of different tumors (Ito et al. 2000, Kato et al. 2001, Kawasaki et al. 1998, Lu et al. 1998, Monzo et. 1999, Saitoh et al. 1999, Satoh et al. 2001, Swana et al. 1999, Tanaka et al. 2000 & Yoshida et al. 2001).

Overexpression of several IAPs has been detected in various hematological malignancies, including acute leukemias, myelodysplastic syndrome (MDS), chronic myeloid leukemia (CML), and many types of lymphoid malignancies, such as chronic lymphocytic leukemia (CLL) and diffuse large B-cell lymphoma (DLBCL). Many publications revealed significant correlation between a high level of IAPs, especially of XIAP and survivin, and tumor progression contributing to leukemogenesis due to deregulated apoptosis (Wrzesień-Kuś et al. 2004 & Troeger et al. 2007). The expression of survivin may be a general feature of cancer and survivin alone or with other antiapoptosis genes such as Bcl-2 may

extend the viability of transformed cells and regulate their susceptibility/resistance to apoptosis-based therapy. For this reason survivin may provide an ideal therapeutic target for its selective expression in neoplasia (Ambrosini et al. 1997). Various strategies have been developed to target IAPs for therapeutic purposes in leukemia and lymphoma cells, including small-molecule inhibitors and antisense oligonucleotides (Fulda 2009).

The aim of this study is to assess the level of expression of the antiapoptotic signal "Survivin" in Egyptian patients with acute leukemias and to delineate any significant correlation between the level of Survivin with the clinical and haematological findings in those patients. Hence we can throw some lights upon the prognostic impact of survivin

## **Patients and Methods**

Thirty patients with untreated de' novo acute leukemia were included in the analysis for survivin expression. Patients were selected from the out patient clinic of medical and pediatric oncology departments of the National Cancer Institute (NCI), Cairo University and followed up for one year. According to the French-American-British (FAB) standard there were 15 patients with acute myeloblastic leukemia (AML) and 15 patients with acute lymphoblastic leukemia (ALL) consisting of 25 males and 5 females. The leukemic patients were divided into two age groups; pediatric group (<18 years): 16 patients and adult group ( $\geq 18$  years): 14 patients. The control group included 20 age and sex matched normal healthy volunteers, they were 14 males and 6 females. All patients of this study were treated according to the institute ongoing induction and consolidation regimens. Patients were followed up for one year and outcome was designated either favorable or unfavorable. Favorable outcome was considered when complete remission (CR) was achieved; CR status was determined 4 weeks after induction chemotherapy, it is defined by neutrophil count at least ( $1.5 \times 10^3/\mu\text{L}$ ), platelet count ( $> 100 \times 10^3/\mu\text{L}$ ), BM aspiration and biopsy that demonstrate at least 20% cellularity, < 5% blasts and no auer rods as well as absence of extramedullary infiltration.

Unfavorable outcome was considered with failure of remission, occurrence of relapse or death.

An informed consent was obtained after complete description of the study from each adult participant and from parents or legal guardians of the children participants at the time of enrolment, according to guide line of the local ethical committee of the National Research Center.

Diagnosis of de novo' acute leukemias was made based on morphological and cytochemical assessment [Myeloperoxidase (MPO), Nonspecific esterase (NSE) and NSE with sodium fluoride inhibition] of peripheral blood and bone marrow smears. Complete Immunophenotyping was routinely performed using Flowcytometer Patec III DAKO cytometry for evaluation of acute leukemia. In addition, all patients were subjected to CSF examination, chest x-ray and pelvi-abdominal ultrasound for extramedullary involvement detection. Sera were analyzed for lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and uric acid levels using Synchro CX9PRO.

*Survivin Gene expression* was quantitatively determined by real-time PCR technique. Extra peripheral blood samples were taken into tubes with K<sub>3</sub>EDTA from the patients (study group) and from healthy subjects (control group).

*RNA Extraction and complementary(c) DNA synthesis:* Total RNA was extracted from whole blood using QIAamp RNA Blood Mini kit supplied by QIAGEN worldwide companies (Cat No. 52304) according to manufacturer's protocol. The concentration and purity of RNA was determined by measuring its absorbance at 260 nm (A<sub>260</sub>) and 280 nm (A<sub>280</sub>) in a UV visible spectrophotometer. Total RNA (2  $\mu\text{g}$ ) was reverse-transcribed to complementary DNA (cDNA) according to manufacturing instructions using Thermal Cycler (Biometra, Germany) with kit provided from Applied Biosystems. cDNAs were stored at -20°C until real-time quantitative PCR was performed.

*Gene expression relative quantitation:* Polymerase chain reaction (PCR) amplification reaction mixtures (50  $\mu\text{L}$ ) contained cDNA, survivin forward primer (SF) 5'-ACCAAGGTGAGAAGTGAGGG-3' and Survivin Reverse Primer (SR) 5'-AACAGTAGAGGAGCCAGGG-3'. The Survivin RNA Genbank accession number is NM001168. The TaqMan Probe sequence was GCACCCCGGAGCGGAT. GAPDH primers were: GAPDH Forward Primer (GF) 5'-ACATCGCTCAGACACCATTG-3' and GAPDH Reverse Primer (GR) 5'-GTA GTTGAGGTCAATGAAGGG-3'. GAPDH RNA Genbank accession number is NM33197. The TaqMan Probe sequence CAAGCTTCCCCTCTCAGCC. All primers and probes were synthesized by Applied Biosystems-PerkinElmer.

Thermal cycle conditions included holding the reactions at 50°C for 2 minutes and at 95°C for

10 minutes and cycling for 40 cycles between 95°C for 15 seconds and 60°C for 1 minute. Results were collected and analyzed by Applied Biosystems Perkin Elmer 7300 sequence detection system. Relative quantitation using comparative threshold ( $C_t$ ) was used to determine the change in expression of a nucleic acid sequence (target) in the test and control samples. The  $C_t$  value for any sample was normalized to the endogenous housekeeping gene GAPDH. A negative control was included in each experiment. Survivin Gene expression levels were expressed as copy number per cell (c/cell).

### Statistical Methods:

All data were collected from the patient charts and entered into a computerized spreadsheet. The fit of the data to the normal distribution were tested with Kolmogorov-Smirnov Test. Data was statistically described in terms of range, mean, standard deviation (SD), frequencies (number of cases) and relative frequencies (percentages). Comparisons were made using Student's t-test, One-way ANOVA test and Pearson's correlation coefficients were calculated between the assessed variables. Statistical Package for Social Science SPSS Inc., Chicago, IL, U.S.A. version 9.0 was used for analysis of data. The null hypothesis was rejected with a two sided P value of  $< 0.05$ .

### Results

Frequency distribution of demographic and clinical data of ALL and AML patients are shown in Table 1. Median percentage of leukemic blast cells in peripheral blood was 80% in ALL patients and 53% in AML patients. Frequency distribution of FAB subtypes and therapy response data of AML and ALL patients included in the study are shown in Table 2.

Comparisons between hematological and biochemical data in the pediatric ALL patients, AML patients and control subjects are demonstrated in Table 3. Comparisons between hematological and biochemical data in the adult ALL patients, AML patients and control subjects are demonstrated in Table 4.

Survivin gene was expressed in 60.0% of ALL patients and in 73.3% of AML patients. Statistically significant higher expression was noted in both ALL and AML groups when compared to the control group (p-value = 0.0001) as shown in Figure 1 and Table 5. Survivin gene expression was significantly higher in pediatric groups of both ALL and AML patients than pediatric group of control subjects with a p-value = 0.004 (Figure 2). Also Survivin gene expression was significantly higher in adult groups of leukemia

patients than adult group of control subjects with a p-value = 0.0001 (Figure 3).

In the correlation studies, no statistically significant correlation was found between Survivin gene expression and demographic and clinical data of patients.

A statistically significant negative correlation was detected between Survivin gene expression and RBCs count, HB level and Platelet count with p-values = 0.01, 0.01 and 0.0001 respectively. Positive correlations were found with T.L.C, peripheral blood blasts, bone marrow malignant cells, LDH, ALP and uric acid levels with p-values = 0.0001, 0.0001, 0.03, 0.0001, 0.006 and 0.001 respectively (Table 6).

No statistically significant difference could be determined between L<sub>1</sub> and L<sub>2</sub> FAB subtypes (p-value = 1.0). Regarding immunophenotyping, Survivin expression level in C-ALLA patients showed a mean value of  $1.3 \pm 0.7$  c/cell, whereas Pro B and Pre B patients showed a mean Survivin expression level of  $0.6 \pm 0.8$  c/cell and  $0.8 \pm 0.7$  c/cell respectively with a p-value = 1.0 which is statistically insignificant.

In an attempt to correlate between Survivin expression and some prognostic factors of AML: AML patients with normal LDH level showed a mean Survivin level of  $0.4 \pm 0.3$  c/cell, whereas AML patients with elevated LDH level showed a mean Survivin level of  $1.6 \pm 0.6$  c/cell with a p-value = 0.003 which is statistically highly significant.

During the post-induction phase, Survivin gene expression showed a statistically insignificant difference between leukemic patients achieving complete remission and those showing unfavorable response with a p-value = 0.7. After the follow up period, the difference between mean Survivin gene expression in patients achieving complete remission and those showing unfavorable response was statistically insignificant with a p-value = 0.6 (Table 7).

### Discussion

Survivin has been identified as one of the top 4 transcripts among 3.5 million human transcriptomes uniformly up-regulated in cancer tissues but not in normal tissues (Sugahara et al. 2004). Therefore, we quantitatively determined the expression of survivin by a real-time polymerase chain reaction technique in 30 patients with acute leukemias; acute lymphoblastic leukemia (ALL), and acute myeloblastic leukemia (AML) in two age groups; pediatric group (<18 years) and adult group ( $\geq 18$  years) and in age and sex matched control healthy subjects. Age-related acute leukemia groups might be associated with distinct expression patterns of apoptosis-related molecules

leading to the distinct prognosis in adult and childhood acute leukemia.

In the present study statistically significant higher survivin expression was noted in both ALL and AML groups when compared to the control group. We found statistically insignificant change in Survivin expression level between ALL and AML groups.

Thus, the results of this study provide further evidence that survivin plays a role in the malignant process of acute leukemias. Survivin gene was expressed in 60.0% of ALL patients and in 73.3% of AML patients. This could be attributed to dysregulation of Survivin gene which is normally controlled by p53 and transcriptional factors as the Kruppel-like factor 5 (KLF5). Zhu et al. (2006) demonstrated that the transcription factor KLF5 is widely expressed in childhood ALL and is a positive regulator inducing the expression of the anti-apoptotic protein Survivin. Suppression of apoptosis contributes to leukemogenesis by different mechanisms, including prolonging cell life span, thus facilitating the accumulation of gene mutations, permitting growth factor-independent cell survival, promoting resistance to immune-based cytotoxicity, and allowing disobedience of cell cycle checkpoints which would normally induce apoptosis (Tamm et al. 2004). Carter et al. (2001) demonstrated that Survivin is highly expressed and is cytokine regulated in myeloid leukemias. In their study cytokine stimulation increased survivin expression in leukemic cell lines and in primary AML samples. In cultured primary samples, single-cytokine stimulation substantially increased survivin expression in comparison with control cells, and the combination of G-CSF, GM-CSF, and SCF increased survivin levels even further. Also Badran et al. (2003) reported that the analysis of Ph (+) blastic crisis CML patients (4 patients) revealed positive Survivin expression in all patients (100%). Data collected by Mori et al (2002) showed that Survivin was detected in 17 out of 31 AML patients (54.8%) and 5 out of 7 patients with CML blastic crisis (71.4%). The variation in the frequency of Survivin expression among different investigators is a consequence of variation in the procedures used and the number of cycles performed in PCR assays which may influence the sensitivity and specificity of the detection method. However Tamm et al. (2004) put few limitations and concluded that cryopreservation may alter protein expression in samples. Freezing and DMSO are proapoptotic stimuli potentially influencing expression levels of the proteins measured. Also the average leukocyte count could influence the expression level.

We did not find significant correlation between Survivin expression level and sex, organomegaly and

lymphadenopathy. Survivin expression level showed statistically significant negative correlations with RBCs count, HB level and platelets count. Significant positive correlations with T.L.C, peripheral blood blasts, bone marrow malignant cells, LDH, ALP and uric acid were identified. This could be explained by the antiapoptotic function of Survivin thus offering a survival advantage to malignant cells that dominate the bone marrow at expense of other hematological elements with high cell destruction. These results are in accordance with Oto et al (2007).

With respect to blast immaturity, a significant variation of Survivin expression level could not be identified in ALL patients. Also Troeger et al. (2007) showed that Survivin expression levels were comparable between the different immunophenotypes.

Concerning the outcome of our studied patients, difference in Survivin expression level during the postinduction period between patients who achieved CR and those who didn't was statistically insignificant.

After the follow up period of one year, the leukemic patients who achieved CR showed insignificant change of expression when compared to those with unfavourable response. Tamm et al. (2004) could not determine a prognostic role for survivin in adult AML. Survivin is a negative prognostic marker in a variety of solid tumors and diffuse large B-cell lymphomas (Adida et al. 2000a) For example, it has been reported that survivin expression in neuroblastomas correlates with clinically more aggressive, histologically unfavorable disease (Adida et al. 1998). Adida et al. (2000b) found no significant difference in remission rate or survival in adult AML patients expressing high versus low levels of survivin. Also difference in outcome correlations between investigators could be attributed to various treatment strategies and duration of follow up.

*In summary,* The previous data emphasized important correlations between Survivin expression level and established risk factors in acute leukemia patients. Thus Survivin could be used as a marker for assessment of bone marrow infiltration that in future could be used to refine treatment stratification. Elevated survivin levels could be cytokine regulated, thus explaining the controversy in its prognostic impact. In future Survivin may even serve as a therapeutic target itself. Patients should be assessed for a considerable period of follow up to compare between Survivin level before, during and after therapy and to correlate between patients' outcome and Survivin level on a larger scale.

**Table (1): Frequency distribution of demographic and clinical data of ALL and AML patients included in the study**

Variables	ALL patients N (%)	AML patients N (%)
<b>Sex:</b>		
Males	13 (86.7)	12 (80)
Females	2 (13.3)	3 (20)
<b>Organomegaly:</b>		
<b>Hepatomegaly</b>		
Negative	3 (20)	9 (60)
Positive	12 (80)	6 (40)
<b>Splenomegaly</b>		
Negative	2 (13.3)	8 (53.3)
Positive	13 (86.7)	7 (46.7)
<b>Lymphadenopathy</b>		
Negative	5 (33.3)	11 (73.3)
Positive	10 (66.7)	4 (26.7)
<b>Extramedullary involvement:</b>		
<b>CNS manifestations</b>		
Negative	15 (100)	15
Positive	0 (0.0)	0
<b>Testicular enlargement</b>		
Negative	14 (93.3)	-
Positive	1 (6.7)	-

CNS : Central Nervous System

N : Number

% : Percentage

**Table (2): Frequency distribution of FAB subtypes and therapy response data of AML and ALL patients included in the study**

Variables	ALL Patients N (%)	AML Patients N (%)
<b>FAB Subtypes:</b>		
M <sub>1</sub>		2 (13.3)
M <sub>2</sub>		10 (66.7)
M <sub>3</sub>		2 (13.3)
M <sub>5a</sub>		1 (6.7)
L <sub>1</sub>	2 (13.3)	
L <sub>2</sub>	13 (86.7)	
<b>I.P.T:</b>		
C-ALLA		
ProB	8 (53.4)	
PreB	2 (13.3)	
	5 (33.3)	
<b>Postinduction response:</b>		
CR	10 (66.7)	
PR	4 (26.7)	
Death	1 (6.6)	
<b>Follow up after one year:</b>		
CR	8 (53.3)	
Relapse	3 (20)	
Death	4 (26.7)	
	7 (50)	
	3 (21.4)	

N.B : one AML patient was lost, so 14 AML patients only were assessed after 1 year of follow up.

<b>FAB</b>	: French American British
<b>I.P.T</b>	: Immunophenotyping
<b>C- ALLA</b>	: Common Acute Lymphoblastic Leukemia Antigen
<b>CR</b>	: Complete Remission
<b>PR</b>	: Partial Remission
<b>N</b>	: Number
<b>%</b>	: Percentage

**Table (3): Comparisons between haematological and biochemical data in pediatric groups (< 18 years) of ALL patients, AML patients and control subjects included in the study**

Variables	AML pediatric patients Mean ± SD N = 5	ALL pediatric patients Mean ± SD N = 11	Control pediatric subjects Mean ± SD N = 9	P-value
Age (years)	11.4 ± 4.9	6.4 ± 5.6	8.8 ± 4.7	0.4
Sex (M/F)	5/-	9/2	7/2	
RBCs (x10 <sup>6</sup> /uL)	1.9 ± 0.4a	2.5 ± 0.8a	5.0 ± 0.4b	0.0001*
HB (gm/dL)	5.4 ± 1.4a	6.5 ± 2.0a	13.8 ± 1.5b	0.0001*
T.L.C. (x10 <sup>3</sup> /uL)	110.3 ± 128.4a	39.8 ± 54.6a	9.0 ± 2.0b	0.04*
Platelets (x10 <sup>3</sup> /uL)	32.0 ± 30.5a	46.0 ± 28.0a	281.1 ± 85.1b	0.0001*
P.B blasts (%)	48.4 ± 27.4	52.8 ± 38.6	-	0.9
Bone Marrow malignant cells (%)	56.4 ± 26.5	86.6 ± 17.4	-	0.06
LDH (U/L)	1636.0 ± 1313.9a	798.0 ± 566.7b	297.2 ± 37.6b	0.007*
ALP (U/L)	161.2 ± 61.1	153.0 ± 106.0	89.0 ± 19.7	0.1
Uric acid (mg/dL)	6.8 ± 1.3a	6.9 ± 1.7a	4.6 ± 0.7b	0.001*

RBCs Red Blood Corpuscles

HB Haemoglobin

T.L.C Total Leucocytic Count

P. B Peripheral Blood blasts  
blasts Lactate Dehydrogenase

LDH Alkaline Phosphatase

ALP Number

N Standard Deviation

SD

P-value is significant if <0.05\*

Different symbol indicates significance

**Table (4):** Comparisons between hematological and biochemical data in adult groups (> 18 years) of ALL patients, AML patients and control subjects included in the study

Variables	AML adult patients Mean ± SD N = 10	ALL adult patients Mean ± SD N = 4	Control adult subjects Mean ± SD N = 11	P-value
Age (years)	35.0 ± 12.4	51.3±8.5	35.5 ± 12.3	0.4
Sex (M/F)	7/3	4/-	7/4	-
RBCs ( $\times 10^6/\mu\text{L}$ )	2.3 ± 0.7a	2.1 ± 0.4a	4.7±0.5b	0.0001*
HB (gm/dL)	6.5±1.5a	5.2±0.9a	13.9±1.2b	0.0001*
T.L.C. ( $\times 10^3/\mu\text{L}$ )	44.8±41.4a	126.0 ± 115.5b	6.7±1.8a	0.002*
Platelets ( $\times 10^3/\mu\text{L}$ )	39.3 ± 24.6a	28.8 ± 15.4a	227.2 ± 49.2b	0.0001*
P.B blasts (%)	50.7 ±27.7	77.0 ± 17.2	-	0.3
Bone Marrow malignant cells (%)	71.5±21.4	89.8 ±4.0	-	0.3
LDH (U/L)	973.5 ± 848.3a	2218.8±1259.7b	311.6±45.2C	0.001*
ALP (U/L)	146.0 ±57.8a	291. 8 ± 146.0b	80.4 ± 20.0C	0.0001*
Uric acid (mg/dL)	7.2±1.7a	8.6±1.1a	4.9 ± 1.3b	0.0001*

RBCs Red Blood Corpuscles

HB Haemoglobin

T.L.C Total Leucocytic Count

P.B blasts Peripheral Blood blasts

LDH Lactate Dehydrogenase

ALP Alkaline Phosphatase

N Number

SD Standard Deviation

P-value is significant if < 0.05\*

Different symbol indicates significance

**Table (5):** Comparison Between mean Survivin Gene expression levels (c/cell) in ALL patients, AML patients and Control subjects included in the study

Variables	Minimum	Maximum	Mean ± SD	P-value
ALL patients	0.02	2.3	1.0±0.8a	0.0001*
AML patients	0.03	2.4	1.2 ± 0.8a	
Control subjects	undetectable	0.6	0.2 ± 0.2b	

c/cell copy number per cell

SD standard deviation

P-value is significant if < 0.05\*

Different symbol indicates significance

Table (6): Correlation of Survivin Gene expression level to biochemical and haematological data of AML patients included in the study

Variables	r	P-value
LDH (U/L)	0.8	0.0001*
ALP (U/L)	0.3	0.2
Uric acid (mg/dL)	0.7	0.002*
RBCs ( $\times 10^6/\mu\text{L}$ )	- 0.7	0.004*
HB (gm/dL)	- 0.7	0.002*
T.L.C. ( $\times 10^3/\mu\text{L}$ )	0.7	0.004*
Platelets ( $\times 10^3/\mu\text{L}$ )	- 0.4	0.1
P.B blasts (%)	0.8	0.0001*
Bone Marrow malignant cells (%)	0.7	0.008*

LDH : Lactate Dehydrogenase

ALP : Alkaline Phosphatase

RBCs : Red Blood Corpuscles

HB : Haemoglobin

T.L.C : Total Leucocytic Count

P.B blasts : Peripheral Blood blasts

% : Percentage

**P-value is significant if < 0.05\***

**Table (7): Relation of Survivin Gene expression level (c/cell) to therapy response data of acute leukemia patients (n=30)**

Variables	Mean ± SD	P-value
Postinduction response :		
Favourable (CR) n = 19	1.2 ± 0.8	
Unfavourable (PR, Relapse, Death ) n = 11	1.1 ± 0.8	0.7
Follow up after one year:		
Favourable (CR) n = 12	± 0.8	
Unfavourable (PR, Relapse, Death ) n = 17	1.2 ± 0.7	0.6

*CR* : Complete Remission

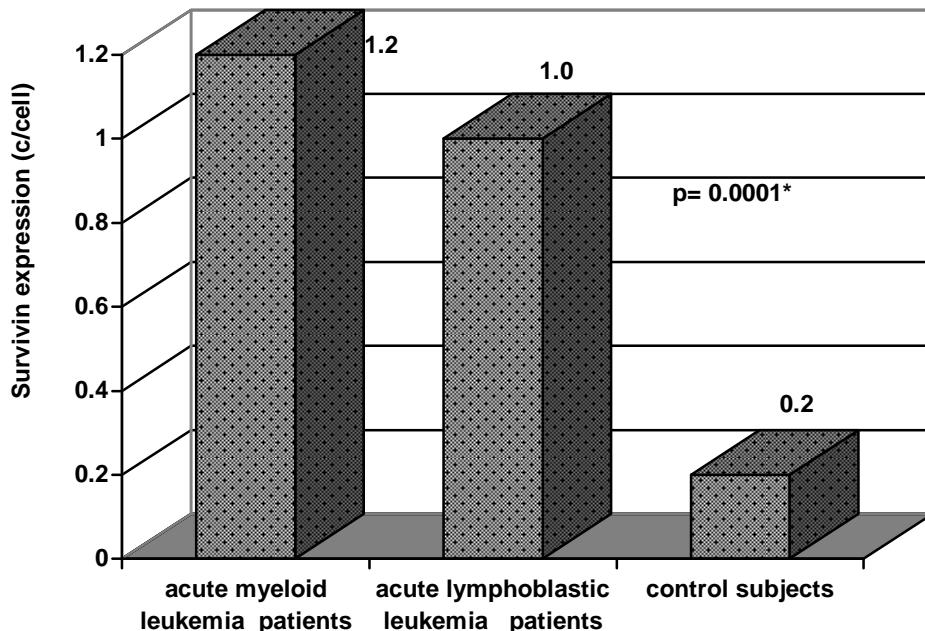
*PR* : Partial Remission

*n* : number

*SD* : Standard Deviation

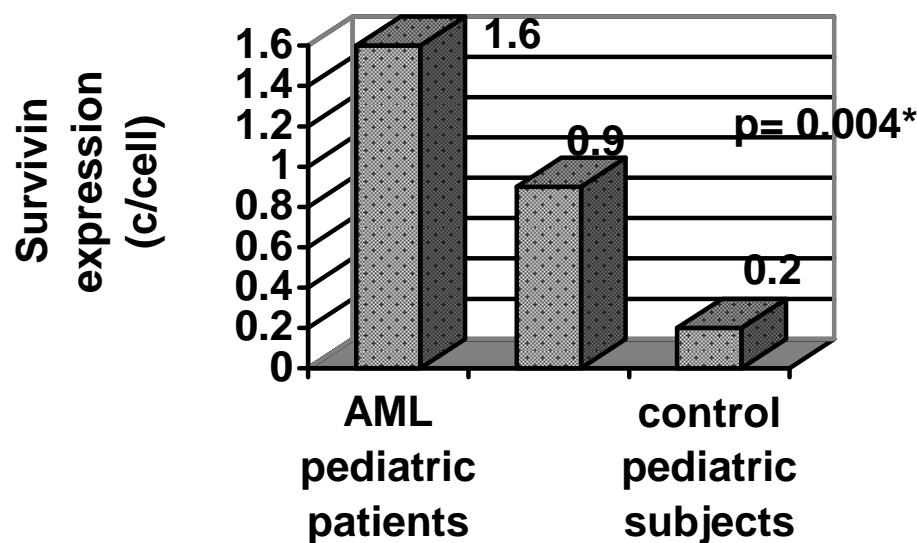
*c/cell* : copy number per cell

**P-value is significant if < 0.05\***



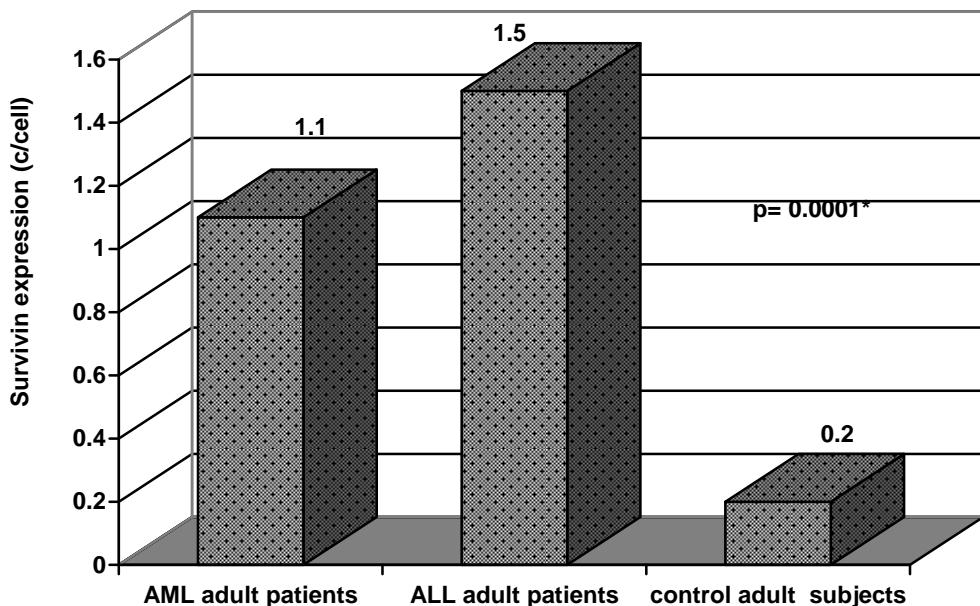
Statistically significant higher expression was noted in both ALL and AML groups when compared to the control group (p-value = 0.0001)

**Figure 1.** Comparison between mean Survivin gene expression levels (c/cell) of AML patients, ALL patients and Control subjects



Survivin gene expression was significantly higher in pediatric groups of both ALL and AML patients than pediatric group of control subjects with a p-value = 0.004

**Figure 2.** Comparison between mean Survivin gene expression levels (c/cell) in pediatric groups (<18 years) of AML patients, ALL patients and control subjects



Survivin gene expression was significantly higher in adult groups of leukemia patients than adult group of control subjects with a p-value =0.0001

**Figure 3.** Comparison between mean Survivin gene expression levels (c/cell) in adult groups (>18 years) of AML patients, ALL patients and control subjects

**\*Corresponding Author:**

Hanaa Rasmy M. Attia  
Clinical and Chemical Pathology Department,  
National Research Center, Egypt  
E-mail: [hanarasmy2000@yahoo.com](mailto:hanarasmy2000@yahoo.com)

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## Biochemical and molecular characterization of three colored types of roselle (*Hibiscus sabdariffa* L.)

Refaei M. Hussein<sup>a</sup>, Yasser E. Shahein<sup>b</sup>, Amr E. El Hakim<sup>b</sup> and Hanem M. Awad<sup>c,\*</sup>

<sup>a</sup>Genetics and Cytology Department; <sup>b</sup>Molecular Biology Department and <sup>c</sup>Department of Tanning Materials and Leather Technology, National Research Centre, El-Behouth St., Dokki; P. Box; 12622; Cairo; Egypt  
[hanem\\_awad@yahoo.com](mailto:hanem_awad@yahoo.com)

**ABSTRACT:** Roselle (*Hibiscus sabdariffa* L.) is a plant which has a considerable industrial, pharmaceutical and economic values in Egypt and many other countries around the world, mainly for its pleasant sepals. There are many colored types of Roselle depends on sepals color. The biochemical and molecular characterization of three roselle types, green (G), light red (LR) and dark red (DR), were studied. RAPD-PCR patterns for their genomic-DNA were significantly different. The total protein electrophoretic profile of their seeds was similar except for some inter-individual variation in band density. Their total protein contents were 46.0, 66.5 and 68.1 mg/g seed, respectively. In addition to the water-soluble antioxidant capacity, the total polyphenolic-content and the antioxidant activity of 12 roselle extracts, three colored types in 2 solvent systems (aqueous, A and 30% ethanolic, E) and 2 extraction temperatures (hot, H and cold, C), were determined by Folin-Ciocalteu reagent and 1,1-diphenyl-2-picrylhydrazyl (DPPH) methods, respectively. The ability of these roselle extracts to inhibit the formation of nitrous acid-induced tyrosine nitration decreases in the order of LREC > DREC > LREH > GEC > DREH > GEH > GAC > DRAH > LRAC > GAH > LRAH > DRAC.

[Refaei M. Hussein, Yasser E. Shahein, Amr E. El Hakim and Hanem M. Awad. Biochemical and molecular characterization of three colored types of roselle (*Hibiscus sabdariffa* L.). Journal of American Science 2010;6(11):726-733]. (ISSN: 1545-1003).

**KEYWORDS:** Antioxidant activity; DPPH; HPLC; molecular characterization; polyphenol content; reactive nitrogen species (RNS); sepals, Roselle (*Hibiscus sabdariffa* L.).

### 1. Introduction

Roselle (*Hibiscus sabdariffa* L.) belongs to *Malvaceae* family and generally regarded as safe (GRAS) as foodstuff. It is commonly used to make jelly, jam, juice, wine, syrup, gelatin, pudding, cake, ice cream and flavors. The brilliant red color and unique flavor make it a valuable food product <sup>(1)</sup>. Many medicinal applications of this plant have been developed around the world. In China, it is used to treat hypertension, pyrexia, and liver damage <sup>(2)</sup>; and lately its sepal extract has been used as an effective treatment against leukemia, due to its high content in polyphenols, particularly protocatechic acid <sup>(3)</sup>. Roselle seeds, which until now do not have any commercial applications, are a source of a vegetable oil that is low-cholesterol and rich in other phytosterols and tocopherols, particularly  $\beta$ -sistosterol and  $\gamma$ -tocopherol. The global characteristics of roselle seed oil allow important industrial applications for this oil. These characteristics represent an added value for the culture of this plant <sup>(4)</sup>.

The fleshy calyces of roselle flowers (sepals) have a pleasant acid taste and very attractive red color, for which roselle is used as a beverage crop in many countries. The beverage drink is known in Egypt as Karkadi and recommended as a mild laxative in the form of acidulous drink. The anthocyanins (**Figure 1**) are responsible for the red color, while the acid taste is due to the presence of some organic acids. Sepals'

acidity may also contribute to their color variation. The dark red colored type has the highest content of anthocyanins followed by the light red colored type, while the green colored type has no or just traces of anthocyanins <sup>(5)</sup>.

Many chemical components present in roselle have potential health benefits and support the ethnomedicinal use of roselle in promoting cardiovascular health and preventing hypertension <sup>(2)</sup>. The red varieties of roselle had greater overall antioxidant and cyclooxygenase inhibitory activity than the white variety, and therefore they would potentially have greater health benefits. However, the white variety could also be used in antihypertensive applications, since its ethyl acetate extract had similar cyclooxygenase inhibitory activity as aspirin and Ibuprofen <sup>(6)</sup>. Nevertheless, further *in vivo* research is required to confirm the specific health benefits in biological systems, before increased consumption of any of the three varieties of roselle could be recommended.

There are few studies on roselle at the molecular level. In this study, we present a molecular characterization based on total protein and DNA level of three colored types of roselle [green (G), light red (LR) and dark red (DR)] (**Figure 2**). The variability of antioxidant capacity of these three colored types of roselle using different biochemical approaches was determined.

## 2. Materials and Methods

### 2.1. Plant Material

Seeds and sepals from three colored types of roselle were collected from previous experiments of our research group at the National Research Center, Egypt.

### 2.2. Chemicals

All chemicals were obtained from Sigma-Aldrich Chemical Co., USA. All reagents and other chemicals were of high analytical grade. The buffers were prepared according to Gomorri<sup>(7)</sup> and Blanchard<sup>(8)</sup> and the final pH was checked by pH meter (Hanna, pH 211 Microprocessor pH meter). *Taq* polymerase and PCR reagents were from Fermentas, Latvia.

### 2.3. DNA Extraction and PCR Amplification Conditions

Genomic DNA of *H. sabdariffa* was extracted from fresh young leaves using the CTAB (cetyltrimethylammonium bromide) extraction method<sup>(9)</sup>. PCR was carried out using a BioCycler TC-S thermal cycler from HVD, Austria. The PCR reactions were developed in a total volume of 50 µl with the following components: 5 µl of 10X reaction buffer (75 mM Tris-HCl, pH 9.0, 50 mM KCl, 20 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and 0.001 % bovine serum albumin), 2 µl of 25 mM MgCl<sub>2</sub>, 4 µl of 2.5 mM/each dNTP mix, 2.5 µl of 20 mM of each primer, 1 µl of *Taq* DNA polymerase (1 U/µl), and template DNA. The volume was completed to 50 µl with deionized, diethylpyrocarbonate (DEPC), water. The following PCR program was used: an initial denaturation of DNA was carried out at 94°C for 2 min followed by 40 cycles including denaturation at 94°C for 1 min, annealing at 37°C for 1 min, extension at 72°C for 2 min and a final extension at 72°C for 12 min. Template DNA was prepared from fresh young roselle leaves. The tubes containing PCR product were stored at -20°C till the next use. For RAPD analysis, the following random primers were used: B05 (5'-TGC GCC CTT C-3'), B08 (5'-GTCCACACGG-3'), B09 (5'-TGGGGGACTG-3'), B11 (5'-GTAGACCCGT-3'), B14 (5'-TCCGCTCTGG-3') and C15 (5'-GACGGATCAG-3').

## 2.4. Protein Determination

### 2.4.1. Protein Extraction

Protein extraction from *H. sabdariffa* seeds was carried out according to Rao and Pernollet<sup>(10)</sup> with minor modifications: 1 g of different types of seeds each was incubated overnight in 10 ml of 0.15 M phosphate-buffered saline (PBS) and 1 mM disodium EDTA, pH 7.2, containing cocktail protease inhibitors (1 mM ethylenediamine tetraacetic acid, ethylene glycol bis *N,N*, *N*, *N*-tetraacetic acid, *N*-ethylmaleimide and phenylmethylsulphonyl fluoride). The mixture was homogenized, filtered, sonicated and then centrifuged at 15,000 × g for 60 min. at 4°C. Supernatants were collected as roselle seed extracts.

### 2.4.2. SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Electrophoretic analysis was performed in the Mini-Protean II Dual-Slab Cell (BioRad, USA). Preparation of

gels, samples and electrophoresis was performed according to Laemmli<sup>(11)</sup>.

### 2.4.3. Estimation of Protein Concentration

Protein concentration was determined according to Bradford<sup>(12)</sup>, using bovine serum albumin as standard protein.

## 2.5. Determination of Total Polyphenol Content (TPC)

The content of total phenolic compounds in each of the 12 extracts was determined according to the Folin-Ciocalteu method<sup>(13)</sup> with some modification to minimize the volume of the reactants used to microlitres. An aliquot of 10 µl of each extract (1 mg/ml) was mixed with 50 µl of Folin-Ciocalteu phenol reagent (10 × dilutions) and allowed to react for 5 min. Then 40 µl of 20% saturated Na<sub>2</sub>CO<sub>3</sub> solution was added and allowed to stand for 1 h in the dark before the absorbance of the reaction mixture was read at 725 nm using a microplate ELISA reader (BioRad). A gallic acid standard curve was obtained for the calculation of phenolic content. The total polyphenol content (TPC) of each extract was expressed as mg gallic acid equivalents per gram of plant material on a dry-weight basis.

## 2.6. Antioxidant Activity Determination

### 2.6.1. Determination of Water-Soluble Antioxidant Capacity (HACT)

Water-soluble antioxidants were extracted as follows: Plant sepals were frozen in liquid nitrogen and later, they were crushed in a hand mortar until obtaining a fine powder, to which a volume of distilled water was added (10 ml/3 g). The homogenized suspension was transferred to polypropylene tubes then shaken for 1 h at room temperature in the dark. The suspension was transferred to Eppendorf tubes and centrifuged at 6000 × g for 10 min; the supernatant was transferred to new Eppendorf tubes and filtered through a centricron (5 kDa cut-off) to eliminate proteins and other macromolecules from the water extract. Later, the resulting supernatant was kept at 4°C until analysis.

Samples of water extracts (5 to 200 µL) were supplemented with 1 ml phosphomolybdenum reagent, homogenized then incubated at 95°C for 90 min. Pure water was used in control reactions. Finally, absorbance at 695 nm was measured. HACT is expressed as equivalents of L-ascorbic acid. Standard curves were constructed with different amounts of L-ascorbic acid dissolved in water. An average extinction coefficient of 213 µM<sup>-1</sup> (*r*<sup>2</sup> = 0.9996) was used for quantitation. Total HACT per gram of wet plant material was obtained with the following formula:

$$\text{HACT} (\mu\text{mol L-ascorbic acid/g}) = A_{695} \times \epsilon^{-1} RV \times SV^{-1} \times EV \times M^{-1}$$

where A<sub>695</sub> is the absorbance at 695 nm, ε<sup>-1</sup> is the inverse of the extinction coefficient (213 µM<sup>-1</sup>), RV is the overall reaction volume, SV is the sample volume used in the reaction, EV is the volume of solvent used in the extraction of the plant material analyzed and M is the amount of fresh plant material extracted in grams. When the assays were performed at 37°C, the total antioxidant capacity was

obtained due to strong water-soluble antioxidants, HACT<sup>37</sup> (14). All determinations were done in triplicate.

#### 2.6.2. Preparation of the Extracts and Standard

A weighed quantities of three colored roselle sepals (G, LR and DR) were extracted with 30% aqueous ethanol (E) and distilled water (A) either at room temperature (C) or boiling temperature (H). This gave 12 samples to analyze: GEH, GEC, GAH and GAC; LREH, LREC, LRAH and LRAC; DREH, DREC, DRAH and DRAC (**Table 1**). A solution of ascorbic acid in water was used as a standard in this study. Serial dilutions of all these solutions were done with their respective solvents.

#### 2.6.3. DPPH Radical Scavenging Assay

The antioxidant activity of the 12 roselle extracts and standard were assessed on the basis of the radical scavenging effect of the stable DPPH free radical (15). 10 µl of each extract or standard (from 0.0 to 100 µg/ml) was added to 90 µl of a 100 µM methanolic solution of DPPH in a 96-well microtitre plate (Sigma-Aldrich Co., St. Louis, MO, US). After incubation in the dark at 37°C for 30 min, the decrease in absorbance of each solution was measured at 520 nm using an ELISA micro plate reader (Model 550, Bio-Rad Laboratories Inc., California, USA). Absorbance of blank sample containing the same amount of either water or 30% aqueous ethanol and DPPH solution was also prepared and measured. All experiments were carried out in triplicate. The scavenging potential was compared with a solvent control (0% radical scavenging) and ascorbic acid. Radical scavenging activity was calculated by the following formula:

$$\% \text{ Reduction of absorbance} = [(A_B - A_A) / A_B] \times 100, \text{ where: } A_B - \text{absorbance of blank sample and } A_A - \text{absorbance of tested extract solution (t = 30 min)}^{(16)}$$

#### 2.6.4. Reaction of Roselle Extracts with Reactive Nitrogen Species

The abilities of different roselle extracts to inhibit nitrous acid-mediated tyrosine nitration were investigated. Equimolar concentrations (400 µM) of tyrosine and NaNO<sub>2</sub> were co-incubated with each extract (0 to 300 µg) in 0.5 M HCl at 37°C for 4 h (17). Snap freezing reaction mixtures prior to HPLC analysis successfully terminated reactions. We monitored the formation of 3-nitrotyrosine (3-NT) from nitrous acid-mediated tyrosine nitration by HPLC analysis with photodiode array detection (see below). 3-NT formed was characterized and quantified by use of an authentic standard (retention time and unique spectral characteristics). Trolox was used as positive control. Artefactual formation of 3-NT during the preparation stage was found to be negligible.

#### 2.6.5. HPLC Analysis

Reaction mixtures were analyzed using reverse-phase HPLC. Analysis was performed on an Agilent 1100 system with a Zorbax ODS C18 column (150 × 4.6 mm i.d., 4 µm) and guard column (15 × 4.6 mm i.d., 4 µm). Mobile phase A consisted of methanol/water/5 N HCl (5/94.9/0.1, v/v/v) and mobile phase B of acetonitrile/water/5 N HCl (50/49.9/0.1, v/v/v). The following gradient system was

used (min/% acetonitrile): 0/0, 5/0, 40/50, 60/100, 65/100, and 65/10 with a flow rate of 0.7 ml/min. The eluent was monitored by photodiode array detection at 280 nm for 3-NT measurements with spectra of products obtained over the 220–600 nm range.

#### 2.7. Statistical Analysis

All experiments were conducted in triplicate (n=3). All the values were represented as mean ± SD. Significant differences between the means of parameters as well as IC<sub>50</sub> were determined by probit analysis using SPSS software program (SPSS Inc., Chicago, IL); with p values < 0.05 considered statistically significant.

Correlation coefficients (R) to determine the relationship between two variables (radical scavenging tests and content of total phenolic compounds) were calculated using MS Excel software (CORREL statistical function).

### 3. Results and Discussion

#### 3.1. RAPD Analysis of Roselle Types

PCR was used for genotypes analysis of roselle (**Figure 3**). The PCR products, which amplified using the random primer B05, were around 1 kb for genotypes green (G) and light red (LR) (**Figure 3A**) and dark red (DR) (**Figure 3B**). The primer B05 showed more specific and distinct bands in case of LR and DR genotypes. LR genotype showed a specific band with molecular size around 700 bp while the DR genotype showed a band around 1.5 kb and two faint amplification products at 800 and 700 bp, respectively. The other random primers B08, B09, B11, B14 and C15 used in this study, to differentiate between roselle genotypes, did not show any visible amplification products.

#### 3.2. Determination of Protein Content of Roselle Seed Extracts

The protein content of three colored roselle seeds revealed that the DR-colored type has the highest protein content (68.1 mg/g) followed by the LR-colored type (66.5 mg/g), while the G-colored type has the lowest protein content (46.0 mg/g).

#### 3.3. Characterization of Protein Content of Roselle Seed Extracts

Protein electrophoretic profiles of roselle seed extracts were very similar, except for some quantitative inter-individual variations. The SDS-PAGE analysis showed approximately 14–15 major protein bands, with relative molecular masses ranging from 14 to 100 kDa (**Figure 4**).

#### 3.4. Total Polyphenol Content (TPC)

As plant polyphenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators, the total amount of phenolic compounds in the 12 roselle extracts was determined using the Folin-Ciocalteu method. The Folin-Ciocalteu phenol reagent is used to obtain a crude estimate of the amount of phenolic compounds present in an extract. Phenolic compounds undergo a complex redox reaction with phosphotungstic and phosphomolybdic acids present in the

reagent. Phenolic compounds respond differently to the Folin-Ciocalteu reagent, depending on the number of phenolic groups they have<sup>(18)</sup>. As shown in **Table 1**, all extracts contain high amount of polyphenolics, to which their antioxidant activity may be ascribed. DREC, GEC, DREH, GEH and DRAC extracts which showed the highest antioxidant activities also had the highest amount of polyphenols. GAC, DRAH, LREH, LREC and GAH extracts which showed moderate antioxidant activities, had moderate TPC as well. LRAC and LRAH extracts showed the lowest TPC and the lowest antioxidant activities. Further studies are in progress to isolate and elucidate the structure of main components present in each extract.

### 3.5. Determination of Antioxidant Activity of Extracts

#### 3.5.1. Water-Soluble Antioxidant Capacity

Water-soluble antioxidant capacity was determined by the phosphomolybdenum method. HACT<sup>37</sup> and HACT represented strong water-soluble antioxidants and total water-soluble antioxidants, respectively<sup>(14)</sup>. It is known that roselle sepals are very rich in vitamin C, anthocyanins, polyphenols and other water-soluble antioxidants<sup>(19)</sup>. In Egypt, however, roselle farmers discard the roselle G-colored type plants, because roselle consumers prefer and demand the DR-colored type sepals<sup>(20, 21)</sup>. As shown in **Figure 5**, sepals of DR type showed the highest levels of total water-soluble antioxidant capacity followed by G type then LR type. These results are consistent with recent study on roselle sepals' water-soluble antioxidant capacity<sup>(4)</sup>.

On the contrary, G type showed the highest levels of strong water-soluble antioxidant capacity, followed by LR then DR types. These results are in agreement with the study of Rady et al.<sup>(22)</sup>. A high level of strong water-soluble antioxidant capacity in sepals of G type suggests that, there are abundant levels of vitamin C, and other strong water-soluble antioxidants, this fact would support the recommendation of consuming both DR- and G-colored types of roselle sepals.

#### 3.5.2. DPPH Radical Scavenging Activity

The stable DPPH radical has been used widely for the determination of primary antioxidant activity, that is, the free radical scavenging activities of pure antioxidant compounds, plant and fruit extracts and food materials. The assay is based on the reduction of DPPH radicals in methanol which causes an absorbance drop at 520 nm. Radical scavenging activities of the 12 roselle extracts were measured using model colorimetric test, DPPH radical scavenging test. The results are summarized in **Table 2**. GEH, DREH, DREC, GEC and DRAC showed the highest DPPH free radical scavenging activities. GAH, LREH, LREC, DRAH and GAC also showed relatively high DPPH free radical scavenging activities. Both LRAH and LRAC showed the lowest DPPH free radical activities. These results are in agreement with both HACT<sup>37</sup> and HACT results.

#### 3.5.3. Reaction of Different Roselle Extracts with Reactive Nitrogen Species (RNS) Derived from Nitrous Acid

Acidification of nitrite is known to generate a number of RNS, including N<sub>2</sub>O<sub>3</sub>, which is capable of nitrosating secondary amines and amides. The gastric formation of N-nitrosocompounds has been suggested to be a major source of human exposure to this class of environmental carcinogens<sup>(23)</sup>. It has been established that compounds such as  $\alpha$ -tocopherol and ascorbic acid react with nitrite, and hence are able to inhibit endogenous nitrosation<sup>(24)</sup>. More recently, the ability of certain polyphenols to inhibit tyrosine nitration mediated by nitrite in acid has been demonstrated *in vitro*<sup>(25)</sup>. This prompted our interest in studying the potential of 12 different roselle extracts as potential scavengers of RNS derived from nitrous acid. In order to monitor the potential of different extracts to react with RNS derived from nitrous acid, a model system was employed. In this study the ability of 12 different roselle extracts, to inhibit the formation of nitrous acid-induced tyrosine nitration, was investigated, for the first time, under conditions akin to those in the gastric milieu of humans. Exposure of tyrosine to nitrous acid in the presence of different extracts resulted in a significant inhibition of 3-NT formation in a dose-dependent manner (**Figure 6**). The IC<sub>50</sub>'s for the inhibition of 3-NT formation by different extracts are shown in **Table 2**. LREC, DREC, LREH and GEC extracts are very effective at a relatively low concentration. DREH, GEH, GAC and DRAH extracts showed moderate inhibition. However, LRAC, GAH, LRAH and DRAC extracts showed relatively low inhibition activities.

### 3.6. Statistical Analysis

With reference to Table 1, the correlations of TPC against the antioxidant activity based on the DPPH assay involving all 12 extracts were very high reflected by the high correlation coefficient (0.86), confirming that phenolic compounds contribute to the radical scavenging activity of these extracts. While, there were no correlations of TPC against IC<sub>50</sub> of 3NT-inhibition and DPPH against IC<sub>50</sub> of 3NT-inhibition reflected by their negative correlation coefficients (-0.49 and -0.42, respectively).

In Summary, the molecular studies on three colored types of roselle (*Hibiscus sabdariffa*) presented in this article suggested that the total protein electrophoretic profiles of these three types of roselle seeds were similar, while, their RAPD-PCR patterns were significantly different. The biochemical studies on these three roselle colored types revealed that, all three types could be used as a considerable source of strong antioxidants. The sepal extracts of these three types of roselle showed important abilities to react with the biologically relevant reactive nitrogen species (RNS) by inhibiting nitrous acid-mediated tyrosine nitration, using different solvents and extraction temperatures. The characteristics of the green sepals roselle type revealed that, it could not be less important than the other roselle colored types in the industrial and pharmaceutical applications. These characteristics support the ethnomedicinal use of roselle in Africa and the Caribbean and suggest that all the three types of roselle could potentially provide health benefits. In addition, our findings present helpful information to food markets and small farmers and may help increasing the roselle

marketability and profitability. Further studies are required to evaluate the contribution of these three types of roselle to the specific health benefits in biological systems, before

increased consumption of any of these three colored types could be recommended.

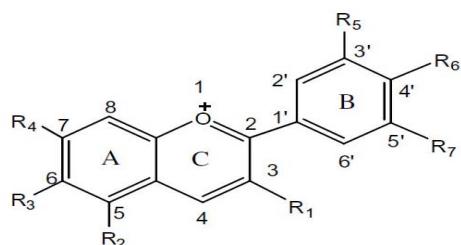
**Table 1. Full description of the abbreviations used in the text.**

Abbreviation	Description
DREH	Dark red roselle sepals (DR) extracted with 30% aqueous ethanol (E) at boiling temperature (H).
DREC	Dark red roselle sepals (DR) extracted with 30% aqueous ethanol (E) at room temperature (C).
DRAH	Dark red roselle sepals (DR) extracted with distilled water (A) at boiling temperature (H).
DRAC	Dark red roselle sepals (DR) extracted with distilled water (A) at room temperature (C).
LREH	Light red roselle sepals (LR) extracted with 30% aqueous ethanol (E) at boiling temperature (H).
LREC	Light red roselle sepals (LR) extracted with 30% aqueous ethanol (E) at room temperature (C).
LRAH	Light red roselle sepals (LR) extracted with distilled water (A) at boiling temperature (H).
LRAC	Light red roselle sepals (LR) extracted with distilled water (A) at room temperature (C).
GEH	Green roselle sepals (G) extracted with 30% aqueous ethanol (E) at boiling temperature (H).
GEC	Green roselle sepals (G) extracted with 30% aqueous ethanol (E) at room temperature (C).
GAH	Green roselle sepals (G) extracted with distilled water (A) at boiling temperature (H).
GAC	Green roselle sepals (G) extracted with distilled water (A) at room temperature (C).
HAECT	Hidrosoluble Antioxidant Capacity Total

**Table 2. Antioxidant activity, total polyphenol content and IC50 of roselle sepal extracts. Antioxidant activity was based on the ability to scavenge DPPH free radicals. Total phenolic content (TPC) was determined by the Folin-Ciocalteu method and IC50 was determined by the inhibition of nitrous acid-mediated 3-nitrotyrosine formation.**

IC50 ( $\mu$ M) Means $\pm$ SD (n = 3)	TPC mg/g plant extract Means $\pm$ SD (n = 3)	Decrease of DPPH absorbance Means % $\pm$ SD (n = 3)	Extract name
84.801 $\pm$ 1.796*	29.363 $\pm$ 0.597	64.678 $\pm$ 4.546	DREH
33.864 $\pm$ 0.986*	32.691 $\pm$ 1.873	63.761 $\pm$ 3.032	DREC
139.583 $\pm$ 2.553*	21.763 $\pm$ 1.509	48.165 $\pm$ 7.392	DRAH
191.065 $\pm$ 1.926*	26.867 $\pm$ 2.316	61.926 $\pm$ 3.301	DRAC
38.655 $\pm$ 1.224*	20.683 $\pm$ 1.438	48.969 $\pm$ 2.547	LREH
29.011 $\pm$ 0.967*	21.377 $\pm$ 1.702	48.323 $\pm$ 4.893	LREC
180.451 $\pm$ 2.893*	17.354 $\pm$ 0.639	29.893 $\pm$ 3.179	LRAH
163.836 $\pm$ 1.859*	19.604 $\pm$ 1.692	29.012 $\pm$ 2.972	LRAC
86.412 $\pm$ 1.132*	28.201 $\pm$ 2.641	65.596 $\pm$ 4.013	GEH
60.645 $\pm$ 2.219*	31.033 $\pm$ 1.205	62.385 $\pm$ 4.065	GEC
174.586 $\pm$ 5.262*	20.035 $\pm$ 2.724	53.758 $\pm$ 3.891	GAH
107.173 $\pm$ 5.663*	22.042 $\pm$ 1.481	48.073 $\pm$ 8.078	GAC
--	--	83.027 $\pm$ 1.079	ascorbic acid
--	--	81.651 $\pm$ 2.098	rutin
15.260 $\pm$ 1.280*	--	--	trolox

\* = P < 0.001



R<sub>1</sub> = O-Sugare (glucose, arabinose, galactose)

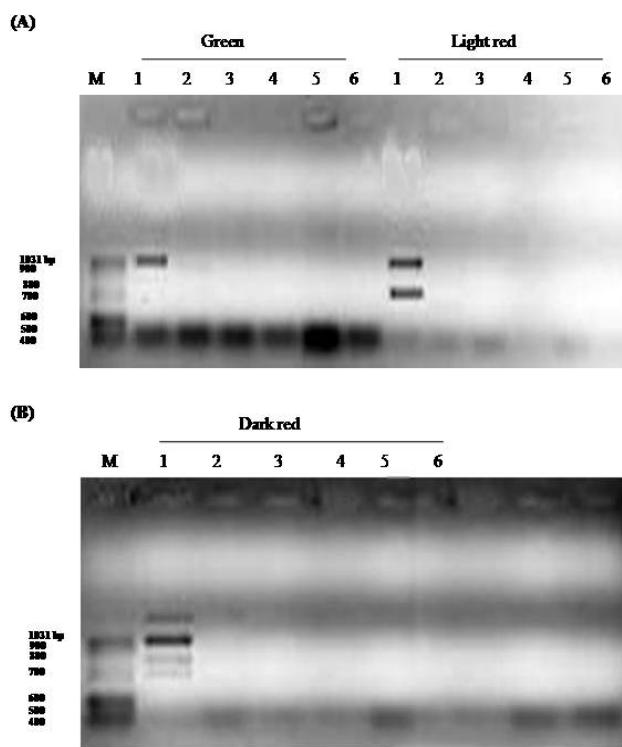
R<sub>2</sub>, R<sub>4</sub>, R<sub>6</sub> = OH

R<sub>3</sub> = H and R<sub>5</sub>, R<sub>7</sub> = H, OH, OCH<sub>3</sub>

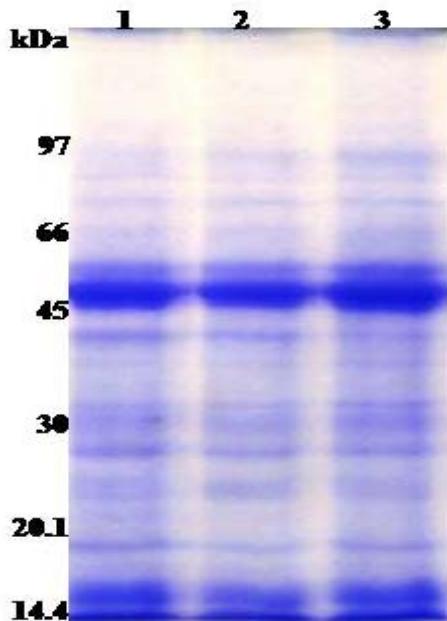
**Figure 1.** Structure of anthocyanins



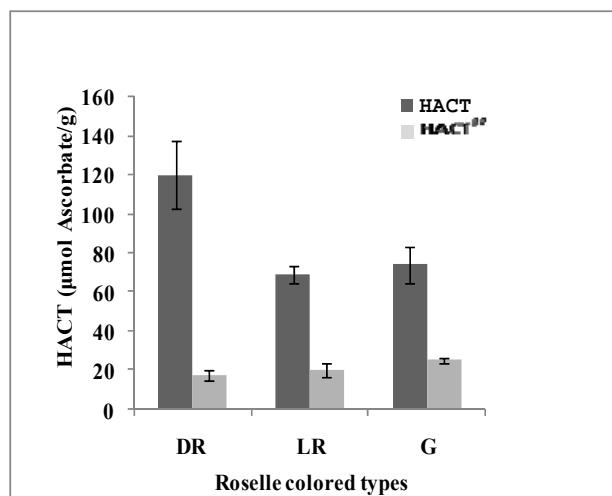
**Figure 2.** Sepals of three colored types of roselle (*H. sabdariffa*). Green (G), light red (LR) and dark red (DR).



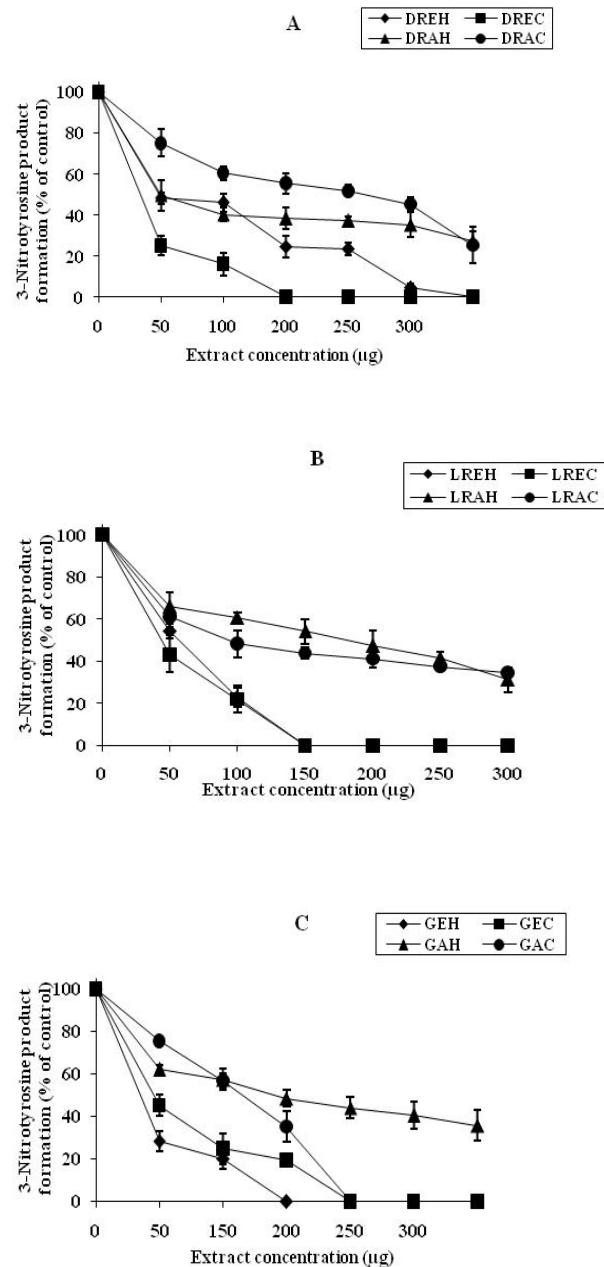
**Figure 3.** RAPD-PCR amplification products of three colored types of roselle genomic DNA. (A) G and LR genotypes and (B) DR genotype. 1 to 6 refers to the random primers (B05, B08, B09, B11, B14 and C15, respectively). M is the 100 bp DNA ladder.



**Figure 4.** SDS-PAGE analysis of different protein extracts from roselle seeds. Gel was stained with Coomassie Brilliant blue R-250. The different lanes correspond with the following samples: G (1); LR (2) and DR seeds (3).



**Figure 5.** Water-soluble antioxidant capacity. Samples of three colored types of roselle plant sepals were extracted with water, and aqueous extracts were analyzed for their water-soluble antioxidant capacity (HACT and  $\text{HACT}^{37}$ ) by the phosphomolybdenum method. Data are the average of 3 independent determinations. Standard deviation was always below 10%.



**Figure 6.** Inhibition of nitrous acid-induced formation of tyrosine nitration by 12 roselle extracts. Each roselle extract (0 to 300  $\mu\text{g}$ ) was exposed to 400  $\mu\text{M}$  nitrite in 0.5 N HCl for 4 h at 37 °C in the presence of tyrosine (400  $\mu\text{M}$ ) to mimic the environment of the stomach. Results are expressed as percentage inhibition of 3-NT formation and were obtained from three independent experiments performed in triplicate and presented as means  $\pm$  SD.

**Acknowledgements**

This work was supported financially by the Science and Technology Development Fund (STDF), Egypt, Grant No 260.

\* Corresponding author. Tel: 002-012 5523 802; Fax: 002-02-337 0931.

E-mail address: [hanem\\_awad@yahoo.com](mailto:hanem_awad@yahoo.com) (H. M. Awad).

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## Management of obstructive sleep apnea using oral appliance with magnetic versus increase vertical dimension

Mohamed. A. Saad-Eldeen<sup>1</sup>, Shawky M. Elmorsy<sup>2</sup>, Shaza. M. Hammad<sup>3</sup>

<sup>1</sup> Ass. Prof., Prosthodontic Department, Faculty of Dentistry, Mansoura University

<sup>2</sup> Ass. Prof, ENT Department, Faculty of Medicine, Mansoura University

<sup>3</sup>Lecturer, Orthodontic Department, Faculty of Dentistry, Mansoura University.

[mohamed.elkhodary@hotmail.com](mailto:mohamed.elkhodary@hotmail.com)

### Abstract

**Statement of Problem:** Oral devices may be helpful in the management of obstructive sleep apnea by improving upper airway potency. **Purpose:** Management of obstructive sleep apnea using oral appliance with magnetic versus oral appliance with increased vertical dimension. **Material and Methods:** 12 patients with mild to moderate obstructive sleep apnea were evaluated in this study before and after wearing devices for six months. The patients randomly divided into two equal groups(A and B ). Group A used oral appliances with magnetic for six month Patients in group B wear oral appliances with increased vertical dimension, Evaluation was done by Polysomnograph, clinical findings and cephalometric x-rays. **Results:** The results of this study revealed that improvement of clinical finding, symptoms and apnea index for patients wearing two types of oral appliance. **Conclusions:** It can be concluded that oral appliance, with magnetic and increase vertical dimension, make improvement for OSA patients oral appliances with magnets are more effective in management of mild and moderate obstructive sleep apnea in comparison to appliances with increased vertical dimension.

[Mohamed. A. Saad-Eldeen, Shawky M. Elmorsy, Shaza. M. Hammad. Management of obstructive sleep apnea using oral appliance with magnetic versus increase vertical dimension. Journal of American Science 2010;6(11):734-741]. (ISSN: 1545-1003).

**Keywords:** Management; obstructive; sleep; apnea; oral appliance; magnetic; dimension

### Introduction

Obstructive sleep apnea (OSA) is repetitive episodes of upper air way obstruction during sleep, arterial oxygen desaturation, daytime hypersomnolence and snoring.<sup>(1)</sup> In addition, these patients are at increased risk for hypertension, heart failure myocardial infarction and stroke and road and traffic accidents when compared with general population.<sup>(2,3)</sup> The site of sleep dependent obstruction in OSA is usually in the retro palatal or the retroglossal region or both, the upper air way lumen.

Several studies demonstrate that oral appliances can be a useful alternative to positive air way pressure with mild to moderate sleep apnea .<sup>(4,5)</sup> There is also robust evidence of the efficacy of oral appliances for improving polysomnographic indices and modifying the health risk associated with OSA.<sup>(6)</sup>

Oral device may be helpful in the management of OSA by; improving upper air way potency, increasing the cross sectional area or decreasing the upper air way collapsibility by increasing the muscle tone .<sup>(7)</sup>

Orthodontists who get involved in this form of therapy are often surprised at how grateful their

patients are after only a few nights of sleep without interruption and the subsequent restoration of adequate rapid eye movement (REM) sleep.<sup>(8)</sup>

The main advantages of oral devices are their relative simplicity of the treatment, reversibility , cost effectiveness and providing a genuine non surgical alternative to patients who can't tolerate continuous positive airway pressure devices ( CPAP) or who represent a poor surgical risk . Also, they enhance craniofacial growth.<sup>(9)</sup>

The US FDA approved 16 devices for use in sleep apnea oral appliances as an alternative to CPAP therapy. They are designed to keep upper air way open.<sup>(10)</sup>

Although Mandibular Advancement Device (MAD) has positive effect in treatment of OSAS, it has multiple complications. These may include craniofacial change in maxillomandibular relationship and bony dimensions, overbite alteration, tooth pain and TMJ problems.<sup>(11)</sup>

In 2003, Iranhoe<sup>(12)</sup> suggested other devices claimed to increase vertical jaw separation. Frantz, 2001<sup>(13)</sup> mentioned that the effectiveness of such appliance improved when increasing vertical opening exceeding the rest position up to 5mm opening and

had comparable success to MADS. A magnetic appliance were used for treatment of obstructive sleep apnea.<sup>(14)</sup>

The aim of this study was to evaluate the effect of magnetic appliance versus increase vertical dimension on management of obstructive sleep apnea

#### **Material and methods:-**

This study was carried out on 12 patients selected from E.N.T clinic, Faculty of Medicine, Mansoura university. The patients suffered from mild or moderate OSA as evaluated by polysomnographic (PSG).

The following steps were done for each patient:

- 1) Apnea hypopnea index (AHI) (average number of apnea events per hour of sleep) was evaluated by PSG, clinical findings and symptoms were recorded. Any caries and perioral infection were also treated.
- 2) Lateral cephalogram was taken for each patient in centric occlusion, then traced and examined.
- 3) Upper and lower primary and secondary impression were made to obtain master cast. The patients were then classified into 2 groups; including 6 patients each, according to the appliance used:

#### **Group A:**

For this group, oral appliance with magnets was constructed as follows:

- 1) Mounting upper cast by using face bow and lower cast by centric occlusion record was done, undercuts and large diastema were blocked out with a thin mix of plaster.
- 2) The upper & lower teeth were waxed up occlusally, buccally and lingually to the height of contour of teeth then packing, curing and finishing to obtain upper and lower acrylic appliance device.
- 3) Fixation of magnets, in each splint two parylene coated (poly-para-xylene 250 $\mu$ m) neodymium-iron-boron magnets (Size 6.4mmØ x 2mm) (Ortho Organizers-Hanover- Germany) of the same pole in area of premolar & molar using self cure acrylic resin (to obtain repulsion force and prevent mouth closing) was done. Fig 1
- 4) A polysomnograph evaluation for one night and recording clinical findings and symptoms during insertion and after 4, 8 months of device insertion were done. Cephalometric radiograph was also taken after insertion, then after 8 months of appliance wear.

#### **Group B:**

For this group, oral appliance with increased vertical dimension of occlusion (about 5mm exceeding the rest dimension) was done as follows.<sup>(15)</sup>

1) After adapting one layer of warm modeling wax over standing teeth, it was converted into clear acrylic resin templates.

2) Accurate adaptation of acrylic template inside the patient mouth after determining the vertical dimension of rest was done.

3) While the patient was closing, the upper and lower acrylic templates were sealed in his mouth at the vertical dimension of rest using soft compound.

4) Converting the modeling compound into chemical cured acrylic resin by using Heaper duplicate was followed.

5) After finishing and polishing, insertion in patient's mouth (Fig 2) and checking adaptation, instructions were given to the patient to use appliance during sleep and to have good home care.

A polysomnograph evaluation for one night, cephalometric radiograph and clinical finding and symptoms were recorded.

6) After one month from appliance insertion, upper and lower templates were separated and reinserted into patient mouth with softened compound for recording the new increased vertical dimension (5mm from rest).

8) The modeling compound was again converted into chemical cured acrylic resin and steps 5 and 6 were made, then follow up after 2, 4, 8 months as in group A.

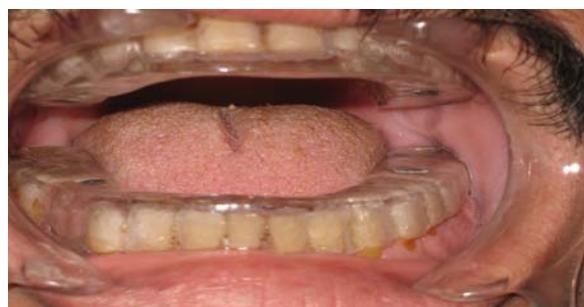
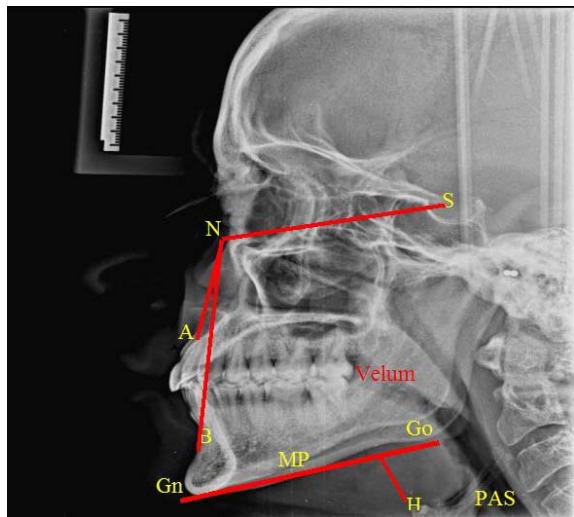


Fig 1: Oral appliance of group A with magnets.



Fig 2: Oral appliance with increased vertical dimension of group B.

### Evaluation of patients:-



**Figure 3:** Lateral cephalometric radiograph showing the Cervico-craniofacial skeletal reference points and lines used for linear and angular measurements. (A: subspinale : the most posterior midline point in the concavity between the anterior nasal spine and the lowest point on the alveolar bone overlying the maxillary incisors, B: Supramentale : the most posterior midline point on the anterior concavity of the mandibular symphysis, N: Nasion : the most anterior point of the frontonasal suture in the median plane, SNA: Maxilla protrusion angle, SNB: mandibular protrusion angle, ANB: maxilomandibular discrepancy, ANS-PNS: sagittal length of maxilla, GN-GO: length of the body of the mandible , MP-H: mandibular plane hyoid distance , P: The most lower point of soft palate, PAS: posterior airway space retroglossal).

OSA is heterogeneous disorder rather than a single disease, therefore, evaluation by multi parametric procedure successively including a clinical findings items, resolution of symptoms and the course of a simple indicator of the quality of breathing sleep were done.

#### a) Cephalometric evaluations:

Lateral cephalometric radiographs with the teeth in occlusion were obtained for all subjects before the start of treatment and after 8 months. All cephalometric films were traced.

According to De Almeida et al.<sup>(16)</sup>, Cervico-craniofacial skeletal reference points and lines were used for linear and angular measurements as follow:

SNA angle measures the projection, anterior or posterior, of the maxilla. The reference range value is  $82 \pm 2^\circ$ . SNB angle measures the position of the mandible. The reference range value is  $80 \pm 2^\circ$ . Less than this is considered retrognathia. ANB angle measures the position of the maxilla with the mandible. The reference range value is  $2^\circ$ . This measures prognathism. PAS or retroglossal space; the reference range is 10-16 mm. MP-H is the distance between the mandibular plane (MP) and the hyoid bone (H). The reference range is 11-19 mm. The longer the distance, the higher the possibility of the patient having OSA (Fig. 3).

b) Clinical findings which help in the subjective assessments of OSA. Patients with high risk of sleep apnea were those who met two of the following three criteria :- Snoring(S), persistent day time sleepiness or drowsiness while driving(P) and obesity or hypertension(H)<sup>(17)</sup>.

c) Frequency of sleep apnea- hypopnea index before and after treatment using polysomnograph. (AHI) Apnea hypopnea index: (the number of apnea and hypopneas average per hour of sleep; which is the total number of apnea during sleep divided by the total number of hours of sleep). This index measures the severity of the apnea.

Traditionally an AHI of 5 or more has been used to define the presence of OSA by dividing the sum of hours.

The American academy of sleep medicine<sup>(18)</sup> classified the severity of sleep apnea as follow :

Mild: RDI score between 5 and 15 apneas or hypopneas per hour of sleep.

Moderate: RDI score between 16 and 30.

Severe: RDI score higher than 30.

### Results:

#### Clinical finding & symptoms:-

The score of clinical finding ranged from 0 to 3: Score: 0= never, 1=slight chance, 2 = moderate chance, 3=high chance of clinical finding symptoms. The patients were evaluated during insertion of the devices, after 4months and after 8 months.

Table 1: Cephalometric parameters of group A and group B patients after appliance insertion and after 8 months of oral appliance wear.

Patients		1	2	3	4	5	6
group A							
-SNA	1	85	80	82	82	85	81
	2	83	80	82	82	84	80
SNB	1	78	75	75	81	81	79
	2	81	79	79	82	83	83
-ANB	1	4	5	5	1	5	4
	2	2	2	1	0	2	3
-GoGN	1	6	6.2	7	5	5	6
	2	6.2	6.5	6.5	5.2	5	6.1
MPH	1	2	2.5	2.3	2.8	3	2.3
	2	1.5	2.2	1.5	1.8	1.6	1.7
-PAS	1	7	1.1	0.9	1.9	1.9	1.6
	2	11	1.8	1.1	2.4	2.3	1.8
group B							
-SNA	1	90	82	83	82	82	82
	2	87	82	82	82	82	81
SNB	1	88	78	77	81	80	75
	2	89	81	79	80	81	79
-ANB	1	2	4	6	2	2	7
	2	1	2	4	1	1	4
-GoGN	1	6.2	6.7	6	6.2	5	5.1
	2	6.5	6.5	6.1	6	5.5	5.5
MPH	1	3.1	2.2	3	3.2	2.6	3
	2	2.7	1.5	1.6	1.5	2	2.7
-PAS	1	1	1	1	2	1.5	1.3
	2	1.5	1.1	2	2.5	2.2	1.9

1-after appliance insertion.

2-after 8 months from appliance wear.

Table 1 Shows clinical findings and symptoms for group A,B during insertion. There were 3 patients with high chance of snoring and persistent sleep. 3 patients had moderate chance of snoring and persistent sleep. 2 patients had high chance of hypertension and 4 patients had moderate chance of hypertension.

Table 2 demonstrates also the result of apnea hypopnea index . It was found that for group A : during insertion 4 patients had moderate OSA, 2 patient had mild OSA. After 4 months, 2 patients had moderate OSA and 4 patients suffered from mild OSA. After 8 months, 3 patients had moderate OSA and 3 patients had no apnea. For group B: during insertion 3 patients had moderate OSA and 3 patients had mild OSA. After 4 months, 2 patients had moderate OSA and 4 patients had mild OSA. After 8 month, only one patient did not suffer from OSA while the other 5 patients had mild OSA.

Table 2: Clinical findings and symptoms: Snoring (S), persistent day time sleepiness or drowsiness while driving (P), hypertension (H) and Apnea hypopnea index (AHI), for group A, B during insertion, after 4 months and after 8 months:

Clinical finding		S	S4	S8	P	P4	P8	H	H4	H8	AHI	AHI4	AHI8
GroupA	1	3	2	2	3	2	0	3	2	20	15	10	
	2	2	1	1	2	1	0	2	1	25	20	9	
	3	3	2	1	2	1	1	2	2	22	17	7	
	4	3	1	1	3	1	0	3	2	10	7	2	
	5	2	1	1	3	2	1	2	1	15	8	3	
	6	2	1	1	2	1	0	2	1	18	9	4	
GroupB	1	3	2	2	3	2	1	3	2	25	20	15	
	2	2	1	1	2	1	2	2	2	15	12	10	
	3	3	2	1	3	2	1	3	2	20	17	12	
	4	2	2	1	3	2	2	2	2	12	10	7	
	5	3	2	1	2	1	1	2	2	10	7	4	
	6	2	2	2	2	1	1	2	2	17	10	7	

Apnea hypopnea index (AHI):- Mild = 5-15, Moderate = 15 -30, Severe < 30.

Table (3) Mann Whitney U test for comparison between group A and B clinical finding and Cephalometric analysis ( Snoring (S), persistent day time sleepiness or drowsiness while driving (P) , obesity or hypertension (H) and Apnea hypopnea index (AHI) , for group A , B during insertion, after 4 months and after 8 months:

variable	Mean± SD		Z	P
	Group A	GroupB		
S04	1.17±.41	.67±.52	-1.687	.092
S04p	47.22±12.55	25.00±20.41	-1.950	.051
P04	1.17±.41	1.00±.00	-1.000	.317
P04p	47.22±12.55	41.67±9.13	-.802	.423
H04	.83±.41	.33±.52	-1.682	.093
H04p	36.11±19.48	11.11±17.21	-2.047	.041*
AHI04	5.67±2.07	3.83±1.83	-1.580	.114
After 8 months				
S08	1.33±.52	1.17±.75	-.365	.715
S08p	52.78±12.55	44.44±25.09	-.424	.672
P08	2.17±.75	1.17±.75	-1.950	.051
P08p	86.11±22.15	44.44±25.09	-2.316	.021*
H08	.83±.41	.67±.52	-.638	.523
H08p	36.11±19.48	27.78±22.77	-.682	.495
AHI08	12.50±3.08	7.33±2.34	-2.347	.019*
Cephalometric analysis				
SNAb	82.50±2.07	83.50±3.21	-.685	-.685*
SNAa	81.83±1.60	82.67±2.16	-.343	.732
SNAc	.67±.82	.83±1.17	-.087	.930
SNAcp	.81±.99	.98±1.35	-.085	.932
SNBb	78.17±2.71	79.83±4.54	-.407	.684
SNBa	81.17±1.83	81.50±3.78	-.494	.622
SNBc	-3.00±1.26	-1.67±1.75	-1.401	.161
SNBcp	-3.71±1.60	-2.07±2.21	-1.212	.226
ANBb	4.00±1.55	3.83±2.23	-.164	.870
ANBa	1.67±1.03	2.17±1.47	-.333	.739
ANBc	2.33±1.21	1.67±.82	-1.012	.312
ANBcp	138.89±141.68	87.50±20.92	-.665	.506
GoGnb	5.87±.77	5.87±.67	-.327	.744
G0Gna	5.92±.66	6.02±.45	-.082	.935
GoGnc	-.05±.29	-.15±.30	-.645	.519
Gognep	-.94±4.54	-2.70±5.22	-.643	.520
MPHb	2.48±.37	2.85±.38	-1.615	.106
MPHa	1.72±.26	2.00±.57	-.573	.567
MPHc	.77±.39	.85±.57	-.080	.936
MPHcp	46.44±25.25	50.57±41.42	-.241	.810
PASb	2.40±2.29	1.30±.40	-.969	.332
PASA	3.40±3.75	1.87±.50	-.402	.688
PASC	-1.00±1.48	-.57±.29	-.405	.686
PAScp	-23.80±11.21	-29.30±13.79	-.480	.631

The statistical analysis of data obtained in the present study was done by using excel program and spss program statistical package for Social Sciences version 10.<sup>(19)</sup> For all statistical analyses, the significance level was set at  $P < .05$ .

By using Mann Whitney U test, the polysomnographic findings revealed that the mean percent changes in AHI values for the 2 groups before and after 4 and 8 months following the insertion of oral appliances. There was a significant ( $P=.041$ ) in H04p in group A patients in comparison to group B patients. Also, P08 was significantly higher in group A patients in comparison to group B patients. Ah08 was significantly higher in group A patients compared to group B patients. On the other hand, SNA<sub>b</sub> was significantly higher in group B patients in comparison to group A patients.

Fig 4 shows improvement Apnea hypopnea index for group A more than group B.

Fig 5,6,7 show improvement hypertension (H), persistent day time sleepiness or drowsiness while driving (P) , Snoring (S) for group A more than group B.

**Fig. 4**

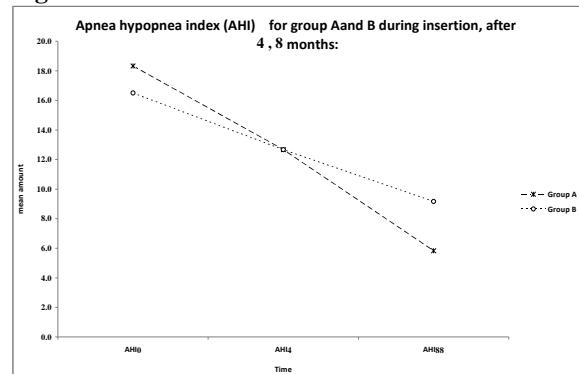
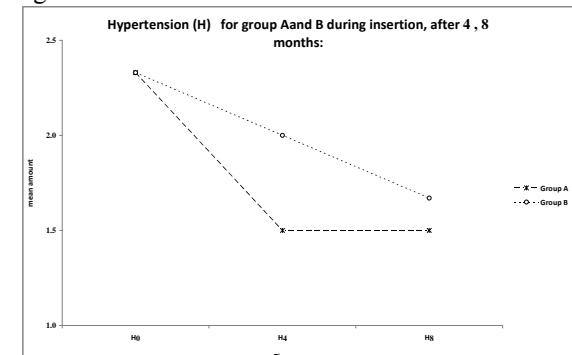
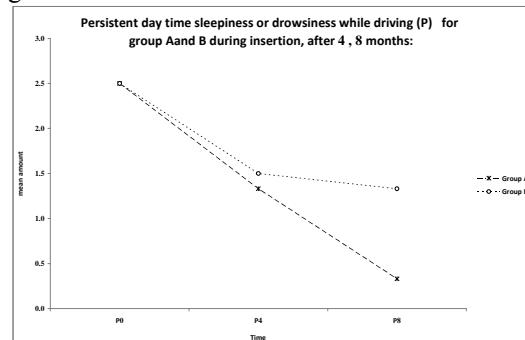


Fig 4. Improvement Apnea hypopnea index for group A more than group B

**Fig 5**



**Fig. 6**



**Fig. 7**

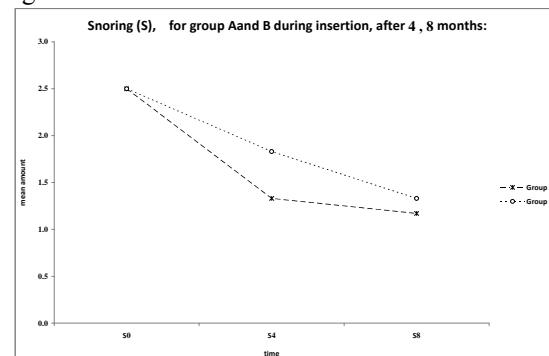


Fig 5,6,7. Improvement hypertension (H), persistent day time sleepiness or drowsiness while driving (P), Snoring (S) for group A more than group B.

### Discussion:

OSA has been described as a public health problem comparable to smoking in its effect upon society.<sup>(20)</sup>

Patients awareness regarding the problem is low especially in mild and moderate cases. An outline of the diagnostic approach to OSA is very important

The diagnosis of OSA is completed by two main:

- 1) Subjective assessments which involved focus sleep questioner clinical finding and classic symptoms
- 2) Objective assessment as physical examination sleep test polysomnograph and portable recording device.<sup>(21)</sup>

Chan A. et al.<sup>(22)</sup> mentioned that major advances in the field of oral appliances have provided a solid evidence for the use of oral appliances in the clinical management of OSA. OA are preferred by the patients; this has the potential of translating into better patient compliance and an equivalent health outcome.

Cephalometric studies have shown subtle retro positioning and shortening of the mandible and maxilla, even in the absence of distinct craniofacial abnormalities. In OSA patients compared with normal subjects<sup>(23,24,25)</sup> shorter and more posteriorly displaced mandible have been confirmed in up to two third of OSA patients and correlated with decreased pharyngeal size.<sup>(26)</sup>

Neodymium-iron-boron alloy was selected as the magnetic material of choice because of its high energy product value (260 kJ/m<sup>3</sup> as compared with 190 kJ/m<sup>3</sup> for the often used samarium-cobalt alloy, Sm<sub>2</sub>Co<sub>17</sub>), and because the neodymium-iron-boron alloy shows even better biocompatibility than samarium-cobalt magnets. The high energy product implies a possibility of stronger attractive forces in the rest position for the same size and shape of magnetic units. In this study, two magnets were inserted in each splint, producing an inter jaw force of 2.5 to 3.0 N (250 to 3250g). One shortcoming of the rare earth magnets, particularly the neodymium-iron-boron alloy, is that the alloy is very susceptible to corrosion assault by the saliva. When a magnet corrodes, there is considerable risk of destroyed magnetic properties and loss of force. Furthermore, there is a risk of liberation of cytotoxic components. To avoid inter oral corrosion, the paryene coated magnets have to, as in this study, be further embedded in acrylic.<sup>(27,28)</sup>

Interestingly, in the present investigation, improvements in clinical, cephalometric and Apnea index record by polysomnographic parameters were observed for most patients of the two groups included. Consequently this could confirm that OA should be a satisfactory and an alternative treatment modality in large proportions of patients with mild and moderate OSA who could not get benefit from other treatment options. This observation appears to be in agreement with the findings of Hoffstein.<sup>(29)</sup> and Jayan et al.<sup>(30)</sup> who supported OA therapy for OSA patients.

This study can firms that OA can be a satisfactory treatment in a large proportion of patients with slight to moderate OSAS as this response consists of a reduction in AHI(<15 events per hour) combined improvement with clinical finding together with improvement in the quality of sleep.<sup>(31)</sup>

Also psychology behavior relative had improved. Another finding in the present work showed statistically significant decrease in daytime sleepiness. There was also an overall subjective improvement after the initial use of OAs.

This may be due to for group A and B made minimum upper air way space. This agree with george zoal.<sup>(32)</sup> who suggest that bite opening should

be kept to minimum for improve at upper air way potency by stretching the palatoglossus and superior pharyngeal constrictor muscle.

An effective appliance for sleep apnea treatment should be able to move lower jaw horizontally to attempt to open the air way and rotate the lower jaw clock wise with a resultant further closing of pharyngeal space.

But more improvement for group A (magnetic appliance) than group B in spite of non significance occur. This can be explained by the fact that continuous repelling force from inherent magnetic forces which lead to improve at upper air way potency and allows freedom of function and, consequently, patient compliance is improved these agree with Bernhold M. et al.<sup>(14)</sup> who concluded that obstructive sleep apnea responded well to treatment with intra-oral magnetic appliance.

The most interesting point is decreased snoring, this may be due to the return of patients to normal state with continuous airflow; thus no vibration of soft Tissue occurred. This agrees with Jayan et al<sup>(30)</sup>, who found that as a result of OSA, a reduction of the air flow occurs, so the patient increase the speed of the air flow in an attempt to maintain the required oxygen to the lungs. The increase in the air flow velocity causes vibration of soft tissues which is the sound of snoring.

### Conclusion:

Oral appliance, with magnetic and increase vertical dimension, make improvement for OSA patients.

Oral appliance with magnetic more effective and help the management of patient with mild or moderate OSA patient in comparing with increase vertical separation of jaws

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# Biochemical Changes in Glutathione Redox System and Glucose Regulation in Late Pregnant Ossimi Ewes

Ali Hafez El-Far<sup>1</sup>, Mohamed K. Mahfouz<sup>2</sup>, Hussein A. Abdel maksoud<sup>2</sup>

<sup>1</sup> Department of Biochemistry, Faculty of Veterinary medicine, Alexandria University, Damanhour Branch (Al-Bostan), Egypt.

<sup>2</sup>Department of Biochemistry, Faculty of Veterinary medicine, Moshtohor, Banha University, Egypt.  
[aboufares90@yahoo.com](mailto:aboufares90@yahoo.com)

**Abstract** –Pregnancy is the more prevalent stress in under feeding small ruminant with multiple bearing. Fifty Ossimi ewes of two years old and their body weight ranging between 35 and 50 kg were allotted into three groups; Group I: contains ten non pregnant non lactating ewes were used as control group. Group II: contains twenty single pregnant ewes\* and Group III: contains twenty twin pregnant ewes used as experimental animals. Our study focused on the comparison between single and twin bearing ossimi ewes in the last three weeks of pregnancy and the day of parturition by measurement of reduced glutathione (GSH) level and the activities glutathione peroxidase (GSH-Px); glutathione reductase (GR-ase); glutathione-S-transferase (GST) and total superoxide dismutase (t-SOD) in erythrocytic haemolysate. In addition, glucose, non esterified fatty acid (NEFA), Beta hydroxyl butyric acid (BHBA), cortisol, insulin and protein electrophoretic patterns were measured in serum. Our results concluded that, In erythrocytic haemolysate the mean values of GSH-Px and GST in group II and III during the period of 2<sup>nd</sup> and one week before parturition and at the day of parturition were high significantly increased. While, GSH and t-SOD were high significantly decreased ( $P<0.01$ ) and GR-ase activities were significantly decreased. While serum insulin level decreased while serum NEFA, BHBA and cortisol were increased in single and twin but in twin the values is more significant. The data showed that twin bearing ewes are more susceptible to pregnancy toxemia than single bearing that may be decrease the productivity and performance of those animals.

[Ali Hafez El-Far, Mohamed K. Mahfouz, Hussein A. Abdel maksoud. Biochemical Changes in Glutathione Redox System and Glucose Regulation in Late Pregnant Ossimi Ewes. Journal of American Science 2010;6(11):742-748]. (ISSN: 1545-1003).

**Keywords**– pregnancy, glutathione, single bearing, twin bearing, ewes

## 1. INTRODUCTION

Pregnancy, parturition, and lactation represent a physiological load to the female body. Where pregnancy toxemia (gestational ketosis) caused by negative energy balance in late gestation is commonly observed in ewes and does (Kulcsar et al., 2006), in beef cows (Rook, 2000), and also in monogastric species as rabbits, guinea pigs, dogs and in ferrets (Bell, 1997; O'Rourke, 1997; Dalrymple, 2004 and Lewington, 2007). The background of the disease is the result of the fetal carbohydrate- or energy-demand exceeding maternal supply during the last trimester of pregnancy.

In ruminants, dietary carbohydrates provide well over one half of the energy needs for maintenance, growth, and production. Glucose is a primary energy source for certain animal tissues and a precursor for lactose synthesis in the mammary gland. Consequently, understanding carbohydrate digestion and absorption, dietary glucose availability, and the involvement of gluconeogenesis in the regulation of glucose homeostasis is essential for the manipulation of the production and quality of agricultural foods (Rafael and Donald, 2007). Lipid

digestion in ruminants is unique in that after ingestion feed lipids are placed into a hydrolytic and reductive environment. The result is that glycerol from triacylglycerols and phospholipids are fermented to VFA and those unsaturated fatty acids which are hydrogenated to mostly saturated fatty acids before absorption (Jenkins and Palmquist, 1984 and Van Saun, 2000). In ewes, number of fetuses plays role in keeping the homeostasis. The last trimester of pregnancy is very demanding for that homeostasis, because fetuses gain over half of their weight in this period (Seidal et al., 2006). Pregnancy toxemia is a metabolic disease that commonly affects pregnant ewes with multiple fetuses and does during late gestation. It is characterized by hypoglycemia, increased concentrations of ketone bodies in the blood and elevated plasma concentrations of free fatty acids is the result of energy demand exceeding maternal supply during last trimester of pregnancy (Kabakci et al., 2003 and Kulcsar et al., 2006).

The endocrine system especially the pancreas probably is involved in the development of ruminant ketosis. Insulin inhibits ketogenesis when free fatty acids levels are high, as well as growth hormone

secretions inhibited by cortisol and free fatty acids. Insulin also appears to be important in regulating the utilization of ketone bodies as the uptake of  $\beta$ -hydroxybutyrate and acetate (**Abd-Elghany et al., 2010**).

Cortisol is a regulator of glucose in ruminants, which acts to increase gluconeogenesis from amino acids. In starving ruminants the gluconeogenesis is maintained by elevated levels of glucocorticoids (**Azab and Abdel-Maksoud, 1999**). In lactating ruminants the rate of hepatic gluconeogenesis and the relative concentrations of glycogenic precursors regulate the level of milk production (**Huntington, 1990**).

Ketone bodies serve as an alternative fuel for many tissues, but they probably do not or only to a minor extent contribute to the energy supply of the fetus (**Battaglia and Meschia, 1988**). Glucose remains the most important metabolite for fetal and placental growth. The ability of the ewe to provide a sufficient amount of glucose to the fetus from dietary sources is limited because about 70 to 75% of the dietary carbohydrate is converted in the rumen into nonglycogenic products. The remaining fraction of digestible carbohydrate provides 40 to 60% of the circulating glucose through propionate. During periods of a negative energy balance and increased demand for glucose, up to 23% of the glucose may be synthesized from liberated glycerol from the adipose tissue. Along with this glycogenic precursor, a larger amount of fatty acids is released into the circulation that may give rise to an increased rate of ketone body formation (**Weekes, 1979 and Schlumbohm and Harmeyer, 2004**). Our study aimed to investigate carbohydrate and fat metabolic changes in single and twin bearing ossimi sheep.

## 2. Material and methods

### *A. Experimental design*

The present study was carried out in field farm of Veterinary medicine, Moshtohor, Banha University. Fifty apparently healthy, multiparous Ossimi sheep, of two years old and their body weight ranging between 35 and 50 kg. All animals were kept at the same environmental and nutritional conditions. All over the experimental period, the ewes were allotted into three groups as following:

*Group I:* included ten ewes (non pregnant non lactating) were used as control group.

*Group II:* included twenty single pregnant ewes used as experimental animals.

*Group III:* included twenty twin pregnant ewes used as experimental animals.

Animals were fed free in feedlot. Concentrate feed mixtures were adjusted to the changing of body weight every two weeks. Concentrate mixtures were

given twice daily at 10 a.m. and 2 p.m. while wheat straw was offered (*ad lib.*).

Table (1) Chemical and cell wall constituents of feed concentrate mixture and corn stalks (on DM basis)

Items	Feed concentrate mixture <sup>a</sup>	Wheat straw
<b>Chemical composition</b>		
<b>DM</b>	91.51	93.48
<b>OM</b>	89.64	89.58
<b>CP</b>	14.34	3.26
<b>CF</b>	8.47	40.23
<b>EE</b>	2.24	1.32
<b>NFE</b>	64.59	44.77
<b>ASH</b>	10.36	10.42
<b>Cell wall constituents</b>		
<b>NDF</b>	34.62	78.24
<b>ADF</b>	16.24	54.13
<b>Hemicellulose</b>	18.38	24.11
<b>NFC<sup>b</sup></b>	8.47	6.76

\*NFC: Non fibrous carbohydrates= 100 - % (CP+ NDF + EE + ASH) (Calsamiglia et al., 1995).

<sup>a</sup> Feed concentrate mixture consists of 18% undecorticated cotton seed meal, 4% soybean meal, 36% yellow corn, 36% wheat bran, 3% Vinass, 1.5 % limestone, 1.4% NaCl and 0.1% common salts.

### *B. Blood samples*

The blood samples were collected from jugular vein of all animals in the examined groups in the early morning with one week interval during the last month of pregnancy and the day of parturition. Blood samples were divided into two portions; The first portion was collected in heparinized tube contained 20 I.U. heparin for one mL blood for preparation of haemolysate by using digitonin and washing by physiological saline according to (**Kornburg and Korecker 1955**). This was used for estimation of erythrocytic GSH (**Sedlak and Lindsay, 1968**); t-SOD (**Misra and Fridovich, 1972**); GSH-Px (EC: 1.11.1.9) (**Chiu et al., 1976**); GR-ase (EC: 1.6.4.2) (**Bergmayer, 1983**); GST (EC: 2.5.1.18) (**Vessey and Boyer, 1984**). The second one was collected without anticoagulant for obtaining a clear non-hemolyzed serum by centrifugation of the blood sample at 3000 r.p.m for 5 minutes. The clear sera were freshly used for determining of blood glucose (**Trinder, 1969**), non esterified fatty acid (NEFA) and Beta hydroxyl butyric acid (BHBA) (**Duncombe, 1964**). Commercial radioimmunoassay kits were used to measure concentration of cortisol and insulin (**Tietz, 1968 and Wilson and Miles, (1977)**).

*C. Electrophoretic pattern of serum protein by SDS-PAGE* which performed according to the method of (**Laemmli, 1970**).

*D-Statistical analysis* was done by (**SAS, 1996**).

### 3. RESULTS

The data presented in (Table 2) revealed a high significant increase ( $P<0.01$ ) in the mean values of GSH-Px and GST in group II and III during the period of 2<sup>nd</sup> and one week before parturition and at the day of parturition. In contrast, GSH and t-SOD were high significantly decreased ( $P<0.01$ ) and GR-ase activities were significantly decreased at the same period of experiment.

Serum glucose level (Table 3) of single pregnant ewes showed significant ( $P<0.05$ ) decrease than control during the last 3 weeks of pregnancy. But of twin pregnant ewes was decreased significantly ( $P<0.05$ ) during the last 4 weeks of pregnancy.

Concentration of serum non esterified fatty acid (Table 3) of single pregnant ewe showed significant ( $P<0.05$ ) increase than the control during the last 3 weeks of pregnancy as well as at the day of parturition. But of twin pregnant ewes showed significant ( $P<0.05$ ) increase during the last 4 weeks of pregnancy.

Serum BHBA level (Table 3) of single pregnant ewes showed significant ( $P<0.05$ ) increase

than the control during the last 3 weeks of pregnancy and the day of parturition. And twin pregnant ewes showed significant ( $P<0.05$ ) decrease than the control during the last 4 weeks of pregnancy and the day of parturition.

Serum insulin level (Table 3) of single and twin pregnant ewes showed significant ( $P<0.05$ ) decrease than the control during the last 4 weeks of pregnancy and the day of parturition.

Serum cortisol level (Table 3) of single and twin pregnant ewes showed significant ( $P<0.05$ ) increase than the control during the last 2 weeks of pregnancy as well as the day of parturition.

The electrophoretic pattern of serum protein revealed that albumin, alpha ( $\alpha$ )-1-globulin, alpha ( $\alpha$ )-2-globulin and gamma ( $\gamma$ ) globulin of single pregnant ewes (Table, 4 and Figure, 1) were significantly decreased during the last week of pregnancy and the day of parturition. But, the concentration of serum beta ( $\beta$ ) globulin showed significant ( $P<0.05$ ) decrease during the last week of pregnancy as well as the day of parturition.

Table (2): Mean values of some biochemical parameters of group I (control), group II (single bearing ewes) and group III (twin bearing ewes)

Duration	Parameters		GSH ( $\mu\text{mol}/\text{mg}$ protein)	t-SOD (U/g protein)	GSH-Px (U/g protein)	GR-ase (U/g protein)	GST (U/g protein)
	Groups		Control	0.89 $\pm 0.08$	13.01 $\pm 1.11$	3.19 $\pm 0.27$	0.81 $\pm 0.02$
Gestation period	4 <sup>th</sup> week	Group II	0.71 $\pm 0.11$	13.01 $0.47\pm$	4.01 $\pm 0.32$	0.61 $\pm 0.10$	0.59 $\pm 0.01$
		Group III	0.81 $\pm 0.12$	11.20 $\pm 0.88$	4.91 $\pm 0.19^*$	0.73 $\pm 0.09$	0.67 $\pm 0.09$
	3 <sup>rd</sup> week	Group II	0.79 $\pm 0.11$	10.11 $\pm 0.49$	4.19 $\pm 0.31$	0.60 $\pm 0.11$	0.91 $\pm 0.11^*$
		Group III	0.77 $\pm 0.09$	9.75 $\pm 0.17$	6.11 $\pm 0.32^*$	0.62 $\pm 0.09$	1.11 $\pm 0.09^*$
	2 <sup>nd</sup> week	Group II	0.57 $\pm 0.11^*$	8.75 $\pm 0.40$	7.01 $\pm 0.27^*$	0.59 $\pm 0.11$	1.10 $\pm 0.11^*$
		Group III	0.61 $\pm 0.10^*$	8.97 $\pm 0.51$	7.33 $\pm 0.29^*$	0.49 $\pm 0.10^*$	1.25 $\pm 0.21^*$
	1 <sup>st</sup> week	Group II	0.42 $\pm 0.11^{**}$	8.19 $\pm 0.31^*$	7.41 $\pm 0.11^{**}$	0.41 $\pm 0.02^*$	1.28 $\pm 0.12^{**}$
		Group III	0.55 $\pm 0.10^{**}$	7.70 $\pm 0.21^*$	7.51 $\pm 0.31^{**}$	0.39 $\pm 0.03^*$	1.39 $\pm 0.20^{**}$
Day of parturition	Group II		0.39 $\pm 0.10^{**}$	7.12 $\pm 0.16^{**}$	8.31 $\pm 0.70^{**}$	0.35 $\pm 0.01^*$	1.75 $\pm 0.21^{**}$
	Group III		0.40 $\pm 0.11^{**}$	6.11 $\pm 0.13^{**}$	9.55 $\pm 0.29^{**}$	0.25 $\pm 0.03^*$	1.90 $\pm 0.13^{**}$

\* Indicate significant difference from control at ( $P<0.05$ ).

\* Indicate high significant difference from control at ( $P<0.01$ ).

GSH (reduced glutathione); t-SOD (total superoxide dismutase); GSH-Px (glutathione peroxidase); GR-ase (glutathione reductase) and GST (glutathione-S-transferase).

Table (3): Mean values of some biochemical parameters of group I (control), group II (single bearing ewes) and group III (twin bearing ewes)

Duration	Parameters		Glucose (mg/dl)	NEFA (g/dl)	BHBA ( $\mu\text{mol/L}$ )	Insulin ( $\mu\text{U/dl}$ )	Cortisol ( $\mu\text{g/dl}$ )
	Groups	Control	66.41 ±0.36	11.53 ±0.22	3.57 ±0.07	2.54 ±0.02	2.04 ±0.04
Gestation period	4 <sup>th</sup> week	Group II	42.88 ±0.85*	29.20 ±0.55*	10.35 ±0.89*	0.78 ±0.03*	3.64 ±0.07*
		Group III	41.56 ±0.70*	32.64 ±1.37*	12.72 ±0.95*	0.67 ±0.06*	3.73 ±0.13*
	3 <sup>rd</sup> week	Group II	44.59 ±0.80*	22.70 ±0.73*	7.85 ±0.29*	0.94 ±0.05*	2.78 ±0.04*
		Group III	43.44 ±0.36*	22.63 ±1.31*	8.92 ±0.84*	0.87 ±0.12*	2.80 ±0.11*
	2 <sup>nd</sup> week	Group II	47.57 ±0.49*	22.68 ±0.43*	7.10 ±0.33*	1.10 ±0.07*	2.32 ±0.06
		Group III	46.48 ±0.46*	21.87 ±0.37*	7.62 ±0.39*	1.17 ±0.03*	2.40 ±0.05
	1 <sup>st</sup> week	Group II	52.29 ±0.53	18.92 ±0.18	6.21 ±0.25*	1.31 ±0.01*	2.04 ±0.02
		Group III	50.30 ±0.47*	19.11 ±0.36*	7.82 ±0.77*	1.29 ±0.006*	2.10 ±0.01
Day of parturition	Group II		40.50 ±0.63*	25.62 ±0.81*	7.52 ±0.32*	1.15 ±0.04*	5.50 ±0.18*
	Group III		40.12 ±0.25*	25.19 ±1.07*	9.36 ±0.27*	1.23 ±0.03*	8.02 ±0.11*

\* Indicate significant difference from control at (P<0.05).

Table (4): Mean vales of serum protein fractions (g/dl) in control and twin ewes

The fractions	Control	The last week of pregnancy	The day of parturition
Albumin	3.15 ± 0.06	1.82 ± 0.11*	2.25 ± 0.17*
(α)-1-globulin	0.17 ± 0.004	0.08 ± 0.004*	0.08 ± 0.008*
(α)-2-globulin	0.56 ± 0.008	0.37 ± 0.006*	0.41 ± 0.006*
(β)-globulin	0.87 ± 0.006	0.74 ± 0.01*	0.75 ± 0.01*
(γ)-globulin	2.92 ± 0.01	1.87 ± 0.013*	2.17 ± 0.05*

\* Indicate significant difference from control at (P<0.05).

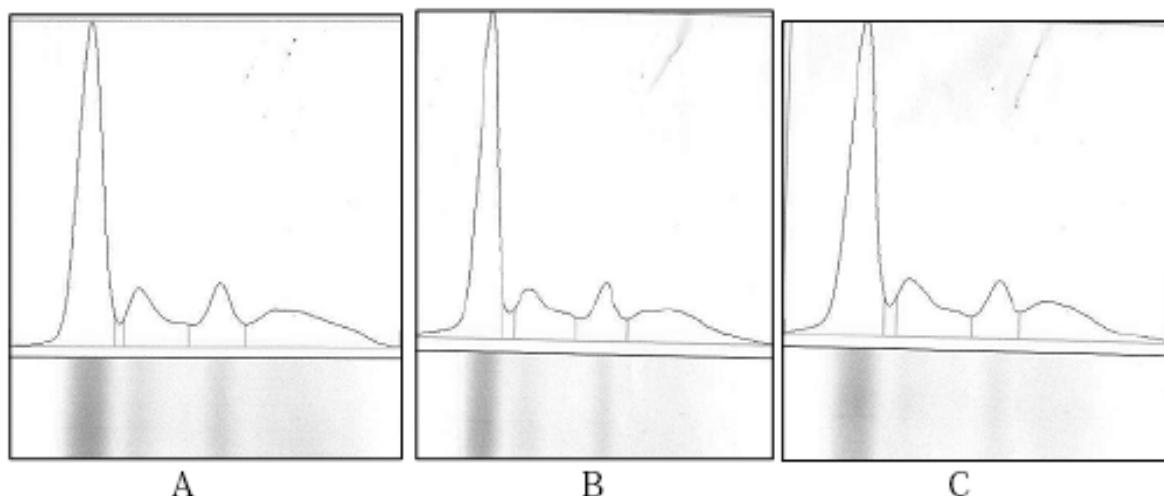


Figure (1): show the electrophoretic serum pattern of Control (A), the last week of pregnancy (B) and the day of parturition (C). In each picture, bands were arranged Albumin, Alpha (α)-1- globulin, Alpha (α)-2- globulin, Beta (β) globulin and Gamma (γ) globulin (From left to right).

#### 4. Discussion

Our study revealed a high significant increase in the mean values of GSH-Px and GST in group II and III during the period of 2<sup>nd</sup> and one week before parturition and at the day of parturition. And showed high significantly decreased in GSH and t-SOD and a significant decrease in GR-ase. This result indicated that t-SOD activity decreased as it is a first line in antioxidant enzymes defense. In the second line, GSH-Px and GST were consuming GSH as a reductant cofactor. For that reason GSH-Px and GST activities were increased and GSH level was decreased. In addition, GR-ase activities were decreased because of GR-ase enzyme generates GSH (**Mandour and Abou-El-Ela, 1999 and Abdel-Maksoud et al., 2000**). As the glutathione assumes pivotal roles in bioreduction, protection against oxidative stress, detoxification of xenobiotics and endogenous toxic metabolites, transport, enzyme activity, and sulfur and nitrogen metabolism. Its biological significance comes from the free sulfhydryl moiety of the cysteine residue and nucleophilic properties. In cells, glutathione mainly exists in the reduced form (GSH), as the oxidized form (GSSG) (**Taisuke et al., 2009**). Erythrocytes are permanently in contact with potentially damaging levels of oxygen, but their metabolic activity is capable of reversing this injury under normal conditions. Erythrocytes are equipped by many defence systems representing their antioxidant capacity. This protective system includes superoxide dismutase (SOD), catalase (CAT), reduced glutathione, glutathione peroxidase (GPx), glutathione-S-transferase, and glutathione reductase (GR). However, the cellular antioxidant action is reinforced by the presence of dietary antioxidants (**Nakbi et al., 2010**).

The present study showed that a significant decrease in the mean values of glucose of single and twin that lower plasma glucose levels came in accordance with (**Balikci et al., 2007 and Seidal et al., 2006**). The observed decrease in serum glucose level may be due to the, negative energy balance increases lipid mobilization, which results in hepatic lipidoses with subsequent impairment of hepatocellular function, glucose deficiency with intermittent hypoglycemia and accumulation of ketone bodies. The hypothesis that cows suffering from stress and/or painful diseases have elevated blood glucose levels due to an increase in serum cortisol (**Forslund et al., 2010**).

NEFA and BHBA concentrations in single and twin pregnant ewes were significantly increased than control but in twin were more significantly increased; these results were proved by (**Nazifi et al., 2002 and Moghaddam and Hassanpour, 2008**).

Serum cholesterol level of single and twin pregnant ewes showed significant increase than the control during the last 2 weeks of pregnancy as well as at the day of parturition. The high cortisol level inhibits the growth of the axial skeleton in the sheep fetus during the late pregnancy which enhances the parturition process (**Fowden et al., 1996**).

Serum insulin level of single and twin pregnant ewes showed significant decrease than the control during the last 4 weeks of pregnancy as well as at the day of parturition. The decrease of insulin level may be attributed to negative energy balance which leads to decrease in glucose level and increase the lipolysis (**Faulkner and Pollock, 1990**). The shift of energy metabolism in a catabolic direction is characterized by a wide range of endocrine changes, such as insufficient pancreatic β-cell function with a coinciding increase in insulin resistance.

The data illustrated in table (3) showed significant increase in cortisol level than the control during the last 2 weeks of pregnancy as well as the day of parturition in Single and twin pregnant ewes. This observation may be due to a hypothesis that the known relation between stress and/or painful diseases in high yielding dairy cows and pregnant ewes may be mediated through a concurrent increased cortisol secretion leading to hyperglycaemia (**Rohrbach et al., 1999**). On the other hand, **Forslund et al., (2010)** reported significantly low levels of cortisol in Cows with ketonemia (BHBA > 1.5 mmol/l). The significant increase in cortisol and presence of significant negative correlation between plasma glucose concentration and cortisol level and the significant positive relationship with β-hydroxybutyrate may be due to increased adrenal output or to impaired ability of the fatty liver, which was a consistent finding in pregnancy toxemia, to mobilize and excrete the hormone (**Ford et al., 1990 and Abd-Elghany et al., 2010**).

The concentrations of serum albumin, alpha-1-globulin, alpha-2- globulin, beta globulin and gamma globulin of single pregnant ewes showed significant decrease than the control during the last week of pregnancy as well as the day of parturition. These results may attributed to consequence increase in the mother's basal metabolic rate, the maximal nutrient requirements of the placenta and the growing fetus, together with the transfer of serum albumin, immune globulins, and amino acids from the blood stream to the mammary gland for synthesis of colostrums (**Batavani et al., 2006**).

#### 5. CONCLUSION

Late pregnancy in ewes is a very stressful period specially the late period in which in erythrocytic haemolysate the mean values of GSH-Px

and GST were high significantly increased; GSH and t-SOD were high significantly decreased ( $P<0.01$ ) and GR-ase activities were significantly decreased. While, serum glucose, total protein, albumin, globulin and insulin were decreased while serum NEFA, BHBA and cortisol were increased in single and twin but in twin the values are more significant. Our research results recommended that twin bearing ewes need a special care during pregnancy and after parturition by supplementation of ewes by a demands of appositive energy balance.

**Corresponding author:**

Hussein A. Abdel maksoud

Present address; Department of Biochemistry, Faculty of Veterinary Medicine (El-Bostan, Damanhur Branch), Alexandria University. Tel; +20109612452; E-mail; [aboufares90@yahoo.com](mailto:aboufares90@yahoo.com)

The single or twin pregnancy is determined in sonography unit of surgery department in Fac. Vet. Med. Moshtohor, Banha University, Egypt.

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10/5/2010

# Study the Efficiency of Investment and its Determinants in the Agricultural Sector

Khairy Hamed Eleshmawy, Enaam Abd elFattah Mohamed, Laila Mustafa ELShrif, Haitham Bauomy Hassan

Department of Agricultural Economics - National Research Center

**Abstract:** The promotion of increased rates of the investment growth is the main priority of the economic development, where there can be no development without adequate levels of investment. The problem has been narrowed to study in lower volume of investment goes to agriculture in spite of the importance of this sector to increase the rate of economic growth, the study aims to identify the relative importance of the investment total agricultural and agricultural domestic and foreign farm, as well as identify the most important factors affecting each. In addition to, identify the efficiency of agricultural investment. The results indicated that, overall agricultural investment and agricultural domestic and foreign farm represented about 9.38%, 7.98%, 1.4% of the total investments, and investments amounted to local agriculture, and foreign to 84.88%, 15.12% of the total agricultural investment. Estimating the efficiency indicators of agricultural investments shows that, there is efficiency in agricultural investment despite lower Kimpalastmarat directed to the agriculture sector during the study period. The results showed that, the agriculture sector capital intensive, in addition to increasing the coverage of agricultural savings to agricultural investment as much as about 46% in 2008. While the share of one acre of agricultural investment from 283.65 pounds in 1999 to about 194 pounds in 2008. The results showed that, the most important factors affecting the local agricultural investments are in value-added farm income and saving agricultural and domestic liquidity and interest rate on farm loans. While the GDP and the budget deficit and non-agricultural investments, the most important factors affecting foreign investments in agriculture. Therefore, the study recommends the need to increase investments directed to the agricultural sector given the importance of this sector and its contribution to economic growth. You need to follow monetary policies that reduce the interest rate on agricultural loans to encourage investment in agricultural projects, in addition to the need to reduce taxes on agricultural projects as a means to stimulate the agricultural investor.

[Khairy Hamed Eleshmawy, Enaam Abd elFattah Mohamed, Laila Mustafa ELShrif, Haitham Bauomy Hassan. Study the Efficiency of Investment and its Determinants in the Agricultural Sector. Journal of American Science 2010;6(11):749-755]. (ISSN: 1545-1003).

**Keywords:** Agricultural. Investments, Domestic Agricultural. Investments, Foreign Agr. Investments. Gross Investments.

## Introduction:

Investment is the primary focus of economic growth on the national level. Agricultural investment is also one of the tools essential to the success of agricultural development, which is the basic foundation to increase production and income and create new jobs. Increasing investment means the addition of productive projects and contributes to increasing production and thereby increase exports and reduce imports, thereby improving the trade balance, and increase national income and the individual and which is reflected in increased savings which in turn results in the creation of new investments, and therefore the investment is considered a variable stream has an effective role in finding solutions to the problems of the Egyptian economy as well as to absorb as much of the manpower is not working as well as reducing the unemployment rate of about 8% in 2008 (7).

Agriculture also contributes about 25% of GDP, has targeted the state would increase the rate of

growth in the agriculture sector to around 5% annually, which requires increased investments aimed at this sector to about 14% (9).

This investment has reached about 104.65 billion national pounds, which represents investments in agriculture by about 6.7% during the period (2002-2008).

**Study Problem:** The problem with the study in the low volume of investment goes to agriculture relative to the investment to other sectors of non-agricultural despite the importance of that sector to increase the rate of economic growth through increased agricultural output and boost agricultural development, and also reduced the relative importance of agricultural investment in general and agricultural investments domestic and foreign, in particular.

**The Aim of the Research Subject:** The study aims to identify the efficiency of agricultural investment

through some of the economic indicators, as well as identifying the size of the total agricultural investment and agricultural investment, foreign and domestic the most important factors influencing them.

**Methodology and Source of Data:** The study relied on methods of descriptive statistics and quantitative data analysis to achieve the objective of research, as well as some measures of efficiency and methods of time trend in the public and multiple regression and gradual. The study was based on data published and unpublished, which have been collected from various sources such as the National Bank of Egypt, Ministry of Economic Development, and the device center for Public Mobilization and Statistics.

### Research results and discussion

#### The relative importance of agricultural investment

Table (1), shows the relative importance of agricultural investment fluctuates from year to year, ranging between a maximum of about 14.2% in 2001 and a minimum of about 4.8% in 2006 and an average of about 9.38% of the total investments of national, which indicates a decline in importance relative to agricultural investments.

As seen from Table (2), the agricultural investments increased annually by 20.4 million pounds and with an annual increase of statistically

significant estimated 0.28% of average, amounting to around 7.21 million pounds during the period (1995-2008).

With regards to, the investments of domestic agriculture, ranged from a maximum of about 8.07 billion pounds in 2001, a relative importance of about 11.9%, 84.2% of the total national and agricultural investments, respectively, a minimum of about 3.8 billion pounds in 1995 represented about 7.01%, 84.8% of the national and agricultural investments, respectively, representing an annual average of about 7.98%, 84.9% of the national investment, agricultural, respectively, during the study period. The data in Table (2), shows that the local agricultural investments increased annually by 6.5 million pounds and an annual increase of about statistically significant 0.11% from an average of about 6.121 billion pounds.

With regards to, the agricultural investments in foreign, data in Table (1) indicates that, it reaches a maximum of 1.52 billion pound in 2001, representing about 2.3%, 18.8% national and agricultural investments, respectively, as a minimum to 0.69 billion pounds in 1995 representing about 0.8%, 11.5% of the national and agricultural investment, respectively, representing an annual average of 1.4%, 15.12% of the national and agricultural investment, during the period (1995-2008).

Table (1): Relative Importance of Agricultural (total, domestic and Foreign Investments) (1995-2008)

year	Gross Investments	Agr. Investments		Domestic Agr. Investments			Foreign Agr. Investments		
		Value	% of Gross Investments	Value	% of Gross Investments	% of total Agr. Inv.	Value	% of Gross Investments	% of total Agr. Inv.
1995	54.9	4.48	8.2	3.8	7.01	84.8	0.69	1.2	15.2
1996	68.5	5.19	7.6	4.45	6.5	85.7	0.74	1.1	14.3
1997	61.3	8.16	13.3	7.13	11.6	87.5	1.02	1.7	12.5
1998	64	8.42	13.2	7.22	11.3	85.8	1.2	1.9	14.2
1999	64.4	8.13	12.6	6.84	10.6	84	1.3	2.0	16
2000	63.6	8.2	12.9	6.81	10.7	83.2	1.38	2.2	16.8
2001	67.5	9.6	14.2	8.07	11.9	84.2	1.52	2.3	15.8
2002	68.1	7.4	9.4	5.49	8.1	85.8	0.91	1.3	14.2
2003	79.6	7.6	9.5	6.74	8.5	88.5	0.88	1.0	11.5
2004	96.5	7.4	7.7	6.34	6.6	85.5	1.08	1.1	14.5
2005	115.7	8.04	6.9	6.71	5.8	83.5	1.33	1.1	16.5
2006	155.3	7.55	4.8	6.21	3.9	81.2	1.42	1.0	18.8
2007	124.2	6.11	4.9	5.13	4.1	84	0.98	0.8	16
2008	93.1	5.71	6.1	4.83	5.2	84.6	0.88	0.9	15.4
Average	75.24	7.21	9.38	6.12	7.98	84.88	1.095	1.4	15.12

**Source:** Compiled and calculated from:

1 – Egyptian National Bank: varians bullitins.

2 – CAPMAS- Statistical Data Base.

As it turns out that, foreign investments in agriculture increased annually by about 13.84 million pounds, with an annual rate estimated statistically significant 1.27% on average, amounting to around 1.092 billion pounds during the study period, Table (2).

Estimating the coefficient of variation to the relative variation for each of the national and agricultural investments of local and foreign during the study period, which means differ from year to year.

Above it is a clear decline in the relative importance of agricultural investment, despite the importance of agriculture to increase production and farm income and create jobs and reduce unemployment, and then push the wheel of development.

Table (2): Statistical Estimation of National, Agricultural, Agricultural domestic and Agricultural foreign investments (1995-2008).

Variable		Average	Change rate %	F TEST	R <sup>2</sup>	Variation coefficient
Gross Investments	20.36	132.63	1.54	30.7**	0.53	11.5
Agr. Investments	20.4	7.21	0.28	12.9**	0.62	19.8
Domestic Agr. Investments	6.5	6.121	0.11	15.3**	0.69	19.8
Foreign Agr. Investments	13.84	1.092	1.27	17.2**	0.72	24.6

(\*\*) Significant at 0.01; **Source:** Compiled and calculated from data Table (1)

**Efficiency of investment in the agricultural sector:** The process of allocating investments to the agricultural sector and optimize the distribution is the main determinant for long-term stability and make economic growth of this sector, with the consequent increase in productivity of factors of production and the growth rate of agricultural GDP. There are various standards and indicators used to measure the efficiency of investment it takes up in this part of the research together.

**1 - The investment rate:** expresses the amount of investment spending necessary to add one unit of agricultural output and is calculated from the following formula:

$$\text{Investment rate} = \text{Agriculture Investment} / \text{Agricultural local output}$$

**Reflects the decline in the rate of one right on the efficiency of agricultural investment and vice versa.**

It is clear from Table (3) that, the investment rate fluctuates from year to year, the maximum is reached about 0.714 million pounds in 1997, the minimum was about 0.332 million pounds in 2008, an average of about 0.554 million pounds during the period (1995-2008), which has already seen that the rate of agricultural investment decreased from the correct one during the study period, which indicates the efficiency of agricultural investment in order to lower the value of the investments needed to increase GDP by one unit.

**2 - Return on Investment:** This indicator reflects the units of the GDP generated from one unit of expenditure Alastmary, and is calculated from the following equation:

**Return on Investment**=Agricultural local output/ total Agriculture Investment

It reflects the high value of the index for the right one on the efficiency of agricultural investment and estimating the return on agricultural investment during the period (1995-2008), shown in table (3)

that ranges from a low of around 1.4 million pounds in 1997, the ceiling was about 3.013 million pounds in 2008 and an estimated annual average of 1.89 million pounds, Which has already seen efficiency in agricultural investment because of the high unit value of GDP generated from investment spending.

### 3 - per acre of agricultural investment:

Calculated by dividing the investment of the agricultural cultivated area. Data in table (3), show that, the high per acre of agricultural investments ranging from a low of around 127.72 L.E in 1995, the ceiling was about 283.65 L.E in 1999, an average of an estimated 204 L.E during the study period (1995 - 2008), which has already seen the success of the investment policy in the mobilization of investment in the agriculture sector.

**4 - Coefficient of Endemism:** Indicates the coefficient of endemism to the contribution of the agriculture sector in generating gross domestic product, according to the investment of this sector and is calculated dividing the proportion of agricultural investment from the National Investment on the percentage of agricultural gross domestic product of the national gross domestic product, as in the following equation:

**Coefficient of Endemism** = (agricultural investment/ National Investment) ÷ (agricultural gross domestic product/ national gross domestic product)

Low this standard for the right one means that the agricultural sector has attracted investments of less than its contribution to GDP of the agricultural sector. The data in Table (3) that the minimum coefficient of endemism was about 0.24% in 1998, while the upper limit of about 0.87% in 2003, and an average annual estimated 0.58%. Notes from the table that the coefficient of endemism at least one order for each year of the study. This indicates the importance of directing more investments to the agricultural sector because of its importance in increasing gross domestic product.

Table (3): Criteria of agricultural investment efficiency (1995-2008)

year	Agr. Invest. coeff	Agr. Invest. returns	Agr. Invest. Per feddan (L.E)	Coefficient of Endemism	Capital intensification coeff.	Agr.savings /investment	Average propensity to save in agrionltwe%	Agr. Investment productivity
1995	0.474	2.108	127.72	0.31	0.963	32	9.6	3.28
1996	0.511	1.957	163.09	0.24	1.106	31	10.4	2.97
1997	0.714	1.4	187.29	0.54	1.718	23	11.2	2.05
1998	0.685	1.459	282.88	0.53	1.753	26	12.5	2.03
1999	0.628	1.591	283.65	0.76	1.674	30	13.5	2.24
2000	0.597	1.674	269.19	0.78	1.668	33	14.4	2.27
2001	0.673	1.485	268.54	0.77	1.929	28	13.9	2.02
2002	0.47	2.126	276.27	0.79	1.276	42	13.8	3.07
2003	0.589	1.696	155.42	0.87	1.487	32	12.5	2.57
2004	0.588	1.699	166.26	0.57	1.439	32	11.5	2.75
2005	0.597	1.675	158.27	0.59	1.534	29	10.4	2.82
2006	0.535	1.868	167.32	0.48	1.415	33	10.5	3.14
2007	0.375	2.662	156.74	0.47	1.126	44	10	4.38
2008	0.332	3.013	194.09	0.39	1.03	46	10.2	4.84
Average	0.554	1.886	204.05	0.58	1.437	33	11.7	3.1

Source: Compiled and calculated from data

1 - Ministry of Economic Development, a plan of economic and social development issues separate.

**5-"Capital Condensation Coefficient (Coefficient of Employment):** Capital intensity factor shows the ratio between agricultural investment and the number of agricultural workers is calculated as follow:

**Capital intensification factor** = agricultural investment/ agricultural workers

The lower the coefficient of employment indicates the number of workers increased by more than increased investment, which requires increasing the volume of investments to recruit more and contribute to solve the problem of unemployment. Coefficient reflects the employment component of the condensation of the capital or the condensation of the work item and thus reflects the contribution of the sector in employment. The data in Table (3) that the coefficient of employment ranges from a low of about 0.963 thousand pounds in 1995, an upper limit of about 1.929 thousand pounds in 2001, and estimated an annual average of 1.43 thousand pounds. Above clearly that employment factor greater than one indicating that the agriculture sector capital intensive except for 1995. This may reflect the tendency for farmers to use modern machinery and modern technologies in agriculture due to the high wages for agricultural labor.

**6 - The coverage rate of saving schemes of agricultural of agricultural investment:** shown in table No. (3) that the coverage rate of saving agricultural to agricultural investment ranging between a minimum of about 23% in 1997, an upper limit of about 46% in 2008, an average year an

estimated 33% during the study period. It is a clear already high rate of savings to cover the agricultural investment during the study period which may be due to higher savings and lower agricultural investments in agriculture, which means an imbalance in the relationship between equilibrium indicates to pass some savings to agricultural investment of non-agricultural.

**7 - The tendency of the average agricultural Savings:** reflects the change in the amount of average propensity to save agriculture and agricultural savings is calculated by dividing the farm income.

**Average propensity to save agriculture I=** save agriculture/agricultural income

Data from Table (3) that the tendency of the average savings ranging from a minimum at about 9.6% in 1995, an upper limit of about 14.4% in 2000, an average year an estimated 11.7% during the study period. Clear from the above average decline in the trend of saving during the study period. This may be due to low savings and increase agricultural income and, which means a relative imbalance in the equilibrium relationship between them.

**8 - Factor productivity of agricultural investment:** This measure reflects the profitability of a unit of money invested in the agricultural sector, calculated by dividing the agricultural income to agricultural investment:

Factor productivity of agricultural investment = agricultural income / agricultural investment

The data table (3) The coefficient of the agricultural productivity of investment between a low of about 2.02 in 2001, and a maximum 4.84 in 2008, and the average annual estimated 3.10 during the study period.

#### **Economic variables affecting the agricultural investment in Egypt:**

**1 - Agricultural GDP Total:** shown in table No. (4) that the total agricultural output fluctuates from year to year from a low of about 11.4 billion pounds, and a maximum of about 17.21 billion pounds, with an average annual rate of about 12.5 billion pounds during the period ( 1995-2008), The amount of annual change by about 0.52 billion pounds, an annual rate of statistically significant estimated 3.98% of the average of the period, reflecting the relative coefficient of variation (\*) variation during the study period, where an estimated 19.9%.

**2 - General deficit:** Data from the previous table that the General deficit in fluctuates from year to year between the lower limit of about 9.6 billion pounds and a maximum of about 89.6 billion pounds, an average annual rate of about 37 billion pounds this, the amount of annual change about 7.16 billion pounds per year statistically significant change is estimated at 19.3%, and a coefficient of variation relative to 85.3 reflecting the differences in the General deficit during the study period.

**3 - Agricultural income:** it is clear from Table (4) that the real agricultural income ranges from a minimum of about 16.71 billion pounds and a maximum of about 27.67 billion pounds, an average annual rate of around 20 billion pounds. This may show that the income of agricultural growing annually by about 0.89 billion pounds and statistically significant annual rate of around 4.5% and the relative difference by a factor is reflecting the variation in per capita income of about 19.6% during the period (1995-2008).

**4 - Evolution of value added:** examine the development of value-added of the agricultural sector in real terms shows that they fluctuate from year to year between a minimum of about 12.8 billion pounds and a maximum of about 20 billion pounds, an average annual rate of about 14.7 billion pounds, this has increased in real value added annually by about 0.63 billion pounds, a statistically significant annual increase of about 4.3% and coefficient of variation reflects the relative differences in real value added of about 19.2% during the period (1995-2008).

**5- Saving agriculture:** Data from the Table No. (4) that saving agricultural real terms ranged from a minimum of about 1.46 billion pounds and a maximum of about 2.7 billion pounds, an average annual rate of around 2.46 billion pounds, this has increased saving agriculture at an annual rate significantly statistically is about a factor of 3.5% and the difference reflects the differences in agricultural savings of about 19.2% during the study period.

**6 - agricultural loans:** Data from the previous table to the relative stability of the value of agricultural loans, ranging from a minimum of about 2.1 billion pounds and a maximum of about 2.7 billion pounds and an average annual rate of around 2.55 billion pounds, this has increased agricultural loans during the study period at a rate statistically significant year of about 1.9% and the relative difference by a factor reflecting the variation in the value of agricultural loans of around 13.6%.

**7 - agricultural workers:** study of the evolution of agricultural employment during the period (1995-2008) shown in Table (4) to range between a minimum of about 4.75 million workers and a maximum of about 5.38 million workers by an average of about 4.58 million workers have taken this trend increased general statistically significant annual rate of change is about a factor of 1.44% and the difference reflects the relative convergence in a number of agricultural labor as an estimated 6.1% during the period of study.

**8 - the interest rate on loans:** The interest rate of the most important determinants of investment if the interest rates have risen, this is the decline of investments, while low interest rates lead to stimulate further investment, it is clear from Table (4) to range from a low of about 11.9% and a maximum of about 17% average annual rate of about 14.5%, this has increased at an annual rate of about statistically significant 3.7% relative difference by a factor reflecting the variation in interest rates on loans of around 11.6% during the period (1995-2008).

**9 - Evolution of the dollar exchange rate of the pound:** reflects the strength of the pound against the dollar and the data indicate the table number (4) that the exchange rate of the dollar is about 3.4 pounds, at a minimum, and about 6.2 pounds with an average maximum of about 4.62 pounds during the study period, and is growing annually by about 0.24 pounds, a statistically significant annual change of about 5.22% and a difference of a factor of about 24.2%, which reflects the differences in the exchange rate.

Table No. (4): Statistical analysis description of the most important Economic Variables affecting the Agricultural: total, domestic and Foreign Investments (1995-2008)

variable	Lower limit	Upper limit	The Average	Change rate %	R <sup>2</sup>	F TEST	Variation coefficient
Agricultural local Output (million L.E)	11421	17206	12954..2	3.98	0.70	27.9**	19.9
General deficit (million L.E)	9623	89642	37012..2	19.33	0.90	105..9**	85.3
Actual Agric. Income (million L.E)	16709	27675	20041..3	4.49	0.92	137**	19.6
Value added (million L.E)	12841	20019	14709	4.31	0.88	85..9**	19.2
Agric. Reserve (million L.E)	141602	2678.3	2458	3.5	0.88	16.6**	19.2
Agric. Loans (million L.E)	2100	2710.5	2553.1	1.9	0.43	6.03**	13.6
Agric. Labours	4747	5380	4578	1.44	0.98	640**	6.1
Profit price	11.9	16	14.5	3.68	0.82	53..3**	11.6
\$ exchange rate	3.4	6.2	4.62	5.22	0.81	51.6**	24.2
Actual Agric. Labour rent (L.E/YEAR)	1136	6841.6	2494..3	15.84	0.76	38.7**	75.9
Unagric. Investment billion L.E.	53.19	147.79	80.18	6.89	0.61	19.12**	36.8
Financial liquidity (million L.E)	52.9	115.23	77.85	6.74	0.89	98.65**	29.8

Source: Compiled and calculated from

1 - Central Agency for Public Mobilization and Statistics - Statistical Database, unpublished data.

2 - National Bank of Egypt, Economic Bulletin numbers sporadic.

3 - Ministry of Economic Development, reports of annual follow-up of the plan the number of sporadic.

**10 - Evolution of the wage of agricultural real:** between a minimum of about 1.14 thousand pounds at a minimum and about 6.84 thousand pounds, a maximum annual average is estimated at 2.5 thousand pounds, this has increased at an annual rate statistically significant estimated 15.8% and a factor of difference relative reflecting the variation in agricultural real wage of the worker is estimated at 75.9%.

**11 - Evolution of investments for non-agricultural:** Data from the table (4) shows, that the investments for non-agricultural fluctuate from year to year, ranging from a minimum of about 53.2 billion pounds and a maximum of about 147.8 billion pounds, an average annual rate of about 80.2 billion pounds during the period (1995 -2008), this has increased at an annual rate of about statistically significant 6.9%, which indicates that the non-agricultural sectors will attract investment, and the relative coefficient of variation was about 36.8, which reflects the differences in non-agricultural investments.

**12 - domestic liquidity:** As indicated in table (4) that, the local fluidity ranging from a minimum of about 0.053 billion pounds and a maximum of about 0.115 billion pounds, an average annual rate of around 0.078 billion pounds, this has increased domestic liquidity during the study period at an annual rate significantly statistically estimated by a factor of 6.74% and the relative difference amounted to about 29.8% reflecting the variation in the value of local liquidity.

**Determinants of agricultural investment:** It has been made several attempts to include various combinations of independent variables of the previous study to measure the impact on total agricultural investment and agricultural investment both domestic and foreign, to the possibility of obtaining estimates on the degree of efficiency using the method of regression staging in different images and select the best images that conform to signals with economic logic.

The most important factors affecting the total agricultural investments: show Equation (1) Schedule No. (5) that changes the equation explain about 86% of the changes in the value of total agricultural investment, as an increase in variables of value added agricultural income, savings and agricultural and domestic liquidity by one million pounds each of them individually leads to increase agricultural investment by 1.9, 2.54, 3.1 0.101400000 pounds, respectively. The effect was a negative interest rate on loans means that the high interest rates on agricultural loans lead to a decrease in agricultural loans and investments in agriculture and about 574 million pounds. The most important factors affecting the local agricultural investments:

Show equation (2) Schedule (5) that, the variables within the model explains about 85% of the changes in investment by local agriculture, and due to the value-added farm income and savings and agricultural domestic liquidity and interest rates on farm loans. As an increase in value-added farm income and saving agricultural and domestic liquidity

by one million pounds each of them individually lead to increased investment by local agricultural 1.54, 2.12, 2.65 0.2670000 pounds, respectively. The effect was negative for a variable interest rate on loans means that the high interest rates lead to decreased agricultural loans, agricultural and domestic investment by about 464 million pounds.

#### The most important factors affecting foreign investments in agriculture:

Show the equation (3) table (5) that the factors affecting foreign investments in agriculture are in the GDP and the budget deficit and non-agricultural investments. Also show a positive

correlation between GDP on foreign investments in agriculture since the increased One million pounds lead to increase foreign investments in agriculture by £ 7.673 million. While showing an inverse relation between the budget deficit and non-agricultural investments on foreign investments in agriculture as enhancing them one million pounds lead to a decrease in foreign investments in agriculture by about 0.024 0.0075000 pounds, respectively. The coefficient of determination shows that 78% of the changes in foreign investments in agriculture due to the previous variables.

Table No. (5): Statistical Estimation of the economic variables affecting the Agricultural: total, domestic and Foreign Investments (1995-2008)

n.q	Variable	F TEST	R <sup>2</sup>	F TEST
1	Total Agr. Investments	$\hat{Y}_i = 20118 + 1.9 X_{1i} + 2.54 X_{2i} + 03.1X_{3i} + 101.4 X_{4i} - 574 X_{5i}$ (2.532) <sup>*</sup> (3.955) <sup>**</sup> (3.22) <sup>**</sup> (2.687) <sup>*</sup> (2.82) <sup>*</sup>	0.86	9.59 <sup>**</sup>
2	Domestic Agr. Investments	$\hat{Y}_i = 17058 + 1.54 X_{1i} + 2.12 X_{2i} + 2.65X_{3i} + 88.8 X_{4i} - 464 X_{5i}$ (3.326) <sup>**</sup> (3.746) <sup>**</sup> (3.116) <sup>**</sup> (2.669) <sup>**</sup> (2.589) <sup>*</sup>	0.85	8.76 <sup>**</sup>
3	Foreign Agr. Investments	$\hat{Y}_i = 1721 + 7.673 X_{6i} - 0.024 X_{7i} + 0.075X_{8i}$ (3.899) <sup>**</sup> (4.378) <sup>**</sup> (2.538) <sup>*</sup>	0.78	6.96 <sup>**</sup>

Where:  $\hat{Y}_i$  = estimated value of the depended variable in i

$X_{1i}$  = Value added (million L.E)in i.  $X_{2i}$  = Actual Agric. Income (million L.E) in i.

$X_{3i}$  = Agric. Reserve (million L.E) in i.  $X_{4i}$  = Financial liquidity (million L.E) in i.

$X_{5i}$  = % Profit price in i.  $X_{6i}$  = GDP (million L.E) in i.

$X_{7i}$  = General deficit (million L.E) in i.

$X_{8i}$  = non-agricultural investments (million L.E) in i.

Figures in parentheses represent the value of (T) calculated. \*: Significant at 5%,

\*\*: significant at 1%

**Source:** Compiled and calculated from research data.

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# Measurement of Family Economic Status

<sup>1</sup>Mehdi Yadollahi & <sup>2</sup>Laily Hj Paim

<sup>1</sup> Faculty of Human Ecolog, Putra University, Malaysia &

Dept. of Management, University of Payam e Noor, Sirjan

E-mail: [mfma155@yahoo.com](mailto:mfma155@yahoo.com)

<sup>2</sup>Dept. of Resources Management & Consumer Studies, Putra University, Malaysia

**Abstract:** The concept of family economic has become important around the world. It has been realized that communities based family economic can play a fundamental role in poverty alleviation. Measuring of family economic status is an important step in developing family economic strategies to achieve poverty reduction. This paper used qualitative approaches to illustrated family economic status. The purpose of this study is to explore the concept and indicators of family economic. The literature derived from my study in family economic management.

[Mehdi Yadollahi & Laily Hj Paim. Measurement of Family Economic Status. Journal of American Science 2010;6(11):756-760]. (ISSN: 1545-1003).

**Key Words:** Family Economic, Income, Physical Assets, Expenditure

## 1. Introduction

Family Economy used to denote the basic structure of production and consumption in the preindustrial Europe. In the family economy there were regional variations, which were how different places were different in family economy (Wikipedia, 2010). Economic status represents the economic capacity of families to meet their material and non-material needs. Income and ownership of physical assets are means that can use to acquire suitable economic status of families. According to Friedman (1957) families with low income levels are disproportionately represented by a provisional reduction in the current income that will usually suggest a high ratio of consumption to income. Expenditure also largely depends on income and assets. It represents an even more direct means to achieve human well-being. Families' perception on availability of money to make ends meet are not uniform, however, it is even closer to indicating overall family economic status. In otherwise, families with high-income levels are representing by those with temporary increases in income and will demonstrate low ratios of consumption to income.

## 2. Indicators of Family Economic Status

The word family raises powerful pervasive images. The institution of the family can be seen as the foundation within our society, the most powerful emotional system to which

we will ever belong. However, the meaning of the world family can vary significantly depending on how it is used and by whom. Bindon and Vitzhum (2003) in their studies have reported a number of significant factors affecting family economic resources. These included education, occupation, and economic behaviour including number of household members involved in family production activities. Some researchers have categorized the family economic status into three categories, poor, average and rich (Dao et al., 2006).

Quality of life is another construct related to family economic status. Several indicators were used by Xavier et al., (2003) to measure quality of life. These indicators were included level of satisfaction and well-being of health, activity, income, social life, and relationship with family members (Xavier et al., 2003). The search for these indicators is an effort to achieve new information that will be valuable to assess the past, direct the activities of the present, and plan for future.

Family economic status also can categorize into two levels. The first is 'not enough to live', and the second is 'enough to live' (Xavier et al., 2008). Conventionally, indicators of socio economic status is measured financially using income or consumption expenditure, based on the proposition that material living standards reflects well-being (Falkingham & Namazie, 2002). The empirical measures of different

levels of family economic status used by different researchers are aimed at the recognition of strengths and weaknesses of the family economic dimensions (Lundberg & Pollak, 2007). There is a long-standing debate about whether income or expenditure is a superior measure of socio economic status. Income is commonly more obtainable than consumption. According to Friedman (1957) permanent income hypothesis confirmed that families are likely to base their consumption on times of income fluctuation, for example, by borrowing or drawing on savings during times of low income (Friedman, 1957). Consequently, it broadly asserted that consumption expenditure is a superior indicator of the long-term socio-economic status than income. This argument holds true in low-income countries, where income maybe derived from a diversity of sources and may vary significantly across seasons. The long-term aspects of the socio economic status take a while before being related to various health outcomes, adding to the reasons for choosing consumption expenditure over income (Laura et al., 2008). Within low-income countries, the measure of consumption expenditure is fraught with problems. There is the inconvenience concerning recall and an unwillingness to reveal information (Deaton & Zaidi, 1999). In addition, collecting consumption expenditure data requires an extended questionnaire that must be done by skilful and experienced interviewers (Laura et al., 2008).

Other indicator to measure family socio economic status is an asset-based approach. It is an option to income and consumption expenditure. In the case of data on income or consumption expenditure lacking, information on possession of a range of durable property can be used (Falkingham & Namazie, 2002; Rutstein & Johnson, 2004). Gathering of data concerning assets has claimed to be more consistent than income or consumption expenditure. This is because, it uses uncomplicated questions or straight observation by the interviewer and, therefore, should suffer less from recall or social desirability related problems (Sahn & Stifel, 2003).

Educational level, occupational status and income are the most widely used indicators of socio-economic status (SES). Though moderately correlated, each of these indicators can capture distinctive aspects of social position, and they are

not interchangeable. Income has employed broadly as an indicator of SES, with the majority of typical income-based measures being a family's total cash income, measured over some period such as a monthly, or yearly preceding measurement. Some researchers suggest that income is perhaps the strongest and most robust predictor of health (Lantz et al., 1998; McDonough et al., 1997), because, to some degree, the impact of other SES variables are mediated through it (House & Williams, 2000). Others would disagree, since a strong case can be made that education alters health-related behaviour as well as some psychosocial factors, and these influence health independently of education's effect on income (John et al., 2002).

In assessing socio-economic status, particularly economic status, measuring variables other than family income may be useful, for example, assets such as inherited wealth, savings, employment benefits, or ownership of homes or motor vehicles (Berkman & Macintyre, 1997). While income represents a flow of resources over some period, wealth captures the stock of assets at a given point in time, and, thus, economic reserves. Wealth is a source of economic security providing an index of a family's ability to meet emergencies or absorb economic shocks such as unemployment. However, the importance of wealth as a source of economic security may vary among societies. Income and wealth are completely correlated, but they are not exchangeable, as revealed by the example of an elderly person with a modest fixed income but substantial accumulated wealth (John et al., 2002).

Socio-economic status typically is divided into three categories – high SES, middle SES, and low SES. When placing a family into one of these categories some or all of the three variables (income, education, and occupation) can be assessed (Werner et al., Goode, 1999; Marmot, 2004; 2007). Other studies have attempted to explain family economic status using two categories –high and low family economic status (Ahmed et al., 2000, 2003). Chuma and Molyneux measured family economic status by using expenditure and assets. They determined that household economic status categorized in rural and urban areas (Chuma & Molyneux, 2009).

Many Americans consider that there are three straight forward class models of family or society that separate the better off, the middle class, and the poor based on economic status (Eichar, 1989). Mainly, definitions of class differentiate people according to wealth, income, education, type of occupation and association in a particular social system. Several explanations of class merely look at

numerical measures such as wealth or income. Additional factors taken into account include qualitative factors, such as education, culture, and social status (Gilbert, 1998).

Salsberry and Reagan (2009) in their research comparing the influence of childhood and adult economic status on midlife obesity used three income categories. An income variable was then used to categorize the sample into three categories (i) below the 33.3 percentile of sample, (ii) between the 33.33 percentile and the 66.7 percentile, and (iii) above the 66.7 percentile (Salsberry & Reagan, 2009). Frederic (2007) also in his research about the anatomy of increasing income inequality of US family used five income categories based on percentile. These categories were from the first income level (0-20), (20.1-40), (40.1-60), (60.1-80) and the last one was (80.1-100) percent (Frederic, 2007; Meyrick & Yusuf, 2006). World Bank (1998) also used five categories of income to investigate world annual consumption. In these categories, per capita income for the first level was less than \$1,000, second level \$1,001 to \$4,000, third category \$4,001 to \$10,000, fourth level \$10,000 to \$20,000, and the last level \$20,000 and above (World Bank, 1998).

## 2.1 Income

Income is the most important indicator of family economic status, as it provides a direct means to acquire goods and services that are considered fundamental to sustaining a healthy lifestyle. Income can be used as a quantitative variable and can be grouped into categories. The categorical approach is more common since individuals tend to be reticent about providing exact income information or they are uninformed. Thus, they are more willing to indicate their placement in categories. Despite the use of the categorical approach to income responses, refusal rates are higher than for the other two commonly used indicators (i.e., education and occupation). Categories often determined by the expected range of income of participants. This fact reduces comparability across studies since the ranges of income levels are affected by the geographic area, characteristics of the respondents, and the time of study. For purposes of analysis, income categories are usually recoded to their midpoints and are often transformed to logarithms (John et al., 2002). An important consideration in the construction of survey questions is the scope of the income sources the respondent should consider when determining "household income". According to John et al., (2002) Questions about income received from jobs, social security, retirement

annuities, unemployment benefits, and public assistance. The income sources such as interest dividends, income from rental properties, child support and alimony also might be considered in a calculation of family income. In addition, family income may also include income earned from the "informal economy" (e.g., jobs that pay cash but have no benefits or job security), particularly in communities of immigrants and minorities, as well as informal transfers (e.g., of goods and services) (John et al., 2002).

Family incomes cannot be compared without knowledge of the family size. The impact of a given income is significantly dependent on family size and composition. A total family income of \$30,000 would mean something quite different to a family of two and a family of eight. It also means something different depending on whether one breadwinner earns all or most of the income while the other is able to attend to other household responsibilities in comparison to two adults having to work full-time to earn an equivalent income. While some researchers ignore the issue of family size and composition, others divide the total household income by the number of household members to produce a per capita income. This tends to overcompensate because the costs of maintaining a given standard of living do not increase proportionately. Other researchers suggested an intermediate adjustment, dividing the family income by the square root of the family size. This approach suggests that a family of four needs about double the income of a single person to have a comparable standard of living (Buhmann et al., 1998). There are a number of limitations in using income as an indicator of FES. Firstly, analysis of income is likely to be open to reverse causation arguments. Secondly, income is a more unstable measure of FES than education or occupation, and is sensitive to changes in life circumstances (thus, the advantage of using, for example, 5-year income). Income information is especially sensitive for some people, resulting in greater errors in reporting and non-response for income questions than for some other FES indicators. In other ways, measuring income can be costly and time consuming.

## 2.2 Ownership of Physical Assets

Historically, property and ownership of physical assets carry an interesting connotation in Iran, since they are mostly immovable as land and buildings and transferred through generations.

Physical assets can classify according to household assets such as whether the family home are owned or rented, and whether there is a car or garden. Wealth in the form of assets maybe offset by accumulated debt, thus, suggesting that getting a sense of the balance of assets to debt is important. Some people's wealth derives from their ability to borrow, or find investors for very large sums of money to invest; and in these less frequent cases they may be "making a living" off borrowed wealth. The distributions of income are related to the division and concentration of wealth – although the two bear no similarity. Wealth is a much broader concept than income, and contains ownership of both monetary (income, savings, investments, stocks and bonds, etc.) and other assets (real estate, home ownership, buildings, land, art, etc.). Assets maybe more strongly linked to social class than earned income. Assets also associated with health independent of other SES indicators. There are some limitations to measure of assets. These limitations are associated with the indexing of income. Given the multiple categories that may contribute to assets, assessment can be difficult for some respondents. The information also is sensitive for some people than for other FES indicators. Lastly, the measuring of assets can be costly and time consuming.

### 2.3 Expenditure

Expenditure includes consumption and non-consumption items such as education and taxes. Usually, to a certain degree, the household expenditure depends on family size; however, some families tend to spend more than others, even with the same size. Therefore, family expenditure also is used to examine the inequality in distribution of expenses. Any consumption estimate largely depends on what items are counted as consumption. It will be the aggregation of family expenditure on food, house rental, utilities, health, clothing, transportation, entertainment, furniture and appliances. The largest component of expenditure for all consumer units in Iran until 2001 has been housing, with compare other expenditures (Ministry of housing and urban, 2010). Magrabi, et al (1992) also confirmed that the housing is the largest expenditure for most of families (Magrabi et al., 1992). In developed countries, family economic status dose not directly affected to the household consumption. Studies on well-being and economic variables have dealt with the relation between income and

assets rather than with consumption. Even though income and assets are not strongly relate to well-being, but the goods and services that families buy with their money affect their satisfaction with consumption.

### 3. Conclusion

The literature review revealed the importance relevance of family economic for poverty reduction in households' communities. This study showed dimensions of family economic status that are involved in processes of family economic development. The findings of this study contribute to family economic literature. The outcome of this study also assists researchers in the field of family management and family economic.

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9/23/2010

# High Efficiency Production of Mosquitocidal Toxin by a novel *Bacillus sphaericus* isolate from Egyptian Soils on Local Agroindustrial Byproducts

M.S.Foda,<sup>1</sup>\*Fawkia M. El-Beih,<sup>2</sup>Maysa E. Moharam,<sup>3</sup>Nora N.A.El-Gamal<sup>4</sup>

Microbial Chemistry Department, Genetic Engineering & Biotechnology Division, National Research Center, Dokki, Giza, Egypt.<sup>1,3,4</sup>Faculty of Science, Ain Shams University, Cairo, Egypt.<sup>2</sup>

[foda302002@yahoo.com](mailto:foda302002@yahoo.com)

**Abstract:** Eighty six cultures were isolated from soil of different Egyptian Governorates including Quina, El-Menofaya, El-Gharbia, El-Sharkia, El-Behera and Kafr EL-Sheikh Governorates. Investigations of the mosquitocidal Egyptian isolates have revealed that isolate No.1 have the ability to form more toxin than the international reference strain *Bs*2362. The selected isolate No.1 exhibited a lower LC<sub>50</sub> and LC<sub>90</sub>values than the International strain *B. sphaericus* 2362 upon bioassay against secondinstars' larvae of *Culex pipiens*. The Egyptian isolate No.1 was identified morphologically and biochemically as *Bacillus sphaericus*. Physiological factors affecting growth and toxin formation in *B. sphaericus*No 1 in comparison to *B.s* 2362 were carried out. THE organism grown on modified Nutrient broth medium yielded the highest larval toxicity against the second instars' of *Culex pipiens* for both *Bacillus sphaericus*isolate No 1 and the international strain *Bacillus sphaericus*. The Optimum air : medium ratio were 9:1 and 4:1 of the flask volume for 4 and 3 days incubation periods using 2%and 3% sizes of inocula for *B. sphaericus* 2362 and the Egyptian isolate *B. sphaericus*No.1, respectively. Sodium acetate was the suitable carbon source for the isolate *B. sphaericus* No.1, while *B. sphaericus* 2362 was capable to utilize both sodium acetate and sodium succinate as carbon sources. The Egyptian isolate *B. sphaericus* No.1exhibited the highest mosquitocidal activity upon growth on kidney beans seeds and sesame meal as nutrient substrates at 3% final concentration, while *B. sphaericus*2362 exhibited the highest mosquitocidal activity by utilizing soy beans, lentils and sesame meal as complete media for growth and mosquitocidal toxin production.

[M.S.Foda., Fawkia M. El-Beih., Maysa E. Moharam., Nora N.A.El-Gamal. High Efficiency Production of Mosquitocidal Toxin by a novel *Bacillus sphaericus*isolate from Egyptian Soils on Local Agroindustrial Byproducts. Journal of American Science 2010;6(11):761-769]. (ISSN: 1545-1003).

**Key words:** *Bacillus sphaericus*, isolation, characterization, mosquitocidal toxin, physiology, agroindustrial byproducts.

## Introduction

The mosquito acts as a vector for many of the world's most serious diseases, both parasitic e.g. malaria (*Anopheles*), lymphatic filariasis (*Anopheles*, *Aedes*, *Culex* and *Mansonia*) and viral e.g. yellow fever, dengue (both *Aedes*) and encephalitis (*Culex*). (Baumann *et al.*, 1991).

*Bacillus sphaericus* has been successfully used for the the biological control of numerous of disease -transmitting mosquitoesmosquito and black fly species (Lacey and Undeen, 1986). The prime advantage of the *B. sphaericus* strain lies in their ability to persist for longer periods in the environment than *Bacillus thuringiensis*var. *israelensis*. This may be due to recycling and amplification of spores in larval cadavers under certain aquatic situations or

may be simply due to the long-term persistence of sufficient and accessible toxin in the environment or a combination of both of the above (Singer, 1990; Correa & Yousten, 1995). Major advantage of these bacterial insecticides are thir safety, biodegradability, and low environmental impact (Maramorosch,1987)

Opotaet *et al.* (2008) reported that the binary toxin (Bin) from *Bacillus sphaericus* exhibits a high insecticidal activity against *Culex* and *Anopheles* mosquitoes. The cytotoxicity of Bin requires an interaction with a specific receptor present on the membrane of midgut epithelial cells in larvae, a direct correlation exists between binding affinity and toxicity. The toxin binds with high affinity to its receptor in its primary target namely, *Culex pipiens*.

The present work paper aims for isolation of new *Bacillus sphaericus* strain with mosquitocidal activity that exceeds the existing international strains e.g. *B. sphaericus* 2362. In the hope to reduce Production costs of mosquitocidal toxin used for biological control of disease-transmitting mosquitoes in the developing countries production physiology of the bacterial toxin was studied on synthetic agroindustrial byproducts.

## MATERIALS AND METHODS

### Microorganisms

The International strain *Bacillus sphaericus* 2362 was kindly obtained from prof. F.G. Priest, school of life sciences, Heriot watt university, UK

A new *Bacillus sphaericus* isolate namely No.1 was isolated from soils of Quina Governorate, Egypt.

### Media used for growth, sporulation and mosquitocidal toxin production in shake cultures.

#### a-Media based on Agroindustrial by-products:

These media included offal's meal, feather meal and cotton seed meal. Most of these agroindustrialby-products are currently used in animals feed and available in Egyptian market.

**b-Media based on cheap, locally available plant proteins:** Certain legume seeds that are locally available in Egypt were examined as protein sources for growth, sporogenesis and mosquitocidaltoxin production. These legumes seeds such as soy beans, kidney bean, black eyed bean, yellow split pea, and lentils were finely grinded and used in conjunction with the standard mineral salt solution at appropriate concentration.

#### Bioassay of bacterial toxins against Mosquitoes larvae.

Bioassay of locally isolated *Bacillus* cultures including *B. thuringiensis* and *B. sphaericus* were carried out as described by **Priest and Youstine (1991)**. Toxicity was determined with laboratory reared *Culex pipiens*. Serial dilutions in distilled water were tested in a preliminary toxicity screen. The range of concentration of full grown whole culture (FWC) which killed 50% and 90% of the larvae were identified. Then further toxicity tests were done in the range recorded to evaluate precisely the LC<sub>50</sub> and LC<sub>90</sub> values for each highly promising bacterial culture.

The corrected mortality was then plotted against culture dilution of cells/ml on log paper to

determine LC<sub>50</sub> and LC<sub>90</sub> values for each highly promising bacterial cultures.

The bacterial dilutions were placed in small cups in duplicates along with 10 second instar larvae. Appropriate controls were run simultaneously using distilled water instead of cultures. The cups kept at room temperature 27±2°C. The mortality percentage was recorded by counting the number of living larvae and corrected by using appropriate control and applying Abbott's formula (**Abbott, 1925**). The medium lethal concentrations LC<sub>50</sub> of potent isolates was computed through probit analysis within 95% confidence limits using propan program.

#### Abbott's formula:

$$\text{Corrected mortality \%} =$$

$$\frac{\text{Observed mortality \%} - \text{Control mortality \%}}{100 - \text{Control mortality \%}} \times 100$$

## RESULTS

### 3.1. Isolation, Identification and Mosquitocidal Toxin Production by *Bacilli* isolated from the Egyptian environments

Eighty six isolates were obtained from soils and mud samples of six different Egyptian Governorates including Quina, El-Menofaya, El-Gharbia, El-Sharkia, El-Behera and Kafr EL-Sheikh. Among these isolates, isolate No.1 obtained from Quina Governorate was the only isolate giving 100% mortality up to culture dilution 10<sup>-5</sup>. Accordingly this isolate obtained from Quina Governorate was selected for further investigation.

### 3.2. Determination of LC<sub>50</sub> and LC<sub>90</sub> values of the Egyptian isolate No.1 obtained from Quina Governorate soils.

LC<sub>50</sub> and LC<sub>90</sub> of isolate No.1 and *B. sphaericus* 2362 bioassayed against second instar larvae of *Culex pipiens* revealed that the Egyptian isolate No.1 is more toxic than the reference strain 2362. **Table (1)**.

### 3.3. Identification of the Egyptian isolate No.1 isolated from Quina Governorate.

The colonies exhibited beige color with medium size colonies, The texture is smooth semi-glistening

with round margin; The appearance of colonies is shiny with little elevation and flat.

Examination of the cells with the electron microscope revealed the rod-shaped of the vegetative cells as shown in Fig (1); sporulated cells (sporangia) with subterminal spores that are round in shape giving the sporangia club shaped appearance as shown in Fig (2). Also it was observed that isolate No.1 produced a spherical spore and round crystals when examined under the electron microscope as shown in Fig (3).

Some biochemical tests for the identification of the Egyptiaisolate No.1 obtained from QuinaGovernoratewere carried out Table (2).

### **3.4. Comparative Physiological studies on factors affecting growth, sporulation, and toxin production of the Egyptian isolate *B. sphaericus*No.1 and the International *B. sphaericus* 2362 strain under submerged fermentation conditions**

#### **3.4.1. Effect of types of media on growth parameters, sporulation titer and mosquitocidal toxicity under submerged conditions.**

Four types of media were used in this study namely Nutrient yeast salt medium, Luria –Bertani medium, Nutrient broth (NB), and modified Nutrient broth (NB+ 0.5% yeast extract)The obtained results showed that modified Nutrient broth medium yielded the highest larval toxicity against the second instars' larvae of *Culex pipiens*for both *Bacillus sphaericus*isolate No 1and the international strain *Bacillus sphaericus*2362(Data not shown).

#### **3.4.2 Effect of aeration level on growth and toxicity of *B. sphaericus***

In this experiment the extent of aeration was altered by varying the air : medium ratio (amount of medium in the culture flask). The effect of aeration extent on growth parameters and toxicity of the mosquitocidal agent produced by the organisms under study are shown in Figures (4,5). It was noted that the viable count and toxicity increased with increasing the air : medium ratio. Furthermore, The sporulation and toxin production gave the highest titers when the medium volume occupied 10% and 20%, i.e. corresponding to air: medium ratio 9:1 and 4:1 of the flask volume for *B. sphaericus* 2362 and the Egyptian isolate *B. sphaericus*No.1, respectively.

#### **3.4.3. Effect of different carbon sources utilized by *B.sphaericus*on growth parameters and mosquitocidal toxin formation.**

It is known that *B. sphaericus*can not utilize carbohydrates for growth due to the lack of many of the enzymes of Embden Meyerhof and hexose monophosphate path ways (**Russelet et al., 1989**).In this experiment different carbon sources were used for testing the ability of the tested organisms*B. sphaericus* No.1 and *B. sphaericus* 2362 to utilize this carbon sources. The results revealed that sod. acetate was utilized by the isolate *B. sphaericus* No.1, at which the sporulation and toxin production yielded the highest titers. On the other hand, *B. sphaericus* 2362 was capable to utilize sod. acetate and sod. succinate, as shown in Figures (6,7).

#### **3.4.5.. Effect of inoculum size on growth parameters and mosquitocidal toxin formation of *B. sphaericus*.**

Different volumes of overnight growing culture were used as inocula for a set of 250 ml conical flasks each containing 25ml of modified nutrient liquid medium . The results of growth parameters and toxin production of tested organisms are illustrated by Figures (8,9).

The increase of inoculum size has led to the increase of sporulation titer and toxin production up to 3% inoculum size, and then decreased with the increasing of inoculums size in case of the Egyptian *B. sphaericus* No.1. However the sporulation and toxin production of *B. sphaericus* 2362 showed a little effect by changing the inoculum size. The highest toxicity were achieved using 3% inoculums size and 2% by isolate *B. sphaericus* No.1 and *B. sphaericus* 2362, respectively.

#### **3.4.6. Effect of incubation period on growth parameters and mosquitocidal toxin formation of *B. sphaericus*.**

This experiment was carried out under standard conditions by using a set of 250 ml conical flasks containing 25ml of modified nutrient liquid medium, then the extent of growth, sporulation titer and toxin production were followed and determined by harvesting after 2, 3, 4 and 7 days of incubation at 28 ± 2°C on a rotary shaker.

The results are shown in Figures (10,11). The mortality increased with increasing the incubation period until 3 days incubation period in case of *B. sphaericus* No.1 and 4 days for*B. sphaericus* 2362.

**Table(1):** Values of LC<sub>50</sub> and LC<sub>90</sub> for mosquitocidal toxins of the Egyptian isolate No.1 in comparison with those of the International strain of *Bacillus sphaericus* 2362 at confidence limits(95%). The bioassay were carried out against second instar larvae of *Culex pipiens*.

**3.4.7. Effect of different by-products and grinded legumes seeds used as complete media on growth, sporulation and toxin production of *Bacillus sphaericus*.**

Isolate	LC <sub>50</sub> (Confidence limits at 95 %) by $\mu$ l	LC <sub>90</sub> (Confidence limits at 95 %) by $\mu$ l
The Egyptian isolate <i>B. sphaericus</i> No.1 from Quina. Egypt	264.4 (155.3-365.8)	725.9 (517.3-1351.7)
The International strain <i>B. sphaericus</i> 2362	359.2 (228.5-479.3)	932.4 (674.3-1818.9)

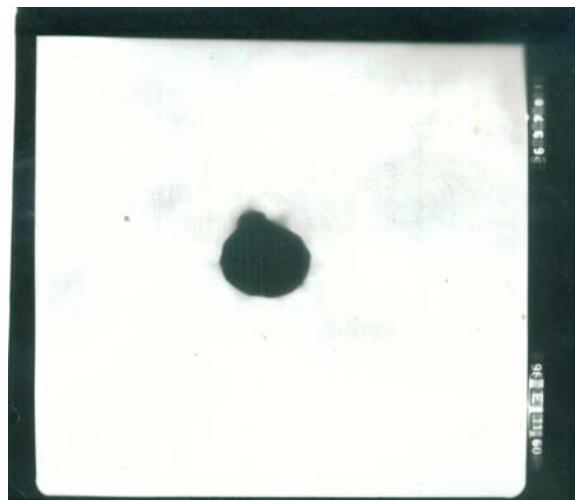
Ten agroindustrial byproducts that are available in Egypt were examined as a complete cost effective media for toxin production. The data in Figures(12,13) illustrated that the Egyptian isolate *B. sphaericus* No.1 exhibited the highest mosquitocidal activity by utilizing kidney beans and sesame meal as nutrient substrate at 3% final concentration, while *B. sphaericus* 2362 exhibited the highest mosquitocidal activity by utilizing soy beans, lentils and sesame meal as growth media for growth and mosquitocidal toxin production.



**Fig (1)** E.M. showing chain of vegetative cells of the Egyptian isolates *B. sphaericus* No.1 isolated from Quina Governorate (X 10.000).



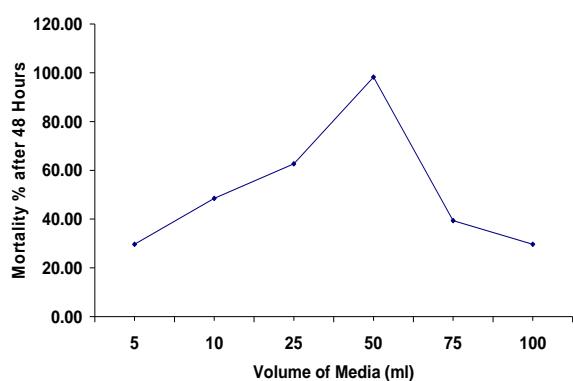
**Fig (2)** E.M. the Egyptian isolates No.1 isolated from Quina Governorate grown on nutrient liquid medium showing the club-shaped cells (X 20.000).



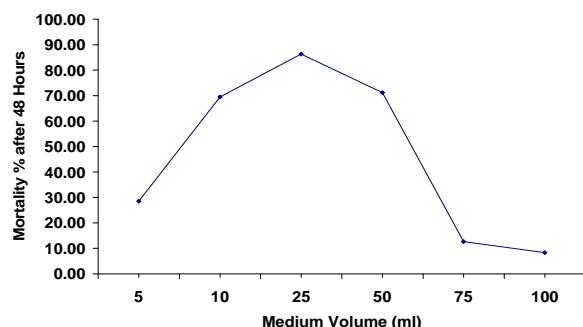
**Fig (3)** Electron Micrograph (E.M.) showing spherical spore and crystal of the Egyptian *B. sphaericus* isolates No.(1) after 3 days of incubation (X 20.000).

**Table (2):** Some biochemical tests for the identification of the Egyptian isolate No.1 obtained from Quina Governorate as compared with *B. sphaericus* 2362.

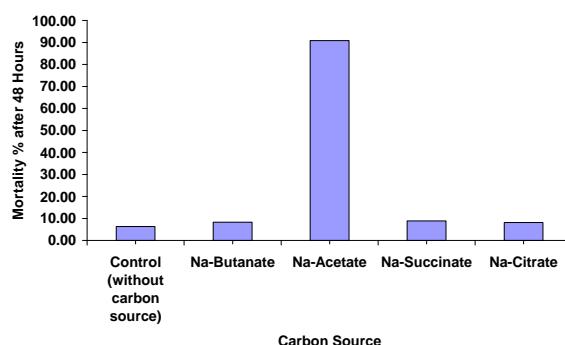
Biochemical tests	Standard strain <i>B. sphaericus</i> 2362	The Egyptian isolate No.1
Tolerance to NaCl 2%	+	+
5%	+	+
7%	-	-
10%	+	+
Degradation of adenine	+	+
Decomposition of urea	+	+
Hydrolysis of casein	+	+
Hydrolysis of Starch	-	-
Hydrolysis of gelatin	+	-
Utilization of citrate	-	-
Methyl red test	-	-
Vogesproskauer test	-	-
Catalase test	-	-
Nitrate reduction test	-	-



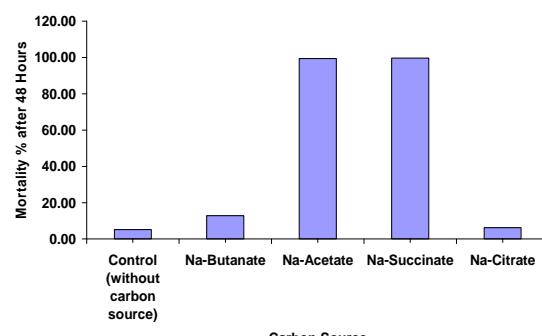
**Fig(4):** Effect of volume of media on toxin production by the Egyptian isolate *B. sphaericus* No.1



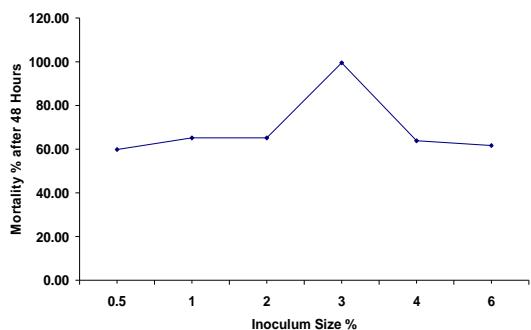
**Fig (5):** Effect of volume of media on toxin production by *B.sphaericus* 2362.



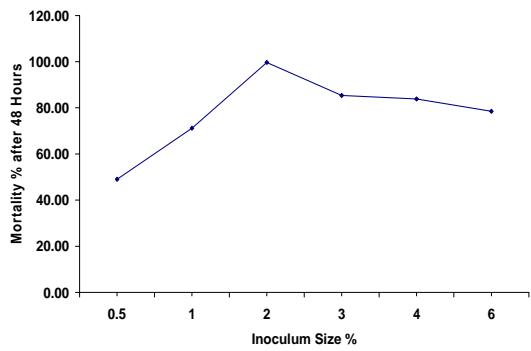
**Fig (6):** Effect of different carbon sources (salts of organic acids)on toxin production of the Egyptian isolate *B. sphaericus*No.1.



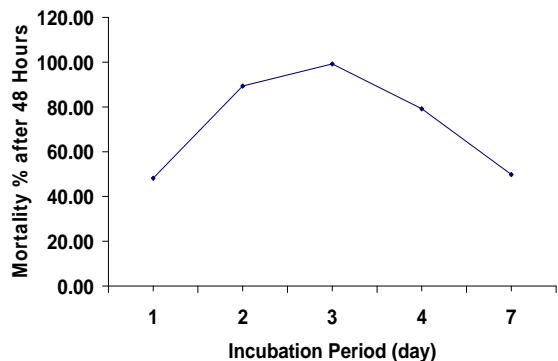
**Fig (7):** Effect of different carbon sources (salts of organic acids) on toxin production by *B.sphaericus* 2362.



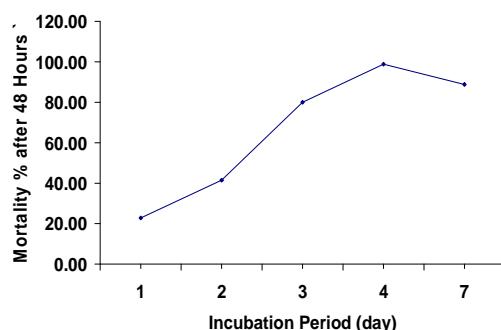
**Fig (8):** Effect of inoculum size on toxin production of the Egyptian isolate *B. sphaericus* No.1.



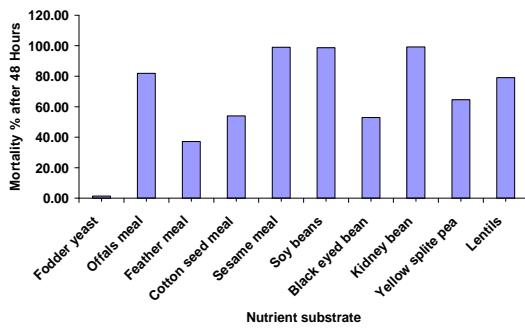
**Fig (9):** Effect of inoculum size on toxin production of *B. sphaericus* 2362.



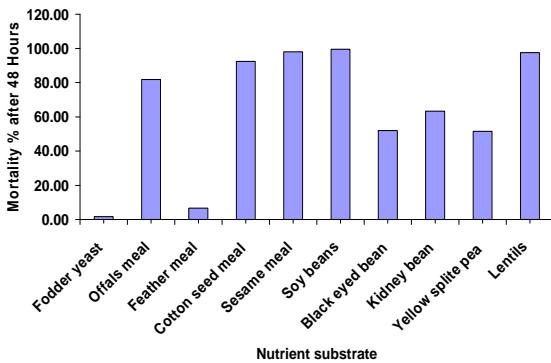
**Fig (10):** Effect of incubation period on toxin production of *B. sphaericus* No.1.



**Fig (11):** Effect of incubation period on toxin production of *B. sphaericus* 2362.



**Fig (12):** Effect of some agroindustrial by-products and grinded legumes seeds used as complete growth media on mosquitocidal toxicity of the Egyptian isolate *B. sphaericus* No.1 using substrate concentration 3%.



**Fig (13):** Effect of some agroindustrial by-products and grinded legumes seeds on mosquitocidal toxicity of *B. sphaericus* 2362 using substrate concentration 3%.

## DISCUSSION

The mosquito acts as a vector for many of the world's mostserious diseases, both parasitic e.g. malaria (*Anopheles*), lymphaticfilariasis (*Anopheles*, *Aedes*, *Culex* and *Mansonia*) and viral e.g. yellow fever, dengue (*Aedes*) and encephalitis (*Culex*). The present work aims to isolate some local isolates of *B. sphaericus*pathogenic to mosquito larvaefrom the Egyptian environment. Itwas also devoted to investigate the growth physiology and various factors that are affecting growth, sporulation and toxins formation. On the other hand, special attention was given to search for suitable media that are low-priced and locally available in Egypt for *B. sphaericus* production on a large scale. The goal stemmed from the fact that the feasibility of economic production of spores and toxin crystals of *B. sphaericus* is dependant to a large extent on production costs and availability of raw materials under the local conditions. Physiological factors affecting growth and toxin formation in *B. sphaericus* revealed that modified Nutrient broth medium yielded the highest larval toxicity against the second instars' of *Culexpipiens*for both *Bacillus sphaericus*isolate No 1 and the international strain *Bacillus sphaericus*2362. The requirements of individual bacterial strain for nutrients may vary for different strains and also of different isolates within the same strain within the same species. Thus optimal concentration of nutrients for one isolate may not necessarily be suitable for another. Therefore, it is impossible to recommend a fermentation medium that will be best for all isolates of the same species (**Fodaet al. 2000**).

It is established that *B. sphaericus* is an obligate aerobe and adequate air supply is needed for growth, initiation of sporulation and toxin synthesis (**Yousten and Wallis, 1987**).

In our studies, it was found that the maximum sporulation and toxicity were acheived when the medium volume to air ratio was 1:4 for the Egyptian isolate *B. sphaericus*No.1 and 1:9 for the International strain *B. sphaericus* 2362 that was used for comparative purposes. The increase in medium volume to air ratio has lead to the decrease in sporulation and toxicity. This result agrees with what reported by **Yousten and Wallis (1987)**. They found that oxygen was required for toxin production by *B. sphaericus* strain 2362. However, they reported that increasing the level of dissolved oxygen (DO) in the medium by use of pure oxygen in the gas stream lowered toxin production, while in case of strain 1593, (another *B. sphaericus*International strain), increased

(DO) produced a block in sporulation, but toxin synthesis was normal (**Youstenet al., 1984**).

The result of growth parameters of tested organisms indicated that *B. sphaericus* isolate No.1 gave high sporulation titer and toxicity at inoculum size 3% and a decrease in toxicity was recorded by increasing the inoculum size, However the highest sporulation and toxin production levels of strain 2362 were achieved by inoculum size 2%. This result agrees with that reported by **Fodaet al. (2000)**, they reported that the sporulation of the Egyptian isolate No. 69 increased by decrease in the inoculum size to reach  $7.5 \times 10^6$ /ml viable count whereas the sporulation of strain *B. sphaericus*2362 exhibited a little effect by changing the inoculum size.

*B. sphaericus*can not use carbohydrates for growth due to the lack of many of the enzymes of Embden Meyerhof and hexose monophosphate pathways(**Russelet et al., 1989**).

In the present study, it has been found that the Egyptian isolate *B. sphaericus* No.1 grew well using acetate as sole carbon source. On the other hand the International strain *B. sphaericus*2362 grew with acetate or succinate as sole carbon source. This result agrees with what reported by (**Gordon et al., 1973; De BarJacet et al., 1980; Klein et al., 1989; Widjayaet al., 1992 and Ahmed et al., 1993 and 1996**). They reported that numerous strains of *B. sphaericus*grew with acetate, Pyruvate, lactose, glutamate, succinate, histidine and arginine, as sole major carbon and energy sources.

In the present study, ten leguminous seeds and agroindustrial by-products were used as nutrient substances at concentration 3% and the result indicated that soy beans, kidney beans and sesame seed meal could be used efficiently as nutrientssources to support growth, sporulation and toxin production of the Egyptian isolate *B. sphaericus*No.1. High levels of toxicity were obtained even at low concentration of diluted culture ( $3 \times 10^6$ ), as inocula. On the other hand, *B. sphaericus* 2362 grew well on a medium contained soy beans, lentils and sesame seed meal and the growth, sporulation and high levels of toxin production were achieved at the same culture dilution. Uses of such various by-products as well as legume seeds have shown that local production from inexpensive ingredients available in different regions is possible. Such studies may pave the way for mass production on industrial scales.**Dulmageet al. (1970)**culturd*B. sphaericus*1593 and 2362 separately in a fermentor on peptonized milk medium with yeast

extract and mineral supplements. The fermentor beer was centrifuged and then resuspended in lactose solution and precipitated with acetone. These powders were highly insecticidal to *Culex quinquefasciatus* larvae producing LC<sub>50</sub> values in the range of 10<sup>-2</sup> µg/ml. **Obeta and Okafor (1983)** formulated five media from dried cow blood, mineral salts and seeds from four species of legumes (ground nut cake, cowpea, mambara beans and soy beans) for production of *B. sphaericus* 1593. Good growth, sporulation and toxin activity of *B. sphaericus* 1593 were obtained with all tested media. **Dharmsthiti et al. (1985)** grew *B. sphaericus* on a medium containing 7% hydrolyzed liquor by-product from a monosodium glutamate factory. **Klein et al. (1989)** used hydrolyzed industrial peptones (waste product of industry) for constructing seven media for production of *B. sphaericus* larvicides. These media contained 5 g/l industrial peptone in 50 mM phosphate buffer (pH 7.0) in combination with other carbon and nitrogen sources. Industrial peptone medium supplemented with glycerol was the most efficient medium for growth and larvicides production by *B. sphaericus* 2362. The local availability of proteinaceous materials is vitally important for *B. sphaericus* fermentation. For example, one of the most useful nitrogen sources is cotton seed flour (**Dulmageet et al. 1990b**). They reported that several nitrogen sources are used in *Bt* fermentation, including soybean flour, cotton seed flour and fish meal. The soy flour and cotton seed flour were both very good sources of nutrients for both *Bt* and *B. sphaericus* production. **Gangurde and Shethna (1995)** concluded that mustard seed meal (MSM) contains 40% protein, with glutamic acid and arginine as a major amino acids. Therefore, growth and larvicidal activity of *B. sphaericus* 2362 and 1593 produced in MSM can be attributed in part to the presence of these amino acids. **Ampofo (1995)** used some local raw-materials for production of *Bs* insecticides in Ghana. He tested anchovy, spent grain from breweries, bambara beans and sprout maize as media for production of *B. sphaericus* IAB 881. He reported that larvicidal activity of *Bs* IAB 881 grown in anchovy, spent grain, bambara beans and sprout maize, was similar to that obtained in synthetic medium with LC<sub>50</sub> ranging from 0.3×10<sup>-5</sup> to 0.68×10<sup>-6</sup> (dilution). Cell counts were in the range of 11×10<sup>8</sup> – 36×10<sup>8</sup> CFU/ml and spore counts were between 29×10<sup>7</sup> and 61×10<sup>7</sup> CFU/ml. **El-Bendary (1999)** used ground agroindustrial by-products and leguminous seeds at 2% final concentration as media for production of *B. sphaericus* in distilled water with or without addition of NYSM salts. The obtained results indicated that most of the tested substances supported

formation of highly efficient media for *Bs* toxin production of appreciably high sporulation yield and toxicity. She also reported that the most efficient media for *B. sphaericustoxin* production were soy flour, cotton seed flour, corn steep solids and offals meal. Furthermore, it was observed that addition of NYSM salts to these substances increased the *B. sphaericus* toxicity. Moreover, the toxicity of *B. sphaericus* increased about 1.5-4.5 times when these agroindustrial by-products were partially hydrolyzed by nuclease or alkalase enzymes before using as media. **El-Bendary et al. (2008)**, used whey permeate (WP) for production of mosquitocidal toxin by *B. sphaericus* 2362 and the Egyptian isolate, *B. sphaericus* 14N1 under both submerged and solid state fermentation conditions. Under submerged fermentation, high mosquitocidal activity was produced by *B. sphaericus* 2362 and *B. sphaericus* 14N1 at 50% -100% and 25% -70% whey permeate, respectively.

Corresponding author:

Email: [foda302002@yahoo.com](mailto:foda302002@yahoo.com)

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9/28/2010

# Global Analysis of an Epidemic Model with General Incidence Rate.

**M. M. A. El-Sheikh and S. A. A. El-Marouf**

Department of Mathematics, Faculty of Science, Taibah University, Kingdom of Saudi Arabia  
 Permanent Address: Department of Mathematics, Faculty of Science, Menoufia University,  
 Shebin El Koom, Egypt.

**Abstract:** A general four-dimensional SIQR epidemic model is considered. Threshold, equilibria and their stability are established. The dynamics of the system is discussed in the case of this general form of incidence rate. The global stability of both free-disease and endemic equilibria are deduced. Hopf bifurcation , boundedness, dissipativity and persistence are studied.

Journal of American Science 2010;6(11):770-783]. (ISSN: 1545-1003).

**Keywords:** Epidemic model; Nonlinear incidence rate; quarantine; uniformaly persistence; global stability; Hopf bifurcation.

## 1. Introduction:

The technique of introducing quarantine in standard *SIS* and *SIR* epidemic models has received great interest in the last two decades (see for example [1], [5], [6], [7], and [21]). Over the centuries quarantine, which means forced isolation or stoppage of interactions with others, succeeded to reduce the transmission of human and animal diseases. In their recent paper Feng and Thieme [5] considered an *SIQR* model with a quarantine class and showed that the quarantine can lead to periodic solutions. They considered in [6] a more general endemic model that includes *SEIQR* models with arbitrary distributed periods of infection including quarantine. They proved extinction and persistence results. In [12], Hethcote et al discussed six endemic models with quarantined class. In this paper we consider an *SIQR* model with an incidence term more general than those used by [3], [10] , [11], [12], [14], and [16]. Following [12],we assume that the total host population is partitioned into susceptible, infectious, quarantine and recovered which

densities denoted respectively by  $S(t), I(t), Q(t)$  and  $R(t)$ . The natural birth rate denoted by  $A$ . Assume that infectious confers permanent immunity, so that, individuals can move from the  $I$  and  $Q$  classes to the  $R$  class, where  $R(t)$  is the number of individuals with permanent immunity and  $N(t) = S(t) + I(t) + Q(t) + R(t)$ . In this paper we assume that the general incidence rate term  $H(I, S)$  is differentiable, with  $\frac{\partial H}{\partial I}$  and  $\frac{\partial H}{\partial S}$  are nonnegative and finite for all  $I$  and  $S$ . More precisely we consider the 4-dimensional system of differential equations,

$$\begin{aligned}
 S' &= A - \beta IH(I, S) - dS, \\
 I' &= \beta IH(I, S) - (\gamma + \delta + d + \alpha_1)I, \\
 Q' &= \delta I - (d + \alpha_2 + \varepsilon)Q, \\
 R' &= \gamma I + \varepsilon Q - dR,
 \end{aligned} \tag{1.1}$$

where  $A$  and  $d$  are positive constants and  $\gamma, \delta, \varepsilon, \alpha_1$  and  $\alpha_2$  are non-negative

constants. The constant  $A$  is the recruitment rate of susceptible corresponding to birth and immigration,  $\beta$  is the average number of adequate contact,  $d$  is the per capita natural mortality rate,  $\delta$  is the rate constant for individuals leaving the infective compartment  $I$  for the quarantine compartment  $Q$ ,  $\gamma$  and  $\varepsilon$  are the rates at which individuals recover and return to susceptible compartment  $S$  from compartment  $I$  and  $Q$ , respectively, and  $\alpha_1$  and  $\alpha_2$  represent the extra disease-related death rate constants in classes  $I$  and  $Q$ , respectively. The total population size  $N(t)$  satisfies  $N'(t) = A - dN - \alpha_1 I - \alpha_2 Q$ , so that the population size  $N(t)$  approached the carrying capacity  $\frac{A}{d}$  when there is no disease. The differential equation for  $N$  implies that the solutions of (1.1) starting in the positive orthant  $R_+^3$  either approach, enter, or remain in the subset

$$D = \left\{ (S, I, Q, R) : S \geq 0, I \geq 0, Q \geq 0, R \geq 0, S + I + Q + R \leq \frac{A}{d} \right\}.$$

The model (1.1) is more general epidemiological model than those discussed in ([11],[14],[16], and [17]). It is known (see [11]) that the system (1.1) always has the disease-free equilibrium  $P_0 = (\frac{A}{d}, 0, 0, 0)$ .

We define the quarantine reproduction

$$\text{number in the form } R_q = \frac{\beta(\frac{A}{d})}{(\gamma + \delta + d + \alpha_1)}$$

where if  $R_q > 1$ , the region  $D$  contains also the endemic equilibrium  $P^* = (S^*, I^*, Q^*, R^*)$ , where

$$\begin{aligned} S^* &= \frac{A}{d} - \frac{(\gamma + \delta + d + \alpha_1)}{d} I^*, Q^* = \frac{\delta I^*}{d + \alpha_2 + \varepsilon}, \\ H(I^*, \frac{A}{d} - \frac{(\gamma + \delta + d + \alpha_1)}{d} I^*) &= \frac{(\gamma + \delta + d + \alpha_1)}{\beta}. \end{aligned} \quad (1.2)$$

The aim of this paper is to study the dynamic of (1.1) by different techniques with a generalized incidence term. We show that some of our obtained results may be applied for many special forms of  $H(I, S)$ . The organization of this paper is as follows. In section 2, we discuss the stability properties of the reduced 3-dimensional  $SIQ$  epidemic model. In section 3, we study the boundedness, dissipativity, persistence, global stability and Hopf bifurcation of solutions of the 4-dimensional model (1.1). Our technique in this section depends on [20]. The paper ends with numerical justifications and brief discussion in section 4.

## 2. A reduced $SIQ$ epidemic model

Since the last equation in (1.1) is independent of the other equations ( see[10], [11], [12] and [13]), we may start by discussing the 3-dimensional system,

$$S' = A - \beta I H(I, S) - dS,$$

$$I' = \beta I H(I, S) - (\gamma + \delta + d + \alpha_1) I,$$

$$Q' = \delta I - (d + \alpha_2 + \varepsilon) Q.$$

The dynamic of (1.1) in  $D$  is equivalent to that of (2.1) in the feasible region

$$\Gamma = \{(S, I, Q) \in R_+^3 : S + I + Q \leq \frac{A}{d}\}, \quad (2.2)$$

which can shown to be closed and positively invariant set with respect to (2.1) (see [14]). Letting  $\partial\Gamma$  denotes the boundary of  $\Gamma$  and  $\Gamma^0$  its interior , the system (2.1) always has

disease-free equilibrium point  
 $P_0 = \left(\frac{A}{d}, 0, 0\right) \in \partial\Gamma$ .

**Lemma 2.1.** If  $R_q \leq 1$ , then there exists a unique equilibrium point  $P_0 = \left(\frac{A}{d}, 0, 0\right)$ .

If  $R_q > 1$ , then there exists an endemic nontrivial equilibrium point  $P^* = (S^*, I^*, Q^*)$  in  $\Gamma^0$ .

**Proof.** The uniqueness of the endemic nontrivial equilibrium point can be guaranteed by [25]. From the isocline equations, it is clear that the coordinates of the endemic equilibrium  $P^* = (S^*, I^*, Q^*)$  satisfy (1.2). Hence since  $S^*$  exists, then  $H$  must be less than  $\frac{A}{d}$ , i.e. when  $R_q > 1$ ,

$S^*$  does not exist and the only equilibrium point is  $P_0 = \left(\frac{A}{d}, 0, 0\right)$ . Now the local stability of  $P_0$  can easily deduced by inspection of the eigenvalues of the following Jacobian matrix at  $P_0$

$$M_{P_0} = \begin{pmatrix} -d & -\beta H(0, \frac{A}{d}) & 0 \\ 0 & \beta H(0, \frac{A}{d}) - (\gamma + \delta + d + \alpha_1) & 0 \\ 0 & \delta & -(d + \alpha_2 + \varepsilon) \end{pmatrix}$$

which has the eigenvalues  $\lambda_1 = -d$ ,  $\lambda_2 = (\gamma + \delta + d + \alpha_1) - \beta H(0, \frac{A}{d})$ ,

and  $\lambda_3 = -(d + \alpha_2 + \varepsilon)$ . This completes the proof.  $\square$

**Lemma 2.2.** The disease-free equilibrium point  $P_0 = \left(\frac{A}{d}, 0, 0\right)$  is globally

asymptotically stable in  $\Gamma$  if  $R_q \geq 1$ , while it is an unstable saddle point if  $R_q \leq 1$ .

**Proof.** Constructing the Liapunov function  $V = I$ ,

then

$$\begin{aligned} V' &= [\beta H(I, S) - (\gamma + \delta + d + \alpha_1)]I \\ &< [\beta(\frac{A}{d}) - (\gamma + \delta + d + \alpha_1)]I, \end{aligned}$$

since  $H < \frac{A}{d}$ . Thus

$$V' \leq I\{R_q - 1\}.$$

Consequently, if  $R_q \leq 1$ , then

$$V' \leq 0.$$

Moreover

$$V' = 0 \text{ iff } V = 0.$$

Thus the largest compact invariant set in  $\{(S, I, Q) \in \Gamma : I = 0\}$  in the case of  $R_q \leq 1$  is the singleton  $\{P_0\}$ . Consequently by La salle's invariance principle, it follows that the disease-free point  $P_0 = \left(\frac{A}{d}, 0, 0\right)$  is

globally asymptotically stable in  $\Gamma$  ( see [23]). Now in the case  $R_q > 1$ ,  $P_0 = \left(\frac{A}{d}, 0, 0\right)$  is an unstable saddle point because as stated in Lemma 2.1 the eigenvalues will be  $\lambda_1 = -d < 0$ ,  $\lambda_2 = (\gamma + \delta + d + \alpha_1) - \beta H(0, \frac{A}{d}) > 0$

and  $\lambda_3 = -(d + \alpha_2 + \varepsilon) < 0$  i.e. if  $R_q > 1$ , the nontrivial equilibrium emerges, two roots have negative real parts and one is positive, so  $P_0$  is an unstable saddle point.  $\square$

Now we may note that the case when  $R_q = 1$  cannot be discussed here by linear analysis. However the above Lyapunov

technique covered this case in the case  $R_q \leq 1$ .

**Remark 2.1.**

(1) heorem 2.1 completely determines the local dynamics of (2.1) in  $\Gamma$  depending on the reproduction rate  $R_q$ . Its epidemiological implication is that the infected population (the sum of the latent and infectious population) vanish in time so the disease dies out.

(2)The quarantine reproduction number

$$R_q = \frac{\beta \frac{A}{d}}{(\gamma + \delta + d + \alpha_1)} \quad \text{represents the}$$

product of  $\beta H(0, \frac{A}{d})$ , and the average

residence time  $\frac{1}{(\gamma + \delta + d + \alpha_1)}$  in infective

class  $I$ . i.e.  $R_q$  is the average number of secondary infectious in a completely susceptible population when one infectious entries the population in the situation where the average infectious period decreased by the quarantining of some infectives.

It was stated in Lemma 2.1 that the system (2.1) has a unique endemic nontrivial equilibrium  $P^* = (S^*, I^*, Q^*)$ . Now we discuss the global asymptotic stability of this unique endemic equilibrium  $P^* = (S^*, I^*, Q^*)$  using the method of higher-order generalization of the Bendixon criterion (see [16],[17],and [23]).The main theorem of the method depends on the use of Lozinski Logarithmic norm. For a general  $3 \times 3$  matrix  $J = (J_{ij})$ . Following [23],we consider the Lozinskii measure  $\mu$  of  $B = P_f P^{-1} + P J^{[2]} P^{-1}$  with respect to a vector norm  $\|\cdot\|$  in  $R^N$ ,  $N = \binom{n}{2}$ ,

$$\mu(B) = \lim \frac{|I + hB| - 1}{h}.$$

The following auxiliary result is a basis for most of the work of global stability for an autonomous system

$$Y' = f(Y).$$

**Lemma 2.3.** Assume that

(I<sub>1</sub>)  $\Gamma$  is simply connected,

(I<sub>2</sub>) There exists a compact absorbing set  $\Omega \subset \Gamma$ ,

(I<sub>3</sub>) The system  $Y' = f(Y)$  has a unique equilibrium  $Y^*$  in  $\Gamma$ .

Then  $Y^*$  is globally asymptotically stable in  $\Gamma$  provided that a function  $B(x)$  and a Lozinski measure  $\mu$  exists such that

$$\limsup_{t \rightarrow \infty} \frac{1}{t} \int_0^t \mu(B(x(s, r_0))) ds < 0.$$

**Theorem 2.4.** If  $R_q > 1$ , then the unique endemic equilibrium  $P^* = (S^*, I^*, Q^*)$  is globally asymptotically stable in  $\Gamma^0$ .

**Proof.** Setting the diagonal matrix

$$p(S, I, Q) = \text{diag}\left(1, \frac{I}{Q}, \frac{I}{Q}\right),$$

then  $P$  is  $C^1$  and nonsingular in  $\Gamma^0$ . Letting  $f$  to represent the vector field of (2.1). Then

$$p_f p^{-1} = \text{diag}\left(0, \frac{I'}{I} - \frac{Q'}{Q}, \frac{I'}{I} - \frac{Q'}{Q}\right), \text{ where}$$

the matrix  $p_f$  is

$$(p_{ij}(x))_f = \left( \frac{\partial p_{ij}(x)}{\partial x} \right)^T \cdot f(x) = \nabla p_{ij} \cdot f(x).$$

(2.3)

Setting  $k_1 = \gamma + \delta + d + \alpha_1$  and  $k_2 = k_1 + d + \alpha_2 + \varepsilon$  the Jacobian matrix  $J$  at  $p^*(S, I, Q)$  is

$$J_{p^*} = \begin{pmatrix} (-\beta I \frac{\partial H}{\partial s} - d) & (-\beta H - \beta I \frac{\partial H}{\partial I}) & 0 \\ \beta I \frac{\partial H}{\partial s} & \beta I \frac{\partial H}{\partial I} & 0 \\ 0 & \delta & k_2 \end{pmatrix}$$

Now it is known (see [17], [19], [22]) that its second additive compound is

$$J_{p^*}^{[2]} = \begin{pmatrix} -\beta \left( \frac{\partial H}{\partial I} + \frac{\partial H}{\partial s} \right) - d & 0 & 0 \\ \delta & k_2 - d - \beta I \frac{\partial H}{\partial s} & -k_1 - \beta I \frac{\partial H}{\partial I} \\ 0 & \beta I \frac{\partial H}{\partial s} & k_2 + \beta I \frac{\partial H}{\partial I} \end{pmatrix}$$

Moreover

$$p^* J_{p^*}^{[2]} p^{-1} = \begin{pmatrix} -\beta \left( \frac{\partial H}{\partial I} + \frac{\partial H}{\partial s} \right) - k_1 - d & 0 & \frac{Q}{I} \left( k_2 + \beta I \frac{\partial H}{\partial I} \right) \\ \frac{\partial I}{Q} & \left( k_2 - d - \beta I \frac{\partial H}{\partial s} \right) \left( -k_1 - \beta I \frac{\partial H}{\partial I} \right) & 0 \\ 0 & \beta I \frac{\partial H}{\partial s} & k_2 + \beta I \frac{\partial H}{\partial I} \end{pmatrix}$$

Consider the matrix  $B = p_f p^{-1} + p^* J_{p^*}^{[2]} p^{-1}$

where the matrix  $p_f$  is as in (3.1) can be written in the matrix form

$$B = \begin{pmatrix} B_{11} & B_{12} \\ B_{21} & B_{22} \end{pmatrix},$$

where

$$B_{11} = -\beta I \left( \frac{\partial H}{\partial I} + \frac{\partial H}{\partial s} \right) - k_1 - d,$$

$$B_{12} = (0, 0), \quad B_{21} = \begin{pmatrix} \frac{\partial I}{Q} \\ 0 \end{pmatrix},$$

and

$$B_{22} = \begin{pmatrix} \frac{I'}{I} - \frac{Q'}{Q} + \left[ k_2 - d - \beta I \frac{\partial H}{\partial s} \right] & -k_1 - \beta I \frac{\partial H}{\partial I} \\ \beta I \frac{\partial H}{\partial s} & \frac{I'}{I} - \frac{Q'}{Q} - k_2 + \beta I \frac{\partial H}{\partial I} \end{pmatrix}.$$

Choosing a vector norm

$$\|(u, v, \omega)\| = \max\{|u|, |v| + |\omega|\},$$

where  $(u, v, \omega)$  be a vector in  $R^3$ . Let  $\mu$  be

the Lozinski measure with

$$\mu(B) \leq \max\{\mu_1(B_{11}) + |B_{12}|, |B_{21}| + \mu_1(B_{22})\}, \quad (2.4)$$

where  $|B_{12}|$  and  $|B_{21}|$  are matrix norm with

respect to the  $L^1$  vector norm ,and  $\mu_1$

denotes the Lozinski measure with respect to

the  $L^1$  norm. Here  $\mu_1$  is given by

$$\mu_1(B_{11}) = -\beta I \left( \frac{\partial H}{\partial I} + \frac{\partial H}{\partial s} \right) - k_1 - d,$$

$$|B_{12}| = 0, \quad |B_{21}| = \frac{\delta I}{Q},$$

$$\mu_1(B_{22}) = \frac{I'}{I} - \frac{Q'}{Q} + k_2 - d.$$

$$\text{Thus } g_1 = -\beta I \left( \frac{\partial H}{\partial I} + \frac{\partial H}{\partial s} \right) - d, \quad \text{and}$$

$$g_2 = \frac{\delta I}{Q} + \frac{I'}{I} - \frac{Q'}{Q} + k_2 - d. \quad \text{But since by}$$

(2.1)

$$\frac{I'}{I} = \beta H(I, S) + k_1 \quad \text{and} \quad \frac{Q'}{Q} = \frac{\delta I}{Q} + k_2.$$

(2.6)

Then by (2.3), (2.4) and (2.5), we get

$$g_1 = -\frac{I'}{I} \left( \frac{\partial H}{\partial I} + \frac{\partial H}{\partial s} \right) \frac{I}{H} - \frac{k_1}{H} - d, \quad \text{and}$$

$$g_2 = \frac{I'}{I} - d.$$

Since by assumptions  $\frac{\partial H}{\partial I}, \frac{\partial H}{\partial S}$  and  $k_1$  are

all nonnegative ,then  $\mu(B) \leq \frac{I'}{I} - d$ .

Thus along each solution  $(S(t), I(t), Q(t))$  of (2.1) such that  $\begin{pmatrix} S \\ (0), I(0), Q(0) \end{pmatrix} \in \Gamma$ , the absorbing set, we have

$$\lim_{t \rightarrow \infty} \frac{1}{t} \int_0^t \mu(B) ds < \lim_{t \rightarrow \infty} \frac{1}{t} \int_0^t \left( \frac{I'}{I} - d \right) ds = \frac{1}{t} \ln \frac{I(t)}{I(0)} - d,$$

$$\text{Then } \limsup_{t \rightarrow \infty} \frac{1}{t} \int_0^t \mu(B(x(s, r_0))) ds < 0.$$

Thus by Theorem 2.1 the unique endemic equilibrium  $P^*$  is globally asymptotically stable in  $\Gamma^0$ . This completes the proof.  $\square$

Now we consider the nontrivial equilibrium  $P^* = (S^*, I^*, Q^*)$  of the system (2.1) where

$$S^* = \frac{A}{d} - \frac{A}{d} I^* (\gamma + \delta + d + \alpha_1), \text{ and } Q^* = \frac{\delta}{(d + \alpha_2 + \varepsilon)} I^*.$$

The Jacobian matrix at  $P^*$  is

$$M_{P^*} = \begin{pmatrix} -\beta^* H_{S^*} - d & -\beta^* - \beta^* H_{I^*} & 0 \\ \beta^* H_{S^*} & \beta^* H_{I^*} & 0 \\ 0 & \delta & -(d + \alpha_2 + \varepsilon) \end{pmatrix}, \quad (2.8)$$

where  $H_{S^*} = \frac{\partial H}{\partial S}|_{S=S^*}$ ,  $H_{I^*} = \frac{\partial H}{\partial I}|_{I=I^*}$  and

$H^* = H(I^*, S^*)$ . Assume that  $H_{S^*}$ ,  $H_{I^*}$

and  $H^*$  are positive. The characteristic equation at  $P^* = (S^*, I^*, Q^*)$  is given by

$$(\lambda + a_0)[\lambda^2 + a_1 \lambda + a_2] = 0, \quad (2.9)$$

where

$$a_0 = d + \alpha_2 + \varepsilon,$$

$$a_1 = (\beta^* H_{S^*} + d) + \beta^* H + H^* H_{I^*} (1 - \beta^* H_{S^*}) - (\gamma + \delta + d + \alpha_1), \quad (2.10)$$

$$a_2 = -(\beta^* H_{S^*} + d)(\beta^* H + H^* H_{I^*}) (1 - \beta^* H_{S^*}) - (\gamma + \delta + d + \alpha_1).$$

Since by the Routh-Hurwitz criterion (see [9]) it is known that  $P^* = (S^*, I^*, Q^*)$  is locally asymptotically stable if the roots of the characteristic equation (2.9) lie strictly in the left half-plane, then we have the following theorem.

**Theorem 2.5.** Suppose that the conditions

$$(A_1) \quad \beta^* H_{S^*} \neq 1, \quad (A_2)$$

$$H^* H_{I^*} < H^*.$$

be satisfied. Then the equilibrium point  $P^* = (S^*, I^*, Q^*)$  is locally asymptotically stable.

**Proof.** The proof is similar to the proof of Theorem 2.1, so it is omitted.  $\square$

### 3. The SIQR epidemic model

In this section we show that the system (2.7) is bounded, positively invariant, and dissipative.

**Definition 3.1.** ([10], pp. 394) A differential equation  $X' = f(X)$  is said to be dissipative if there is a bounded subset  $B$  of  $R^2$  such that for any  $X^* \in R^2$  there is a time  $t_*$ , which depends on  $X^*$  and  $B$ , so that the solution  $\phi(t, X^*)$  through  $X^*$  satisfies  $\phi(t, X^*) \in B$  for  $t \geq t_*$ .

**Theorem 3.1.** Let  $\Gamma$  be the region defined by

$$\Gamma = \left\{ (S, I, Q, R) \in R_+^4 : S + I + Q + R \leq \frac{A}{d} \right\}. \quad (3.1)$$

Then

- (i)  $\Gamma$  is positively invariant,
- (ii) All solutions of the system (1.1) are uniformly bounded,
- (iii) System (1.1) is dissipative.

**Proof.** Let  $S(t_0) = \bar{S}_0 > 0$ . Since

$$\dot{S} = A - \beta IH(I, S) - dS,$$

$$< A - dS - S \min_{S \in \Gamma} \overline{H}(I, S),$$

where  $IH(I, S) = S\overline{H}(I, S)$ . Letting

$$\mu = -(d + \min_{S \in \Gamma} \overline{H}(I, S)), \text{ then}$$

$$\dot{S} < A + \mu S, \quad \mu < 0. \quad (3.3)$$

Thus

$$S \leq \frac{-A}{\mu} + \bar{S}_0 e^{\mu t}, \quad (3.4)$$

so that

$$S \leq \max\left(\frac{-A}{\mu} + \bar{S}_0\right). \quad (3.5)$$

Thus

$$\limsup_{t \rightarrow \infty} S \leq \frac{-A}{\mu}, \quad \mu < 0, \quad \bar{S}_0 \geq 0. \quad (3.6)$$

Hence  $S(t)$  is uniformly bounded. Since  $S(t) = N(t) - I(t) - Q(t) - R(t)$ , and  $S(t)$  is uniformly bounded, then the solutions of (1.1) are uniformly bounded. Dissipativity of the system (1.1) follows by Definition 3.1. Thus the proof is completed.  $\square$

Now, we discuss the existence and global stability of the equilibria of (1.1). By solving the system of isocline equations  $A - \beta IH(I, S) - dS = 0$ ,

$$\beta IH(I, S) - (\gamma + \delta + d + \alpha_1)I = 0,$$

$$\delta I - (d + \alpha_2 + \varepsilon)Q = 0,$$

$$\gamma I + \varepsilon Q - dR = 0,$$

thus the possible equilibrium points of (1.1)

$$\text{are } P_0 = \left(\frac{A}{d}, 0, 0, 0\right), \text{ and } \bar{P} = (\bar{S}, \bar{I}, \bar{Q}, \bar{R}).$$

The Jacobian matrix due to linearizing (1.1)

at the equilibrium point  $P_0 = \left(\frac{A}{d}, 0, 0, 0\right)$  is

$$J_{P_0 = \left(\frac{A}{d}, 0, 0, 0\right)} = \begin{pmatrix} -d & \beta H(0, \frac{A}{d}) & 0 & 0 \\ 0 & \beta H(0, \frac{A}{d}) - (\gamma + \delta + d + \alpha_1) & 0 & 0 \\ 0 & \delta & -(d + \alpha_2 + \varepsilon) & 0 \\ 0 & \gamma & \varepsilon & -d \end{pmatrix} \quad (3.2)$$

The eigenvalues of  $P_0 = \left(\frac{A}{d}, 0, 0, 0\right)$  are given by

$$\lambda_1 = \lambda_2 = -d < 0, \quad \lambda_3 = -(d + \alpha_2 + \varepsilon) < 0 \text{ and } \lambda_4 = \beta H(0, \frac{A}{d}) - (\gamma + \delta + d + \alpha_1) \quad (3.8)$$

The above discussion leads to the following results.

**Theorem 3.2.**

(i) If  $R_q \leq 1$ , then the disease-free equilibrium point  $P_0 = \left(\frac{A}{d}, 0, 0, 0\right)$  is locally asymptotically stable.

(ii) If  $R_q > 1$ , then the equilibrium point  $P_0 = \left(\frac{A}{d}, 0, 0, 0\right)$  is a hyperbolic saddle and is repelling in the both directions of  $Q$  and  $R$ . In particular, the dimensions of the stable manifold  $W^+$  and unstable manifold  $W^-$  are given by

$$\text{Dim } W(P_0) = \left(\frac{A}{d}, 0, 0, 0\right) \nparallel 1, \quad \text{Dim } W(P_0) = \left(\frac{A}{d}, 0, 0, 0\right) \nparallel 3, \quad (3.9)$$

respectively.

**Proof.** The proof of(i) follows by Lemma 2.1 and the Routh-Hurwitz theorem [ 9], so it is omitted. The proof of (ii) follows directly from inspection of the eigenvalues of the

Jacobian matrix at  $P_0 = \left(\frac{A}{d}, 0, 0, 0\right)$  and

examples from Freedman and Mathsen [8].

□

Now to give sufficient conditions for existence of a positive interior equilibrium  $\bar{P} = (\bar{S}, \bar{I}, \bar{Q}, \bar{R})$ , we discuss the uniform persistent of (1.1). To show a uniform persistence in the set

$$R_{SIQR}^+ = \left\{ (S, I, Q, R) : S > 0, I > 0, Q > 0, R > 0, S + I + Q + R \leq \frac{A}{d} \right\}$$

(3.10)

we assume the following hypotheses for system (1.1).

( $A_3$ ) All dynamics are trivial on  $\partial R_{SIQR}^+$  (the boundary of the set  $R_{SIQR}^+$ ).

( $A_4$ ) All invariant sets (equilibrium points) are hyperbolic and isolated.

( $A_5$ ) No invariant sets on  $R_{SIQR}^+$  are asymptotic stable.

( $A_6$ ) If an equilibrium point exists in the interior of any 3-dimensional subspace of  $R_{SIQR}^+$  it must be globally asymptotically stable with respect to orbits initiating in that interior.

( $A_7$ ) If  $M$  is an invariant set on  $\partial R_{SIQR}^+$  and  $W^+(M)$  and it is strong stable manifold, then  $W^+(M) \cap \partial R_{SIQR}^+ = \emptyset$ .

( $A_8$ ) All invariant sets are cyclic.

Here, we drive criteria for the global stability hypothesis ( $A_7$ ) to be valid.

Now, we discuss the global stability of  $\bar{P}_* = (\frac{A}{d}, 0, 0)$ . In  $R_+^4$  consider the Liapunov function

$$V = I. \quad (3.11)$$

Thus

$$\begin{aligned} \frac{dV}{dt} &= [\beta H(I, S) - (\gamma + \delta + d + \alpha_1)]I \\ &= (\gamma + \delta + d + \alpha_1) \left[ \frac{\beta H(I, S)}{(\gamma + \delta + d + \alpha_1)} - 1 \right] I. \\ &\leq 0. \end{aligned}$$

Now we give the following result.

**Theorem 3.3.** If

$$\frac{\beta H(I, S)}{(\gamma + \delta + d + \alpha_1)} \leq 1, \quad (3.13)$$

then the disease-free equilibrium point

$\bar{P}_* = (\frac{A}{d}, 0, 0)$  is globally asymptotically stable with respect to solution trajectories initiating from  $\text{int } R_S^+$  (the interior of the set  $R_S^+$ ).

**Proof.** The proof is similar to the proof of Theorem 3.1 in ([19], p. 197) so it is omitted.

□

Now, to discuss the global stability of the point  $\bar{P} = (\bar{S}, \bar{I}, \bar{Q})$ , choose the Liapunov function

$$V = \frac{1}{2} k_1 (S - \bar{S})^2 + \frac{1}{2} k_2 (I - \bar{I})^2 + Q - \bar{Q} - \bar{Q} \ln \frac{Q}{\bar{Q}}$$

(3.14)

where  $k_i \in R^+, i = 1, 2$ .

The derivative of (3.14) along the solutions curve (2.1) in  $R_{SIQ}^+$  is given by,

$$\frac{dV}{dt} = k_1 (S - \bar{S}) [(A - IH(I, S) - dS]$$

$$+ k_2 [\beta IH(I, S) - (\gamma + \delta + d + \alpha_1)]]$$

(3.15) (3.15)

$$+ \left(1 - \frac{Q}{\bar{Q}}\right) [\delta I - (d + \alpha_2 + \varepsilon) Q]$$

where

$$A = \beta \bar{I} H(\bar{I}, \bar{S}) + d \bar{S}, \quad (3.16)$$

$$\beta H(\bar{I}, \bar{S}) = (\gamma + \delta + d + \alpha_1),$$

$$\delta \frac{\bar{I}}{Q} = d + \alpha_2 + \varepsilon.$$

Hence

$$\frac{dV}{dt} = -k_1 d(S - \bar{S}) + k_1 [\bar{I} H(\bar{I}, \bar{S}) - I H(I, S)] \quad (3.17)$$

$$+ \beta k_2 (I - \bar{I}) [I H(I, S) - \bar{I} H(\bar{I}, \bar{S})] + \delta [\bar{I} Q - I \bar{Q}]$$

$$\text{Let } X = \begin{pmatrix} v_1 \\ v_2 \\ v_3 \end{pmatrix} \text{ such that } v_1 = (S - \bar{S}), \quad v_2 = (I - \bar{I}), \quad v_3 = (Q - \bar{Q})$$

set

$$a_{11} = -k_1 d,$$

$$a_{12} = a_{21} = k_1 \frac{\beta [\bar{I} H(\bar{I}, \bar{S}) - I H(I, S)]}{(I - \bar{I})} + k_2 \frac{\beta [\bar{I} H(\bar{I}, \bar{S}) - I H(I, S)]}{(S - \bar{S})} \quad \text{number, where } x_* \text{ is the equilibrium point.}$$

$$a_{22} = k_2 \frac{\beta [I H(I, S) - \bar{I} H(\bar{I}, \bar{S})]}{(I - \bar{I})}, \quad a_{13} = a_{31} = 0, \quad a_{33} = \delta \frac{[\bar{I} Q - I \bar{Q}]}{(\bar{Q} - Q)} \quad \text{(iii) } a_{ij} \text{ are bounded for all } i, j = 1, 2, 3.$$

The characteristic roots of the matrix  $A$  are given by

$$\det(A - \lambda I_{3 \times 3}) = 0 \quad (3.21)$$

$$(\bar{Q} - Q)^3 \lambda^3 + m_1 \lambda^2 + m_2 \lambda + m_3 = 0,$$

where

(3.19)

Thus

$$m_1 = -\text{trace} A = -(a_{11} + a_{22} + a_{33}),$$

$$\frac{dV}{dt} = a_{11} v_1^2 + a_{22} v_2^2 + a_{33} v_3^2 \quad (3.19)$$

$$= a_{11} v_1^2 + \frac{1}{2} a_{12} v_1 v_2 + \frac{1}{2} a_{13} v_1 v_3 + \frac{1}{2} a_{12} v_1 v_2 + \frac{1}{2} a_{23} v_2 v_3$$

$$+ a_{22} v_2^2 + \frac{1}{2} a_{23} v_2 v_3 + \frac{1}{2} a_{13} v_1 v_3 + a_{33} v_3^2, \quad \text{where } a_{ij} = a_{ji} \quad \text{with}$$

$$a_{13} = a_{23} = 0, \quad i, j = 1, 2, 3. \quad \text{But}$$

$$\frac{dV}{dt} = X^T A X = X A X^T = \langle A X, X \rangle$$

(quadratic form), where  $A$  is an  $3 \times 3$  real symmetric matrix such that  $A = \frac{1}{2}(A + A^T)$

and given by

$$A = \begin{pmatrix} a_{11} & \frac{1}{2} a_{12} & \frac{1}{2} a_{13} \\ \frac{1}{2} a_{12} & a_{22} & \frac{1}{2} a_{23} \\ \frac{1}{2} a_{13} & \frac{1}{2} a_{23} & a_{33} \end{pmatrix}. \quad (3.20)$$

Let  $a_{ij}, i, j = 1, 2, 3$  are such that

$$(i) a_{ij} \in C^1(R^+ \times R^+ \times R^+, R),$$

$$(ii) \lim_{x \rightarrow x_*^-} a_{ij} \text{ exists as a finite}$$

$$a_{12} = a_{21} = k_1 \frac{\beta [\bar{I} H(\bar{I}, \bar{S}) - I H(I, S)]}{(I - \bar{I})} + k_2 \frac{\beta [\bar{I} H(\bar{I}, \bar{S}) - I H(I, S)]}{(S - \bar{S})} \quad \text{(iii) } a_{ij} \text{ are bounded for all } i, j = 1, 2, 3.$$

The characteristic roots of the matrix  $A$  are given by

$$\det(A - \lambda I_{3 \times 3}) = 0 \quad (3.21)$$

$$(\bar{Q} - Q)^3 \lambda^3 + m_1 \lambda^2 + m_2 \lambda + m_3 = 0,$$

where

(3.19)

Thus

$$m_1 = -\text{trace} A = -(a_{11} + a_{22} + a_{33}),$$

$$\frac{dV}{dt} = a_{11} v_1^2 + a_{22} v_2^2 + a_{33} v_3^2 \quad (3.19)$$

$$m_2 = \det \begin{vmatrix} a_{11} & \frac{1}{2}a_{12} \\ 1 & a_{22} \\ \frac{1}{2}a_{12} & a_{22} \end{vmatrix} + \det \begin{vmatrix} a_{11} & \frac{1}{2}a_{13} \\ 1 & a_{33} \\ \frac{1}{2}a_{13} & a_{33} \end{vmatrix} + \det \begin{vmatrix} a_{22} & \frac{1}{2}a_{23} \\ 1 & a_{33} \\ \frac{1}{2}a_{23} & a_{33} \end{vmatrix},$$

$$m_3 = -\det A.$$

But since  $a_{13} = a_{23} = 0$ , then

$$m_1 = -(a_{11} + a_{22} + a_{33}), \quad (3.22)$$

$$m_2 = a_{11}(a_{22} + a_{33}) - \frac{1}{4}a_{12}^2,$$

$$m_3 = a_{33}\left(\frac{1}{4}a_{12}^2 - a_{11}a_{22}\right)$$

Hence by the Routh-Hurwitz criteria and Lemma 6.1 of ([15], pp. 177), it follows that  $A$  is negative definite if

$$m_1 < 0, m_3 < 0, \text{ and } m_1m_2 > m_3. \quad (3.23)$$

Thus we have the following theorem.

**Theorem 3.5.** Suppose that the two conditions,

$$(i) a_{ii} < 0, i = 1, 2, 3,$$

$$(ii) a_{11}a_{22} - \frac{1}{4}a_{12}^2 < 0,$$

hold, then the equilibrium point  $\bar{P} = (\bar{S}, \bar{I}, \bar{Q}) \in R_{SIQ}^+$  is globally asymptotically stable with respect to solution trajectories initiating from  $\text{int } R_{SIQ}^+$

**Proof.** The proof follows the lines of those of Nani et al[20, Lemma 6.1] and Frobenius Theorem.  $\square$

The following Lemma due to Butler-McGehee (cf. [20]) be needed for our later results.

**Lemma 3.6.** Let  $P$  be an isolated hyperbolic equilibrium in the omega limit set  $\Omega(X)$  of an orbit  $\mathcal{O}(X)$ . Then either  $\Omega(X) = P$  or there exist points  $Q^+, Q^-$  in  $\Omega(X)$  with  $Q^+ \in M^+(P)$  and  $Q^- \in M^-(P)$ .

Now, we discuss persistence, uniformly persistence and give sufficient conditions for the existence of a positive interior equilibrium point  $\bar{P} = (\bar{S}, \bar{I}, \bar{Q}, \bar{R})$ .

**Theorem 3.7.** Assume that,

(i)  $P_\circ = (\frac{A}{d}, 0, 0, 0)$  is a hyperbolic saddle point and is repelling in both  $Q$  and  $R$  direction (see Theorem 3.4).

(ii) System (1.1) is dissipative and solutions initiating in  $\text{int } R_{SIQR}^+$  are eventually uniformly bounded.

(iii) The equilibrium points  $\bar{P} = (\frac{A}{d}, 0, 0)$

and  $\bar{P} = (\bar{S}, \bar{I}, \bar{Q})$  are globally asymptotically stable. Then the system (1.1) is uniformly persistence.

**Proof.** The proof depends on Lemma 3.6. Let

$$\Gamma = \{(S, I, Q, R) \in R_{SIQR}^4 : S + I + Q + R = 1\} \subset R_+^4.$$

We have shown in Theorem 3.2 that  $\Gamma$  is positively invariant, and any solution of system (1.1) initiating at a point in  $\Gamma \in R^4$  is eventually uniformly bounded. However

$\bar{P}_\circ = (\frac{A}{d}, 0, 0)$  is the only compact

invariant set on  $\partial R_+^4$ . Let

$M = \bar{P} = (\bar{S}, \bar{I}, \bar{Q}, \bar{R})$  be such that

$M \in \text{int } \partial R_+^4$ . The proof will be completed by showing that no points  $Q_i \in \partial R_+^4$  belongs to

$\Omega(M)$ . Suppose the contrary that  $P_\circ \in \Omega(M)$ .

Since  $P_\circ$  is a hyperbolic,  $P_\circ \notin \Omega(M)$ . By Lemma 3.6, there exists a point

$Q_0^+ \in W^+(P_\circ) \setminus \{P_\circ\}$  such that

$Q_0^+ \in \Omega(M)$ . But since

$W^+(P_\circ) \cap (R_+^4 \setminus \{P_\circ\}) = \emptyset$ , this contradicts

the positive invariance property of  $\Gamma$ . Thus

$P_\circ \notin \Omega(M)$ . We also show that

$P_1 = (\bar{S}, \bar{I}, \bar{Q}, 0) \notin \Omega(M)$ . If  $P_1 = (\bar{S}, \bar{I}, \bar{Q}, 0) \in \Omega(M)$ , then there exists a point  $Q_1^+ \in W^+((P_1) \setminus \{P_1\})$  such that  $Q_1^+ \in \Omega(M)$ . But  $W^+(P_1) \cap (R_+^4) = \emptyset$  and  $P_1 = (\bar{S}, \bar{I}, \bar{Q}, 0)$  is globally asymptotically stable with respect to  $R_{SIQ}^+$ . This implies that the closure of the orbit  $\vartheta(Q_1^+)$  through  $Q_1^+$  either contains  $P_1$  or be unbounded. This is a contradiction. Hence

$P_1 = (\bar{S}, \bar{I}, \bar{Q}, 0) \notin \Omega(M)$ . Thus we see that if  $P_1$  is unstable, then  $W^+(P_1) \cap (R_+^4 \setminus \{P_1\}) = \emptyset$ . Also, we deduce that if  $P_1$  is unstable, then

$$W^+(P_1) \cap (Int R_+^4) = \emptyset, \text{ and}$$

$$W^-(P_1) \cap (R^4 \setminus R_+^4) = \emptyset.$$

Now, we show that  $\partial R_+^4 \cap \Omega(M) = \emptyset$ . Let  $E \in \partial R_+^4$  and  $E \in \Omega(M)$ . Then the closure of the orbit through  $E$ ,  $\vartheta(E)$  either contains  $P_1$  and  $P_1$  or be unbounded, and the uniformly persistence result follows since  $\Omega(M)$  must be in  $int R_+^4$ . This completes the proof.  $\square$

Now, we discuss Hopf bifurcation for the system of equations (1.1) with bifurcation parameter  $\delta$ . The system (1.1) can be recast into the form

$$\dot{X} = F(X, \delta),$$

where  $X \in R^4 = \begin{pmatrix} S \\ I \\ Q \\ R \end{pmatrix}$  and  $\delta$  is the bifurcation parameter.  $F(X, \delta)$  is a  $C^r$  ( $r \leq 5$ ) function on an open set in  $R^4 \times R^1$ . Let

$B_\delta = \left\{ P_\circ = \left(\frac{A}{d}, 0, 0, 0\right), \bar{P} = (\bar{S}, \bar{I}, \bar{Q}, \bar{R}) \right\}$  be the set of equilibrium points of (1.1) such that  $F(B_\delta) = 0$ , for some  $\delta \in R^1$  on a sufficiently large open set  $G$  containing each member of  $B_\delta$ . The linearized problem corresponding to (1.1) about any  $\delta$  is give by

$$\dot{y} = J_\delta(F(B_\delta))y, \quad y \in R^4.$$

(3.24)

Here, we are interested in studying how the orbit structure near  $B_\delta$  changes as  $\delta$  is varied.

**Theorem 3.8.** If

$$\beta H\left(\frac{A}{d}, 0\right) > (\gamma + \delta + d + \alpha_1),$$

then the Hopf bifurcation can not occur at

$$P_\circ = \left(\frac{A}{d}, 0, 0, 0\right).$$

### Examples.

1- Consider the special case of incidence rate  $\beta I^P S^q$  considered by [18], and [19] for  $q = 1$ , with the choices  $\gamma = 0.8$ ,  $\mu = 0.3$ ,  $A = d = 0.00027473$  and  $\beta = 0.3$ , then a simple calculations , leads to the  $R_q$  is very close to 0.3 i.e. the condition  $R_q \leq 1$  be satisfied .Moreover in this case the condition  $(A_1)$  be satisfied .This because in this case  $IH_s = I^P$ , where  $q = 1$  may only equal one in two uncosiderable cases,  $p = o$  or  $I = 1$ . In the special case of incidence rate  $\beta IS$  considered by [ 23 ], the conditions of Theorem 3.10 are not satisfied in this special case of the incidence rate. This is consistent with the conclusion of [12].

### 4. Discussion

In this paper, we discussed a generalized *SIQR* epidemic system with vertical transmission for the dynamics of an infectious disease. The generalized incidence term of the form  $\beta IH(I, S)$  is of nonlinear form and the immunity is assumed to be permanent. It has endemic equilibrium that are asymptotically stable so that no periodic solutions arise by Hopf bifurcation. We established local asymptotic stability of the disease free-equilibrium  $\bar{P}_o = (\frac{A}{d}, 0, 0)$  and

$P_o = (\frac{A}{d}, 0, 0, 0)$  for the systems (2.1) and (1.1), respectively. Our results are consistent with those obtained by Korobeinikov et al [13], when conditions  $(A_1)$  and  $(A_2)$  of Theorem 2.1 be satisfied. We have shown that if the condition  $(A_1)$  of Theorem 2.1 is satisfied, then the disease free-equilibrium point  $\bar{P}_o = (\frac{A}{d}, 0, 0)$  is locally asymptotically stable in the interior of the feasible region and the disease always dies out. The main theorem of the method depends on Lozinski Logarithmic norm. We have shown that if the two conditions  $(A_2)$  and  $(A_3)$  of Theorem 2.2 hold, then a unique endemic equilibrium point  $P^* = (S^*, E^*, I^*)$  exists and is locally asymptotically stable in the interior of the feasible region. Moreover, once the disease appears, it eventually persists at the unique endemic equilibrium level. The local stability of  $\bar{P}_o = (\frac{A}{d}, 0, 0), P = (\frac{A}{d}, 0, 0, 0)$ , and  $P = (S^*, E^*, I^*)$  are obtained using the Routh-Hurwitz criteria, which has been widely used in the literature ([2] and [9]). The global stability of  $\bar{P}_o = (\frac{A}{d}, 0, 0)$  and

$P = (S^*, E^*, I^*)$  in Theorem 3.4 and Theorem 3.5 are established using Liapunov functions a similar approach to those in Li ([14], [15]) and Freedman [20]. We employ the mathematical tools of differential analysis, persistence theory and a technique similar to that used by Nani and Freedman [20]. We discuss uniform persistent and Hopf bifurcation of system (1.1) at  $P_o = (\frac{A}{d}, 0, 0, 0)$ . We give some numerical examples that ensure our results. Our obtained results improve and partially generalize those obtained in [3], [4], [11] [13] and [24].

### Corresponding author

M. M. A. El-Sheikh

1-Department of Mathematics, Faculty of Science, Taibah University, Kingdom of Saudi Arabia

Permanent Address: Department of Mathematics, Faculty of Science, Menoufia University, Shebin El Koom, Egypt.

This work is supported by Deanship of Scientific Research, Taibah University, Kingdom of Saudi Arabia No.451-430.

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9/28/2010

# The Effect of Combining Herbal Therapy with Conventional Chemotherapy on the Incidence of Chemotherapy Side Effects in 2nd Stage Breast Cancer Patients

Nagla Hamdi Kamal Khalil, Sanaa Alaa El- Din, Maha Adel Salem

Medical-Surgical Nursing Department, Faculty of Nursing,

Ahmed Adel Seif El-Din, Pharmacognosy Department, Faculty of Pharmacy

Waleed Osman Arafat, Oncology department, Faculty of Medicine, Alexandria University

[Mahaadel52@yahoo.com](mailto:Mahaadel52@yahoo.com)

**Abstract:** The purpose of this study is to identify the effect of the combination of herbal mixture and conventional chemotherapy on minimizing the incidence of chemotherapeutic side effects for 2<sup>nd</sup> stage breast cancer. Forty adult female patients aging 20 to 65 years old diagnosed with breast cancer stage (II), receiving chemotherapy for at least one month and will continue to receive it for 3 months- were selected randomly and divided equally into study and control groups. They were free from any associated co-morbid diseases as diabetes, renal, cardiac. The patients were interviewed in the oncology outpatient clinic. Study group patients were instructed about the importance of taking herbal capsules regularly with chemotherapeutic cycles, on a scheduled dose of 1 capsule three times per day for 3months. Complete assessment for both groups as baseline data to assess the chemotherapeutic side effects, laboratory investigations and the nutritional status of the patients were done, and then after 45days and after 3 months. The results revealed that (45%) of cancer breast women were in age group 49-65 years. The greater proportion of the sample (62.5%) breast fed and lactated for three times and more through their life. The least affected systems with chemotherapeutic side effects and the most affected systems when combined herbal to conventional therapy were: liver functions and endocrine studies, renal functions, reproductive system, urinary system, and weight changes. While psychological status, nervous system, and skin, hair, and nails were the most affected systems with the side effects of chemotherapy, and they were the least affected systems when combined herbal to conventional chemotherapy. Also it was found that there was significant difference between the study and control groups in relation to second and third assessments related to all body systems. It is recommended that herbal education should be introduced in nursing and medical curriculum. Further researches related to these herbal components to measure its efficacy on minimizing the side effects of chemotherapy for breast cancer and/ or other types of cancer. Further researches are also needed on larger number of sample. Clinical studies should be done to identify the effect of these herbs on different cancer therapies, different chemotherapeutic protocols, specifically pre or post mastectomy.

[Nagla Hamdi Kamal Khalil, Sanaa Alaa El- Din, Maha Adel Salem. The Effect of Combining Herbal Therapy with Conventional Chemotherapy on the Incidence of Chemotherapy Side Effects in 2nd Stage Breast Cancer Patients. Journal of American Science 2010;6(11):784-801]. (ISSN: 1545-1003).

## Introduction

Breast cancer is the second most common type of cancer after lung cancer, and the fifth most common cause of cancer death. Egyptian National Cancer Institute (NCI)<sup>(9)</sup>, reported that breast cancer is the most common cancer among women, representing 18.9% of total cancer cases (35.1% in women and 2.2% in men) among series of 10556 patients during the year 2001, with an age-adjusted rate of 49.6 per 100 000 population. Breast cancer in Egyptian patients has a younger age distribution with the majority of patients occurring at 30–60 years of age. In Alexandria over the year 2006, 1183 patients

diagnosed with breast cancer (1132 patients were females and 51 patients were males)<sup>(10)</sup>.

The type of treatment for breast cancer varies with the location, type and severity of the tumor. Therapy options for breast cancer include surgery, chemotherapy, radiation therapy, biological therapy and hormonal therapy<sup>(63)</sup>. The treatment can be physically exhausting for the patient. Current chemotherapeutic techniques have a range of side effects mainly affecting the fast-dividing cells of the body. Important common side-effects include: Bone marrow suppression, digestive system changes as nausea and vomiting, appetite loss and weight

changes, taste changes, constipation, diarrhea, hair loss, fatigue, heart damage and nervous system changes<sup>(14, 15)</sup>. Recently, there is a call for returning to the nature by using herbals with proven efficacy in prevention, treating, and minimizing the consequences of many diseases. Health care in the twenty-first century requires that nurses recognize the shift of thinking toward the incorporation of alternative and complementary approaches to care. Nurses at all levels and in every area of practice are answering the call to use new methods to care for the ill patients and to enhance the health of those who are well<sup>(7)</sup>.

Complementary and alternative medicine (CAM) is widely embraced by cancer patients due to largely patients' demands for integration of herbal therapies into their cancer treatment. Integrated medicine is a holistic approach to cancer care, with some herbal medicines showing proven efficacy as adjuvant to conventional medical treatments. At the present time there is little evidence of a systematic process of evaluation or dialogue between mainstream medicine and herbal medicine practitioners<sup>(16)</sup>. Herbal Medicine is one of the oldest types of medicine which is dating back to the ancient cultures of Egypt, China, and India, and possibly even pre-historic times for healing purposes<sup>(17)</sup>. It encompasses the use of natural sources as plants and natural products for healing or therapeutic purposes<sup>(16, 17)</sup>.

In Egypt, there is a lack in clinical researches investigating the real effect of herbals as a complementary and / or alternative medicine, despite of its safety and benefits in curing chronic diseases or conditions. So the approach today is to use herbals in dealing with these chronic disabilities with other conventional or traditional therapy for managing these disorders<sup>(22)</sup>.

This study emphasizes on identifying the effect of a herbal mixture in a capsule form containing a blend of 5 herbals used in culinary edible purposes. The herbal mixture composed of Ginger rhizome, Nigella Sativa seeds, Boswellia, Curcuma rhizome, Cardamom seeds. This study aims to identify the effect of the combination of herbal mixture to conventional chemotherapy on minimizing the incidence of chemotherapeutic side effects for 2nd stage breast cancer. When patients integrate these complementary therapies as herbals into their medical and surgical care, they are creating a more comprehensive treatment plan and helping their own bodies to regain health and vitality<sup>(22)</sup>.

David (2008), reported that, *nigella sativa* (Black Cumin) helps treat a broad array of diseases,

including some immune and inflammatory disorders, anticancer activity in prostate and colon cancers, as well as antioxidant. Much of the biological activities of its seeds have been reported to provide protection against nephrotoxicity and Hepatotoxicity induced by either disease or chemicals<sup>(90)</sup>.

Ginger is the rhizome of *Zingiber officinale* (*Zingiberaceae*) contains effective compounds known as gingerols, and volatile oils with antimicrobial effects. The root has antiemetic effects from its carminative and digestive properties which has the ability to enhance GI motility and it reduces the severity and duration of nausea during chemotherapy<sup>(94,95)</sup>. Ginger has anti-inflammatory effects; by inhibit prostaglandins and thromboxane, antimigraine effects; which inhibit platelet aggregation, antithrombotic effects. Ginger is used most commonly to treat colic, flatulence, and indigestion<sup>(95)</sup>. It is claimed to treat hypercholesterolemia, burns, ulcers, depression, impotence, and liver toxicity and as an anti-inflammatory for those with arthritis and as an antispasmodic.

*Boswellia* is a natural oleo-gum resin which consists of resinous portion contains about 60% of Boswellic Acids (BA) (alpha-and beta) which are the active constituents in boswellia<sup>(97)</sup>. Studies have shown that boswellic acids have an anti-inflammatory action by having an antimicrobial activity and inhibit the complementary system. This acts much like the conventional nonsteroidal anti-inflammatory drugs (NSAIDS). *Boswellia* inhibits pro-inflammatory mediators in the body, such as leukotrienes and also prevents the breakdown of connective tissue. The mechanism is similar to the action of non-steroidal groups of anti-arthritis drugs. Long-term use of *boswellia* does not lead to irritation or ulceration of the stomach<sup>(98)</sup>.

*Curcuma* is a dried rhizome of *Curcuma longa*, family *Zingiberaceae*. The main active constituent of which is known as curcumin. It protects against free radical damage because it is a strong antioxidant. It also reduces inflammation by reducing histamine levels and possibly by increasing production of natural cortisone by the adrenal glands. It protects the liver from a number of toxic compounds and, it reduce platelets from clumping together, which in turn, improves circulation and helps protect against atherosclerosis (figure 15)<sup>(100)</sup>. Numerous studies have also shown cancer-preventing effects of curcumin. This may be due to its powerful antioxidant activity in the body. A symbol of prosperity, it was considered as a cleansing herb for the whole body<sup>(101)</sup>. Medically, it was used as a digestive aid and treatment for fever,

infections, dysentery, arthritis, jaundice and other liver problems.. Curcumin is used for the treatment of anorexia, liver disorders, cough, diabetic wounds, rheumatism, and sinusitis. It has been evaluated for its anticarcinogenic and antimutagenic properties<sup>(102)</sup>.

Cardamoms are used to sooth the stomach and treat dyspepsia for its antispasmodic, antiflatulent, and motility- enhancing effects<sup>(105)</sup>. It is also used for respiratory disorders, asthma, common cold, cough, bronchitis, headache, hoarseness, indigestion, diarrhea, nausea, vomiting and stomach complaints<sup>(105, 106)</sup>. Cardamom seeds, with their sweet and spicy aroma, are used in aromatherapy to stimulate energy. A few drops of the oil in the bath help fight fatigue. The standardized dose of cardamom is 400 mg-600mg three times per day<sup>(106)</sup>.

The nurse has an important role in assessing and managing many of the problems experienced by the patient undergoing chemotherapy. Therefore, nursing assessment and care focus on identifying and modifying factors that further increase the patient's risk. Suppression of the bone marrow and immune system increase the risk for anemia, infection, and bleeding disorders. Aseptic technique and gentle handling are indicated to prevent infection and trauma. Laboratory test results, particularly blood cell counts, are monitored closely. Any changes in blood test results and signs of infection and bleeding must be reported promptly. The patient and family members are instructed about measures to prevent these problems at home<sup>(107)</sup>.

**Aim of the study:** The aim of the present work is to identify the effect of the combination of herbal mixture to conventional chemotherapy on minimizing the incidence of chemotherapeutic side effects for 2<sup>nd</sup> stage breast cancer.

#### **Keywords:**

**Herbal Mixture:** in form of capsules containing 5 natural herbals (Nigella Sativa–Cardamom m–Ginger–Curcuma–Boswellia serrata gum ).

#### **Material & Methods**

##### **Materials:**

**Research design:** A Quasi experimental research design was utilized in this study.

##### **Settings:**

**Oncology out-patient clinics of Alexandria Main University Hospital.**

#### **Sample :**

Forty adult female patients who were diagnosed with breast cancer and were receiving chemotherapy for at least one month and will continue to receive it for 3 months were included in the study. The subjects were selected randomly and divided equally into study and control groups, 20 patients in each group. Patients age ranged from above 21 to below 65 years , they were diagnosed with breast cancer of the same type, stage II, on chemotherapy for at least 4 months basis. The subjects included were one month post 1st chemotherapeutic cycle of the same protocol. Either pre-or post operative and they were free from any associated co-morbid diseases as diabetes, renal, cardiac, hypertension.

**Tools:** Two tools were used:

#### **Tool: Breast cancer women's assessment questionnaire sheet:**

This questionnaire was developed by the researchers based on literature review and specialist opinion. It was divided into three parts.

**(A). Chemotherapeutic side effects schedule sheet;** it was adopted from Sitizia, (1999)<sup>(116)</sup>.This part includes a list of chemotherapeutic side effects. Its grade of severity ranged from one to four (not present, mild, moderate, and severe). These side effects were divided according to body parts and systems.

**Facio-maxillary, integumentary system, gastrointestinal system, musculoskeletal system, neurological system, cardiovascular system, respiratory system, urinary system, reproductive system, psychological status**

**(B). Laboratory investigations:** Complete blood count (CBC), renal and liver functions tests, and fasting blood glucose level.

**(C). Quality of life standard scale:** This part includes two questions which assess the standard of life for all patients -control and study groups- over the three months of the study. For study group they assess the standard of life pre and post administering the herbal capsules. These two questions are part of The European Organization for Research and Treatment of Cancer quality of life questionnaire version 3,0 (EORTC QLQ-C30 Version 3.0): it is the most recent version, composed of 30 questions, for assessing the health related quality of life (QOL) of cancer patients participating in international clinical trials research, developed by The European Organization for Research and Treatment of Cancer (EORTC) in Arabic language in 1995.these two questions (likert

scale) are scored positively (i.e. 7 indicated excellent is the best and 1 indicated very poor is the worst)<sup>(47), (117)</sup>.

\***In addition to:** Patient's Sociodemographic data as:

Name ,age, educational level, occupation, marital status, number of children, type and number of child's feeding, date and type of first dose of chemotherapy.

Chemotherapeutic protocol prescribed by oncologist for both groups, for each cycle, includes:

- Endoxane with 500 cc glucose 5%.
- Adriamycin with 100 cc saline 9%.
- Fluorouracil with 500 cc saline 9%.

\* The doses of these drugs are calculated according to the patient's weight and height.

\* Before starting these drugs, there was a starting bottle contains 100 cc saline 9% with one ampoule of Zantac, one ampoule Decadron and another one of Emax

#### **Tool II: Nutritional assessment check list:**

It was developed to detect nutritional health status of the breast cancer patients which includes:

\* **Anthropometric measures:** anthropometric measurements provide an objective assessment of nutritional status it includes patient's body weight, height, triceps skin fold (TSF) mid – upper arm circumference. (MAC) and mid upper arm muscle circumference (MAMC) as well as body mass index (BMI). Each of these measurements was taken according to the standard procedures recommended by Jelliffe (1998)<sup>(118)</sup>.

**1- Body weight:** - it should be recorded on admission and monitored regularly. Weight was taken by asking every patient to stand on the center of a bath spring scale without moving or touching any thing. The reading was recorded to the nearest 1 kilogram<sup>(119)</sup>.

**2- Standing height:** - Height was measured by asking the patients to stand on the floor bare head and feet .she stands erect with shoulders and back of the head in the upright position and looking straight ahead, both heels and scapula are in contact with the wall. The measurement was taken by a measuring tape. The reading was recorded to the nearest 0.1 cm<sup>(112)</sup>.

**3- Body mass index (BMI):** - calculation of body mass index, is a way of classifying weight. It was estimated by the following equation:  $BMI \text{ (kg/m}^2\text{)} = \{\text{weight (kg)}/\text{height (meter)}^2\}$ <sup>(119)</sup>.

**4- Triceps skin fold thickness (TSF):**-it is an index of total body fat, the assessment of subcutaneous body fat by skin fold measurements is accurate. The measurement was done using the skin fold calipers. The site of measurement selected is the same mid point used to measure mid – upper arm circumference<sup>(112)</sup>.

**5- Mid-upper arm circumference (MAC):-**mid – arm circumferences measures muscle mass and subcutaneous fat, although it is not a useful measurement by it self, it is used as a part of the procedure for calculating arm muscle circumference (MAMC).<sup>(119)</sup>.

**6- Mid arm muscle circumference (MAMC):-** it was calculated from the MAC and TSF measurements by the following equation:  
 $MAMC \text{ (cm)} = MAC \text{ (cm)} - \{3.14 \times TSF \text{ (cm)}\}$ .

The value is recorded and compared with standard reference value<sup>(119)</sup>.

#### **II-METHODS:**

**1. Permission to conduct** the study was obtained from responsible authorities of the general director of the Alexandria University Hospital and the head of oncology department in the Main University hospital.

**2. An ethical approval** was taken from the ethical committee of the Faculty of Nursing Alexandria University for carrying out this study.

**3. The researchers have undergone a special training** about the route for preparing and fixing the herbal capsules about one month, under the supervision of a specialist trainer in the pharmacognosy department who was one of the supervisors of the thesis, the herbal mixture was mixed then prepared in the form of capsules. The dose was adjusted to be 1 capsule three times per day for 3 months for the study group in conjunction with chemotherapy protocol .Control group will be on chemotherapy protocol only.

**4. Aflatoxins study:** The mixture was subjected to aflatoxins test to insure its safety and puerility.

**5. Herbal materials:** The dose of herbal calculated and fixed according to the reference's recommended doses as *nigella sativa* seeds is 500 mg /three times per day, curcumin is 500 mg /three times per day, cardamom is 500 mg /three times per day, boswellia is 300 mg /three times per day, ginger is 2 gm /three times per day.

**6. Research Tools** The tools were developed based on the recent relevant literature. Tool I was developed by the investigators. Content validity was tested by ten experts in the filed of nursing and oncology field. Tool II was taken according to the standard procedures recommended by jelliffe (1998)<sup>(118)</sup>. Reliability for Tool I and Tool II were done by using test- re test.

**7. Pilot study** A pilot study was carried out before starting the data collection. It was applied on 10 patients with breast cancer who fulfill the study criteria to test feasibility and applicability of both tools and necessary modification were done.

**9. Patient's consent** Patient's written consent to participate in the study was then obtained. Every patient was informed that confidentiality will be assured, and she had the right to discontinue from the study at any time she wants.

The researchers interviewed every patient from the first day of the second cycle of receiving chemotherapy. Each patient was interviewed individually and each interview took approximately one hour. For the study group patient and her family were instructed about the importance of taking herbal capsules regularly, and illustrate the aim of the study as well as the scheduled dose which is 1 capsule three times per day for 3months on an individual basis. Numbers of sessions were adopted according to chemotherapeutic protocol till 3 months.

**11. Assessment phase (for both groups)** firstly General data about the patient has been collected as name, age, address, marital status, level of education, occupation, and date of first dose of chemotherapy.

**12. Implementation Phase (for both groups)** two tools (I&II) were used for both groups as baseline

## **II. The standard ranges that used for laboratory investigations are<sup>(66)</sup>:**

RBC (4.2 – 6.1) x 10<sup>6</sup> cells /ul  
HCT (37 -52) %  
MCH (27 – 31) pg  
WBC (5.2 – 12.4) x 10<sup>3</sup>cells/ul  
Lympho (19- 48) %  
Eso (0 – 7) %  
Platelets count (150 – 450) x 10<sup>6</sup> cells /ul  
Blood urea Nitrogen (7 – 18) mg/dl  
Total Bilirubin (.3-1.9)mg/dl  
SGOT (30 – 65) u/l  
Fasting Blood Glucose (70 -110).

data to assess the chemotherapeutic side effects, laboratory investigations as well as the nutritional status of the patients. Then these two tools were also applied for both groups after 45days then after 3 months of administering the herbal capsules to detect their effect on chemotherapeutic side effects and the nutritional status of the patients. Laboratory investigations data was obtained from the patient's hospital sheet at the scheduled times. Nutritional assessment weight has been measured at the first assessment then at the second and third one; percent of weight changes is calculated using the following formula. {(first weight – current weight) / first weight} x 100 =% weight change.

## **13. Evaluation Phase (for both groups):**

**I. Side effects of chemotherapy** in relation to all body systems were evaluated by using tool I & II after 45 days and then repeated after 3 months.

HGB (12- 18) g/dl  
MCV (80-99) fL  
MCHC (33- 37) g/dl  
Neutro (40 -74) %  
Mono (3.4 – 9) %  
  
Baso (0.5 -1.5) %  
Creatinine (0.6- 1.3) mg/dl  
SGPT (15 – 37) u/l

## **IV. Interpretation and evaluation of anthropometric measurements:**

### **(1) Evaluation of Body Mass Index:<sup>(125)</sup>**

Under weight	<19
Normal weight	19 - < 25
Over weight	25 - < 30
Mild obesity	30 - < 35
Moderate obesity	35 - < 40
Severe obesity	40

### **(2) Evaluation of MAC, TSF, and MAMC:<sup>(119)</sup>**

Measurement	Standard
MAC (cm)	29.0

TSF (mm)	16.5
MAMC (cm)	23.5

#### 14. Statistical analysis:

Data was analyzed using PC with statistical Package for Social Sciences version 13.0. The 0.05 level was used as the cut off value for statistical significance and the following statistical measures were used.

#### A. Descriptive statistics:*Count and percentage and Minimum, Maximum, Arithmetic mean ( $\bar{X}$ ), Standard deviation (SD):*

Analytical statistics:the following were used:

##### 1. Chi square: ( $\chi^2$ ):

Fisher Exact Test:

Monte Carlo Exact Test:

##### 2. Student t-test:

a. The pooled variance t-test: If the variances of the two groups are equal.

b. The separate variance t-test: If the variances of the two groups are not equal.

**Levene test** is used to test the hypothesis that the two population variances are equal.

##### 3. One way Analysis Of Variance (ANOVA):

It is used for testing the difference between more than 2 groups mean. In case of significant ANOVA f test the Post Hoc Sheffe test was used for multiple comparison of each of couple of groups.

## RESULTS

Table (I): Distribution of the study and control groups regarding to biosociodemographic characteristics.

Item	Study		Control		Total		Test of significant
	n	%	n	%	n	%	
<b>Age:</b>							
27-	6	30%	2	10%	8	20%	
38-	7	35%	7	35%	14	35%	
49-65	7	35%	11	55%	18	45%	<b>FET</b> = 0.274 <b>P</b> = 2.793
<b>Education:</b>							
- High level	0	0%	0	0%	0	0%	<b>FET</b> = 0.443 <b>P</b> = 1.951
- Moderate	4	20%	6	30%	10	25%	
- Lower than moderate	1	5%	3	15%	4	10%	
- illiterate	15	75%	11	55%	26	65%	
<b>Occupation:</b>							
- Working	1	5%	7	35%	8	20%	<b>FETp</b> = 0.044*
- Non working	19	95%	13	65%	32	80%	
<b>Marital status:</b>							
- Married	18	90%	12	60%	30	75%	<b>FET</b> = 0.025* <b>P</b> = 7.371
- single	2	10%	2	10%	4	10%	
- widowed	0	0%	6	30%	6	15%	
<b>type of breast feeding:</b>							
- breast feeding	12	60%	13	65%	25	62.5%	<b>FET</b> = 0.744 <b>P</b> = 0.107
- bottle feeding	8	40%	7	35%	15	37.5%	
<b>Frequency of breast feeding:</b>							
- less than 3 times	8	40%	7	35%	15	37.5%	<b>FET</b> = 0.744 <b>P</b> = 0.107
- 3 times and more	12	60%	13	65%	25	62.5%	
<b>Total</b>	20	100	20	100	40	100	

n: number of patients

FET: fisher's exact test

P: level of significant  $\leq 0.05$

Table (II): Effect of herbal and conventional therapy on the side effects of chemotherapy regarding to all body systems among the study and control groups.

Item	<u>Study</u>			<u>Control</u>			<u>t- test</u>		
	$X \pm SD$			$X \pm SD$					
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>

Eye, ear, mucous membranes.	52.3 $\pm$ 8.5	37.0 $\pm$ 8.8	28.0 $\pm$ 6.7	47.3 $\pm$ 9.4	55.4 $\pm$ 8.0	64.5 $\pm$ 5.6	1.760	6.902	18.502
Skin and nails.	47.2 $\pm$ 12.6	39.4 $\pm$ 11.1	33.1 $\pm$ 8.9	40.6 $\pm$ 7.5	49.4 $\pm$ 9.7	62.8 $\pm$ 6.9	2.008	3.029	11.841
Weight changes.	18.1 $\pm$ 8.6	13.1 $\pm$ 4.9	16.9 $\pm$ 6.1	6.3 $\pm$ 9.5	13.8 $\pm$ 9.0	18.1 $\pm$ 12.5	4.146	0.273	0.402
Gastro intestinal system.	49.8 $\pm$ 7.7	32.0 $\pm$ 5.2	17.3 $\pm$ 3.4	38.0 $\pm$ 7.7	44.3 $\pm$ 9.2	53.3 $\pm$ 7.1	4.836	5.170	20.365
Musculo skeletal system.	47.2 $\pm$ 11.0	34.7 $\pm$ 10.4	24.4 $\pm$ 8.3	47.8 $\pm$ 8.9	55.3 $\pm$ 9.6	68.1 $\pm$ 6.0	0.197	6.515	18.996
Nervous system.	47.2 $\pm$ 13.8	37.5 $\pm$ 12.5	32.5 $\pm$ 10.3	38.3 $\pm$ 9.0	45.6 $\pm$ 7.6	53.8 $\pm$ 7.1	2.289	2.483	7.603
Respiratory system.	46.7 $\pm$ 11.2	31.3 $\pm$ 7.2	23.5 $\pm$ 4.9	45.2 $\pm$ 3.6	50.6 $\pm$ 8.6	65.8 $\pm$ 4.8	0.555	7.728	27.506
Urinary system.	34.2 $\pm$ 7.8	22.7 $\pm$ 6.3	15.6 $\pm$ 6.6	31.9 $\pm$ 6.1	38.5 $\pm$ 6.3	48.3 $\pm$ 6.3	1.033	7.956	16.082
Reproductive system.	26.0 $\pm$ 8.2	18.5 $\pm$ 5.8	13.5 $\pm$ 5.9	19.4 $\pm$ 4.9	22.1 $\pm$ 4.5	27.9 $\pm$ 5.3	3.114	2.154	8.159
Psychological status.	51.6 $\pm$ 9.3	38.8 $\pm$ 5.2	30.3 $\pm$ 6.5	43.8 $\pm$ 8.1	50.0 $\pm$ 7.6	65.0 $\pm$ 9.2	2.837	5.467	13.813

t: t-test

1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> : assessments

**Table (II) cont.**

Item	n=20 <u>Study</u>			n=20 <u>Control</u>			t- test		
	(X $\pm$ SD)			(X $\pm$ SD)					
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>
Hematological tests	48.8 $\pm$ 16.3	41.9 $\pm$ 17.7	25.8 $\pm$ 15.6	36.2 $\pm$ 13.7	50.0 $\pm$ 12.0	57.3 $\pm$ 12.3	2.652	1.635	7.070
Renal functions studies	45.0 $\pm$ 22.4	2.5 $\pm$ 11.1	10.0 $\pm$ 22.5	37.5 $\pm$ 39.3	52.5 $\pm$ 19.7	92.5 $\pm$ 18.3	0.742	9.871	13.413
Liver functions and endocrine studies	51.3 $\pm$ 18.9	25.0 $\pm$ 18.1	6.3 $\pm$ 11.1	37.5 $\pm$ 23.6	35.0 $\pm$ 20.5	45 $\pm$ 15.4	2.028	1.633	9.131
<b>Total</b>	43.0 $\pm$ 5.6	30.9 $\pm$ 4.4	23.2 $\pm$ 3.4	37.4 $\pm$ 4.9	43.9 $\pm$ 5.3	54.3 $\pm$ 3.6	T <sub>1</sub> 3.369	P 0.002*	
							T <sub>2</sub> 8.420	P 0.000*	
							T <sub>3</sub> 27.781	P 0.000*	

Significant relation at P level  $\leq$  0.05

1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> : assessments

**Table (III): Ranking of all body systems that affected with the chemotherapeutic side effects post herbal and conventional therapies (3<sup>rd</sup> assessment) among two groups.**

study		control	
Body system		(X $\pm$ SD) of 3 <sup>rd</sup> assessment	
Liver functions and endocrine studies	6.3 $\pm$ 11.1	Weight changes	18.1 $\pm$ 12.5
		Reproductive system	27.9 $\pm$ 5.3

Renal functions studies	10+ 22.5		
Reproductive system.	13.5 ± 5.9	Liver functions and endocrine studies	45.0± 15.4
Urinary system.	15.6 ± 6.6	Urinary system.	48.3 ± 6.3
Weight changes.	16.9 ± 6.1	Gastro intestinal system	53.3 ± 7.1
Gastro intestinal system.	17.3 ± 3.4	Nervous system.	53.8 ± 7.1
Respiratory system.	23.5 ± 4.9	Hematological tests	57.3±12.3
Musclo skeletal system.	24.4 ± 8.3	Skin and nails.	62.8 ± 6.9
Hematological tests	25.8± 15.6	Eye, ear, mucous membranes.	64.5 ± 5.6
Eye, ear, mucous membranes.	28.0 ± 6.7	Psychological status.	65.0 ± 9.2
Psychological status.	30.3 ± 6.5	Respiratory system	65.8 ± 4.8
Nervous system.	32.5 ± 10.3	Musclo skeletal system.	68.1 ± 6.0
Skin and nails	33.1 ± 8.9	Renal functions studies	92.5 ±18.3

**Table (IV) :** Effect of herbal and conventional therapy on the side effects of chemotherapy regarding to health and life style among the study and control groups.

Item	n=20 Study			n=20 Control			FET / P
	1 <sup>st</sup> %	2 <sup>nd</sup> %	3 <sup>rd</sup> %	1 <sup>st</sup> %	2 <sup>nd</sup> %	3 <sup>rd</sup> %	
<b>Health grade:</b>	30%	0%	0%	0%	5%	45%	<b>FET<sub>1</sub>= 23.356    P<sub>1</sub> = 0.000*</b> <b>FET<sub>2</sub>= 25.761    P<sub>2</sub> = 0.000*</b> <b>FET<sub>3</sub> = 44.879    P<sub>3</sub>= 0.000*</b>
	- Bad						
	- Accepted	70%	0%	0%	35%	65%	
	- Moderate	0%	65%	0%	65%	30%	
	- Good	0%	35%	20%	0%	0%	
<b>Life style:</b>	0%	0%	80%	0%	0%	0%	<b>FET<sub>1</sub>= 28.429    P<sub>1</sub> = 0.000*</b> <b>FET<sub>2</sub>= 23.356    P<sub>2</sub> = 0.000*</b> <b>FET<sub>3</sub> = 44.691    P<sub>3</sub>= 0.000*</b>
	- Bad						
	- Accepted	65%	0%	0%	25%	65%	
	- Moderate	0%	70%	0%	75%	35%	
	- Good	0%	30%	30%	0%	0%	
	- Very good	0%	0%	70%	0%	0%	

Significant relation at P level  $\leq 0.05$

1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> : assessments

**Table (V): Effect of herbal and conventional therapy on the side effects of chemotherapy regarding to anthropometric measurements among the study and control groups.**

Item	Assess.	Study group		Control group		t- test	P
		Range	Mean ± SD	Range	Mean ± SD		
Weight	1 <sup>st</sup>	54.0 – 95.0	73.6 ± 11.0	60.0 – 86.0	74.6 ± 8.9	0.317	0.753
	2 <sup>nd</sup>	55.0 – 96.0	74.8 ± 11.1	59.0 – 85.0	73.2 ± 8.4	0.514	0.610
	3 <sup>rd</sup>	56.0 – 98.0	76.9 ± 11.1	58.0 – 83.0	71.1 ± 7.8	1.915	0.063
Height	1 <sup>st</sup>	150.0 - 173	163.6 ± 6.3	150.0 – 175.0	166.1 ± 5.9	2.904	0.006*
	2 <sup>nd</sup>	150.0 - 173	163.6 ± 6.3	150.0 – 175.0	166.1 ± 5.9	2.904	0.006*
	3 <sup>rd</sup>	150.0 - 173	163.6 ± 6.3	150.0 – 175.0	166.1 ± 5.9	2.904	0.006*
Mid arm circumference	1 <sup>st</sup>	26.0 – 36.0	31.0 ± 2.6	23.0 – 31.0	28.6 ± 2.0	3.312	0.002*
	2 <sup>nd</sup>	26.0 - 37	31.5 ± 2.7	23.0 – 30.5	28.0 ± 1.8	4.650	0.000*
	3 <sup>rd</sup>	27 – 37.5	32.1 ± 2.7	23.0 – 30.0	27.6 ± 1.8	6.284	0.000*
Mid arm muscle circumference	1 <sup>st</sup>	21.1 – 29.7	25.4 ± 2.1	18.2 – 25.3	23.3 ± 1.6	3.517	0.001*
	2 <sup>nd</sup>	21.2 – 30.4	25.8 ± 2.2	18.2 – 25.0	22.9 ± 1.5	4.845	0.000*
	3 <sup>rd</sup>	20.9 – 30.7	26.3 ± 2.3	18.2 – 24.5	22.4 ± 1.6	6.212	0.000*
Triceps skin fold	1 <sup>st</sup>	15.5 - 21	17.5 ± 1.9	15.0 – 20.0	16.8 ± 1.1	1.526	0.137
	2 <sup>nd</sup>	15.0 – 22.0	17.8 ± 2.1	15.0 – 19.5	16.5 ± 1.0	2.564	0.016*
	3 <sup>rd</sup>	16.0 – 23.0	18.4 ± 2.2	15.0 – 19.0	16.2 ± 1.0	4.185	0.000*
Body mass index	1 <sup>st</sup>	22.4 – 34.4	27.3 ± 3.0	21.7 – 29.7	26.2 ± 2.7	1.307	0.199
	2 <sup>nd</sup>	22.8 – 34.8	27.8 ± 3.0	22.4 – 29.0	25.6 ± 2.5	2.432	0.020*
	3 <sup>rd</sup>	23.3 – 35.5	28.6 ± 3.0	22.2 – 28.3	25.0 ± 2.3	4.280	0.000*

Significant relation at P level  $\leq 0.05$

**Table (XXI): Relation between age and chemotherapeutic side effects of all body systems among breast cancer women post herbal and conventional therapy.**

Item	Side effects ( X ± SD)					
	n = 20      Study			n = 20      Control		
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>
<b>Age :</b>						
27-	42.8 ± 2.7	29.3 ± 4.3	22.8 ± 3.6	36.9 ± 10.3	42.4 ± 11.4	53.3 ± 6.2
38-	44.4 ± 3.1	32.0 ± 2.1	23.9 ± 2.6	35.5 ± 2.7	41.9 ± 3.9	52.9 ± 3.9
49-65	41.8 ± 8.9	31.0 ± 6.0	22.7 ± 4.2	38.6 ± 5.1	45.3 ± 5.1	55.2 ± 3.0

F	0.364	0.576	0.238	0.854	0.908	0.900
P	0.700	0.573	0.790	0.443	0.422	0.425

Significant relation at P level  $\leq 0.05$ ; 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>: assessments

**Table (XX): Relation between occupation and chemotherapeutic side effects of all body systems among breast cancer women post herbal and conventional therapy.**

Item	Side effects ( X $\pm$ SD)					
	n = 20      Study			n = 20      Control		
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>
<b>Occupation:</b>						
- Working	43.2	30.7	22.9	$34.0 \pm 3.5$	$40.3 \pm 5.0$	$51.9 \pm 3.8$
- Non working	$43.0 \pm 5.7$	$30.8 \pm 4.5$	$23.2 \pm 3.5$	$39.2 \pm 4.7$	$45.8 \pm 4.7$	$55.5 \pm 2.9$
t-test	0.037	0.029	0.083	2.525	2.429	2.350
P	0.971	0.977	0.935	0.021*	0.026*	0.030*

Significant relation at P level  $\leq 0.05$

1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> : assessments

**Table (XXI): Relation between type and frequency of breast feeding and chemotherapeutic side effects of all body systems among breast cancer women post herbal and conventional therapy.**

Item	Side effects ( X $\pm$ SD)					
	n = 20      Study			n = 20      Control		
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>
<b>Type of feeding:</b>						
- Bottle feeding	$42.6 \pm 2.7$	$30.4 \pm 4.0$	$23.8 \pm 3.4$	$38.0 \pm 6.3$	$44.5 \pm 7.2$	$54.8 \pm 4.3$
- Natural feeding	$43.3 \pm 7.0$	$31.1 \pm 4.8$	$22.8 \pm 3.6$	$37.0 \pm 4.3$	$43.5 \pm 4.4$	$54.0 \pm 3.4$
t-test	0.242	0.373	0.583	0.391	0.384	0.434
P	0.812	0.714	0.567	0.705	0.705	0.669
<b>Frequency of breast feeding:</b>						
- Less than three						
- Three and more	$42.7 \pm 3.1$	$29.6 \pm 3.9$	$23.2 \pm 3.8$	$36.2 \pm 4.6$	$43.4 \pm 5.2$	$53.1 \pm 4.1$
	$43.2 \pm 6.9$	$31.7 \pm 4.6$	$23.1 \pm 3.4$	$38.1 \pm 5.1$	$44.1 \pm 5.6$	$54.9 \pm 3.4$
t-test	0.200	1.093	0.039	0.834	0.286	1.038
P	0.844	0.289	0.969	0.415	0.778	0.313

Significant relation at P level  $\leq 0.05$ ; 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> : assessments

**Table (I):** shows distribution of the patients among the study and control groups regarding to biosociodemographic characteristics. It was observed that the majority of patient's age (45%) were in the age group of (49 – 65) years, while (20%) only were in the age group of (27 – 38) years. Regarding to educational level, it is evident that illiterate patients formed the greatest proportion of the sample (65%). There was no statistically significant difference among the study and control groups in relation to age and educational level. P= (0.274 and 0.443), respectively.

As regard the occupation the majority of the patients (80%) were found to be non - working, while (20%) were working. There was a statistically significant difference among the two groups in relation to occupation P= (0.044). In relation to marital status, it was observed that the majority of patients (75%) were married, while (10%) were single. there was a statistically significant difference among the two groups in relation to marital status P= (0.025). Concerning the type and frequency of breast feeding. It was found that the majorities of the sample (62, 5%) were breast fed and lactated their children for three times and more through their life. There was no statistically significant difference among the study and control groups in relation to type and frequency of breast feeding where, P= (0.744).

**Table (II):** shows the effect of herbal and conventional therapy on the side effects of chemotherapy regarding to all body systems among the study and control groups. This table illustrated that, the side effects of chemotherapy regarding to all body systems for the study group shows a decrease on the second and third assessments ( $30.9 \pm 4.4$ , and  $23.2 \pm 3.4$ ), respectively compared with the first assessment ( $43.0 \pm 5.6$ ). In contrast for the control group, it was observed that these side effects showed an increase of its severity on the second and third assessments ( $43.9 \pm 5.3$ , and  $54.3 \pm 3.6$ ), respectively compared with the first assessment ( $37.4 \pm 4.9$ ). It was found that there was a significant difference between study and control groups in relation to first, second and third assessments related to all body systems. P = (0.002, 0.000, 0.000), respectively.

**Table (III):** shows the ranking of all body systems that affected with the chemotherapeutic side effects post herbal and conventional therapies (3<sup>rd</sup> assessment) among two groups. This table illustrated the actual effect of herbal therapy in relation to the third assessment, it was found that, liver functions and endocrine studies, renal functions studies, reproductive, urinary system, and weight changes were the least affected systems with the side effects

of chemotherapy, and they were the most affected systems with combining herbal to conventional chemotherapy ( $6.3 \pm 11.1$ ), ( $10.0 \pm 22.5$ ), ( $13.5 \pm 5.9$ ), ( $15.6 \pm 6.6$ ), and ( $16.9 \pm 6.1$ ), respectively. Gastro intestinal, respiratory, musclo skeletal system, hematological tests, and eye, ear, mucous membranes were moderately affected systems with the side effects of chemotherapy, and they were also moderately affected systems with combining herbal to conventional chemotherapy ( $17.3 \pm 3.4$ ), ( $23.5 \pm 4.9$ ), ( $24.4 \pm 8.3$ ), ( $25.8 \pm 15.6$ ), and ( $28.0 \pm 6.7$ ), respectively. It was also found that, psychological status, nervous system, and skin , hair, and nails were the most affected systems with the side effects of chemotherapy, and they were the least affected systems with combining herbal to conventional chemotherapy ( $30.3 \pm 6.5$ ), ( $32.5 \pm 10.3$ ), and ( $33.1 \pm 8.9$ ), respectively. On the other hand, for control group, it was also found that, weight changes, reproductive system, liver functions studies , urinary system, and gastro intestinal were the least affected systems with the side effects of chemotherapy, ( $18.1 \pm 12.5$ ), ( $27.9 \pm 5.3$ ), ( $45.0 \pm 15.4$ ), ( $48.3 \pm 6.3$ ) and ( $53.3 \pm 7.1$ ), respectively. Nervous system , hematological tests, Skin, hair, and nails, eye, ear, mucous membranes and psychological status were moderately affected systems with the side effects of chemotherapy, ( $53.8 \pm 7.1$ ), ( $57.3 \pm 12.3$ ), ( $62.8 \pm 6.9$ ), ( $64.5 \pm 5.6$ ) and ( $65.0 \pm 9.2$ ), respectively. Concerning, respiratory systems, musclo skeletal and renal functions studies were the most affected systems with the side effects of chemotherapy, ( $65.8 \pm 4.8$ ), ( $68.1 \pm 6.0$ ) and ( $92.5 \pm 18.3$ ), respectively.

**Table (IV):** reveals the effect of herbal and conventional therapy on the side effects of chemotherapy regarding to Health and Life style grades among the study and control groups. Concerning, health grades and life style of the patients, the table revealed that, all the study group have bad and accepted degrees of health grade and life style grades, and all the control group have bad and moderate degrees of health grade and life style grades on the first assessment. In relation to the third assessment post herbal and conventional therapy it was found that the entire study group has good and very good degrees, while all of the control groups have bad and accepted degrees.

In relation to significant difference between the three assessments among the study and control groups in relation to health and life style grades. It was found that there was a significant difference between study and control groups in relation to first, second and third assessments P = (0.000).

**Table (V):** Reveals the effect of herbal and conventional therapy on the side effects of chemotherapy regarding to anthropometric measurements among the study and control groups. Concerning patient's weight, there was an increase in patient's weight of the study group on the third assessment ( $76.9 \pm 11.1$ ), compared with the first assessment of weight ( $73.6 \pm 11.0$ ). While there was a decrease in patient's weight of the control group on the third assessment ( $71.1 \pm 7.8$ ), compared with the first assessment of weight ( $74.6 \pm 8.9$ ). There was no statistical significant difference between both groups in relation to assessment of weight in first, second and third assessment  $P= (0.753, 0.610, 0.063)$ , respectively. The table revealed that, there was statistical significant difference between both groups in relation to assessment of height in first, second and third assessments.  $P=0.006$ .

In relation to mid arm circumference, the mean of mid arm circumference in study group was increased in the 2<sup>nd</sup> and 3<sup>rd</sup> assessments ( $31.5 \pm 2.7$ ,  $32.1 \pm 2.7$ ), respectively, compared with the first assessment of mid arm circumference ( $31.0 \pm 2.6$ ). While in the control group there was a slightly decrease on the 2<sup>nd</sup> and 3<sup>rd</sup> assessments ( $28.0 \pm 1.8$ ,  $27.6 \pm 1.8$ ), respectively, compared with the first assessment of mid arm circumference  $28.6 \pm 2.0$ . There was statistical significant difference between both groups in relation to assessment of mid arm circumference on first, second and third assessments  $P= (0.002, 0.000, 0.000)$ , respectively.

Moreover, the mean of triceps skin fold in study group was increased on the 2<sup>nd</sup> and 3<sup>rd</sup> assessments ( $17.8 \pm 2.1$ ,  $18.4 \pm 2.2$ ), respectively, compared with the first assessment of triceps skin fold ( $17.5 \pm 1.9$ ). While in the control group there was a decrease on the 2<sup>nd</sup> and 3<sup>rd</sup> assessments ( $16.5 \pm 1.0$ ,  $16.2 \pm 1.0$ ), respectively, compared with the first assessment of mid arm circumference ( $16.8 \pm 1.1$ ). There was no statistical significant difference between both groups in relation to first assessment of triceps skin fold  $P= (0.137)$ . While on the second and third assessments There was statistical significant difference between both groups  $P= (0.016, 0.000)$ , respectively.

Finally the table shows that, there was an increase in study group in relation to body mass index on the 2<sup>nd</sup> and 3<sup>rd</sup> assessments ( $27.8 \pm 3.0$ ,  $28.6 \pm 3.0$ ), respectively, compared with the first assessment of body mass index ( $27.3 \pm 3.0$ ). While in the control group there was a decrease on the 2<sup>nd</sup> and 3<sup>rd</sup> assessments ( $25.6 \pm 2.5$ ,  $25.0 \pm 2.3$ ), respectively, compared with the first assessment of body mass

index ( $26.2 \pm 2.7$ ). There was no statistical significant difference between both groups in relation to first assessment of body mass index  $P= (0.199)$ . While in the second and third assessments There was statistical significant difference between both groups  $P= (0.020, 0.000)$ , respectively.

**Table (VI):** shows the relation between age and chemotherapeutic side effects of all body systems among breast cancer women post herbal and conventional therapy. This table revealed that, the chemotherapeutic side effects of all body systems for the study group by its age categories were decreased on the 2<sup>nd</sup> and 3<sup>rd</sup> assessment ( $29.3 \pm 4.3$ ,  $22.8 \pm 3.6$ ), respectively, compared with the first assessment ( $42.8 \pm 2.7$ ) for the age group 27-38.while for the control the side effects of chemotherapy were increased on the 2<sup>nd</sup> and 3<sup>rd</sup> assessments ( $42.4 \pm 11.4$ ,  $53.3 \pm 6.2$ ), respectively, compared with the first assessment ( $36.9 \pm 10.3$ ) for the same age group. There was no significant difference between age and all the side effects of chemotherapy among the three assessments of the study group  $P= (0.700, 0.573, 0.790)$  respectively. And also for the control group  $P= (0.443, 0.422, 0.425)$ , respectively.

**Table (VII):** shows the relation between occupation and chemotherapeutic side effects of all body systems among breast cancer women post herbal and conventional therapy. It was found that, the chemotherapeutic side effects of all body systems of the study group was slightly increased between the working group (43.2),compared with the non - working group (43.0) on their first assessment. This result was changed on the third assessment, working group (22.9) and non - working (23.2), while in the control group it was a decrease between the working groups (34.0) compared with the non - working group (39.2) on their first assessment. This result wasn't changed on the third assessment, working group (34.0) and non - working (55.5).The table also revealed that, for non- working group of the study group, the chemotherapeutic side effects of all body systems were decreased on the second and third assessments ( $30.8 \pm 4.4$ ), ( $23.2 \pm 3.5$ ), respectively, compared with the first assessment ( $43.0 \pm 5.7$ ). While for the control group, there was an increasing in the chemotherapeutic side effects of all body systems on the second, and third assessments ( $45.7 \pm 4.6$ ), ( $55.5 \pm 2.9$ ), respectively, compared with first the assessment ( $39.2 \pm 4.6$ ).

There was no significant difference between occupation and all the side effects of chemotherapy among the three assessments of the study group  $P= (0.971, 0.977, 0.935)$ , respectively. While for the

control group there was a significant difference between occupation and all the side effects of chemotherapy among the three assessments of the control group  $P= (0.021, 0.026, 0.030)$ , respectively.

**Table(VIII):** shows the relation between type and frequency of breast feeding and chemotherapeutic side effects of all body systems among breast cancer women post herbal and conventional therapy. This table illustrated that, the chemotherapeutic side effects of all body systems of the study group was slightly increased between the patients have a history of natural breast feeding for three times and more ( $43.2 \pm 6.9$ ), ( $43.2 \pm 6.8$ ), respectively, compared with patients who have history of bottle feeding for less than three times ( $42.6 \pm 2.7$ ), ( $42.7 \pm 3.0$ ), respectively on their first assessment. This result was changed on the third assessment; patients have a history of natural breast feeding for three times and more ( $22.8 \pm 3.5$ ), ( $23.1 \pm 3.3$ ), respectively and patients who have history of bottle feeding for less than three times ( $23.7 \pm 3.3$ ), ( $23.3 \pm 3.7$ ), respectively.

Concerning, the relation between the type of breast feeding it was found that, the chemotherapeutic side effects of all body systems was decreased on the second , and third assessment ( $t=0.37$ ), ( $t=0.58$ ), respectively, compared with the first assessment ( $t=0.24$ ). While for the control group, there was an increasing in the chemotherapeutic side effects of all body systems on the second, and third assessment ( $t=0.38$ ), ( $t=0.43$ ), respectively, compared with first the assessment ( $t=0.39$ ). On the other hand, for the control group, there was an increasing in the chemotherapeutic side effects of all body systems on the second, and third assessment ( $t=0.38$ ), ( $t=0.43$ ), respectively, compared with the first assessment ( $t=0.39$ ).There was no significant difference between type of breast feeding and all the side effects of chemotherapy among the three assessment of the study group  $P= (0.812, 0.714, 0.567)$ , respectively. Also for the control group  $P= (0.705, 0.705, 0.669)$ , respectively.

Regarding, significant difference between frequency of breast feeding and all the side effects of chemotherapy, it was found that, there was no significant difference among the three assessment of the study group  $P= (0.84, 0.28, 0.96)$ , respectively. Also for the control group  $P= (0, 41, 0.77, 0.31)$ , respectively.

## DISCUSSION:

The use of complementary and alternative medicine using herbals and their natural products for

the prophylaxis, protection, and treatment of various diseases has become more popular in recent years than ever before. Surveys indicate that 64% of cancer patients use alternative medicine, either for treating cancer or relieving symptoms that are associated with different cancer treatments<sup>(123)</sup>.

This study was carried out to show the effect of the combination of herbal mixture to conventional chemotherapy on minimizing the incidence of chemotherapeutic side effects for 2<sup>nd</sup> stage breast cancer.

Geoff, (2006) illustrated that; integrated medicine is a holistic approach to cancer care. Some herbals showed proven effectiveness as adjuvant to conventional medical treatments<sup>(124)</sup>. Janelle et al, (2007) mentioned that, complementary and alternative medicine is widely used by cancer patients due to largely patient's demands for integration of herbal therapies into cancer treatment<sup>(83)</sup>. This was congruent with the findings of the present study which revealed that, patients with herbal and conventional therapies experienced less chemotherapeutic side effects of all body systems than patients with only conventional therapy.

In the present study, as regards to age, it was found that, the majority of patient's age was ranged from 49-65 years old. This was congruent with the American Cancer Society, (2008), which reported that about 80% of all breast cancers arise in women over age 50<sup>(125)</sup>. In addition, Winsock (2004) stated that the most important risk factor for development of breast cancer is increasing age. As any woman ages, her risk of breast cancer increases, especially over 50 years<sup>(58)</sup>.Also this is in line with Thom et al, (2006) who indicated that people over the age of 50 years old are more risky for developing breast cancers<sup>(126)</sup>. DeMichele et al, (2007) reported that, Breast cancer occurs more often in women over 50 and is less common in premenopausal women. Nearly 80 percent of all newly diagnosed invasive breast cancer cases occur in women aged 50 and older<sup>(127)</sup>.

Regarding to educational level, the study illustrated that, the majority of the patients were illiterate this result is similar to Ebrahim's findings, (1999) on his study to identify the preoperative stressors among surgical patients, emphasized that, the majority of the studied subjects were illiterate; he stated that the overwhelming majority of the Egyptian population are illiterate or does not have any formal education<sup>(128)</sup>. The present findings may be due to the setting of the study is a free governmental hospital and the majority of patients have a low education and

economical standards. This result is not in agreement with Jan and Sundquist, (2007) who mentioned that, highly educated women run a greater risk of developing breast cancer especially noninvasive breast cancer than women with less education. At the same time, highly educated women have better chances of surviving in various types of cancer than those with a low level of education<sup>(129)</sup>. They said that; it is naturally not education itself that causes breast cancer. It is most likely that highly educated women attend mammography in greater numbers. Such screening is the most reliable way to find the earliest stages of breast cancer<sup>(129)</sup>. Furthermore, the present study revealed that the non working patients formed the greatest proportion of the sample. This may be due to their lower educational level and being females which decrease their chance of getting a job, they were house wives which are the greatest proportions of the Egyptian's females<sup>(131)</sup>, also it may be due to the setting of the study does not provide health insurance services and there is other hospital for working patients. This result disagree with Ali and Hussain, (2003) who reported that occupations are increasing the risk of getting cancer especially some types of occupations as , teachers who had the highest rates of having breast cancer, and other three occupational groups had statistically significant for cancer of the ovary as printing machine, launderers, and dry cleaners<sup>(132)</sup>.

In relation to marital status the present study showed that, married represented higher percentage than single and widowed, this is opposite to Hayes, (2005) who concluded that, the risk for breast cancer, treatment recommendations and follow-up should not be altered based on a woman's marital status<sup>(133)</sup>.

In agreement with the present study, Frank (2005) mentioned that, current chemotherapeutic techniques have a range of side effects mainly affecting the fast-dividing cells of the body. These side-effects can be reduced by several agents and therapeutic modalities<sup>(138)</sup>. One of these managements which reduce these side effects is the using of herbal medicine as a complementary therapy to eliminate the side effects of cancer therapy<sup>(138)</sup>. This is in line with the present findings, that the herbal components of the study are composed of *nigella sativa*, ginger curcuma, boswella gum, and cardamom. These components have different actions and properties, the commonest actions of these components are; anti-inflammatory, immunomodulator, anti-oxidant, anti- emetic, anti-arthritics, analgesic. Therefore, the aim of this study is decreasing the side effects of the chemotherapy by

using these effects and actions of the herbal components to minimizing these side effects.

Braun and Cohen, (2007) stated that, long term side effects of chemotherapeutic drugs, are due to the formation of free radicals that lead to oxidative organ damage<sup>(139)</sup>. Herbal and its ingredients antioxidants have been investigated in humans and several studies have shown improvement or prevention of some side effects and possibly increased treatment effectiveness<sup>(140, 141)</sup>.

Altman and Marcussen, (2001) high lights the study findings regard to musclo skeletal system, which is a moderately affected system by the combining herbal to conventional therapy, the result illustrated that, there was no significant difference between study and control groups in relation to first assessment. While, in the second and third assessments there was a statistically significant difference between the two groups. This may be attributed by the effect of ginger inhibits the production of immune-system components called cytokines. These chemicals are believed to create a long-term tendency toward inflammation. Stated that, Ginger stimulates blood circulation. These effects of ginger are taken advantage in treating a number of disorders marked by swelling and pain, such as arthritis<sup>(95)</sup>. The finding is congruent with Cassiani et al, (2002) who reported that, studies have also shown that ginger can relieve pain without the side effects typically found when using nonsteroidal anti-inflammatory drugs (NSAIDs) and steroids<sup>(145)</sup>.

This view is also supported with Goldberg et al, (2000) who mentioned that ginger can decrease inflammation. In fact, many health care professionals today use ginger to help treat health problems associated with inflammation, such as arthritis and muscle stiffness. In a study of 261 people with osteoarthritis of the knee, those who received a ginger extract twice daily experienced less pain and required fewer pain-killing medications compared to those who received placebo<sup>(146)</sup>. Davis et al, (2007) reported that, Boswella can reduce painful symptoms of arthritis on dozens of patients, who received the herb and showed better movement and less pain and stiffness<sup>(98)</sup>.

Moreover, the study also revealed that, there was a significant difference between study and control groups in relation to first, second and third assessments of the nervous system, This result were supported by Fahs and Kinny, (2005) who reported that the main effect of curcuma, in which curcumin is the main constituent, is to protect against free radical damage because it is a strong antioxidant, and reduces inflammation. It accomplishes this by reducing histamine levels and

possibly by increasing production of natural cortisone by the adrenal glands by these mechanisms curcumin can inhibits headache, and suppress the unpleasant effects of the nervous system<sup>(147,148)</sup>.

As regard to respiratory systems it was found that, there is no significant difference between study and control groups in relation to first assessment. While in the second and third assessments there was a significant difference between the two groups. This result was supported by Vanbree et al (2002). Who noted that boswellia was believed to support the respiratory and immune systems during respiratory attacks as flu, bronchitis, coughs and decreasing restlessness<sup>(149)</sup>. It is also in line with Hadid et al, (2003) who noted that Cardamom used to sooth the respiratory tract, and it is used also for asthma, cold, cough, bronchitis<sup>(150)</sup>.

Recent studies as indicated by Ashraf and Ali, (2005) who support the action of fixed and essential oils of the *nigella sativa* seeds which has anti-inflammatory, an immunomodular activity, and analgesic effects<sup>(151)</sup>. So; the present findings is due to the combined effects of boswellia and cardamom as well as *nigella sativa* seeds.

Furthermore, the current study also revealed that , there is a significant difference between study and control groups in relation to first , second and third assessments of the reproductive and psychological status. This result is congruent with Basil, (2007) who reported that, due to the analgesic effect and / or the pain reliving properties of ginger; it can reduce discomfort associated with breast cancer. Also added that, ginger also helps control inflammation, due to the presence of gingerols, which is the main constant of ginger, which work like the older anti-inflammatory drugs, such as aspirin. Ginger unlike aspirin, has a calming effect on the intestinal tract<sup>(152)</sup>. Gingerols prevent the aggregation of platelets, as well as reducing inflammation; they can help to minimizing platelets aggregation.<sup>(152)</sup>.

This was congruent with the present findings, especially related to a menorrhea and decreasing breast and vaginal discharge which may be due to inflammatory effects. Ahmed et al, (2003) added that, in addition to these benefits; Ginger can decrease the risk of heart disease by "platelet inhibition" and by lowering cholesterol. It is a strong antioxidant and can inhibit certain bowel infections (*Salmonella*) and it can be effective against vaginal Trichomonas infections, which is the commonest cause of vaginal discharge<sup>(153)</sup>.

Jane et al, (2004) mentioned that, ginger has a long history of use for treating anxiety and depression<sup>(154)</sup>. Heller et al, (2001) recommended the use of ginger as anti depressant and for managing or treating insomnia, especially before the bed time<sup>(155)</sup>. Emphasized that, ginger works in a similar way to some prescription antidepressants by increasing the brain chemical serotonin, involved in controlling mood.

Adesson, (2007) patients are more concerned about the occurrence and management of side effects than the actions of particular chemotherapeutic agents. Identifying the side effects or patient's problems, will assist in achieving the desired outcome. As well as the time sequence in which side effects generally occur may allay patient anxiety and will assist nurses in selecting the appropriate interventions<sup>(156)</sup>.

Related to the hematological studies, renal, and liver and endocrine studies, the study illustrated that, these systems are the latest affected systems with the side effects of chemotherapy. And they are the most affected systems by the combining herbal to conventional chemotherapy as well as there is a significant difference between the study and control groups in relation to first assessment except for renal functions. While in relation to second and third assessment there was a significant difference between the tow groups. This result was congruent with Elassafy, (2001) who mentioned that; cancer patient should continue the intake of *nigella sativa* during chemotherapy. This can be effective way of limiting the side effects of chemotherapy, because *nigella sativa* supports maintenance of trileanial erythropoiesis (red blood cells, white blood cells and platelets). The guiding principles is the adherence to 6 sessions of chemotherapy which usually are the hemoglobin level, the white cell count, the platelet count with administering of *nigella sativa* help in maintaining these normal levels during chemotherapy<sup>(157)</sup>.

Nagi et al, (2008) concluded that, *nigella sativa* seeds are effective in protecting against hypertension and renal damage especially serum creatinine level possibly via its antioxidant activity of thymoquinone (TQ), the main constituent of the volatile oil of *nigella sativa* seeds<sup>(158)</sup> . Also it is in line with Elkahtany et al, (2007) who added that, ginger has been used to reduce the toxic side effects of some chemotherapeutic drugs, as showed in protective effects against nephrotoxicity induced by chemotherapeutic agents<sup>(159)</sup>. Ahmed et al, (2006) reported that, there is a significantly reduction in the elevated levels of blood glucose, lipids, plasma insulin

and improved altered levels of lipid peroxidation products and antioxidant enzymes which minimizing the harmful effects of the liver and kidney<sup>(160)</sup>. These results confirm the antidiabetic activity of *nigella sativa* seeds extract and suggest that because of its antioxidant effects its administration may be useful in controlling the diabetic complications<sup>(160)</sup>.

Block & Mead, (2003) added that, curcumin and boswellia, combination have been used in cancer therapy, not only to reduce the associated side effects but also to enhance the effectiveness of chemotherapy<sup>(161)</sup>. On a systematic review of these herbals for chemotherapy induced side-effects in cancer patients, Taixiang et al, (2005), analyzed the results of four trials that used a formulation containing Curcumin can stimulate immuno-competent cells and decrease side effects in patients treated with chemotherapy.<sup>(162)</sup>

One of the more problematic side effects of chemotherapy is the incidental damage to normal tissues and all body systems. This damage to normal tissues of the body systems, in some patients, can be sufficiently severe to stop chemotherapy. Ginger and *nigella sativa* have a powerful anti inflammatory effects and immunomodulatory effects; so they can reduce these side effects<sup>(93, 95)</sup>.

It is imperative to assess accurately the patient's physical and emotional status before therapy is initiated. This information assists the health-care team to identify risk factors that could contribute to the occurrence or severity of side effects. Other factors that may affect the patient's response to therapy are age, general condition, coexisting illnesses, and nutritional status<sup>(163)</sup>.

Concerning to health and life style grades, it was found that there was a significant difference between study and control groups in relation to first, second and third assessments. This result is opposite to Ganz, (2004) who mentioned that, patients survivors older than the age of 65, less educated individuals and people living in urban areas were less likely to make or maintain healthy lifestyle changes after awarning they had cancer<sup>(164)</sup>. While it is in line with Petter, (2006) who concluded that, being diagnosed with cancer can be a "teachable moment," when people are very open to suggestions about eating better, exercising and other healthy habits. So, in the present study, when the patients had low chemotherapeutic side effects of all body systems, they became more healthy and their life style became better<sup>(165)</sup>.

In relation to body mass index (BMI), there was no statistical significant difference between study and control groups in relation to first assessment. While in the second and third assessment there was significant difference between both groups. This result is congruent with Massimo, (2005) who indicated that, when nausea and vomiting decreased; body weight and body mass index eventually can be also increased. He also added that, high BMI has been considered as a prognostic indicator for managing cancer patients<sup>(166)</sup>. This findings are not in agreement with Kifeli, (2005) who mentioned that, high BMI is known as an important risk factor for development of breast cancer this is at the pre diseased period<sup>(167)</sup>. But, in the present study, the patients are already cancer patients so; high BMI has considered as a prognostic indicator for managing cancer.

Concerning to the relation between age and all the side effects of chemotherapy, there is no significant difference among the three assessments of the study and also for the control group. It is observed that, breast cancer's women of the study group at the age of (49-65) experienced minimal side effects than other age groups. This result is congruent with Lancet, (2001) who reported that, the effects of chemotherapy are more pronounced in younger women than elders, emphasized this by younger women who are likely to need chemotherapy because their cancer often has a worse prognosis, and she experienced a severe degree of the chemotherapeutic side effects<sup>(168)</sup>.

Darry, (2002) mentioned that, cancer patients who continue to work are as productive on the job as other workers. Most cancer patients who are physically able to work do go back to their jobs. Returning to work can help them feel they are getting back to the life they had before being diagnosed with cancer<sup>(169)</sup>. This is in line with the study findings regarded to the relation between occupation and all the side effects of chemotherapy, it was found that there is no significant difference among the three assessments of the study while for the control group there was a significant difference among the three assessments. This result was opposite to Ashing, (2004) findings who documented that breast cancer and it's treatment have varying effects on women employment, several women choose to change jobs or stop working, others lost their function and experience serious job disruptions<sup>(170)</sup>.

Recent studies revealed that herbal components, the subject of the current study, have anti inflammatory, anti oxidant and immunomodulator effects, which support the present findings in decreasing and minimizing the side effects of conventional chemotherapy. Robbson, (2006) stated

that, oncology nurses are engaged in a collaborative practice with all members of the care team to provide optimal management of patients with cancer. Their professional practice requires detailed knowledge of the biologic and psychosocial dimensions of the cancer problem. They have key roles not only as caregivers but in patient and family education and clinical cancer research. Oncology nurses also are continuously involved in the enhancement of nursing practice through research, continuing education, and advanced education<sup>(171)</sup>.

Also it was concluded that, when patients integrate these complementary therapies as herbals into their medical and surgical care, they are creating a more comprehensive treatment plan and helping their own bodies to regain health and vitality<sup>(22)</sup>. Therefore, minimizing of the chemotherapeutic side effects requires skillful nursing interventions as well as collaborative work between oncologist, pharmacologist, nutritional therapy, laboratory technicians, and oncology

This study aims at declaring the effect of the herbal combination of herbal mixture to conventional chemotherapy on minimizing the incidence of chemotherapeutic side effects for 2<sup>nd</sup> stage breast cancer patients. Based on the results of this study, it can be concluded that:

The risk of breast cancer was found to be highly among women with age group (49 – 65) years, and common among breastfeeding women who lactated for three times and more through their life.

Patients with herbal and conventional therapies experienced less chemotherapeutic side effects of all body systems than patients with only conventional therapy. It was also concluded that, liver functions and endocrine studies, renal functions, reproductive system, urinary system, and weight changes were the least affected systems with chemotherapeutic side effects and were also the most affected systems when combined herbal to conventional therapy

Gastro intestinal, respiratory, musclo skeletal systems and hematological studies, as well as eye, ear, mucous membranes were moderately affected systems with the side effects of chemotherapy, and also with combining herbal to conventional chemotherapy. While Psychological status, nervous system, and skin, hair, and nails were the most affected systems with the side effects of chemotherapy, and they were the least affected systems with combining herbal to conventional chemotherapy.

Patients with combining herbal and conventional therapy group have good and very good degrees of health grades and life style, after three months of treatment by herbal and conventional therapy combination. While patients with only conventional therapy, have bad and moderate degrees.

Patients with herbal and conventional therapy have increased body mass index, after three months of treating with herbal and conventional therapy. While patients with only conventional therapy, have decreased their body mass index.

It was found that, there was no relation between chemotherapeutic side effects with age, occupation, type and frequency of breast feeding.

The present findings declare that herbal combination of the study has a great action on attenuating, decreasing, minimizing and protecting the body from the usual side effects appears during conventional chemotherapy.

### **Recommendations**

Based on the findings of the present study, the following recommendations are derived and suggested:-

#### **A- Recommendations for oncology team:**

1. Herbal education should be introduced in nursing and medical curriculum as well as health awareness about herbals with proven efficacy, and its health effects should be increased.
2. It is essential to increase the level of awareness among public, patients and health care providers regarding the efficacy and toxicity of these medicinal herbals, through mass media.

#### **B- Recommendations for further researchers:**

1. Further researches are needed for these herbal components to measure its efficacy on minimizing the side effects of chemotherapy for breast cancer and / or other types of cancer.
2. Clinical studies to identify the effect of these herbals on different cancer therapies, different chemotherapeutic protocols, as well as specifically pre or post mastectomy.
3. Further research also needed for larger number of sample, as well as long period of study time to confirm the effect of these herbals on minimizing the chemotherapeutic side effects.

4. Investigate the relation between awareness of educated patients towards herbal medicine and their compliance.
5. Collaboration, guidance and support for relevant research in herbal medicines to test its safety and efficacy for managing other diseases.

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9/28/2010

## Women's Awareness of Danger Signs of Obstetrics Complications

Wafaa A. Rashad \*, Rasha M. Essa \*\*

\* Assistant Professor of Obstetric and Gynecologic Nursing, Faculty of Nursing, University of Alexandria, Alexandria, Egypt

\*\* Lecturer of Obstetric and Gynecologic Nursing, Faculty of Nursing, University of Alexandria, Damnhour Branch, Alexandria, Egypt

[wafaa.rashad@alex-nursing.edu.eg](mailto:wafaa.rashad@alex-nursing.edu.eg); [wafaara@yahoo.com](mailto:wafaara@yahoo.com); [rashaessa111@yahoo.com](mailto:rashaessa111@yahoo.com)

**Abstract:** An exploratory descriptive study was conducted at two Maternal and Child Health Centers (MCH) selected randomly in Albeheira Governorate to assess women's awareness of danger signs of obstetric complications. The study subjects consisted of 200 pregnant women attending the previously mentioned setting for tetanus toxoid immunization during pregnancy was enrolled in the study. (100 from each) A structured interview schedule was developed by the researcher after reviewing of the relevant literature and used to collect the necessary data. It comprised the following parts: Part I: Socio-demographic data such as age, level of education, occupation and number of family members...etc Part II: Obstetric characteristics such as gravidity, parity, abortions, antenatal follow up and presence of any complications. etc. Part III: questions related to knowledge about signs of obstetric complications, complaining of any obstetric complication, what to do if the woman has any of these signs. The study revealed that slightly more than one quarter of the study subjects (26.5 %) were unaware of obstetric danger signs compared to almost the same proportion (26.0 %) that had good awareness about such signs, while 47.5 % of the study subjects exhibited fair awareness. Lack of awareness about obstetric danger signs was related younger age, low level of education, gravidity and parity, previous experiences with any obstetric complications and lack of antenatal care. This study reflects the need for strategic plane to increase the awareness to shape health seeking behavior of the public related to signs of obstetric complications.

[**Wafaa A. Rashad**, Rasha M. Essa, Women's Awareness of Danger Signs of Obstetrics Complications. Journal of American Science 2010;6(11):802-]. (ISSN: 1545-1003).

**Keywords:** obstetric danger signs, awareness, signs of obstetric complications

### Introduction

Pregnancy is a normal process that results in a series of both physiological and psychological changes in expectant mothers. However, normal pregnancy may be accompanied by some problems and complications which are potentially life threatening to the mother and / or the fetus. (Fraser et al, 2003).

Globally, every minute, at least one woman dies from complications related to pregnancy or childbirth – that means 529 000 women a year. In addition, for every woman who dies in childbirth, around 20 more suffer injury, infection or disease – approximately 10 million women each year. (WHO, 2005).

In Egypt, maternal mortality ratio has declined dramatically from 174 / 100 000 live births in 1992-1993 to 67.6 / 100 000 live births in 2005, a further decline to 44.6 / 100 000 was also reported by 2009. (GHC, 2010; WHO, 2010)

Five direct complications account for more than 70% of maternal deaths: hemorrhage (25%), infection (15%), unsafe abortion (13%), eclampsia (very high blood pressure leading to seizures – 12%), and obstructed labor (8%). A total of 99 % of all maternal deaths occur in developing countries, where 85 % of population lives. While these are the main causes of maternal death, unavailable, inaccessible, unaffordable, or poor quality care is fundamentally responsible. (WHO, 2010).

Most maternal deaths are avoidable as the health care solutions to prevent or manage the complications are well known. This includes well functioning health system that provides accessible and high quality care from household to hospital level. Egyptian health officials have long been concerned about the country's preventable maternal deaths, with good reason. According to Egypt Demography and health Survey, slightly more than one quarter of Egyptian pregnant women do not receive antenatal care. However, among those who receive antenatal care only one third of them received advised about signs of obstetric complications and

where and when to seek medical assistance. (El-Zanaty et al, 2008).

Obstetric danger signs include persistent vomiting, severe persistent abdominal pain, vaginal bleeding during pregnancy and delivery, severe vaginal bleeding after delivery, swelling of face , fingers and feet, blurring of vision, fits of pregnancy, severe recurrent frontal headache, high grade fever, marked change in fetal movement , awareness of heart beats, high blood pressure, sudden escape of fluid from the vagina, dysuria, oliguria or anuria, prolonged labor, loss of consciousness and retained placenta. Awareness about the significance of symptoms and signs of obstetrics complications may lead to timely access to appropriate emergency obstetric care. (WHO, 2010) Obstetric nurse/ midwife plays a crucial role in promoting an awareness of the public health issues for the pregnant woman and her family, as well as helping the pregnant woman to recognize complications of pregnancy and where to seek medical assistance.

### **Significance of the study**

Women need not to die in childbirth. Women die from a wide range of complications in pregnancy, childbirth or the postpartum period. These life threatening complications are treatable, and thus most of these deaths are avoidable if women with the complications are able to identify and seek appropriate emergency obstetric care which makes a difference between life and death.<sup>(3)</sup> Lack of awareness of the significance of symptoms of obstetric complications is one of the reasons of failure of women to identify and seek appropriate emergency care. Accordingly, assessment of women's awareness of obstetric danger signs and associated factors contributes to their awareness.

### **Aim of the study**

The aim of this study was to assess women's awareness of danger signs of obstetric complications.

### **Research question**

Are women aware of danger signs of obstetric complications?

### **Material and Methods**

#### **Research design**

This is an exploratory descriptive study.

### **Setting**

The study was conducted at two Maternal and Child Health Centers (MCH) selected randomly in Albeheira Governorate namely Mahmodya and Abu-Matameer.

### **Subjects**

A convenient sample of 200 pregnant women attending the previously mentioned setting for tetanus toxoid immunization during pregnancy was enrolled in the study. (100 from each)

### **Tool of data collection**

A structured interview schedule was developed by the researcher after reviewing of the relevant literature to collect the necessary data. It comprised the following parts: Part I: Socio-demographic data such as age, level of education, occupation and number of family members.....etc Part II: Obstetric characteristics such as gravidity, parity, abortions, antenatal follow up and presence of any complications . etc. Part III: questions related to knowledge about signs of obstetric complications, complaining of any obstetric complication, what to do if the woman has any of these signs.

### **Methods**

1. An official permission was obtained from the administration of the previously mentioned settings.
2. The tool was developed after reviewing of relevant literature and content validity was tested by a jury from 3 experts in the field.
3. A pilot study was carried out to ascertain the clarity and applicability of the tool.
4. The researcher met the participants and explained the purpose of the study, and then an individual interview was carried out with the participants who accepted to participate in the study to collect the necessary data.
5. A list of medically recognized life threatening obstetric signs was obtained from the women's responses.
6. After data collection, responses were categorized, coded and analyzed by computer.

### **Ethical considerations**

An individual interview was carried out with the participants who voluntarily accepted to participate in the study to ascertain privacy and confidentiality.

### **Statistical analysis:**

The collected data was categorized, coded computerized, tabulated and analyzed using SPSS/version 17. Chi square test was used to

demonstrate the difference between study subjects' characteristics and level of awareness about obstetric danger signs. The level of significant selected for this study was p equal to or less than 0.05.

Good awareness about obstetric danger signs is defined as the ability to mention >75.0% recognized danger signs, fair awareness is the ability to mention 50-75% and the ability to mention up to 50% of obstetric danger signs was considered poor awareness.

## Results

Table I shows the general characteristics of the study subjects. It is observed that, 23.5% of the subjects were aged 20 to less than 25 years old, 34.5% aged 25 to less than 30 years old and 30.5% aged 30 to less than 35 years old, while only 11.5% were aged 35 years old and more.

Regarding level of education, only 13.5% of the study subjects had university education, 33.0% finished secondary school, 19.5% finished primary or preparatory school, 14.5% were just able to read and write and 19.5 % were illiterate.

As to their occupation, it is observed that, the majority of the study subjects (83.5%) were housewives and 16.5 % were workers.

As regards number of family member, it is found that, only 8.5% of the study subjects live within large family size and less than one half live within family consists of 4 to five members.

Table II shows that, 38.0 % and 34.5 % of the study subjects were pregnant two and three times respectively, 45.0 % and 29.5 % delivered two and three times respectively, 64.5% of them delivered by cesarean section, only 4.0 % had normal spontaneous vaginal delivery and the majority (81.0%) had no abortion. Places of delivery were home (5.5%), governmental hospital (23.0%), private hospital/ clinic (38.0 %) and health/ maternal and child health centers (32.5).

Regarding their babies' health, it is found that only 9.0 % of the study subjects having babies with health problems. These problems are congenital malformations (5.0%), small for date babies (1.5%) macrosomic babies (1.0%) and mental retardation (1.0 %).

Table III: shows the distribution of the study subjects according to attendance of antenatal care. It is observed that the majority of the study subjects (90.0 %) attended antenatal clinics, 49.4 % seek antenatal care at private hospital / doctor, 45.0 % at health / maternal and child health centers and only 5.0 % attended governmental hospital for antenatal care. Only 20.6 % of the study subjects had more than four antenatal visits, followed by 9.4 % had only

four visits, and 36.7 % had three visits and 22.2 % had only two visits.

Table IV presents the distribution of the study subjects according to presence of complications with last pregnancy. It is observed that, about half of the study subjects (46.5%) had complications with the last pregnancy. During pregnancy 17.2 % had threatened abortion or preeclampsia, and 15.1 % had placenta previa. During labor, 6.5 % had difficult labor and intrapartum bleeding, while during postpartum period 4.3 % had breast problems.

Table V presents the distribution of the study subjects according to their awareness of obstetric danger signs. It is found that, slightly more than one quarter of the study subjects (26.5%) were unaware of obstetric danger signs compared to almost the same proportion (26.0%) that had good awareness about the obstetric danger signs, while 47.5% of the study subjects exhibited fair awareness.

Table VI shows the relation between level of awareness of the study subjects and their general characteristics. It is observed that 68.1 % of the study subjects aged 20 to less than 24 years old had good awareness about obstetric danger signs, compared to 21.7 % of those aged 25 to less than 30 years old. It is also observed that 29.5 % of the study subjects aged 30 to less than 35 and 78.3 % aged 35 or more years old exhibit poor awareness regarding such signs. A statistical significant difference was observed between level of awareness and study subjects' age. ( $p = 0.001$ )

The table also shows that the level of education played a positive role in relation to awareness of obstetric danger signs. Therefore, only 11.1 % of the university graduate unaware of obstetric danger signs compared to more than two thirds (69.2%) of illiterate and the difference was statistically significant between level of awareness and level of education. ( $p = 0.001$ )

Regarding occupation, it is observed that, about one third of housewives (31.7 %) exhibited poor awareness about obstetric danger signs, and the majority (90.9 %) of working women exhibited good awareness and the difference was statistically significant regarding level of awareness about obstetric danger signs and occupation. ( $p = 0.0031$ )

As regards number of family member it is observed that the 8.3% and 5.9% of those who had 6 and 8 or more family members respectively exhibited lack of awareness about obstetric danger signs, compared to 45.8 % and 35.3 % of the same group who exhibit good awareness regarding such signs. A statistically significant difference was observed between awareness of women about obstetric danger signs and number of family members. ( $p = 0.015$ ).

Table VII presents the relation between level of awareness of the study subjects and their clinical characteristics. It is observed that 61.5 % of those who were pregnant for the first time and 40.6 % those who were pregnant for three times exhibited good awareness about obstetric danger signs and 78.9 % of those who was pregnant for the second time had fair awareness about such signs. A statistically significant difference was found between awareness of obstetric danger signs and number of pregnancies. (**p = 0.014**)

Regarding parity it is observed that, 41.7 % of those who had no deliveries had either good or poor awareness about obstetric danger signs, the majority (66.7 %) of those who delivered once exhibited fair awareness about such signs. Almost the same proportions (42.4 % and 40.7 %) of those who delivered twice had either fair or poor awareness about such signs, and 57.1 % and 50.0 % of those who delivered for three and more times exhibited good awareness. The difference was statistically significant between awareness about obstetric danger signs and number of deliveries. (**p = 0.028**)

As regards to place of previous delivery, it is observed that, near one half (43.5 %) who delivered at governmental hospital had poor awareness about obstetric danger signs compared to one half of those who delivered at private hospital/private clinics and 61.5 % of those who delivered at Health centers/MCH centers. The difference was statistically significant between awareness of women about obstetric dnger signs and place of delivery. (**p = 0.001**).

Presence of complications during the last pregnancy did not influence the awareness of women about obstetric danger signs as 50.5 % of those who had complications exhibited poor awareness compared to 5.6 % of those who had no complications, while 32.3 % of those with complications had fair knowledge compared to 60.7 % of those who had no complications. The difference was statistically significant between occurrence of complications and the awareness about obstetric danger signs. (**p = 0.0031**)

Attendance of antenatal care positively affected the awareness of women about obstetric danger signs. The majority of those who attended the antenatal care had more awareness than those who did not. And the difference was statistically significant between the level of the study subjects about obstetric danger signs and attendance of antenatal care. (**p = 0.001**).

**Table I: General characteristics of study subjects**

<b>General characteristics</b>	<b>n=200</b>	<b>%</b>
<b>Age (years)</b>		
20-	47	23.5
25-	69	34.5
30-	61	30.5
35-	23	11.5
<b>Level of education</b>		
Illiterate	39	19.5
Read & write	29	14.5
Primary/preparatory	39	19.5
Secondary	66	33.0
University or more	27	13.5
<b>Occupation</b>		
Housewife	167	83.5
Working	33	16.5
<b>Number of family members</b>		
< 3	72	36.0
4-	87	43.5
6-	24	12.0
8+	17	8.5

**Table II: Obstetric characteristics of study subjects**

<b>Clinical characteristics</b>	<b>n=200</b>	<b>%</b>
<b>Gravidity</b>		
Once	13	6.5
Twice	76	38.0
3 times	69	34.5
More than three times	42	21.0
<b>Parity</b>		
None	12	6.0
Once	90	45.0
Twice	59	29.5
3 times	21	10.5
More than three times	18	9.0
<b>Types of delivery</b>		
Normal	8	4.0
Cesarean section	129	64.5
Instrumental	58	29.0
Others (induction)	5	2.5
<b>Place of delivery</b>		
Home	11	5.5
Governmental hospital	46	23.0
Private hospital / Private doctor's clinic	76	38.0
Health centers / Maternal and child health care center	65	32.5
<b>No of abortions</b>		
None	162	81.0
Once	27	13.5
Twice	9	4.5
3 times	1	0.5
More than three times	1	0.5
<b>Having babies with problems</b>		

Yes	18	9.0
No	182	91.0
<b>Types of problems</b>		
Congenital malformations	10	5.0
Small for date	3	1.5
Macrosomic baby	2	1.0
Mentally retarded	2	1.0
Others	1	0.5

**Table III: Distribution of the study subjects according to attendance of antenatal care:**

Ante natal care	n=200	%
<b>Attendance of antenatal care</b>		
Yes	180	90.0
No	20	10.0
<b>Place of ante natal visits</b>		
Governmental hospital	9	5.0
Private hospital / doctor	89	49.4
Health center / Maternal and child health care center	81	45.0
Other	1	0.6
<b>Number of ante natal visits</b>		
Once	20	11.1
Twice	40	22.2
Tree times	66	36.7
Four times	17	9.4
More than four	37	20.6

**Table IV: Distribution of the study subjects according to presence of complications with last pregnancy**

Complications	n=200	%
<b>Presence of complications</b>		
Yes	93	46.5
No	107	53.5
<b>Types of complication</b>		
<b>During pregnancy</b>		
Threatened abortion	16	17.2
Vesicular mole	6	6.5
Ectopic pregnancy	7	7.5
Placenta previa	14	15.1
Preeclampsia	16	17.2
Intrauterine fetal death	6	6.5
Others	7	7.5
<b>During labor</b>		
Difficult labor	6	6.5
Precipitous labor	2	2.2
Bleeding	6	6.5
Perineal tears	4	4.3
Premature labor	4	4.3
<b>Postpartum</b>		
Bleeding	2	2.2
Puerperal infections	2	2.2
Breast problems	4	4.3

**Table V: Distribution of study subjects according to their awareness of obstetric danger signs**

Awareness of danger signs	No =200	%
Good	52	26.0
Fair	95	47.5
Poor	53	26.5

**Table VI: Relation between study subjects' level of awareness and their general characteristics.**

General characteristics	Good		Faire		Poor		p
	No.	%	No.	%	No.	%	
<b>Age (years)</b>							<b>0.001*</b>
20-	32	68.1	10	21.3	5	10.6	
25-	15	21.7	42	60.9	12	17.4	
30-	3	4.9	40	65.6	18	29.5	
35-	2	8.7	3	13.0	18	78.3	
<b>Level of education</b>							
Illiterate	0	0.0	12	30.8	27	69.2	<b>0.001*</b>
Read & write	3	10.3	16	55.2	10	34.5	
Primary/preparatory education	19	48.7	18	46.2	2	5.1	
secondary education	22	33.3	33	50.0	11	16.7	
University or more	8	29.6	16	59.3	3	11.1	
<b>Occupation</b>							
Housewife	22	13.2	92	55.1	53	31.7	<b>0.0031*</b>
Working	30	90.9	3	9.1	0	0.0	
<b>Number of family members</b>							
<3	25	34.7	22	30.6	25	34.7	<b>0.015*</b>
4-	10	11.5	52	59.8	25	28.7	
6-	11	45.8	11	45.8	2	8.3	
8+	6	35.3	10	58.8	1	5.9	

**Table VII: Relation between study subjects' level of awareness and their clinical characteristics.**

Clinical characteristics	Good		Faire		Poor		P
	No.	%	No	%	No	%	
<b>Gravidity</b>							
Once	8	61.5	2	15.4	3	23.1	0.014*
Twice	5	6.6	60	78.9	11	14.5	
3 times	28	40.6	21	30.4	20	29.0	
More than three times	11	26.2	12	28.6	19	45.2	
<b>Parity</b>							
None	5	41.7	2	16.7	5	41.7	0.028*
Once	16	17.8	60	66.7	14	15.6	
Twice	10	16.9	25	42.4	24	40.7	
3 times	12	57.1	4	19.0	5	23.8	
More than three times	9	50.0	4	22.2	5	27.8	
<b>Place of previous delivery</b>							
Home	1	9.1	3	27.3	7	63.6	0.001*
Governmental hospital	12	26.1	14	30.4	20	43.5	
Private hospital / Private doctor's clinic	18	23.7	38	50.0	20	26.3	
Health centers / Maternal and child health care center	20	30.8	40	61.5	5	7.7	
<b>Presence of complications</b>							
Yes	16	17.2	30	32.3	47	50.5	0.0031*
No	36	33.6	65	60.7	6	5.6	
<b>Attendance of antenatal care</b>							
Yes	49	27.2	92	51.1	39	21.7	0.001*
No	3	15.0	3	15.0	14	70.0	

## Discussion

The current challenge worldwide is to decrease maternal mortality rate. Reproductive health is closely related to maternal mortality. McCarthy & Main (1992) mentioned that health status and reproduction status of women belong to intermediate variables determine maternal mortality. Health status of women concerning nutritional status; history of pregnancy morbidity such as infectious or chronic diseases; it is clear that women's health status plays a significant role in their reproductive function. Reproductive status consists of maternal age, parity and marital status which is directly related to maternal mortality.

The vast majority of maternal deaths could be prevented if women know when and where to seek medical care because if the pregnant women do not have appropriate information about pregnancy and childbirth they would be unable to make a choice that will contribute to their own well-being, the question is are women aware of the warning signs of obstetric complications?

The present study aimed to answer the research question whether women in a certain rural area in Egypt are aware of obstetric danger signs? This is important because in rural areas in Egypt it is generally believed that pregnancy is a natural phenomenon and a part of women's reproductive functions, and problems or complications during pregnancy are being viewed by some women as natural to pregnancy. Such beliefs contribute to lack of utilization of medical services which in turn leads to undetected and untreated complications consequently increased maternal mortality rate.

In the current study only about one-quarter of the study subjects exhibited good awareness about obstetric danger signs. This awareness indicates the existence of knowledge which can be in turn transferred into action. The importance of knowledge to shape health seeking behaviors which contribute to save women's live from preventable causes of maternal deaths is stressed by many authors (Myer &

Hariisson 2003; Smith et al 2004; Stekelenburg et al 2004; Sugiarto 2007)

Unfortunately, almost the same proportion of the study subjects was unaware of obstetric danger signs which reflect lack of knowledge regarding such signs even though such knowledge is very important. This could be explained by poor counseling of pregnancy danger signs among those who attended antenatal clinics as the majority of the study subjects attended antenatal clinics, among them about two-thirds had three visits. This emphasizes the needs to ensure that nurse/midwives inform all antenatal clients about obstetric danger signs

Women's awareness of potential obstetric danger signs is expected to influence their decisions regarding when to decide to seek medical care. Accordingly, awareness of obstetric danger signs is expected to help women to early recognize signs when the complications occur and limit the time to make a faster decision to seek medical care which makes a difference between life and death if all such signs are well known. . This makes it very important to women to be aware of all obstetric danger signs. However, the results of the present study revealed that nearly one half of the study subjects had fair awareness of obstetric danger signs; it means they were able to identify 50-75% of such signs. This could be explained by the fact that 26.4 % of Egyptian women does not receive antenatal care, among them 34.1 % told about signs obstetric complications and 31.4 % told where to go if had any of complications (EDHS 2008). Therefore, this study reflects the needs for strategic plan to increase the awareness to shape health seeking behaviors of the public related to obstetric complications.

The present study revealed that age, education, occupation, gravidity, parity, presence of complication and attendance of antenatal care are associated with awareness of women about obstetric complications. Increased awareness among older and multigravida and multiparous women may be related to their own experiences of pregnancy and delivery which is an important source of their information specially those who had complications associated with their pregnancy. This is in line with Pembe et al (2009) who stated that young women in their first pregnancy may need more consideration when providing counseling and health education.

In the current study education seems to play a positive role in increasing the awareness of women about signs of complications. This is in agreement with Anya et al (2008) who stated that, educated women have better pregnancy outcome compared with uneducated women; this may be partly because they are better informed and make better choices. Moreover, occupation seems to influence the level of

women awareness about signs of obstetric complications. This could be explained by the fact that, working women have better opportunity to share experiences with others than housewives. Furthermore, in rural areas sources of information are limited unlike urban areas in addition to the prevalence of illiteracy which may contribute to this result.

The findings of the present study revealed that attendance of antenatal had positive effects on increasing the awareness of women about the signs of obstetric danger signs. This is online with Anya (2008) who stated that high antenatal coverage and relatively high frequency of visits provides an excellent opportunity for information, education and communication. However, this finding is incongruent with Pembe et al (2010) who reported deficiencies in the counseling of pregnancy danger signs in his study and a significant proportion of the clients were not informed about pregnancy danger signs. Similar results are documented by NBS (2004/5), Nikiema et al (2009), von Both et al (2006) and Boller et al (2003)

#### **Limitation of the study:**

Women were not asked about the source of their information and to whom they should report if they experience any complication in addition to the immediate intervention they should receive.

#### **Conclusion**

Based on the findings of the present study, it can be concluded that lack of awareness about obstetric danger signs was related younger age, low level of education, gravidity and parity, previous experiences with any obstetric complications and lack of antenatal care. These factors stressed the need for a plan to increase the awareness of the public about such signs. This information will help the services providers for improving the quality of antenatal care services.

From this study the need for strategic plan to increase the awareness of women about signs of obstetric complications is highly recommended.

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9/28/2010

## The Use of lemongrass extracts as Antimicrobial and food additive potential in yoghurt

Abd-El fattah<sup>1</sup>; Abo sree, Yahia Hassan<sup>1</sup>; Hala M. Bayoum<sup>2</sup> and Hesham A. Eissa<sup>3</sup>

Food Toxins and contaminants Department<sup>1</sup>, Dairy Department<sup>2</sup>, Food Technology Department<sup>3</sup>, National Research Centre, Cairo, Egypt.  
[shaabanmostafa@yahoo.com](mailto:shaabanmostafa@yahoo.com)

**ABSTRACT:** The following study was conducted to investigate the antifungal and food additive potential of medicinal plants. herbal decoction and essential oil (EO) extracts of *Cymbopogon flexuosus* (lemongrass) leaves and stems were tested for their inhibitory action against spoilage organisms and mycotoxins formation in two separated experiments. In the first experiment, yeast- extract sucrose medium (YES) was used as a basal medium to examine the mold growth and mycotoxin production by three pathogenic fungi: *Aspergillus flavus* (*A. flavus*), *Aspergillus parasiticus* (*A. parasiticus*) and *Aspergillus ochraceus* (*A. ochraceus*). The YES medium was supplemented with four different concentrations of Lemongrass oil, inoculated with 1-mL of a spore suspension containing  $10^5$ - $10^6$  conidia of each test mold and then incubated at 28° C for 14 days. After incubation period, cultures were analyzed for mycelial dry weight and mycotoxin accumulation. In the second experiment, yoghurt medium was used as a basal medium and the same system of study was applied in two different degrees of temperature (5°C and 28°C) for 4 weeks. Evaluation of the Lemongrass oil activity in yoghurt samples focused on the microbial stability of yoghurt, sensory evaluation as well as mold growth and mycotoxin formation. In the 1<sup>st</sup> experiment, the level of 0.1% of the EO extract was effective in inhibition both mold growth and mycotoxin production for all tested molds, and 0.3 % extract completely prevented the growth and toxin production. whereas, 1% of the decoction extract was effective. So, the EO extract was the suitable agent in the second experiment. It is of interest to note that while reduction in mold growth due to increasing extract concentrations was observed, the most striking effect was the reduction of mycotoxin production. The obtained data from the second experiment showed that the EO extract (0.1% concentration) inhibited viable yeasts and preserved yoghurt for over 28 days at 5°C. Also, the inhibitory action of the EO extract against yeasts was concentration dependent. The maximum inhibitory effect of was found when the extract level increased above 0.1%. Incubation temperature had an important role in growth and mycotoxin production in yoghurt medium. During cold storage for 28 days at 5°C, the different concentrations of the EO extract added to the yoghurt samples displayed different titratable acidity and total bacterial cells and pH than the control yoghurt ( $p < 0.05$ ). Overall sensory acceptability of yoghurt supplemented with the EO extract was higher than that of the control yoghurt prepared without the EO extract. Total sensory evaluation of experimental yoghurt used as a control was up to 4.3 scores lower compared to yoghurt samples treated with the EO extract. The results indicate that the addition of the appropriate the EO concentrations (0.1%, w/v) improved the physicochemical properties as well as sensory characteristics of yoghurt, could be used for decontamination of dairy products such as yoghurt from mycotoxicogenic fungi and mycotoxins formation, beside its beneficial properties as a functional food.

[Abd-El fattah; Abo sree, Yahia Hassan; Hala M. Bayoum and Hesham A. Eissa. The Use of lemongrass extracts as Antimicrobial and food additive potential in yoghurt. Journal of American Science 2010;6(11):810-822]. (ISSN: 1545-1003).

**Key words:** Yoghurt, lemongrass, molds, yeasts, mycotoxins, aflatoxins, ochratoxin A, food additives.

### INTRODUCTION

Yoghurt in addition to its high nutritional value, it possesses antagonistic and therapeutic values. The valuable sensory characteristics of yoghurt are due to its content of carbonyls, mainly acetaldehyde, acetone, diacetyl and ethanol, produced by yoghurt bacteria. Yoghurt provides higher levels of protein, carbohydrate, calcium and certain B vitamins than milk (Gurr, 1987; Deeth and Tamime, 1981). The shelf life of yoghurt is short, i.e., 1 day under ambient condition (25–30°C) and

around 5 days at 7°C, which hinders its commercialization (Salji, 1987). Yoghurt defects due to microbial contamination are widely reported in literature; the most frequent contaminants are yeasts and moulds (Spolaor *et al*, 1988; Foschino and Ottogalli, 1988; and Abdel-Fattah and Abdel-Salam 2004) usually causing the swelling of packs, the presence of superficial coloured spots and abnormal tastes (Ottogalli, 1991). The low pH of yoghurt offers a selective environment for the growth of acid tolerant yeasts and molds (Banaquio *et al*, 1981, Spillmann and Geiges, 1983). Therefore, it is

not surprising that various investigators have found that yeasts are the primary spoilage microorganisms for yoghurt and that fruits, flavors, and coloring agents are frequent contamination sources (**Main, 1984; Weber and Broich, 1986**). The spoilage of yoghurt by yeasts has been generally characterized by yeasty offflavors, loss of textural quality due to gas production, and swelling and occasional rupturing of the product containers (**Davis, 1974**). As a result, there is an apparent need for an effective preservation method to control acid-tolerant spoilage yeasts and molds in yoghurt.

Micotoxicogenic Fungi and Pathogenic bacteria can grow at refrigeration temperature to numbers, which can result in an infection. For this reason dairy products should be kept well covered to prevent contamination, should ideally be consumed within two days of opening, or used in cooked foods after that two-day period (**Potter and Hotchkiss, 1995**). Mycotoxins may be found in milk products, originating from three possible sources: raw milk (such as aflatoxin M1 which present as a consequence of aflatoxin B1 metabolism by the animal); growth of a toxicogenic fungal strain on product and mycotoxins synthesis, and production of these toxins in dried milk used to make milk product (**Jose et al., 1988**).

Hence, it is highly desirable to prevent mould growth or to prevent mycotoxins formation in contaminated food. Several chemicals have been used to detoxify mycotoxins but these chemicals can not be added to foods to prevent mycotoxins formation because of their hazardous effect on human health. In recent years, studies on the natural antifungal agents, herbs and spices, have been reported by numerous investigators (**Afrodit, 1995; Hiroshi and Jun Sato, 2002; and Abd-EL Fattah and Abdel-Salam, 2004**). They found that some herbs and spices had antifungal effect against some kinds of mycotoxicogenic fungi, such as *A. flavus*, *A. ochraceous* and *A. parasiticus*, in synthetic medium. Among herbs and spices used, were sage, thyme, rosemary, mint, and Lemongrass.

*Cymbopogon flexuosus* (Lemongrass) is an economically important plant that has been used for centuries, as a medicine because of its wide-ranging therapeutic properties included relief of rheumatic and other pain and healing effect on ulcers (**Fenwick et al., 1990**). Flavonoids extracted from Lemongrass are of considerable interest as natural plant components with antioxidant and antifungal activity (**Pratt and Hudson, 1990; Nieto et al., 1993; and Abu-Seif, et al., 2009**). Of the flavonoids present in Lemongrass, licochalcone A and licochalcone B which have equal antioxidant activity of vitamin E, and

glabrene which is 3 times as active when compared with vitamin E (**Okuda et al., 1989**).

One objective of the present study was to investigate the inhibitory action of Lemongrass oil against spoilage organisms and mycotoxins formation in yoghurt under laboratory conditions. The use of Lemongrass herb in this study was due that Lemongrass is naturally occurring material, widely cultivated, cheap, had a medical functions and safe. These properties and the antifungal activity, if possible, make lemongrass oil may be potential multi-functional food additives. The physico-chemical properties, color characteristics, total phenol content, microorganisms, sensory evaluation and the effect of storage time at 5°C for 2 months of yoghurt were also studied.

## 2- MATERIALS AND METHODS

### 2.1- Experimental design.

Depending on our previous results (**Abdel-Fattah, 2002; Abdel-Fattah and Abdel-Salam, 2004 and Abu-Seif, et al., 2009**), concerning the antimicrobial effects of herbal extracts, this study was achieved. Two separated experiments were carried out during this study. The first experiment was to examine the best of the tow different extracts of Lemongrass, essential oil (EO) extract and decoction extract as antifungal agents. In the second experiment, the best extract selected from the first study was used on yoghurt medium, to test its antifungal effects and to study the physico-chemical properties, color characteristics, total phenol content, as well as microbial stability and sensory evaluation of yoghurt during storage time at 5°C for 4 weeks.

### 2.2- Organisms.

**a- Fungal strains:** *Aspergillus flavus* (A. *flavus*), aflatoxigenic local strain; *Aspergillus parasiticus* (A.*parasiticus*) NRRL 2999 and *Aspergillus ochraceus* (A.*ochraceus*) NRRL 3174, were obtained as lyophilized preparation from the Mycotoxin lab. , National Research Center, Dokki, Giza , Egypt.

**b- Bacterial strains:** Starter culture of Yoghurt *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (1:1) was obtained from HACCP certified and ISO22000: 2005 Dairy Company, and used as a source of the starter culture.

### 2.3- Milk used for making of lab. Yoghurt:

Raw buffalo's milk used for making yoghurt, the milk was obtained from a dairy farm at Agriculture

faculty, Cairo University Governorate. Starter cultures used for making plain yoghurt Old plain yoghurt obtained from HACCP certified & ISO22000: 2005 Dairy Company was used as a source of the starter culture.

#### **2.4- Plant material:**

Lemongrass powder (leaves and stems) was purchased from an Egyptian local market (Harraz Co., Cairo, Egypt).

#### **2.5- Mycotoxins standards:**

All standards of Mycotoxins (Aflatoxins, B1, B1, G1, G2, and ochratoxin A) were purchased from sigma company, USA.

#### **2.6- Analytical methods:**

##### ***2.6.1- Extract preparations of Plant material:***

Lemongrass powder was purchased from a local market and the samples were extracted as follow:

**a- Preparation of herbal decoction:** Decoctions of lemongrass leaves were prepared by boiling the powder material at solid : liquid ratio 1:10 with distilled water for 5 minutes. The vessel containing the decoction and herb was then covered and removed from the heat and allowed to cool for 5 minutes. The herbal material and liquid was then strained through cheese cloth and the resulting decoction placed into 100 mL reagent bottles which had been kept for use as the test decoction (**Abdel-Fattah and Abdel-Salam, 2004**).

**b- Preparation of herbal Oil:** Briefly, 250 g fresh plant material (leaves and stems) of lemongrass plant was put in a round bottom flask and 1000 mL distilled water was added before subjecting to hydro-distillation (**Bankole and Joda, 2004**) for 6 hours. The oil was recovered and dried over anhydrous sodium sulphate.

##### ***2.6.2- Spore suspension of fungal strains:***

These culture strains were grown on (PDA) slants for 10 days at 25 °C Until will sporulated . the spores were washed from the slants with a sterile 0.01% solution of tween 80 as a spore dispersal agent. The final spore preparations were resuspended in the appropriate volume of sterile saline to yield a direct microscopic count of approximately  $10^5$ - $10^6$  spores /mL, of each tested fungus.

##### ***2.6.3- Preparation of yoghurt containing Lemongrass oil:***

Raw buffalo's milk was subjected to a heat treatment at 72°C for 2 seconds to kill microorganisms followed by cooling to 40 – 45°C.

Oil extract of Lemongrass was added to milk before processing with different concentrations. As starter culture yoghurt (*L.bulgaricus* and *S. thermophilus*) was added (1.5%) to the milk, followed by mixing, and packed in sterilized glass capped cups 100 mL capacity, followed by incubation at 40°C for 3 hours till gel forms (pH 4.5). Freshly yoghurt was cooled and stored at refrigeration at 5°C till examination to slow down the physical, chemical and microbiological analysis.

#### ***2.6.4- Antifungal assay:***

**2.6.4.1- With yeast extract sucrose medium (YES):** Yeast extract- sucrose (YES) broth (2% yeast extract, 15% sucrose) was used in the 1st experiment as a basal medium for mould growth and mycotoxin production in stationary cultures (**Davis et al., 1966**). Each medium (50 mL) in 250 mL Erlenmeyer flasks was sterilized at 121°C for 15 min. For each organism used, the appropriate amounts of lemongrass essential oil and lemongrass decoctions were added into sterile YES to obtain the concentrations of 0.05, 0.1, 0.3, 0.5 and 1.0 %. YES without any herbal extract added served as control. Each flask with or without appropriate extract was inoculated with 0.1 mL spore suspension, then cultures were incubated at 28°C in the dark for 14 days. Each concentration of tested extracts was tested twice, sometimes three times.

**2.6.4.2- With yoghurt medium:** The same antifungal assay on YES, was applied on yoghurt medium except that extract used was the EO extract, added to milk before processing and the culture media were incubated at two different temperature, 5 and 28 °C for 35 days. Yoghurt medium were inoculated with mycotoxicogenic fungi and stored for 35 days at 5 and 28 °C. Semi quantitative assay of the tested oil extract was conducted according to **Harboure (1973)**.

#### ***2.6.4.3- Evaluation of antifungal properties of lemongrass extracts.***

**a- With YES broth media:** Contents of flasks, with and without (serve as the control) lemongrass extracts, were analyzed in triplicates for dry weight of mycelium and mycotoxin accumulation. Dry weight of mycelium was determined according the method of **Davis et al., (1966)**. Aflatoxins were extracted according to the modified procedure of **Eleghede, (1978)**. After extraction, aflatoxins (B<sub>1</sub>+G<sub>1</sub>) were determined according to the **AOAC methods, (1980)**. Ochratoxin A (OA) was extracted and determined

according to the method of Scott *et al.*, (1971). Percent reduction or accumulation over control, in growth or mycotoxin production was calculated by the following equation:

$$\text{Percent reduction} = 100 - \{(A_1 / A_0) * 100\}$$

$$\text{Accumulation over control} = \{(A_1 / A_0) * 100\} - 100$$

Were:  $A_1$  = The amount obtained by treatment.

$A_0$  = The amount obtained by control.

**b- With yoghurt medium:** Fungal growth of all tested molds were visually assessed using a semi-quantitative scale, Viz.(0) no growth; (1) very little growth covered the surface of the plate; (2) 25 % of the plate surface covered; (3) 50% of the plate surface covered; (4) 75 % of the plate surface covered and (5) 100% of the plate surface covered. Yoghurt medium were examined for the presence of aflatoxin production as described by the **AOAC methods, (1980)**. For ochratoxin A, cultures were extracted, and OA levels were determined as described by **Valenta and Michael, (1996)**.

#### **2.6.5- Microbiological stability of yoghurt:**

The microbial stability of yoghurt containing the EO extract of lemongrass during storage at refrigerator ( $5^{\circ}\text{C}$ ) for 28 days were investigated. The populations of total bacteria, yeast and molds were determined by the method of **Sadler, et al., (1992)**. The counts of total bacteria (TPC), yeast and molds (Y&M) calculated per one gram of all yoghurt slices using plate count agar and malt extract agar (Merck KGaA, Darmstadt, Germany). The number of colonies (TPC or Y&M) that appeared on the plates was counted and expressed as Colony Forming Unit (CFU/g).

#### **2.6.6- Determination of pH:**

The pH of fruit sample was measured using a combination pH electrode with a digital pH meter (HANNA, HI 902 meter, Germany) standardized with stirring as described in (AOAC, 2000).

#### **2.6.7- Determination of total soluble solids (TSS):**

The percent total soluble solids, expressed as oBrix, were determined with a refractometer (ATAGO, Japan).

#### **2.6.8- Determination of Titratable acidity (TA):**

Titratable acidity were determined as described by (Tung Sung Chung, et al., 1995) by using approximately 10 g portion of yoghurt sample

blended with 100 mL distilled water for 30 sec in blender and was titrated to pH 8.0 with a 0.1N NaOH solution. The end point was determined with a pH-meter. Titratable acids in the sample were calculated as percent of lactic acid or malic acid.

### **2.6.9- Viscosity measurements:**

The viscosity measurements were carried out using a HAAKE viscometers (HAAKE, Mess-Technik Gmbhu. Co., Germany) with thermostatic bath to control the working temperature within the temperature of 25°C. Results of viscosity were expressed in centipoise (cP) according to the method of Ibarz *et al.*, (1994).

#### **2.6.10- Total phenol determination:**

Total phenol content of the untreated and treated samples was measured by the method of **Amerine and Ough (1980)**, the absorbance was measured at 765nm using Spectrophotometer, UVD-3500, Labomed, USA and the results were expressed as milligram of garlic acid as standard equivalent per gram.

#### **2.6.11- Color characteristics determinations:**

Color is one of the more important quality parameters in processed products. Undoubtedly, possible color changes would influence the Organolytic properties of samples and would limit their potential applications.

Hunter  $a^*$ ,  $b^*$  and  $L^*$  parameters were measured with a color difference meter using a spectrophotometer (Tristimulus Color Machine) with the CIE lab color scale (Hunter, Lab Scan XE - Reston VA, USA) in the reflection mode. The instrument was standardized each time with white tile of Hunter Lab Color Standard (LX No.16379):  $X=72.26$ ,  $Y=81.94$  and  $Z=88.14$  ( $L^*=92.46$ ;  $a^*=-0.86$ ;  $b^*=-0.16$ ) (**Sapers and Douglas, 1987**). Color difference, Delta E, was calculated from  $a^*$ ,  $b^*$  and  $L^*$  parameters, using Hunter-Scotfield's equation (**Hunter, 1975**) as follows.

Delta E = (delta a<sup>2</sup> + delta b<sup>2</sup> + delta L<sup>2</sup>) 1/2 ----- (1)  
 where: a - a<sub>o</sub>, b - b<sub>o</sub> and L - L<sub>o</sub>; subscript "o" indicates color of control or untreated sample.

The Hue-Angle ( $H$ )\*, Chroma ( $C$ )\* and Browning Index ( $B_I$ ) was calculated according to the method of **Palou et al.**, (1999) as follows:

$$C^* = \text{square root of } [a^{2*} + b^{2*}] \quad (3)$$

Where:  $X = (a^* + 1.75L^*) / (5.645L^* + a^* - 3.012b^*)$

#### **2.6.12- Sensory Evaluation:**

Sensory evaluation of the studied was carried out included 20 experienced panelists. The attributes such as: flavour intensity, body, texture and color were organolaptically assessed at stated by (Crandall, et al., 1990). The all tested samples subjected to sensory evaluation after 28 days in yoghurt samples.

#### **2.7- Statistical Analysis:**

Analyses for experiments were performed in duplicated, and results were averaged. A Duncan Multiple Range Test was carried out by means of the “shortest significant ranges SSR” (Larmond, 1974) to determine the differences between the treatments using HDSS statistical analysis program.

### **3- RESULTS AND DISCUSSION**

#### **3.1- Antifungal effect of the two different extracts of lemongrass on YES borth medium:**

Data presented in Table (1) clearly indicate that mould growth by all tested strains, were suppressed by lemongrass oil extract or decoctionextract. The inhibitory effect of these extracts was proportional with their concentrations. Slightly effect on fungal growth was observed when low concentration (0.05% and 0.1%, respectively) of the EO extract and decoction extract were applied, whereas high concentrations of these extracts inhibited fungal growth and, consequently, mycotoxin formation. The maximum inhibitory effect of these extracts were recorded at the level 1 % and 0.3% for decoction extract and EO extract, respectively (Table, 1).

In regard to Table (2), *A. ochraceus* was more sensitive one for the two lemongrass extracts than the two other molds. The EO extract was more effective agent on mycelial growth than decoction extract. The inhibitory effect of the two different extracts on mycelial growth according to the mold type, may be rankled as follow: *A. ochraceus* > *A. flavus* > *A. parasiticus*, for the EO extract. However, for decoction extract were: *A. ochraceus* > *A. parasiticus* > *A. flavus*. These differences in the inhibitory effect may be mainly due to interfering some factors: the mold type, incubation temperature and type of extract and subsequently the differences in the chemical composition for each extract. In this respect, many publications indicated that the compositions and concentrations of compounds within the distinct types of herbal extract

preparations would differ and play an important role in its antifungal activity action (Buchanan and Shepard, 1981; Lienert et al., and Nass-Reinhold et al., 1998; and Abdel-Fattah and Abdel-Salsm, 2004). Also, El GendY and Marth, 1980, reported that temperature is one factor affects mold growth and mycotoxin production by toxigenic aspergilli and penecillus and in the presence of lactic acid bacteria. Abdel-Fattah, (2002), found that mold type and incubation temperature were important factors affecting mold growth and aflatoxin production by *A. flavus* and *A. parasiticus* in media contained solvent extracts of licorice.

Data represented in Table (2) clearly indicate that increasing levels of the extract, in YES broth media, resulted in detection of decreasing levels of mycotoxin production. At the lowest level (0.05% extract), reduction in mycotoxin production was slightly decreased in the media supplemented with decoction extract compared to those supplemented with oil extract. Increasing extract concentrations caused a linear depression in mycotoxin formation by the all tested molds, but the maximum inhibitory effect was differ, and this may be referred that organism was more variable in its reaction to the extract. This trend indicates that extract inhibited mycotoxin formation by inhibiting the mould growth. In a similar study, Masood and Ranjan (1991) reported that extracts of *Argemone mexicana* and *cyperus rotundus* inhibited aflatoxin production by inhibiting the growth of *A. flavus*. Also, Mahmoud et al., (1994), found that extracts of lupinus and xanthium punens inhibited aflatoxin production by inhibiting the growth of *A. flavus*.

Reduction of fungal growth and mycotoxin production by the EO extract in our study was due to interference by active principles of these extracts. Such interference may be at the biosynthetic levels. In this respect, Kumar and Prasad (1992) suggested that growth and aflatoxin production by *A. flavus* are proportionate processes. However, Bhatnagar and McCromick (1987) reported that the growth and aflatoxin production by *A. parasiticus* are independent phenomena. On the other hand, Abu-Seif, et al., (2009) reported that oil extract of lemongrass leaves and stems, completely inhibited mycelial growth and mycotoxin production of *A. flavus*, *A. parasiticus* and *A. ochraceus* at level (0.3 %) of YES broth medium.

Data represented in Table (1) showed that there were a considerable differences in mold or mycotoxin inhibition in YES medium supplemented with the EO extract or the decoction extract, in trend to EO extract. Therefore, the EO extract of Lemongrass leaves and stems was selected, as the best, to examine its antimicrobial effect on yoghurt medium.

**Table (1): Mold growth (mg/50 mL media) and percent reduction of the tested molds as affected by the two different extracts of lemongrass on YES broth media for 14 days at 28° C.**

Extract level, %	Mold growth and percent reduction for different toxigenic strains					
	<i>A. flavus</i>		<i>A. parasiticus</i>		<i>A. ochraceus</i>	
	Mould growth	percent reduction	Mould growth	percent reduction	Mould growth	percent reduction
<b>Control, 0.0%</b>	410±13.4 <sup>F</sup>	0.0	305±14.8 <sup>F</sup>	0.0	840±10.5 <sup>F</sup>	0.0
<b>Decoction extract</b>						
0.05						
0.1	325±16.5 <sup>E</sup>	20.73	251.5±19.5 <sup>E</sup>	17.54	599.6±16.3 <sup>E</sup>	28.62
0.3	304±8.3 <sup>E</sup>	25.85	251.5±8.3 <sup>E</sup>	17.54	538±22.4 <sup>D</sup>	35.95
0.5	259±13.0 <sup>D</sup>	36.83	217.5±14.7 <sup>D</sup>	28.70	315±16.5 <sup>C</sup>	62.50
1.0	192±21.5 <sup>C</sup>	53.17	176.5±9.5 <sup>C</sup>	42.13	110±8.7 <sup>B</sup>	86.90
<b>EO extract</b>						
0.05	175±13.5 <sup>C</sup>	57.32	170.0 ±7.3 <sup>C</sup>	44.26	80±7.2 <sup>B</sup>	90.48
0.1	180±11.6 <sup>C</sup>	56.10	65±0.9 <sup>B</sup>	78.69	113±11.4 <sup>B</sup>	86.55
0.3	112±5.8 <sup>B</sup>	72.68	0.0 <sup>A</sup>	100	0.0 <sup>A</sup>	100
0.5	0.0 <sup>A</sup>	100	0.0 <sup>A</sup>	100	0.0 <sup>A</sup>	100
1.0	0.0 <sup>A</sup>	100	0.0 <sup>A</sup>	100	0.0 <sup>A</sup>	100
<b>LSD at (p ≤ 0.05)</b>	28.8	-	21.2	-	36.5	-

Each value represents the mean ± SE of three replicates.

**Table (2): Mycotoxin production and percent reduction of the tested molds as affected by the two different extracts of lemongrass on YES broth media for 10 days at 28° C.**

Extract level, %	Mycotoxin production (µg per 50 mL YES) and percent reduction for different toxigenic strains.					
	<i>A. flavus</i>		<i>A. parasiticus</i>		<i>A. ochraceus</i>	
	Mycotoxin production	percent reduction	Mycotoxin production	percent reduction	Mycotoxin production	percent reduction
<b>Control, 0.0%</b>	265	0.0	345	0.0	270	0.0
<b>Decoction extract</b>						
0.05	180	32.07	295	14.49	150	44.44
0.1	135	49.06	210	39.13	103	61.85
0.3	55	79.24	73	78.84	45	83.33
0.5	56	79.24	31	91.01	0.0	100
1.0	0.0	100	0.0	100	0.0	100
<b>EO extract</b>						
0.05	125	52.83	113	67.25	28	89.63
0.1	105	60.38	93	73.04	0.0	100
0.3	0.0	100	0.0	100	0.0	100
0.5	0.0	100	0.0	100	0.0	100
1.0	0.0	100	0.0	100	0.0	100

Each value represents the mean of three replicates.

### 3.2- Antifungal effect of lemongrass oil on yoghurt medium:

Results obtained from Table (3) showed that increasing levels of EO extract added to yoghurt

either incubated at 5 or 28 °C for 28 days, an inhibitory effect was noted on the growth of the all tested molds. At the lowest concentrations of extract (0.05%), the mold growth by the tested molds, were comparatively no changed over control. However,

increasing concentration level up to 0.1% completely prevented the mold growth, either when the media incubated at 5 or 28 °C. These results also indicate that growth was influenced by both mold type and incubation temperature. Also, these results revealed that 28 °C was the optimum incubation temperature for growth and consequently, mycotoxin formation occurred in this study. The mold growth was higher by *A. flavus* and *A. ochraceus* than *A. parasiticus*. Also, growth was influenced by mold type and temperature degree of incubation. Temperature is one factor which affects mold growth and mycotoxin production. Other publications supported our results (**EL-GENDY and MARTH, 1980; MASHALY and EL-DEEB, 1982, and ABDEL-FATTAH, 2002, Abdel-Fattah and Abdel-Salam, 2004**).

When mycotoxin production was determined in the media incubated at 5 and 28°C, the effect of EO extract was even more striking (Tables 4 and 5). Increasing concentration of extract resulted in detection of decreasing levels of mycotoxin production. At the lowest level of extract (0.05%), a great reduction mycotoxin production were found, and the reduction was especially pronounced with aflatoxins and ochratoxin A. No toxin was detected when the extract level increased to 0.1%.

Regarding results represented in Tables 3, 4 and 5, The lack of mycotoxin production on yoghurt raises questions concerning the reasons for this phenomenon. The possibility that ingredients contained in yoghurt, but not in YES, might be inhibitory to growth and toxin production was considered.

It is possible that antifungal activity of the used lemongrass essential oil in this study is due to an unidentified component of the antioxidants extracted (perhaps phenols, flavonoids, flavones,..etc) . In this respect, **Rosenthal et al., 1997**, reported that phenols play an important role as antifungal agents. They found that ferulic acid, p-coumaric acid and other plant cell wall phenols, had antifungal actions in microorganisms isolated from dairy products. Also, **Abu-seif et al., 2009**, found that phenolic compounds extracted from Lemongrass leaves had antifungal effects on *A. flavus* and *A. parasiticus*. **Hiroshi and Sato (2002)**, reported that lemongrass extract with 80% methanol was found to have high fungicidal effect against *Arthrinium sacchari* M001, and its active compound was identified as glabridin. Strong antioxidant activity has been observed in flavonols such as quercetin, flvonones such as luteolin, and chalcones such as butin (**Hudson and Lwiss, 1983**).

**Table (3): Effect of various concentrations lemongrass oil extract on molds incubated for 28 days at 5 and 28° C.**

Extract ,%	<i>A. flavus</i>		<i>A. parasiticus</i>		<i>A. ochraceus</i>	
	5 °C	28 °C	5 °C	28 °C	5 °C	28 °C
Control, 0.0%	3	5	3	5	2	4
EO extract						
0.05	2	4	3	4	1	3
0.1	0	0	0	0	0	0
0.3	0	0	0	0	0	0
0.5	0	0	0	0	0	0
1.0	0	0	0	0	0	0

(0) no growth; (1) very little growth ; (2) 25 % of the plate surface covered with mycelia ; (3) 50% of the plate surface covered with mycelia; (4) 75 % of the plate surface covered with mycelia and (5) 100% of the plate surface covered with mycelia.

**Table (4): Effect of various concentrations lemongrass oil extract on mycotoxin production in yoghurt medium for 28 days at 5 and 28°C.**

Extract level	Mycotoxin production (µg per 50 mL YES)						
	At 5°C			At 28°C			
	Total Aflatoxins		Ochratxin A	Total Aflatoxins		Ochratxin A	
	<i>A. Flavus</i>	<i>A. parasiticus</i>		<i>A. Flavus</i>	<i>A. parasiticus</i>		
Control, 0.0%							
EO extract	35.3	84.0	116.0	108.6	142.5	185.0	
0.05	0.0	0.0	0.0	65	56.5	0.0	
0.1	0.0	0.0	0.0	43	40.15	0.0	
0.3	0.0	0.0	0.0	0.0	0.0	0.0	
0.5	0.0	0.0	0.0	0.0	0.0	0.0	
1.0%	0.0	0.0	0.0	0.0	0.0	0.0	
	0.0	0.0	0.0	0.0	0.0	0.0	

- Each value represents the mean of three replicates.

- ND: No toxin detected.

**Table (5): percent reduction in mycotoxin production over control by mold with oil extract of lemongrass in yoghurt media for 35 days at 5 and 28° C.**

Extract level	Percent reduction					
	At 5°C			At 28°C		
	Total Aflatoxins		Ochratoxin A	Total Aflatoxins		Ochratoxin A
	A. Flavus	A. parasiticus		A. Flavus	A. parasiticus	
<b>Control, 0.0%</b>	0.0	0.0	0.0	0.0	0.0	0.0
<b>Oil extract</b>						
0.05	100	100	100	40.14	60.35	100
0.1	100	100	100	60.40	71.82	100
0.3	100	100	100	100	100	100
0.5	100	100	100	100	100	100
1.0%	100	100	100	100	100	100

Each value represents the mean of three replicates.

**3.2- The effect of essential oil of lemongrass on the log (CFU/gm) of growth yeast and molds (Y&M) and bacteria in yoghurt during storage at refrigerator for 4 weeks.**

The microbial stability of yoghurt supplemented with extracts of lemongrass during storage at refrigerator (5°C) for 4 weeks were investigated. Tropical spices (lemongrass) may prove useful in preservation of yoghurt by hurdle technology (**Ejichi, et al., 1998**). Total microbial count of different yoghurt treatments with 0.0, 0.05, 0.1, and 0.3 % of lemongrass EO extract, and of untreated yoghurt were followed up through 4 weeks at 5°C. The effect of treating yoghurts with the studied various volatile or essential oil extract and stored at 5°C for 4 weeks on inhibiting the microbial counts are seen in Table (6). It can be observed that the yoghurt treated with low concentration of lemongrass extract have the highest inhibition of yeast and molds (Y&M) and bacteria (B) followed by those treated with high concentration of lemongrass extract after 4 weeks at 5°C. Untreated yoghurt was 6.75 log (CFU/gm) of B compared to 0.00 log (CFU/gm) in case of those Y&M. Whereas, the yoghurt treated with 0.5% lemongrass was 0.00 log (CFU/gm) of Y&M and 6.10 log (CFU/gm) of B.

The results from Table 6 showed that the yoghurt treated with high concentration of lemongrass has also the highest reduction of Y&M and B followed by low concentration of lemongrass, but untreated samples were the lowest reduction of Y&M and B for 4 weeks stored at 5°C.

These results are partially confirmed by those of **Kanako et al., (1998)**. They found that Limon grass and clove exhibiting strong anti-fungal activity for 30 days. Whereas no colonies were seen for 30 days and fungal growth was inhibited for more than 30 days. On the other hand, **Sebti and Tantaoui, (1994)** showed that cinnamon powder although very efficient at inhibiting the fungi, imported a dark color

to the papers and therefore is not recommended. While, cinnamon water extract did not inhibit fungal growth up to concentration of 80 g/kg (8%). Also, results from Table (6) showed that refrigeration temperature 4 oC of yoghurt could enhance the inhibitory effect of volatile or essential oil lemongrass extract. These results nearly in consistent with results given by **Eissa et al., (2003a, b), Eissa et al., (2008)** and **Ting and Deibel, (1992)** who appeared that refrigeration temperature (5°C) could enhance the inhibitory effect of some spices extracts but not others. When 0.5 or 1.0 % cloves were tested, the organism died more rapidly in tryptic soy broth at 24°C than at 5°C. Whereas, > 5 log reduction in CFU was observed after 7 days of incubation at 5°C and after 3 h incubation at 24°C in tryptic soy broth.

In general, the refrigeration of yoghurt effects increased the inhibition of bacteria, yeast and mold counts. Also, the results showed that the yoghurt treated with different concentrations of lemongrass extract were observed no browning and lowest microbial count (T and M and B) during storage at 5°C for 5 weeks. Lemongrass extract as preservatives may be due to it contain aldehydes and volatile compounds that have efficient on inhibition of browning and inhibition of growth microorganisms. **Nakatani, (1994)** proposed inhibitory mechanism that the anti-fungal action of the aldehydes that was due to the ability to form charge transfer complexes with electron donors and reactivity with SH group in cystein or glutathione moieties.

**Zaika, (1988)** reported that food product safety and shelf life depend in some part on the type, quantity, and character of volatile oil spices extracts added to the products. Then, our results showed that refrigerating at 5°C and 0.05% volatile or essential oil extracts treatments caused a marked reduction in yeast and bacteria populations with acceptable taste and extension shelf life of yoghurt up to 5 weeks.

**Table 6. Effect of essential oil extracts on the number of colonies (TPC or Y&M) that appeared on the plates and expressed as the log (CFU/gm) in yoghurt during storage at refrigerator (5 °C) for 4 weeks.**

Extract level,%	Zero time		1 week		2 weeks		4 weeks	
	*M&Y	TPC	M&Y	TPC	M&Y	TPC	M&Y	TPC
0.0	0	6.1	0	6.50	0	6.50	0	6.75
0.05	0	5.95	0	6.20	0	6.30	0	.506
0.1	0	5.90	0	6.00	0	6.10	0	5.95
0.3	0	5.90	0	5.90	0	6.00	0	6.10

- Each value represents the mean of three replicates
- (CFU/gm).means Colony Forming Unit per gream yoghurt.

### **3.3- Effect of lemongrass essential oil on physico-chemical content in yoghurt during storage time:**

#### **Quality evaluation of yoghurt products.**

The following discussion of the chemical characteristics for fresh, products and lemongrass extract pre-treated yoghurt is based on the data given in table (7). The pH of fresh and treated yoghurt ranged from 4.04 to 4.49 showing a increase in pH values. The increase in pH was directly related to increase lemongrass extract concentrations in yoghurt. TSS (g/Kg) of yoghurt products after 28 days storage was lower than the fresh yoghurts. Whereas, the increase of TSS was obvious with increasing of lemongrass extract concentration. This increase of TSS was attributed to the greater degree of tissue breakdown, releasing more components that contribute to soluble solids (**Tung-Sun, et al., 1995**). The TSS / acid ratio is the major analytical measurement for quality in fresh and treated yoghurt. The TSS / acid ratio of fresh, and treated yoghurt was increased by increasing of lemongrass extract concentration. TSS / acid ratio was shown to be correlated with sweetness but not so closely with flavour (**Guyer, et al., 1993**).

Titratable acidity of yoghurt products was lower than fresh yoghurt (Table 7), which may due to enzymatic desertification and degradation of pectin resulting in an increased of total acid.

The viscosity (cP) was selected as a measure of yoghurt quality. However, the viscosity between fresh and treated yoghurt samples were decreased from 2.92 cP to 2.64 by increasing the lemongrass extract concentration in yoghurt than fresh sample (2.86 cP), respectively as seen in Table (7). Total phenol content were decreased also by increasing of lemongrass extract concentration in all yoghurt samples, as seen in table (7).

**Table (7): Effect of Concentrations on physico-chemical properties in yoghurt at zero time and after 21 days.**

Extract level,%	TSS		pH		% acidity		TSS/acidity		Viscosity (cP)		Total Phenols	
	at zero time	after 28 days	at zero time	after 28 days	at zero time	after 28 days						
0.0	7.50	6.00	4.04	4.39	0.94	0.94	7.8	6.38	2.86	2.60	1342.85	1342.85
0.05	7.00	6.00	4.00	4.30	0.96	0.92	7.4	6.51	2.92	2.80	1345.97	1345.97
0.1	7.50	7.00	4.08	4.39	0.97	0.94	7.7	7.44	2.75	2.60	1338.17	1338.17
0.3	8.00	7.00	4.11	4.49	0.98	0.95	8.17	7.36	2.64	2.52	1317.87	1317.87

### **3.4- Effect of lemongrass essential oil concentrations and storage on Color characteristics of yoghurt:**

#### **a- Color parameters during storage time of yoghurt:**

Tristimulus Reflectance Colorimetry (TRC) measuring the reflectance L\*, a\* and b\* values) was used to follow the extent of browning in yoghurt and change of color in foods (**Sapers and Douglas., 1987**). The results in Table (8) showed change of color in yoghurt during storage time up to 28 days at 5°C. These results illustrated the changes in color of yoghurt in terms of redness a\*, yellowness b\* and lightness L\* during 28 days of storage at 5°C. In addition to determination of the lightness L\*, redness a\* and yellowness b\*, for experimented yoghurt. Hue angle (H\*) as well as the chromaticity (C\*) were determined. Hue is the aspect of color that we describe by words such as green, blue, yellow or red. The chroma refers to reflection at given wavelength and indicates how much a color differs from gray (**Eissa and Moharam, 2001**). The equations No. 1, 2, 3 and 4 are showed the DE, B<sub>1</sub>, H\* and C\*.

The H\* values were closely stable in all samples with increasing of storage time up to 28 days at 5°C. The chromaticity (C\*) increased by the increasing of stoarge time in yoghurt up to 28 days. Thereafter, no relation was

noticed. It can be observed that the  $a^*$ -value of the fresh yoghurt was -2.22 compared to -2.27 after 7 days, -2.39 after 14 days and increased -2.18 after 28 days, as seen in Table (8).

Regarding the lightness  $L^*$  and the yellowness  $b^*$ . It is clear that the lightness  $L^*$  as well as the yellowness  $b^*$  were decreased as a result of increasing the time of storage up to 28 days. The effect of storage time on increasing the  $a^*$ -value from -2.22 at zero time days to -2.18 after 28 days was noticed. The change in color may be referred to chemical changes occurred during storage (**Kumar et al., 2006**).

The analysis of variance identified the significant ( $p<0.05$ ) effect of storage time on Hunter values of yoghurt. Although the  $a$ -value showed a definite increased trend throughout storage, the  $L$ -value decreased and the  $b$ -value increased as the yoghurt storage aged, as seen in Table (8).

It can be concluded that the storage of yoghurt slightly inhibited the changes in color yoghurt. The total color differences (DE) increased by the increasing of storage time in yoghurt up to 28 days as presented in Table 1, total colour differences of yoghurt were small, which almost correspond to the sensory difference threshold (**Rohm and Jaros, 1996b**). However, greatly different values of DE were found for yoghurts at 7, 14 and 28 days of storage and at different concentrations lemongrass extract treated samples. The almost identical colour values found in yoghurt could be attributed to their similar structure. The browning index ( $B_1$ ) increased by the increasing of storage time in yoghurt up to 28 days, especially in high concentration treated yoghurt (0.3%). Thereafter, no relation was noticed.

#### ***b- Non-enzymatic browning of yoghurt samples:***

Non-enzymatic browning in yoghurt is only one component that determines overall color and might not be a problem at low levels. The effects of heat treatment of milk and yoghurt products in the inhibition of the browning reaction are listed in Tables (8). It is obvious that the yoghurt product treated and untreated yoghurt samples inhibits the development of A 420 nm and red colour  $a^*$ . For example, the A420 nm and  $a^*$ -value of fresh yoghurt was 64.34 compared to 64.11, 64.36 and 61.56 in case of the different concentrations of lemongrass extract yoghurt samples, respectively. **Crandall et al., (1986)** concluded that two measures of browning were used, color  $a^*$  or  $L^*$  and absorbance at 420nm where the higher numbers indicate increased absorbance due to the formation of brown pigments. Browning is also indicated by a decrease in the color  $L^*$  toward black and an increase in the color  $a^*$  toward brown or red.

**Table (8): Effect of on colour characteristics in yoghurt during storage at 5 oC for 35 days.**

	$L^*$	$a^*$	$b^*$	Delta E	$C^*$	$H^*$	$B_1$	OD 420nm
At zero time								
0.0	92.22	-2.22	11.38	11.65	11.59	78.96	20.15	64.34
0.05	91.89	-2.14	11.04	11.31	11.25	79.03	19.60	64.11
0.1	92.19	-2.25	11.38	11.65	11.60	78.82	20.11	64.26
0.3	91.33	-2.27	11.91	12.22	12.12	79.21	21.46	61.56
Storage after 7 days								
0.0	93.68	-2.27	11.75	12.04	11.97	79.07	20.54	65.83
0.05	93.41	-2.24	11.08	11.35	11.30	78.57	19.20	66.3
0.1	93.97	-2.09	10.21	10.52	10.42	78.43	17.44	68.88
0.3	93.51	-2.38	10.32	10.63	10.59	77.01	17.34	68.34
Storage after 14 days								
0.0	92.4	-2.39	11.94	12.19	12.18	78.68	21.08	64.3
0.05	92.33	-2.09	11.12	11.34	11.31	79.36	19.75	64.77
0.1	92.09	-2.53	13.01	13.27	13.25	79	23.33	61.71
0.3	92.97	-2.23	12.12	12.35	12.32	79.57	21.57	63.56
Storage after 28 days								
0.0	93.2	-2.18	10.47	10.74	10.69	78.24	18.02	67.83
0.05	92.6	-2.25	11.92	12.17	12.13	79.31	21.20	64.47
0.1	91.69	-2.04	11.25	11.52	11.43	79.72	20.26	63.63
0.3	92.4	-2.59	13.75	14.03	13.99	79.33	24.82	60.63

#### **3.5- Sensory evaluation of Yoghurt:**

The results of sensory evaluation of the products based on colour, odour, taste, texture and appearance are shown in Table (9). Sensory evaluation of the yoghurt samples was carried out during 4 weeks of samples at 5°C by

20 experienced panels using 10 points scales. Difference in sensory properties of yoghurt samples due to the effect of different concentrations of lemongrass extract was determined by analysis of variance (ANOVA). Sensory attributes are of great importance to measure consumer attitudes and their influence on food choice and acceptability. The colour of yoghurt is the first quality attribute used to judge acceptability of yoghurt products.

The change in color may be referred to chemical changes occurred during storage of yoghurt samples (**Young-Hee, and Song Sun, 2009**). The mean value of flavor or odour scores for all treatments of yoghurt stored was 8.13, 7.75, 7.00 and 7.38 at 0.0, 0.05, 0.1 and 0.3 % concentration of lemongrass extract, respectively. The texture of the tested samples was affected by refrigeration. The over all mean score for the texture of yoghurt was only 8.13, 7.63, 8.13 and 8.25 at 0.0, 0.05, 0.1 and 0.3 % concentration of lemongrass EO extract, respectively (Table 9). These levels of score indicate the importance of the lemongrass extract in keeping a texture for the tested yoghurt. However, there were significant differences between the individual ripening. Samples pre-treated for 0.3% lemongrass EO extract showed the highest score (8.25) in texture stored at 5°C.

In general, the lemongrass extract pretreated yoghurt samples received higher sensory scores than untreated sample. For example, color values were 8.0 – 8.63 the EO extract pretreated yoghurt samples but were 8.13 – 8.75 in untreated samples, these differences were nonsignificant ( $P<0.05$ ) for all samples. Samples treated with 0.05 and 0.1 % EO extract generally had better score for all sensory characteristics at all samples. On the contrary, the sample treated with 0.3% EO extract had lower score for all sensory characteristics at all samples especially in taste characteristics. However, concentration of lemongrass extract had a positive influence on acceptability of colour and flavor of yoghurt samples. Increased concentration of lemongrass extract also showed that the same odour, colour texture and acceptability characteristics in hoghurt samples. However, all sensory scores were in the acceptable range, which greater than 5 scores.

It is clear that the the lemongrass EO extract pretreated yoghurt gave higher mean panel scores (8.0- 8.5) than the untreated yoghurt sample, which were the most preferred in all the studied characteristics. The lemongrass EO extract pretreated yoghurt samples with different concentrations had a non-significant difference ( $P<0.05$ ) between these samples.

**Table (9): effect of lemongrass concentrations on sensory evaluation of youghurt stored at 5°C for 4 weeks.**

Extract level, %	Appearance	Texture	Colour	Taste	Odour
0.0	8.13 <sup>A</sup>	8.13 <sup>A</sup>	8.75 <sup>A</sup>	8.50 <sup>A</sup>	8.13 <sup>A</sup>
0.05	8.0 <sup>A</sup>	7.63 <sup>A</sup>	8.50 <sup>A</sup>	7.13 <sup>B</sup>	7.75 <sup>A</sup>
0.1	8.50 <sup>A</sup>	8.13 <sup>A</sup>	8.50 <sup>A</sup>	7.13 <sup>B</sup>	7.00 <sup>A</sup>
0.3	8.63 <sup>A</sup>	8.25 <sup>A</sup>	8.38 <sup>A</sup>	6.88 <sup>B</sup>	7.38 <sup>A</sup>
LSD	NS	NS	NS	S	NS

LSD = least significant difference at 0.05 level.

#### 4- CONCLUSION

While the primary function of Lemongrass may not be preservative in nature, it has preservative properties, which may useful in built-in safety systems in food. In addition, Lemongrass herb is cheep, safe, and had a medical functions. Our results show that water extract from Lemongrass, at level 0.1% was effective agent to inactivate mold growth and mycotoxin formation and increasing level to 0.3% completely prevented growth and mycotoxin production , for the all tested molds, in YES broth medium. However, in yoghurt medium its inhibitory effect was different according to mold type, supplemented concentration and constituents of the used medium. Its evident from our data that, if possible, a sufficient amount of lemongrass oil to prevent mold growth needs to be used if one wishes to prevent mycotoxin production. It can be concluded from the results of this work that of lemongrass

volatile oil extract treatments with refrigeration at 5oC may serve as alternative to conventional chemical preservatives in the preservation of yoghurt by hurdle technology.

#### Corresponding author:

Shaaban Mostafa Abdel-Fattah, National Research Center, Food Toxins & Contaminants Department, 33El Buhoth Street, P.O. Box. 12311, Dokki. Giza, Egypt; tel. (00 202) 38670994 ; fax : (00 202) 3337 0931 ; e-mail: [shaabanmostafa@yahoo.com](mailto:shaabanmostafa@yahoo.com)

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# The Assessment Of Mycotic Settlement Of Freshwater Fishes In Egypt

Refai, M.K.<sup>1</sup>, Laila, A. Mohamed<sup>2</sup>, Amany, M. Kenawy<sup>2</sup>, and Shima, El-S.M.A.<sup>\*2</sup>

<sup>1</sup> Microbiology Dept., Faculty of Vet.Medicine, Cairo University, Giza, Egypt.

<sup>2</sup> Hydrobiology Dept., National Research Center. Dokki, Giza, Egypt.

[shimaakhalifa2003@yahoo.com](mailto:shimaakhalifa2003@yahoo.com)

**Abstract:** This study was carried out on 360 freshwater fishes (240 *Oreochromis* species and 120 *Clarias gariepinus*). They were collected from different governorates and during different seasons. Naturally infected fishes showed clinical abnormalities such as skin darkening, exophthalmia, corneal opacity, abdominal distention, ulceration of the skin and cotton wool like growths on various parts of the body. Fishes were then subjected to post mortem examination which revealed many abnormalities. Mycological examination revealed the isolation of 2081 fungal isolates from 150 diseased and 210 apparently healthy fish samples (1658 mould and 423 yeast isolates), of which 1334 were isolated from *Oreochromis* species and 747 isolates from *Clarias gariepinus*. Isolated moulds belonged to the following genera: *Saprolegnia* (4.2%), *Aspergillus* (43.0%), *Fusarium* (14.1%), *Mucor* (14), *Penicillium* (17.2), *Rhizopus* (4.8%), *Scopulariopsis* (1.2%), *Paecilomyces* (1%) and *Curvularia* (0.4%). Yeasts isolated also from both fish species had the following incidence: *Candida albicans* (35.9 %), other *Candida* species (19.1%), *Rhodotorula* species (31.4%) and *Torulopsis* species (13.5%). Experimental infection with the most predominant fungi (*Aspergillus flavus*, *Fusarium* species and *Candida albicans*) was conducted to evaluate the pathogenicity of these isolates. Clinical pictures of experimentally infected fish were similar to those of natural infection. Inoculated fungi were re-isolated from different organs. Results were confirmed with histopathological examination, which revealed the presence of fungal hyphae and spores in different organs.

[Refai, M.K., Laila, A. Mohamed, Amany, M. Kenawy, Shima, El-S.M.A. The Assessment Of Mycotic Settlement Of Freshwater Fishes In Egypt. Journal of American Science 2010;6(11):823-831]. (ISSN: 1545-1003).

**Keywords:** Mycotic infection, *Oreochromis* species, *Clarias gariepinus*, Moulds, Yeasts, *Aspergillus*, *Fusarium*, *Candida*, *Penicillium*.

## 1. Introduction

Many of the fungi that affect fishes are considered opportunists, attacking the fishes when they are stressed or immunocompromised because of unfavorable environmental conditions, or secondary to bacterial or viral infections, or when they have lost their mucus protection because of trauma or excessive handling (Roberts 1989 and Quiniou *et al.*, 1998). Mycotic infections of fishes by Oomycetes are wide spread in freshwater and represent the most important fungal group affecting wild and cultured fishes. The *Saprolegniaceae*, in particular members of the genus *Saprolegnia*, are responsible for significant infections involving both living, dead fishes and eggs. Oomycetes are classical saprophytic opportunities, multiplying on fishes that are physically injured, stressed or infected (Pickering and Willoughby, 1982). Members of this group are generally considered agents of secondary infection arising from conditions such as bacterial infections, poor husbandry, and infestation by parasite and social interaction. However, there are several reports of Oomycetes as primary infectious agents of fishes (Pickering and Christie, 1980) and their eggs (Walser

and Phelps, 1993). Moreover, there are other fungi that have been implicated in fish diseases. Some of the genera involved include *Aspergillus* (Salem *et al.*, 1989b), *Fusarium* (Bisht *et al.*, 2000), *Ichthyophonus* (Faisal *et al.*, 1985), *Branchiomyces* (Easa 1984), *Phoma* (Hatai *et al.*, 1986), *Paecilomyces* (Lightner *et al.*, 1988), *Exophialia* (Langdon and MacDonald 1987), *Phialophora* (Ellis *et al.*, 1983), *Rhizomucor* (Wolf and Smith 1999) and *Candida* (Neish and Hughes 1980). Most of these are multiple case reports or single, and causing systemic disease with high mortality rates in fishes. The objective of this study was to determine the types of fungal pathogens affecting freshwater fishes specially those causing high mortality rates, elucidation of the incidence and distribution of such pathogens in *Oreochromis* species and *Clarias gariepinus*, studying the seasonal variations enhancing fungal diseases of fishes and determination of the pathogenicity of the most prevalent isolated fungi.

## 2. Material and Methods

A total number of 360 fish were observed for their behavior, external lesions prior to autopsy. Then they were killed and examined. The examination included external changes as well as examination of internal organs. Wet mount preparation of fish samples were commonly made in 10% KOH. A simple stain such as lactophenol cotton blue was used. The preparation was examined microscopically after about 30 minutes for fungal elements. Mycological examination was done according to and (1993 Noga). Identification of moulds was carried out according to Refai (1987). Identification of yeasts: Plates were examined microscopically for the presence of chlamydospores, arthrospores and blastospores (Refai, 1987) and the scheme of identification of yeasts given by Terrence (1971). Urease test (Cruickshank *et al.*, 1975). Suspected *Candida* species were scratched onto rice or corn meal agar for pseudohyphae and chlamydospores production (Larone, 1987) and a confirmatory identification was carried out by germ tube test (Martin, 1979).

#### Histopathological examination:

Tissue samples were prepared according to Roberts 1989. and stained by periodic acid Schiff's (PAS) and GMS (Sheehan and Hrapchak 1980).

#### Experimental infection:

A total of 120 *Oreochromis* species with 30-40 g average body weight were used. They were divided into four equal groups (each one contained 30 fish). Each group were subdivided into three sub-groups, each contained 10 fish.

**Preparation of spores suspension for experimental infection:** Inocula were prepared by spreading 5 ml of sterile phosphate buffer saline over the plates containing 7- 10 day old pure cultures of *Aspergillus flavus* and *Fusarium* sp. The spores were harvested by gentle washing of the surface of the colonies with sterile loop, then transferred aseptically to sterile flasks. Two drops of tween 80 were added to avoid clumping of spores in case of *Aspergillus flavus* group. Spores were counted by aid of haemocytometer and suspension was diluted to reach  $9 \times 10^4$  spores/ml for both *Aspergillus flavus* and *Fusarium* sp.

**Preparation of yeast suspension for experimental infection:** A loopfull of one day old pure yeast culture of *Candida albicans* was added to test tube containing 5 ml of sterile phosphate buffer saline and mixed gently to reach equal distribution. Spores were counted by using haemocytometer then suspension was adjusted to reach  $2 \times 10^3$  *Candida* spores per ml.

### 3. Results and Discussion

Mycological examination revealed the isolation of 2081 fungal isolates from 150 diseased and 210 apparently healthy fish samples, of which 1334 were isolated from *Oreochromis* species and 747 isolates from *Clarias gariepinus*. Identification of fungi into yeasts and moulds revealed that the percentage of moulds was slightly higher in *Oreochromis* species (80.5%) in comparison to that in *Clarias gariepinus* (78.2 %). On other hand, the rate of yeast isolates per fish was slightly higher in *Clarias gariepinus*. Isolated moulds belonged to the following genera: *Saprolegnia*, *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus*, *Scopulariopsis*, *Paeciliomyces* and *Curvularia*. The same fungal isolates were reported by Abdel Alim (1992) and Khalil (1993).

The Incidence of moulds in diseased and apparently healthy fishes were recorded in (Fig.1&2), also the incidences of isolated moulds from different organs of *Oreochromis* species (Fig.3) and *Clarias gariepinus* (Fig.4) were detected. Seasonal incidences were seen in (Fig. 5). As these isolates were recovered from apparently healthy and clinically diseased *Oreochromis* species and *Clarias gariepinus* This was expected, as almost all these fungi were categorized by Shaheen (1986) as normal mycoflora. This does not mean that they cannot produce disease. They can better be considered as opportunistic fungi (Refai, 1987) as many of them possess virulence factors, which enable them to cause diseases (Refai *et al.*, 2004), particularly under favourable predisposing condition. Regarding to seasonal incidence *Saprolegnia* species were isolated with high incidence in Winter, followed by early Spring and late Autumn. This result agrees with Naguib (1994), who stated that the seasonal variations play an important role in spreading of the *Saprolegnia* infection among freshwater fishes especially during late Autumn, Winter and early Spring, where the water temperature was low.

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Clinical findings of *Oreochromis* species inoculated with *Aspergillus flavus*, *Fusarium* species and *Candida albicans* revealed that exophthalmia (Photo.21), skin darkening (Photo.22), cotton wool-like growth on various parts of the body (Photo.23&24), moderate abdominal distention (Photo.25) and corneal opacity and haemorrhages all over the body surface (Photo.26). These results are supported by Marzouk *et al.*(2003).

Postmortem finding revealed congestion and ulceration of gills (Photo.27), haemorrhagic abdominal fluids, necrotic foci within liver and distention of gall bladder (Photo.28), multiple

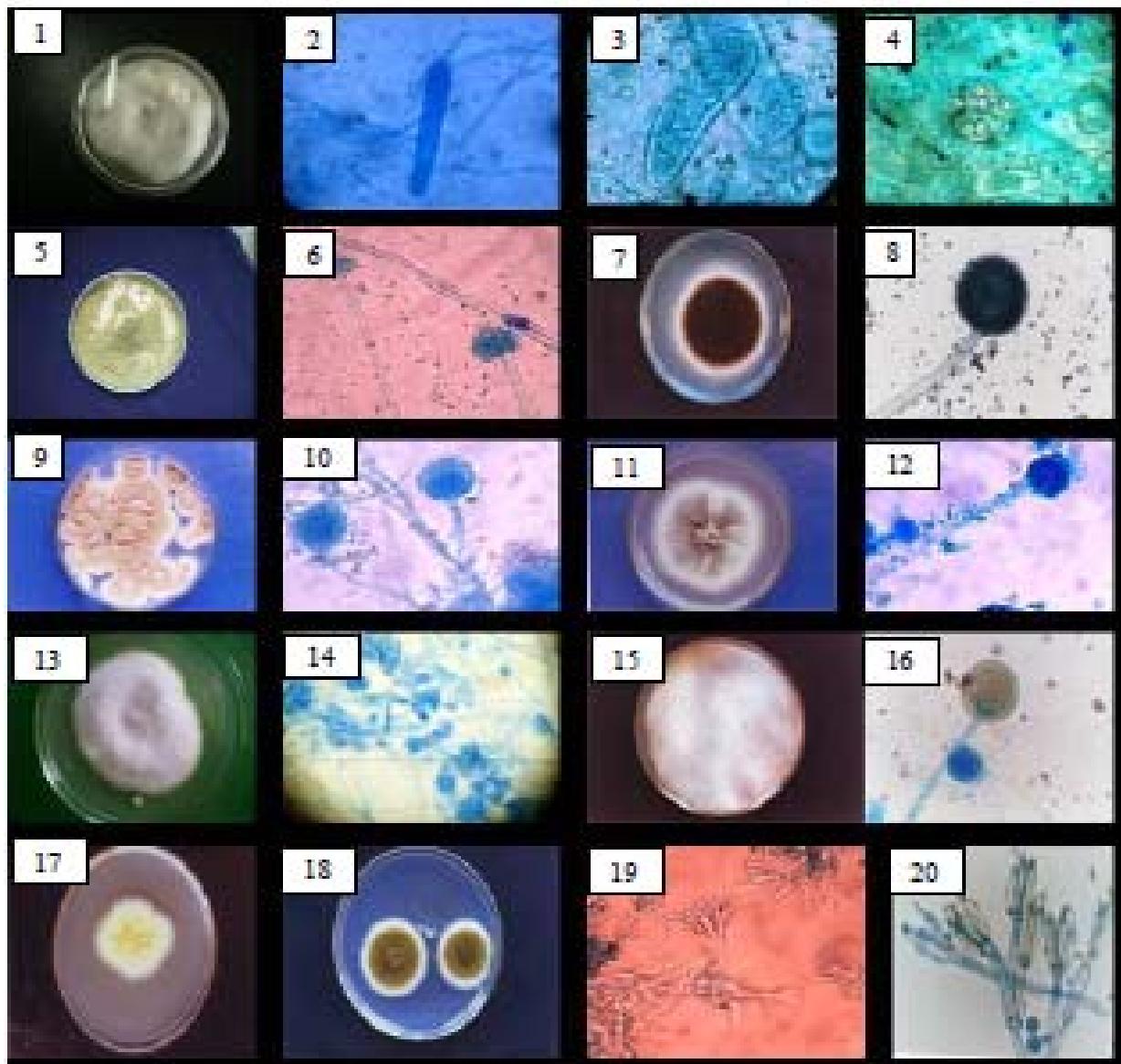
nodules within spleen (Photo.29) and severe intestinal congestion (Photo.30) were also observed. On the other hand, no clinical or postmortem changes were detected on fish groups maintained at 18°C. These findings are in agreement with those of Refai *et al.* (1987).

It can be concluded from the results obtained in the present work that, though most fungi isolated from fishes are considered by several authors as normal mycoflora, yet we could prove in the present study that many fungi can cause natural infections. This was confirmed by histopathological reactions characteristic of fungal infection in naturally infected fishes, and the presence of fungal elements in the lesions. This was substantiated also by experimental infection of fish that induced similar findings as the natural infection, i.e. a clear application of Koch's postulate. This conclusion should direct our attention to the possible role of fungi in affecting fishes industry.

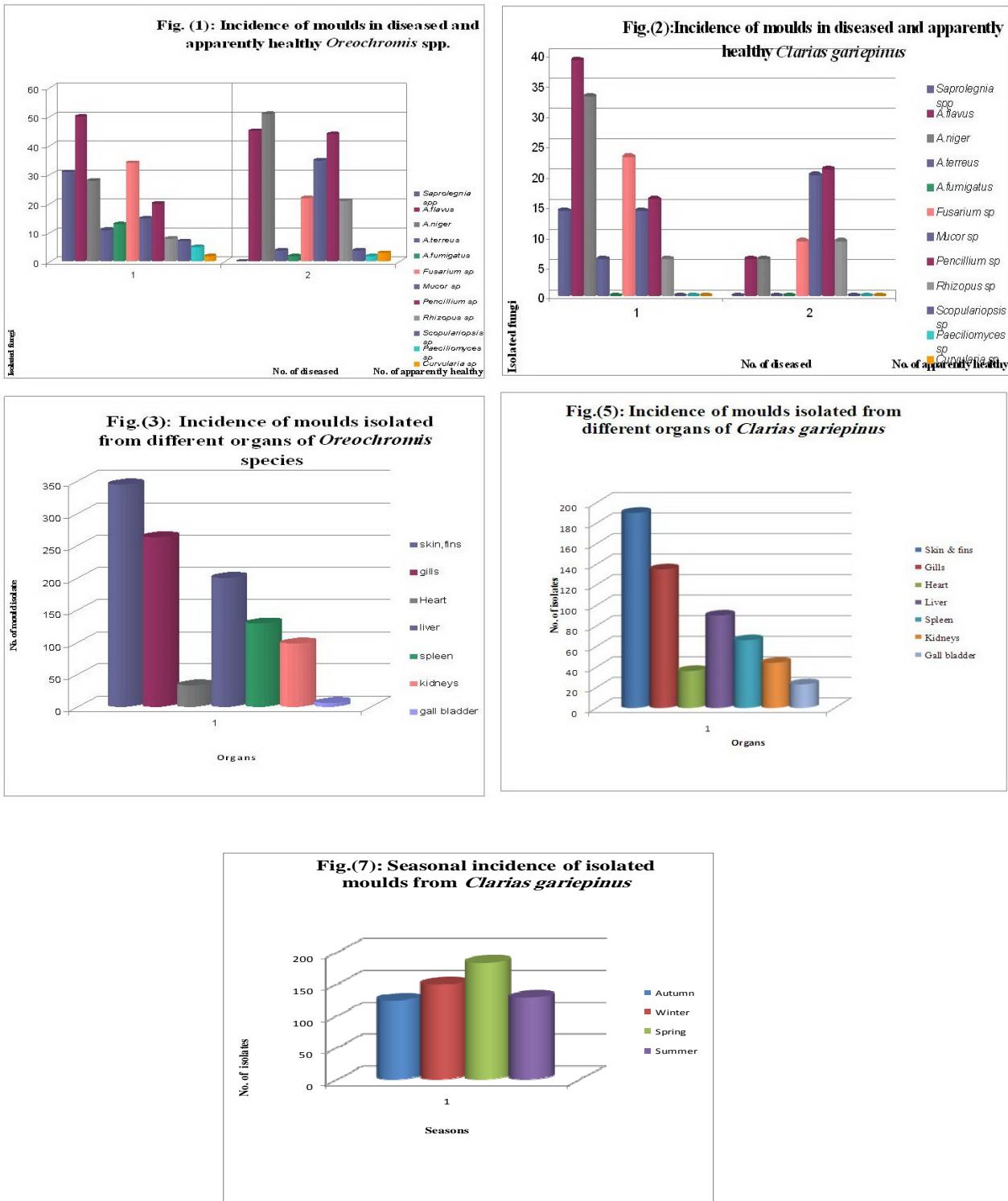
The pathological changes and the fungal elements in tissue sections in naturally infected fishes of various organs are described under each of the following photos (31-39), stained by either PAS or GMS stains.

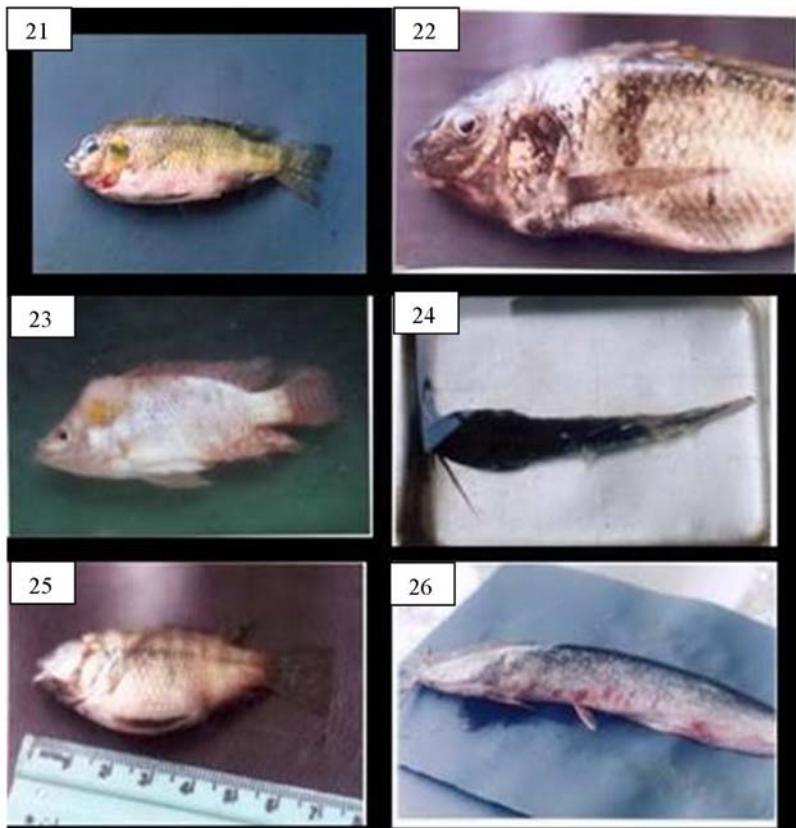
**Table (3): Type, average body weight of fish, spores concentration per ml, dose, route of inoculation and temperature.**

Fish	Body weight	Number of fish in each subgroup	Inoculated fungi	Dose	Conc.	Route	Temp.	References	
Tilapia sp.	30-40 g	10 10	<i>Aspergillus flavus</i>	0.2ml 0.2ml	9x10 <sup>4</sup> 9x10 <sup>4</sup>	I.P I.M	18°C	Olufemi <i>et al.</i> (1983)	
		5 5	Normal saline	0.2ml 0.2ml	— —	I.P I.M			
	30-40 g	10 10	<i>Aspergillus flavus</i>	0.2ml 0.2ml	9x10 <sup>4</sup> 9x10 <sup>4</sup>	I.P I.M	26°C		
		5 5	Normal saline	0.2ml 0.2ml	— —	I.P I.M			
Tilapia sp.	30-40 g	10 10	<i>Fusarium</i>	0.2ml 0.2ml	9x10 <sup>4</sup> 9x10 <sup>4</sup>	I.P I.M	22°C	Muhvich <i>et al.</i> (1989)	
		5 5	Normal saline	0.2ml 0.2ml	— —	I.P I.M			
	30-40 g	10 10	<i>Candida albicans</i>	0.2ml 0.2ml	2x10 <sup>3</sup> 2x10 <sup>3</sup>	I.P I.M	22°C		
		5 5	Normal saline	0.2ml 0.2ml	— —	I.P I.M			

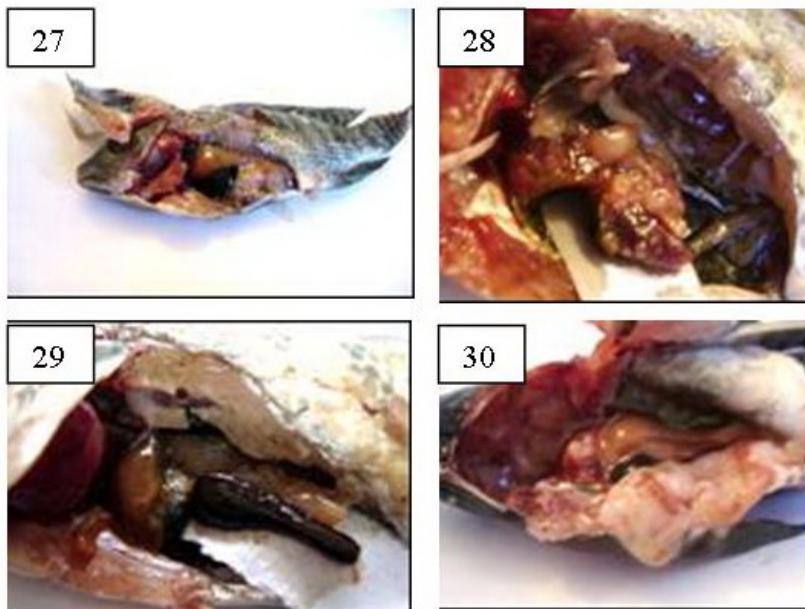


**Photo. (1):** A colony of *Saprolegnia* species with the characteristic cotton-wool like growth. **Photo. (2):** Non-septated broad hyphae of *Saprolegnia* species (X 200). **Photo. (3&4):** Different stages of reproductive structures of *Saprolegnia* species on hemp seeds (X 400). **Photo. (5):** Colonies of *Aspergillus flavus* on SDA, one weak old. **Photo. (6):** Typical heads *Aspergillus flavus* (X 400). **Photo. (7):** A colony of *Aspergillus niger* on SDA. **Photo. (8):** *Aspergillus niger* showing characteristic round head with black conidia (X 400). **Photo. (9):** Colonies of *Aspergillus terreus* on SDA. **Photo. (10):** *Aspergillus terreus* with small hemispherical vesicle (X 400). **Photo. (11):** A colony of *Aspergillus fumigatus* on SDA. **Photo. (12):** *Aspergillus fumigatus* with columnar head (X400). **Photo. (13):** A colony of *Fusarium* species on SDA with rose pigments on the center. **Photo. (14):** *Fusarium* species with characteristic slender, multicelled conidia (X 200). **Photo. (15):** Colonies of *Mucor* species showing spread over the surface of SDA. **Photo. (16):** Round sporangia of *Mucor* species containing sporangiospores (X 400). **Photo. (17):** *Penicillium* species on SDA with different colour and texture. **Photo. (18):** *Penicillium* species showing brush-like arrangement of fruiting head "A" (X400) and "B" (X 200). **Photo. (19):** *Rhizopus* species colony on SDA showing dens woolly mycelia. Sporangia are seen as small black dots. **Photo. (20):** *Rhizopus* species showing long, branched Sporangioophores and terminate with rhizoids (X200).

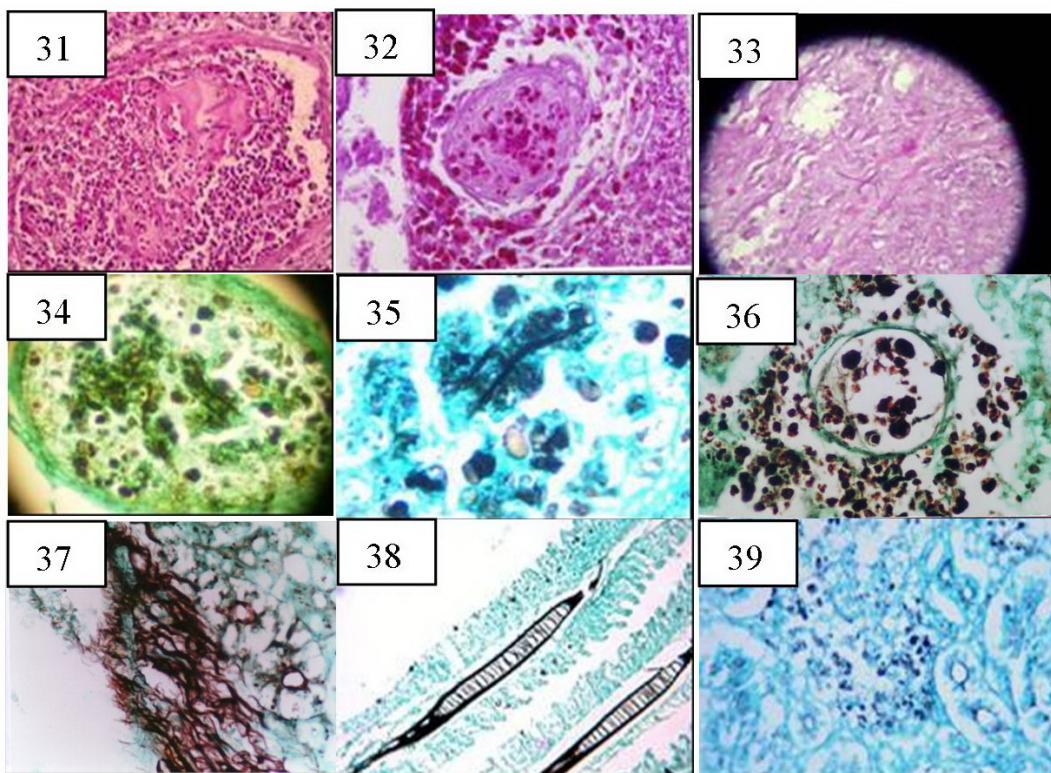




**Photo. (21):** *Oreochromis* species showing exophthalmia. **Photo. (22):** *Oreochromis* species showing skin darkening. **Photo. (23&24):** *Oreochromis* species and *Clarias gariepinus* showing cotton wool-like growth on various parts of the body. **Photo. (25):** *Oreochromis* species showing ascitis. **Photo. (26):** *Clarias gariepinus* showing haemorrhages all over the body surface.



**Photo. (27):** Liver of *Oreochromis* species showing necrotic foci with distention of gall bladder. **Photo. (28):** Spleen of *Oreochromis* species showing multiple nodules. **Photo. (29):** *Oreochromis* species showing severe enteritis. **Photo. (30):** *Oreochromis* species showing severe enlargement of spleen.



**Photo. (31):** Spleen section stained with PAS (X400) showing a granuloma formed of epithelioid cells and macrophages surrounded with fibroblasts and fibrous connective tissue capsule. Fungal hyphae appear within the granuloma. **Photo. (32):** Spleen section stained with PAS (X400) showing granuloma consists of epithelioid cells, macrophages and surrounded with connective tissue capsule. Large number of fungal spores appear within and surrounding granuloma. **Photo. (33):** Liver section showing fungal hyphae between the hepatocytes stained with PAS (X200). **Photo. (34):** Liver section stained by GMS (X400) showing granuloma consists of aggregation of epithelioid cells, macrophages and fibrous connective tissue capsule. Fungal hyphae and spores appear within granuloma. **Photo. (35):** Liver section stained by GMS (X 1000) showing fungal hyphae and spores between the hepatic tissue. **Photo. (36):** Spleen section stained by GMS (X 400) showing focal aggregation of spores surrounded with proliferating fibroblasts and fibrous connective tissue in between. **Photo. (37):** Kidney section stained by GMS (X 400) showing hyphal threads in between the interstitial tissues with marked severe degenerative changes in the tubular epithelium. **Photo. (38):** Gills section stained by GMS (X 400) showing yeast cells investing necrosed areas of epithelial lining the secondary lamellae. **Photo. (39):** Kidney section stained by GMS (X 400) showing yeast cells investing the interstitial tissues.

#### Corresponding Author:

Shimaa, Khalifa

Dept. of Hydrobiology, veterinary research division,  
National Research Center.Dokki, Giza,Egypt. Tel.:  
+202-3371728- Fax: +202-3370931

E-mail: [shimaakhalifa2003@yahoo.com](mailto:shimaakhalifa2003@yahoo.com)

#### Author Information:

Refaï, M.K.<sup>1</sup>, Laila, A. Mohamed<sup>2</sup>, Amany, M.  
Kenawy<sup>2</sup>, Shimaa, El-S.M.A.<sup>2</sup>

1-Prof. Dr. in Dept. of Microbiology, Faculty of  
Vet.Medicine, Cairo University, Giza, Egypt. Tel. &  
Fax: +202-37430683- E-mail: [mohrefai@yahoo.com](mailto:mohrefai@yahoo.com)

2-Dept. of Hydrobiology, veterinary research division,  
National Research Center.Dokki, Giza,Egypt. Tel.:  
+202-3371728- Fax: +202-3370931- E-mail:  
[www.nrc.org.eg](http://www.nrc.org.eg)

\*\*3- Researcher in Department of Hydrobiology,  
National Research Center , Dokki, Giza. Tel.: +202-  
24936882- Fax: +202-3370931- E-mail:  
[Amanyk70@yahoo.com](mailto:Amanyk70@yahoo.com)

4- Assistance Researcher in Department of  
Hydrobiology, National Research Center , Dokki,  
Giza. Tel.: +202-3371728- Fax: +202-3370931-  
E-mail: [shimaakhalifa2003@yahoo.com](mailto:shimaakhalifa2003@yahoo.com)

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10/5/2010

# Impact of Balanced Caloric Diet and Physical activity on Body Composition and Fat Distribution of Obese Egyptian Adolescent Girls

**\*Nayera El-morsi Hassan, \*\*Safaa T. Zaki ,\*Sahar El-masry, \*\*Manal A. Mohsen, \*\*Eman Elashmawy**

\*Biological Anthropology, \*\* Child Health Depts., National Research Centre, Cairo, Egypt

[masrysa@yahoo.com](mailto:masrysa@yahoo.com)

**Abstract:** **Objective:** The aim of this study was to evaluate the effects of 6 months of balanced caloric moderately deficit diet program combined with individualized moderate Physical exercise on the body weight, body composition and fat distribution of adolescent girls. **Subjects & methods:** It was a longitudinal survey comprised 1244 adolescent girls, aged from 14 to 18 years. Their body weight and height were measured, and the BMI was calculated. Of the total sample, only one hundred and eleven girls (8.9%), with mean age was  $15.82 \pm 0.75$  years, were suffering from obesity based on their body mass index; which is greater than the 95<sup>th</sup> percentile for age and gender based Egyptian Growth Reference Charts. These obese girls were undergoing nutritional intervention (specific dietary program, nutritional education and exercise) for 6 months. At the start of this program, the obese girls were assessed for their anthropometric measures: the body weight, body height (or stature), body mass index (BMI), waist and hip circumferences, waist/hip ratio, skin folds thickness at 5 sites and, according to BIA, their body composition. This assessment was repeated after 6 months. Only thirty eight girls completely finished the program till the end. **Results:** The current study showed that after following the dietary program and physical activity, there were highly significant reduction in waist circumference, the skin fold thickness at the 5 sites (triceps, biceps, sub scapular, suprailiac and abdominal), peripheral and central adiposity, and fat mass, and significant reduction in body weight, hip circumference and fat%. The change in BMI was not significant. On the other hand, there was a highly significant increase of the total body water and Basal metabolic rate after following the dietary program and physical activity. **Conclusion:** The nutritional intervention program was succeeded in 38 obese adolescent girls. These girls show highly significant reduction in body composition and body fat distribution. This revealed that the combined program of diet restriction and exercise is necessary.

[Nayera El-morsi Hassan, Safaa T. Zaki, Sahar El-masry, Manal A. Mohsen, Eman Elashmawy. Impact of Balanced Caloric Diet and Physical activity on Body Composition and Fat Distribution of Obese Egyptian Adolescent Girls. Journal of American Science 2010;6(11):832-842]. (ISSN: 1545-1003).

**Keywords:** Egyptian adolescents, obese girls, diet restriction, exercise training, body composition, anthropometry

## Introduction

The prevalence of obesity has reached alarming levels. Obesity is affecting virtually both developed and developing countries of all socioeconomic groups including all age groups thereby posing an alarming problem, described by the World Health Organization (WHO) as an “escalating global epidemic”<sup>(1)</sup>.

Worldwide, over 22 million children under the age of 5 are severely overweight, as are 155 million children of school age. This implies that one in 10 children worldwide is overweight<sup>(2)</sup>. The dramatic increase in the prevalence of obesity in the

past few decades can only be due to significant changes in lifestyle influencing children and adults<sup>(3)</sup>. These obesity-promoting environmental factors are usually referred to under the general term of “obesogenic” or “obesigenic”<sup>(4)</sup>. The current changing nature of this obesogenic environment has been well described in a WHO Technical Report as Changes in the world food economy have contributed to shifting dietary patterns, for example, increased consumption of energy-dense diets high in fat, particularly saturated fat, and low in unrefined carbohydrates. These patterns are combined with a decline in energy expenditure that is associated with a sedentary lifestyle<sup>(5)</sup>.

With changing food habits and increasingly sedentary lifestyles, a potential deluge is evident across the globe with obesity rates increasing more than two fold over the past 25 years in the U.S., almost threefold in the past 10 years in England, and almost fourfold over a similar time frame in Egypt<sup>(6)</sup>. Recently, in Egypt research of National Survey for Diet, nutrition and non-communicable diseases "DNPCNCD" by National Nutrition Institute (2008)<sup>(7)</sup> stated that the prevalence of overweight and obesity among adolescents aged 10-18 years was 20.5 %.

Obesity in children and adolescents is associated with several metabolic and hemodynamic abnormalities: dyslipidemia, high blood pressure (BP), impaired glucose tolerance, insulin resistance and assorted cardiovascular risk factors<sup>(8)</sup>. In addition, atherosclerosis reportedly begins in childhood<sup>(9)</sup>.

So, the need for evidence-based treatment recommendations is a critical health care issue, because obese children and adolescents are at risk for developing many of the co-morbidities seen in obese adults. Studies demonstrated that fasting serum glucose, insulin, and triglyceride levels and the prevalence of impaired glucose tolerance and systolic hypertension increase significantly as children become obese (BMI of ≥95th percentile)<sup>(10)</sup>. Even children and adolescents who are overweight (BMI of 85th to 94th percentile) are at risk for co-morbidities. Therefore, interventions using dietary modifications, increased physical activity, and behavioral therapy may be beneficial for overweight children and adolescents, with more-aggressive intervention directed toward obese children and adolescents<sup>(11)</sup>.

Health care professionals, however, may find it difficult to determine which interventions will be most efficacious for their patients. Clinical trials have failed to determine whether specific dietary modifications alone without behavioral interventions and increased physical activity are effective in reducing childhood overweight and obesity rates. Comprehensive interventions that include behavioral therapy along with changes in nutrition and physical activity are the most closely studied and seem to be the most successful approaches to improve long-term weight and health status<sup>(12)</sup>. However, the clinical trials testing these interventions often are limited in their ability to determine the relative efficacy of individual strategies. Ultimately, children and adolescents (and adults, for that matter) become

overweight or obese because of an imbalance between energy intake and expenditure. Dietary patterns, television viewing and other sedentary activities, and an overall lack of physical activity play key roles in creating this imbalance and therefore represent opportunities for intervention.

**The aim of this study** was to evaluate the effects of 6 -month of balanced caloric moderately deficit diet program combined with individualized moderate physical exercise on the weight, body composition and fat distribution of adolescent girls.

### Subjects and methods:

This study was conducted by the National Research Centre, Egypt, to estimate the prevalence of obesity among secondary school adolescent girls. It was a longitudinal survey, comprised 1244 adolescent girls, with age ranged from 14 to 18 years. They were recruited from "Gamal Abd-El-Naser Secondary Public School", in Giza governorate, for a whole studying year from October, 2008 to April 2009. Girls were excluded if they had a prior major illness, including type 1 or 2 diabetes, took medications or had a condition known to influence body composition, insulin action or insulin secretion (e.g. glucocorticoid therapy, hypothyroidism and Cushing's disease).

Permission to perform the study was granted by the Ministry of Education, and the director of the school included in the research. Parents were informed about the purpose of the study and their permission in the form of written consent was obtained. Another assent from the student's involved in the research was obtained. The protocol was approved by the "Ethical Committee" of the "National Research Centre".

Each girl underwent a complete physical examination, including anthropometric measures which are following the recommendations of the International Biological program<sup>(13)</sup>. The body height was measured to the nearest 0.1 cm on a Holtain portable anthropometer, and the body weight was determined to the nearest 0.01 kg on a Seca scale Balance with the subject wearing minimal clothing and no shoes. Body mass index (BMI) was calculated as body weight (in kilograms) divided by body height (in meters) squared.

Of the total sample, only one hundred and eleven girls or 8.9% with mean age 15.82± 0.75 years, were suffering from obesity based on their

body mass index; which is greater than the 95<sup>th</sup> percentile for age and gender based on the Egyptian Growth Reference Charts<sup>(14)</sup>. These obese girls were undergoing nutritional intervention (specific dietary program, nutritional education and exercise) for 6 months. At the start of this program, the obese girls were assessed for following anthropometric measures and calculated parameters: the body weight and body height; waist and hip circumferences; five skin fold thickness; BMI; waist/hip ratio; and body composition according to BIA. This assessment was repeated after 6 months.

**Anthropometric measures:** Body weight was measured as previously mentioned. Waist circumference was measured at the level of the umbilicus with the subject standing and breathing normally, hip circumference at the level of the iliac crest, using non-stretchable plastic tape to the nearest 0.1 cm. Skin fold thickness were taken at five sites: triceps, biceps, sub scapular, suprailiac and abdominal. Each skin fold was measured three times on the left side of the body with Holtain skinfold caliper to the nearest 0.2 mm, and the mean was recorded. The following indices were calculated:

- Body mass index (Kg/m<sup>2</sup>)
- Waist/ Hip ratio (cm/ cm).
- Peripheral adiposity: as the sum of triceps and biceps skin fold thickness.
- Central adiposity: as the sum of subscapular, suprailiac and abdominal skin fold thickness.

**Body Composition:** Whole body resistance and reactance (capacitance) were measured using a Bioelectrical Impedance Analyzer (HOLTAIN LIMITED). As specified by the manufacturer, the unit was calibrated before testing using 400-ohm resistor, and electrodes were placed on right wrist and ankle. By using girl's sex, age, body weight and body height approximated to the nearest unit, the percentage body fat (Fat %), fat mass (FM), fat free mass (FFM), total body water and basal metabolic rate (kcal) were derived.

**Nutritional intervention** (specific dietary program, nutritional education and exercise): A balanced caloric moderate deficit diet (BCDD), is a reduced-energy diet (- 500 kcal/day) was established by a dietician. The diet was selected from the usual 4 food groups in quantities thought to meet basic requirements of all macronutrients and micronutrients in a healthy proportion: ( 25-30% of total calories from fats, 8-10% from saturated fats, up

to 15% of total calories from monounsaturated fatty acids, up to 10% from polyunsaturated fats ,< 300mg/d cholesterol , approximately 15% of total calories from protein, 55% from CHO. , 20—30gm/d fiber ,no more than 1. 5 gm /d Na , water not less than 2.5 liter /d, 1000—15000 mg/d calcium). These diet regimen supplies enough calories to enable the patients to proceed normally in their life & not feel hungry nor lacking energy .Also, it can be adherent to and safe in long and short time, the diet design was individualized, flexible allowing the patients to exchange items, so as not to be boring, and produce 1 Ib or 0.5 kg loss per week .We spread the food through the day to meet the energy needs, avoiding long periods of no food or hunger. The foods were selected according to the girl's dietary habits. PowerPoint presentations and role-play scripts were designed for trainers to be used during the educational program.

**Exercise:** moderate Physical activity in the form of unsupervised walking for an hour daily or at least 5 times /week, starting by half an hour then gradually increase the duration to an hour after 3 days. This was encouraged and motivated by grouping teams of students.

The anthropometric measurements and body composition were remeasured after 6 months from the start of the dietary program to assess the effect of the nutritional modifications.

**Statistical Analysis:** Graphic presentation of the % of girls with different effect of the program, according to the reduction of the % body fat was drawn. Evaluation of the statistical distribution of the variables was done using Kolmogorov - Smirnov Goodness of Fit Test. Most the variables have asymmetric distribution ( $p<0.05$ );except skin fold thickness at the triceps and suprailiac crest, fat free mass and total body water; where they have normal distribution ( $p>0.05$ ). All values are reported as the mean  $\pm$  SD (the range). The changes in the used parameters before and after the dietary program were calculated. WILCOXSON non parametric t- test was used to examine the differences before and after the dietary program for the variables which had asymmetric distribution, while Paired sample t test (d-statistics) was used for the variables which had normal statistic distribution. The level of significance was set at a probability of less than 5% ( $p<0.05$ ). Statistical evaluation of the results was performed with the SPSS 9.05 computer program.

## Results:

The nutritional intervention program started with 111 girls; but unfortunately; 38 girls only continued strictly with the program for the 6 months. All of them undergo weight reduction, except 3 girls showed very minimal changes in weight but significant changes in body fat distribution and body composition. Their ages ranged between 15 to 16 years (with mean age  $15.45 \pm 0.50$ ).

Percentage of the girls with different effect of the program; according to the reduction in % body fat; are presented in figure (1). It was noticed that 38.6% (14 girls) had no effect on the % body fat; in spite of the reduction in their weight; 26.3% (10 girls) had reduction less than 5%, 18.4% (7 girls) had reduction between 5 - 10%, and 18.4% (7 girls) had reduction between 10 - 20%.,

Anthropometric measurements at the beginning and the end of the nutritional intervention were summarized in table (1) and figure (2). The main baseline of BMI was  $32.39 \pm 3.13$  Kg/m<sup>2</sup>, which indicates that; on average; these girls were obese at the beginning of the nutritional intervention.

After the 6 months nutritional intervention, highly significant reduction in body weight and waist circumference, and significant reduction in hip circumference were recorded after following the dietary program, nutritional education and physical activity. However, the change in BMI was insignificant. Body fat distribution at the beginning and the end of the nutritional intervention program were summarized in table (2) and figure (3). Highly significant reduction in all the skin fold thickness at the 5 sites (triceps, biceps, sub scapular, suprailiac and abdominal), peripheral and central adiposity, waist circumference and waist-hip ratio was recorded.

Body composition at the beginning and the end of the nutritional intervention program were summarized in table (3). It was observed that there were highly significant reduction in fat % and fat mass, while, total body water and basal metabolic rate showed highly significant increase. However, the fat free mass was not significantly increased or even not changed. The nutritional intervention program was succeeded in 38 obese adolescent girls. These girls show highly significant reduction in body composition and body fat distribution.

**Table (1): Anthropometric measurements before and after the 6 months nutritional intervention**

	Before	After	Changes
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
	(Range)	(Range)	(Range)
<b>Weight (Kg)</b>	<b>86.01 <math>\pm</math> 6.79</b>	<b>82.93 <math>\pm</math> 5.93**</b>	<b>3.08 <math>\pm</math> 2.92</b>
	( 76.00 ~ 94.00 )	(73.80 ~ 92.10)	( - 1.60 ~ 8.60 )
<b>BMI(kg/m<sup>2</sup>)</b>	<b>32.39 <math>\pm</math> 3.13</b>	<b>32.36 <math>\pm</math> 2.14</b>	<b>0.029 <math>\pm</math> 1.48</b>
	(28.50 ~ 37.80)	(30.30 ~ 36.40)	( - 1.90 ~ 1.40 )
<b>Waist C(cm)</b>	<b>85.55 <math>\pm</math> 6.70</b>	<b>80.79 <math>\pm</math> 8.08**</b>	<b>4.76 <math>\pm</math> 2.87</b>
	( 77.00 ~ 97.00 )	(74.00 ~ 95.00)	( 2.00 ~ 10.00 )
<b>Hip C (cm)</b>	<b>105.84 <math>\pm</math> 9.39</b>	<b>100.45 <math>\pm</math> 9.01*</b>	<b>5.39 <math>\pm</math> 2.88</b>
	(93.00 ~ 120.00)	(89.00 ~ 116.00)	( 3.00 ~ 11.00 )

N.B.: The significance was tested using WILCOXON non parametric test

\* Significant ( $p < 0.05$ ); \*\*Highly significant ( $p < 0.01$ )

**Table (2): Fat distribution before and after the 6 months nutritional intervention**

	Before	After	Changes
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
	(Range)	(Range)	(Range)
<b>Skinfold Thickness</b>			
+Triceps	<b>32.68 <math>\pm</math> 6.79</b> (22.050 ~ 42.00)	<b>26.87 <math>\pm</math> 6.65**</b> ( 18.00 ~ 35.00)	<b>5.82 <math>\pm</math> 1.65</b> (4.00 ~ 8.50)
#Biceps	<b>24.74 <math>\pm</math> 5.82</b> ( 17.00 ~ 32.00)	<b>19.62 <math>\pm</math> 5.76**</b> (12.00 ~ 27.00)	<b>5.12 <math>\pm</math> 1.65</b> ( 1.80 ~ 7.00)
#Subscapular	<b>31.26 <math>\pm</math> 5.59</b> (23.00 ~ 41.00)	<b>25.68 <math>\pm</math> 6.51**</b> (18.00 ~ 37.00)	<b>5.58 <math>\pm</math> 2.21</b> (3.00 ~ 10.00)
+Suprailiac	<b>26.89 <math>\pm</math> 7.24</b> (19.00 ~ 40.00)	<b>21.95 <math>\pm</math> 6.19**</b> (15.00 ~ 33.00)	<b>4.95 <math>\pm</math> 1.29</b> ( 2.00 ~ 7.00)
#Abdominal	<b>27.32 <math>\pm</math> 7.95</b> (17.00 ~ 38.00)	<b>21.99 <math>\pm</math> 7.16**</b> (15.00 ~ 32.00)	<b>5.33 <math>\pm</math> 1.54</b> ( 1.00 ~ 6.50)
#Peripheral Adiposity	<b>57.42 + 11.72</b> (44.50 ~ 74.00)	<b>46.49 <math>\pm</math> 11.99**</b> (32.00 ~ 61.00)	<b>10.93 <math>\pm</math> 2.95</b> (7.00 ~ 15.50)
#Central Adiposity	<b>85.47 <math>\pm</math> 18.74</b> (70.00 ~ 119.00)	<b>69.62 <math>\pm</math> 18.44**</b> (51.00 ~ 102.00)	<b>15.86 <math>\pm</math> 3.48</b> ( 6.00 ~ 20.00)
#Waist Circumference(cm)	<b>85.55 <math>\pm</math> 6.70</b> ( 77.00 ~ 97.00)	<b>80.79 <math>\pm</math> 8.08**</b> (74.00 ~ 95.00)	<b>4.76 <math>\pm</math> 2.87</b> ( 2.00~ 10.00)
#Waist-Hip Ratio (cm/cm)	<b>0.81 <math>\pm</math> 0.05</b> ( 0.76 ~ 0.91)	<b>0.81 <math>\pm</math> 0.06**</b> (0.74 ~ 0.91)	<b>0.005 <math>\pm</math> 0.01</b> ( - 0.01 ~ 0.02)

N.B.: # The significance was tested using WILCOXON non parametric test

+ The significance was tested using Paired sample t-test

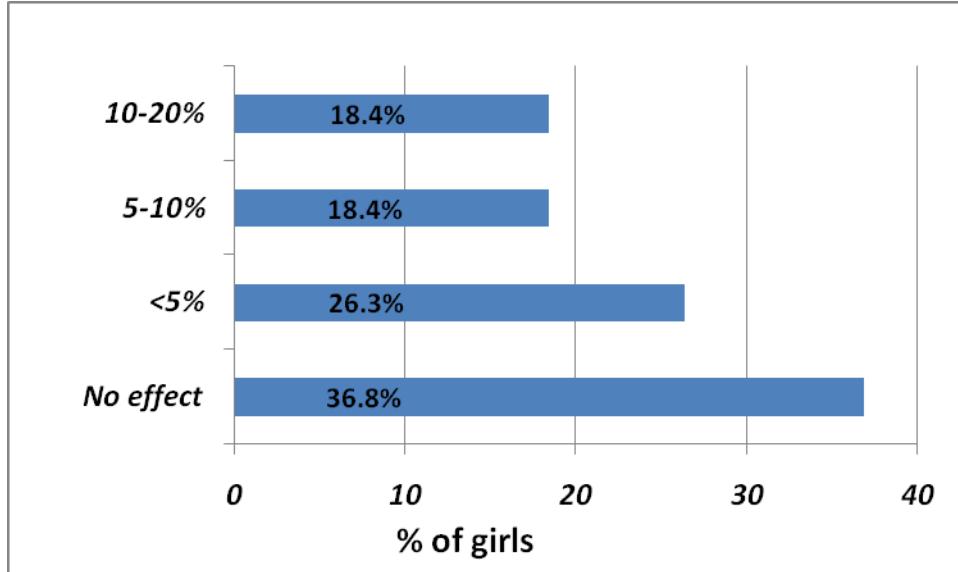
\* Significant ( $p < 0.05$ )      \*\*Highly significant ( $p < 0.01$ )

**Table (3): Body Composition before and after the 6 months nutritional intervention**

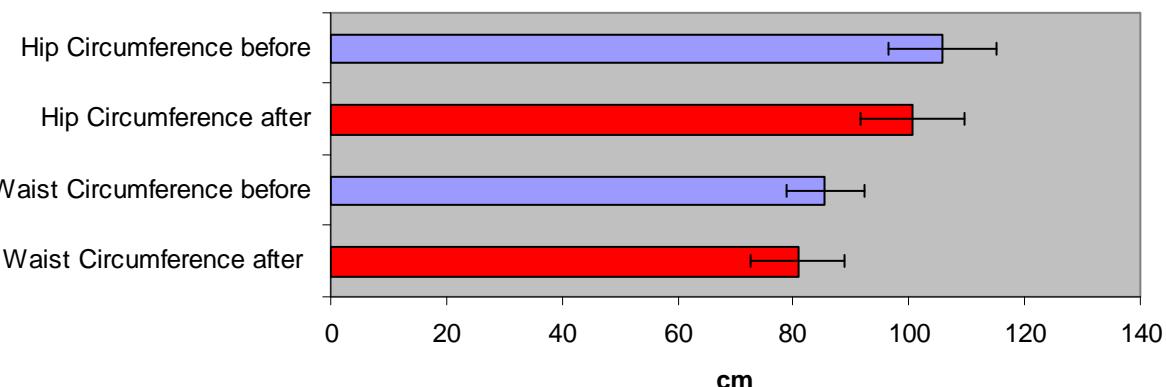
	Before	After	Changes
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
	(Range)	(Range)	(Range)
#Fat %	42.93 $\pm$ 2.96 (37.80 ~ 46.10)	41.40 $\pm$ 3.08** (37.70 ~ 46.30)	1.53 $\pm$ 2.19 ( -1.40 ~ 4.20)
#Fat mass (Kg)	38.04 $\pm$ 3.68 (31.20 ~ 42.40)	34.33 $\pm$ 3.65** (30.70 ~ 39.50)	3.71 $\pm$ 2.61 (0.00 ~ 6.90 )
+Fat free mass(kg)	48.26 $\pm$ 3.57 (42.80 ~ 53.70)	48.60 $\pm$ 4.24 (43.10 ~ 54.80)	- 0.34 $\pm$ 1.24 ( -2.20 ~ 1.60)
#Total body water	28.92 $\pm$ 14.17 (31.30 ~ 39.30)	35.58 $\pm$ 3.09** (31.60 ~ 40.10)	- 0.47 $\pm$ 0.63 ( -1.60 ~ 0.10)
+BMR(KC)	1586.0 $\pm$ 82.21 (1499.00 ~ 1714.00)	1642.32 $\pm$ 62.13** (1542.00 ~ 1714.00)	- 56.32 $\pm$ 34.04 ( - 104.00 ~ 0.00)

N.B.: # The significance was tested using WILCOXON non parametric test

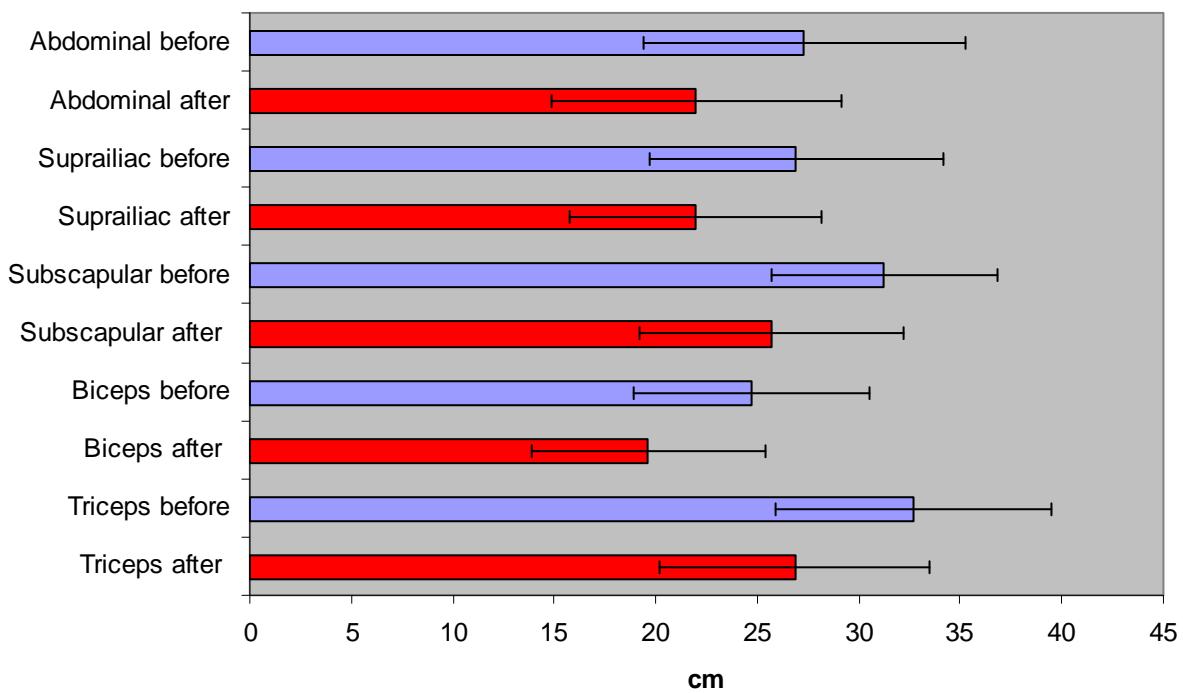
+ The significance was tested using Paired sample t-test

\* Significant ( $p < 0.05$ ); \*\*Highly significant ( $p < 0.01$ )**Fig 1: The different effect of the 6 months nutritional intervention program on the reduction in body fat percentage**

**Fig. 2: Hip and waist circumferences before and after the 6 months nutritional intervention program**



**Fig. 3: Fat distribution before and after the 6 months nutritional intervention program**



## Discussion

Adolescents who are overweight or obese are more likely to remain so in adulthood than pre-adolescents aged 10–14<sup>(15)</sup>, unless the latter obtain treatment.

Treatment for children and adolescents who are overweight or obese seems easy, that is, just counsel children, adolescents and their families to eat less and to exercise more. In practice, however, treatment of childhood obesity is time-consuming, frustrating, difficult, and expensive. In fact, choosing the most effective methods for treating overweight and obesity in children is complex at best. This is especially true for primary care providers, who have limited resources to offer interventions within their offices or programs. The paucity of providers to whom they can refer patients adds to the problem.

A multi-factorial approach has been in use for the treatment of obesity, including dietary modification, exercise, psychotherapy and medication. There are several reports in the literature about exercise programs for adolescents with obesity<sup>(16, 17)</sup>. However, the focus of most programs is on long-lasting endurance activities, which in our opinion are boring for the pediatric population. Furthermore, most programs are not easily reproducible due to lack of detail or requirement of special equipment. Therefore, our aim was to encourage adolescents to do a simple, cheap, cost effective activity which is accessible to everyone and motivates adolescents by walking with their colleagues.

Low physical activity levels may be as important as excess energy intake<sup>(18)</sup>. Although data from previous studies were equivocal, Swinburn et al. (2006)<sup>(19)</sup> found energy intake was a more important determinant of high body weight than low physical activity. TES, 2008<sup>(20)</sup> stated that in the absence of caloric restriction, moderate exercise does not generally cause weight loss. However, in combination with decreased caloric intake, exercise can achieve significant weight loss. This matches our results as with both caloric restriction and regular physical activity over a period of 6 months our patients achieved weight reduction. Wittmeier et al., 2008<sup>(21)</sup>, also, reported that lower durations of both moderate physical activity (MPA) and vigorous physical activity (VPA) are associated with increased odds of overweight and adiposity. They concluded that forty-five minutes of MPA and fifteen minutes of VPA were associated with reduced body fat and

BMI. A total of one hour per day of moderate-intensity activity, such as walking on most days of the week, is probably needed to maintain a healthy body weight<sup>(22)</sup>.

In general, to lose weight, you either have to decrease the amount of calories you are eating and drinking, exercise to burn more calories, or even better, do a combination of both. To lose 1 pound in 1 week, you can decrease your calories by 500 a day or burn 500 extra calories a day. The American Diabetic Association<sup>(23)</sup> stated that: Use of a reduced-energy diet (not less than 1200 kcal/day) in the acute treatment phase for adolescent overweight is generally effective for short-term improvement in weight status; and this is agreed with our data which showed significant reduction in weight of the studied group.

The BMI can be easily assessed at low cost, and has a strong association with body fatness and health risks<sup>(24)</sup>. The subcutaneous skin fold thicknesses have been widely used to estimate body fat. The main advantages are simplicity of use and suitability for epidemiological studies<sup>(25)</sup>. It is used as a measure of nutritional status in children on assumption that increased subcutaneous fat reflects a greater caloric reserve<sup>(26)</sup>. It has been shown to correlate with estimates of total body fat and with lean body mass<sup>(27)</sup>.

In this study, BMI and sum of skin fold thickness were used as indicators of overall adiposity. The sum of skin fold is reported as a reliable estimate of obesity and regional fat distribution<sup>(28)</sup>. WC was considered as an appropriate predictor of abdominal fat in children<sup>(29)</sup> and adolescents<sup>(30)</sup>. Another indicator of abdominal adiposity is WHR, which was reported as a better indicator of adiposity independent of age and sex<sup>(31)</sup>. But in this study the changes of WHR were discrete and not significant, so the future investigations are needed about the sensitivity of WHR in detection of obesity, in the population of Egyptian girls.

Highly significant reduction in waist circumference was also, noticed after following the dietary program and physical activity. However, the change in BMI was not significant. Skin fold thickness at 5 sites (triceps, biceps, sub scapular, suprailiac and abdominal) were significantly decreased. Highly significant reduction in fat mass, peripheral and central adiposity; significant reduction in fat %; and highly significant increase in total body

water were found. There was a highly significant increase of the basal metabolic rate, but the increase of fat free mass was not significant. The BMI didn't show a reduction, and probably one of the possible reasons for this is a highly significant increase of total body water in our investigated girls.

Decrease in the fat percent with preservation of the FFM and increase of the resting metabolic rate (RMR) of the girls was also observed. The weight reduction and maintenance appears to be antagonized by a reduction in the resting metabolic rate (RMR) which comes in agree with our results. As the largest component of daily energy expenditure, RMR comprises approximately 60–70%, Fat-free mass (FFM) is the main factor that accounts for the magnitude of resting metabolism<sup>(32)</sup>. As a heterogeneous compartment, FFM consists of highly metabolically active muscle and organs and low-metabolic rate tissues such as bone and connective tissue<sup>(33)</sup>. Therefore, any diet or exercise interventions, which are capable of maintaining FFM or at least attenuating its decline following weight loss, could have significant effects on total energy balance. The foremost objective of a weight-loss trial has to be the reduction in fat mass leading to a decrease in risk factors for a metabolic syndrome. Both with regard to a reduction in risk factors and long-term weight maintenance the content of adipose tissue in the weight lost has to be maximized, thus preserving FFM<sup>(34)</sup>. A decline in body weight can be achieved while favorably modifying body composition with the maintenance of FFM is through physical activity<sup>(35)</sup>.

Wells and Victoria<sup>(36)</sup>, 2005, also, stated that changes in body composition indicators may have important health implications, as it has been demonstrated that the health risks associated with obesity derive primarily from fat rather than body weight. Moreover, it is not only the total amount of fat that is important, but also the distribution of fat in the body<sup>(37)</sup>, with central fatness being most related to health risks<sup>(38)</sup>. Teixeira et al. (2001)<sup>(39)</sup> showed that measures of central adiposity such as WC and WHR significantly correlated with serum lipid levels in obese children and adolescents but not in leaner individuals. Kelishadi et al. (2007)<sup>(40)</sup> concluded that BMI, WC and WSR were the most appropriate indices in predicting CVD risk factors. Many studies confirming the predictive value of BMI for CVD risk factors<sup>(41, 42)</sup>.

Finally the current research also, revealed that after the 6 months nutritional intervention, reduction in body weight, hip, and waist circumference were significant after following the dietary program. However, the change in BMI was minimal. The reduction in all the measured skin fold thickness at the 5 sites (triceps, biceps, sub scapular, suprailiac and abdominal), reflects a reduction in peripheral and central adiposity. However, the change in waist-hip ratio was minimal.

In summary, it can be concluded from this research that dietary composition can modify the physiological adaptations to energy restriction. A balanced diet with moderate energy restriction together with moderate physical activity and behavioral modification result in body weight loss with preservation of the FFM and a decrease in the fat percentage. This study may contribute in establishing evidence based Egyptian program for obesity and overweight management.

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10/5/2010

# Morphological and Molecular Evidences Among Four Heteroforms of *Avicennia marina* (Forssk) Vierh.

Wafaa M. Said and Nahla O. M. Ehsan\*

Botany Department, Women's College for Arts, Science, and Education, Ain Shams University.

[dr.nahla.osman@gmail.com](mailto:dr.nahla.osman@gmail.com)\*

**Abstract:** Morphological characteristics and random amplified polymorphic DNA (RAPD) marker were used to assess inter-specific relationships among four heteroforms of gray mangrove (*Avicennia marina* (Forssk) Vierh.) grown in Al-Sharm Al-Bahari site, 33Km south Al-Qussier, Red Sea Coast, Egypt. The four heteroforms viz. I, II, III and IV were detected in two distinct habitats (marine and desert). The morphological and molecular data indicated high variation between form I&III and II&IV. On the other hand, low variation between form I&II and III&IV. Dendrogram based on morphological, anatomical and genetic data supported the segregation of the four heteroforms of *Avicennia marina* into two groups; one includes form I & III and the second include form II & IV. The study concluded that the four heteroforms can be classified as two subspecies, *A. marina* subsp. *eucalyptifolia* (form I) and the *A. marina* subsp. *marina* (form II). In addition, forms III and IV considered as phenotypes from I and II, respectively.

[Wafaa M. Said and Nahla O. M. Ehsan. Morphological and Molecular Evidences Among Four Heteroforms of *Avicennia marina* (Forssk) Vierh. Journal of American Science 2010;6(11):843-856]. (ISSN: 1545-1003).

**Keywords:** Mangrove; *Avicennia marina*; Morphology, RAPD; Red Sea

## 1. Introduction:

*Avicennia* L. (F: Avicenneaceae) is a genus of mangrove woody trees or shrubs that grow in coastal habitats. It has the largest longitudinal and latitudinal distribution of all mangrove species, ranging from the east coast Africa from the Red Sea to South Africa to the west pacific from Japan to New Zealand (Le *et al.*, 2003).

Moldenke (1975) and Tomlinson (1986) recorded that the genus *Avicennia* L. shows considerable morphological variation especially in leaves and flowers, and classified based on these attributes. *Avicennia* represents the largest polymorphic genus of the mangrove, where it well known ecologically, systematically, morphologically and genetically in comparison with other taxa (Duke, 1995).

Mabberly (1981) classified the genus *Avicennia* to 14 species according to morphological characters of leaves and flowers. While Tomlinson (1986) and Duke (1991, 1992) classified the *Avicennia* species to four major groups according to their morphological criteria; *A. marina* & *A. alba*, *A. officinalis* & *A. integra*, *A. rhumphiana*, and *A. germinans*, *A. schaueriana* & *A. bicolor*.

*A. marina* (Forssk.) Vierh is an important true mangrove species; it is a halophytic plant, grows as a shrub or tree to a height of three to ten meters. In addition, it has three subspecies; *A. marina australasica*, *A. marina*

*eucalyptifolia* and *A. marina marina* (Schwarzbach and Mc Dade, 2002).

*A. marina* (Forssk.) Vierh represents the dominant mangrove species in Egypt, found along Red Sea Coast ling from Ras-Mohamed to Mersa-Halaib (Zahran, 1993).

Tomlinson (1986) recorded that *A. marina* species characterized by different heteroforms. Four heteroforms of *A. marina* were recorded in Al-Sharm Al-Bahary site, 33 km south Al-Qussier region in two different habitats. Two forms growing in the inundation area, while the other two are found in the desert closed to Red Sea shores. The two habitats have different physico-chemical properties as recorded by khalafallah (2002). Are those four heteroforms, subspecies of *A. marina* oreotypes/phenotypes? These four heteroforms need taxonomical study.

The previous taxonomical studies of genus *Avicennia* L. of *A. marina* species were achieved based on the morphological variations of the vegetative and reproductive organs. The advent of technology that directly examines genes and gene products, it has been found that the morphological characteristics not be completely reliable indicators for genetic variation or taxonomic differences, owing to their tendency to be highly influenced by environmental factors (Brown *et al.*, 1997; Duke *et al.*, 1998 and Bryars & Adam, 1999).

Recently, several studies have been carried out on mangrove species in order to assess genetic diversity using genetic markers, as studies the worldwide genetic diversity of *A. marina* using Allozyme and ALFP & microsatellite markers, RAPD, RLFP, ALFP DNA (Mguire *et al.*, 2000 & 2002 and Le *et al.*, 2003). Gallios & Burrus (1998) and Gauer & Cavalli-Molina (2000) reported that molecular genetic markers can provide a relatively unbiased method of quantifying genetic diversity in plants and their populations.

Said (2008) concluded that DNA based genetic markers have been recently integrated into the important of several plant systems and are expected to play a very important role in the future of molecular genetic and plant taxonomy.

The objectives of this study are: (1) investigation the morphological and anatomical characteristics of the four heteroforms, (b) study the genetic variation of the four heteroforms by application of the RAPD technology and (c) study the taxonomic relationship between the four heteroforms of *A. marina* in Al-Sharm Al-Bahari, Al-Qussier region, Red Sea Coast, Egypt.

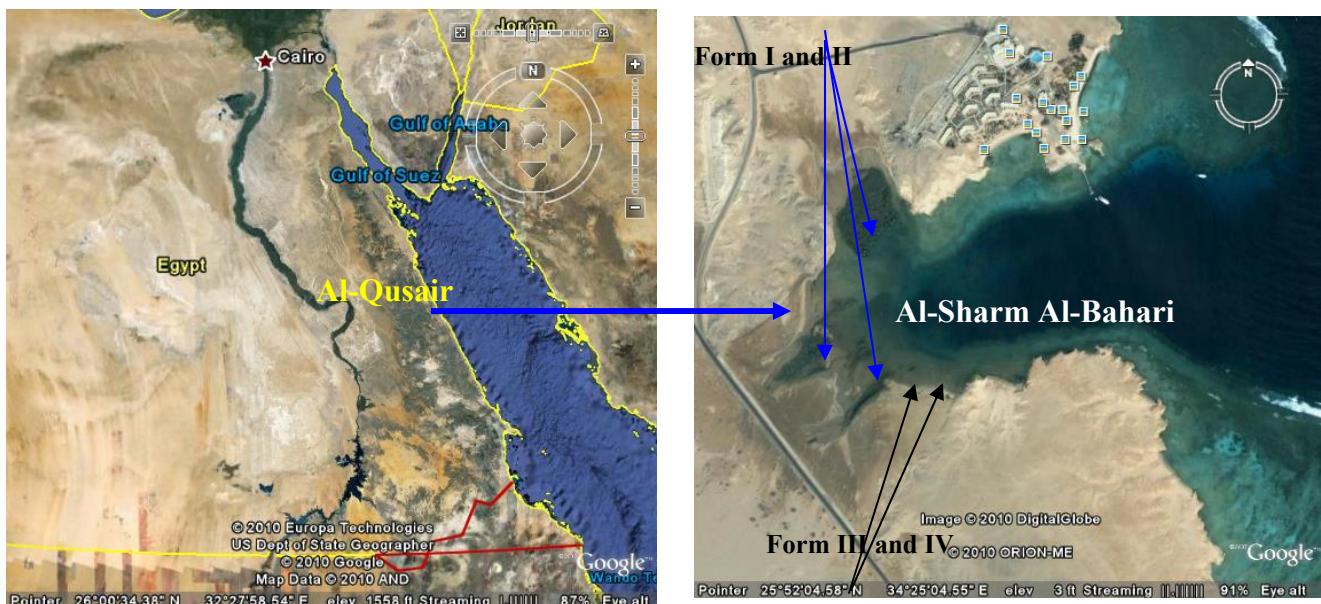
## 2. Materials and methods

### Study Site

Al-Sharm A-Bahari site was chosen as it contains the four heteroforms of *A. marina* plants in two different habitats. Forms I and II are growing in the inundation area, while Forms III and IV are found in the desert closed to Red Sea shores. The site is located at 33km south Al-Qussier city ( $25^{\circ}52'04.58''$  N and  $34^{\circ}25'04.55''$  E, Fig. 1). Physico-chemical properties of the two habitats were analyzed by khallafallh (2002) and recorded in table (1&2). The four forms are photographed and showed in Fig. (2).

### Plant samples collection

Thirteen healthy samples (third internodes, third leaves, flowers and fruits) of the four forms of *A. marina* were collected at May 2008 from Al-Sharm Al-Bahari site, Al-Qusseir Red Sea Coast for the morphological, anatomical and molecular investigations. Voucher specimens are deposited in the herbarium of Botany Department, Women's College for Art, Science and Education, Ain Shams University



**Fig. 1: Location of Al-Sharm Al-Bahari at Al-Qussair region, Red Sea coast, Egypt (Google earth program).**



Photo. 1: Form I found in the inundated area



Photo. 2: Form II found in the inundated area



Photo. 3: Form III found in the desert



Photo. 4: Form IV found in the desert

Fig. 2: The four heteroforms of *A. marina*.

Table 1: Physico-chemical properties of the two habitats soils; A: soil of the inundation area where forms I and II are found and B: soil of the desert where forms III and IV are found

	A	B		A	B	
Granulation %	Gravel	13.8	2.2	Anions (meq/100 g soil)	Cl <sup>-1</sup> 27.9 8.5	
	Coarse sand	20.8	7.4		HCO <sub>3</sub> <sup>-1</sup> 4.35 4.35	
	Medium sand	40.8	61.8		CO <sub>3</sub> <sup>-2</sup> 0.00 0.20	
	Fine sand	17.8	27.8		SO <sub>4</sub> <sup>-2</sup> 32.3 100.4	
	Silt	1.2	0.4	Soluble cations (meq/100 g soil)	Na <sup>+1</sup> 14.78 47.80	
	Clay	5.4	0.4		K <sup>+1</sup> 1.05 2.90	
pH		7.8	8.3		Ca <sup>+2</sup> 1.90 1.90	
EC (dS / m)		18.93	4.18		Mg <sup>+2</sup> 46.7 65.8	
Total soluble salts (g / 100 g)	1.6	Waterlogged	Exchangeable cations (meq / 100 g soil)		Na <sup>+1</sup> 7.83 8.70	
Water content %	21.6	7.2			K <sup>+1</sup> 0.53 050	
NaCl (%)	1.63	0.50			Ca <sup>+2</sup> 18.50 15.60	
CaCO <sub>3</sub> (%)	30.0	19.0			Mg <sup>+2</sup> 245.0 151.7	
Organic mater (g/ 100 g)	0.50	2.77				

Table 2: Physico-chemical properties of water of aquatic habitat of Al-Sharm Al-Bahari site where forms I and II are found

pH	7.8	Cl <sup>-</sup> (meq/L)	609.6	Na <sup>+</sup> (meq/L)	1635
EC (dS / m)	75.1	HCO <sup>3-</sup> (meq/L)	7.30	K <sup>+</sup> (meq/L)	47.9
Total soluble salts (g / L)	47.4	CO <sub>3</sub> <sup>2-</sup> (meq/L)	70	Ca <sup>++</sup> (meq/L)	23.6
Salinity (G/L)	38.1	SO <sub>4</sub> <sup>2-</sup> (meq/L)	1206	Mg <sup>++</sup> (meq/L)	117.6
NaCl (g / L)	35.7				

### Botanical investigation

Plant heights of the four heteroforms were measured in the field. The macro-morphology of the investigated forms was described directly from fresh specimens. Measurements included both numeric attributes (internode length, leaf length, flower length and number of flowers/inflorescence) and coefficient attributes (leaf narrowness and leaf area) as modified from Duke (1990). The micromorphology of stems, petioles and leaves of each form was carried out through hand-microtome cross sections at 10-15 $\mu$ m, stained with safranin and light green according to the methods described by Johanson (1940). The sections were photographed by using light microscope (Olympus) with digital camera (Canon Power Shot S80) connected to computer; the photographs were taken by Zoom Browser Ex program. The dimensions of sections were measured by using Corel Draw program ver. 11.

### Molecular investigation

Total genomic DNA was extracted from 0.5g fresh young leaves of the four *A. marina* heteroforms according to the Dellaporta *et al.* (1983). The extraction examined by RAPD-PCR marker at Genetic Engineering and Biotechnology Center, Ain Shams University to determine inter and intra specific variations.

Based on RAPD markers for amplification of unknown DNA sequences; single and short random oligonucleotide primers were used. Ten-mer random DNA oligonucleotide primers (UBC) were obtained from Operon kit (Operon Tech. Inc., USA). Their code and sequences were listed in table (3). After electrophoresis of the RAPD pattern, amplification was carried out according to Williams *et al.* (1990). Amplification products were size-fractionated on a 1% w/v agarose gel containing 10mg ml<sup>-1</sup> of ethidium bromide. They were visualized under UV light and photographed. The gels were documented using gel documentation advanced software UVP-England Program.

**Table (3): List of primers and their nucleotide sequences**

GC %	Sequences(5' to 3')	Primer code	Number
<b>1</b>	Op- A19	CAA ACG TCG G	60%
<b>2</b>	Op- A3	AGT CAG CCA C	60%
<b>3</b>	Op-A7	GAA ACG GGT G	60%
<b>4</b>	Op- A18	AGG TGA CCG T	60%
<b>5</b>	Op- B17	AGG GAA CGA G	60%
<b>6</b>	Op- Z 7	CCA GGA GGA C	70%
<b>7</b>	Op- D3	GTC GCC GTC A	70%
<b>8</b>	Op- B15	GGA GGG TGT T	60%
<b>9</b>	Op - C2	GTG AGG CGT C	70%
<b>10</b>	Op- C5	GAT GAC CGC C	70%

### Data analysis

Standard deviations of the morphological and anatomical characters were calculated. The data were treated statistically using the one-way analysis of variance (ANOVA) as described by Snedecor and Cochran (1969), the means were compared by LSD using SPSS program version 15. The morphological, anatomical and genetic evidences were scored for presence (1) or absent (0) to the computation analysis under a program using similarity and dissimilarity assessment percentage method ver. 2.02 Rohlf (1998).

### 3. Results

#### Macromorphological investigations

Macromorphological characters of the four heteroforms *Avicennia marina* described or measured and recorded in tables 4&5. The recorded data

indicated that from 59 studied characters, the four heteroforms shared in 51 (86%) characters but differed in 8 (14%) characters (habitat, stem surface, node shape, pneumatophores presence, leaf base, leaf shape, leaf apex, upper surface colour of leaf). Form I&II growing in aquatic and have pneumatophores, while forms III and IV growing in desert without pneumatophors. The main difference focused between I&III as a group and II&IV as other group (table 4).

There is no significant difference between heights of forms I and II also forms III and IV, while it significantly differed between forms I&II as a group and forms III&IV as other group (Table 5). The other measured dimensions; internode length, petiole length, leaf length, leaf width, leaf narrowness, leaf area and no. of flowers/inflorescence significantly differed between forms I&III as a group and forms II&IV as other group.

**Table 4: Macromorphological characters of the four heteroforms of *Avicenna marina***

			I	II	III	IV
<b>Whole plant</b>	<b>Habitat</b>	Aquatic	1	1	0	0
		Desertic	0	0	1	1
	<b>Habit</b>	Shrub	1	1	1	1
	<b>Bark color</b>	Grey	1	1	1	1
	<b>Stem surface</b>	Smooth	1	0	1	0
		Flaky	0	1	0	1
	<b>Node shape</b>	Normal	1	0	1	0
		Swollen	0	1	0	1
	<b>Pneumatophores</b>		1	1	0	0
<b>Petiole</b>	<b>Base</b>	Narrow-grooved	1	1	0	1
		Circle	0	0	1	0
	<b>Texture</b>	Hairy	1	1	1	1
<b>Leaf</b>	<b>Type</b>	Simple	1	1	1	1
	<b>Shape</b>	Lanceolate	1	0	1	0
		Ovate	0	1	0	1
	<b>Color (upper surface)</b>	Dark green	1	0	1	0
		Light green	0	1	0	1
	<b>Color (lower surface)</b>	Grey	1	1	1	1
	<b>Margin</b>	Entire	1	1	1	1
	<b>Apex</b>	Acuminate	1	0	1	0
		Acute	0	1	0	1
	<b>Arrangement</b>	Opposite decussate	1	1	1	1
	<b>Venation</b>	Pinnate	1	1	1	1
	<b>Texture (upper surface)</b>	Hairless	1	1	1	1
	<b>Texture (lower surface)</b>	Hairy	1	1	1	1
<b>Inflorescence</b>	<b>Type</b>	Cymose	1	1	1	1
	<b>Shape</b>	Capitate	1	1	1	1
	<b>Branching</b>	Terminal Axillan on long stocks	1	1	1	1
	<b>Position</b>	Axillary on long staks	1	1	1	1
<b>Flower</b>	<b>Type</b>	Regular	1	1	1	1
	<b>Sex</b>	Bisexual	1	1	1	1
	<b>Size</b>	Small	1	1	1	1
<b>Calyx</b>	<b>Shape</b>	Bell-shaped	1	1	1	1
	<b>Fusion</b>	Gamosepalous	1	1	1	1
	<b>Number of calyx lobe</b>	5-lobed	1	1	1	1
	<b>Texture</b>	Hairy	1	1	1	1
	<b>Nature</b>	Persistent	1	1	1	1
	<b>Color</b>	Green	1	1	1	1
<b>Corolla</b>	<b>Shape</b>	Funnel shape	1	1	1	1
	<b>Fusion</b>	Gamopetalous	1	1	1	1
	<b>Number of petals</b>	Four	1	1	1	1
	<b>Texture</b>	Glabrous	1	1	1	1
	<b>Nature</b>	Deciduous	1	1	1	1
	<b>Color</b>	Yellow	1	1	1	1
<b>Androecium</b>	<b>Stamens type</b>	Epipetalous	1	1	1	1
	<b>Number of stamens</b>	Four	1	1	1	1

**Table 4 cont.**

			<b>I</b>	<b>II</b>	<b>III</b>	<b>IV</b>
<b>Gynoecium</b>	<b>Number of anther locules</b>	Four	1	1	1	1
	<b>Anther fertility</b>	Fertile	1	1	1	1
	<b>Anther Dehiscence</b>	Longitudinal slits	1	1	1	1
	<b>Type</b>	2-Carpelled	1	1	1	1
	<b>Fusion</b>	Syncarpous	1	1	1	1
	<b>Ovary type</b>	Superior	1	1	1	1
	<b>Ovary texture</b>	Hairy above & glabrous beneath	1	1	1	1
	<b>No. of locules</b>	4-locular	1	1	1	1
	<b>No. of ovules/locuoli</b>	One	1	1	1	1
	<b>Placenta Type</b>	Axile	1	1	1	1
<b>Fruit</b>	<b>Style texture</b>	Hairy	1	1	1	1
	<b>Stigma Shape</b>	Bilobed	1	1	1	1
<b>Seeds</b>	<b>Stigma texture</b>	Glabrous	1	1	1	1
	<b>Type</b>	Fleshy	1	1	1	1
	<b>Capsule dehiscence</b>	Bivalved	1	1	1	1
	<b>Type</b>	Endospermic	1	1	1	1
	<b>Texture</b>	Glabrous	1	1	1	1
	<b>Color</b>	Green	1	1	1	1
	<b>Germination</b>	Epicotyle	1	1	1	1
	<b>Embryo type</b>	Crypto-viviparous	1	1	1	1

I: Form I found in the inundated area  
III: Form III found in the desert

II: Form II found in the inundated area  
IV: Form IV found in the desert

**Table 5: Morphological attributes of four heteroforms of *A. marina* plants**

<b>Attributes</b>	<b>I</b>	<b>II</b>	<b>III</b>	<b>IV</b>
<b>Plant height (m)</b>	2.90a ± 0.52	2.85a ± 0.48	1.80b ± 0.32	1.65b ± 0.25
<b>Internode length (cm)</b>	6.6a ± 0.23	2.6b ± 0.08	5.0a ± 0.20	3.0b ± 0.15
<b>Petiole length (cm)</b>	1.5a ± 0.24	1.2b ± 0.13	1.5a ± 0.24	1.1b ± 0.09
<b>Leaf length (cm)</b>	7.3a ± 0.46	6.5b ± 0.87	7.5a ± 0.87	6.5b ± 0.8
<b>Leaf width (cm)</b>	2.1b ± 0.16	3.5a ± 0.46	2.3b ± 0.24	3.6a ± 0.68
<b>No. of flowers/inflorescence</b>	21b ± 1.07	27a ± 1.18	24b ± 1.28	27a ± 1.01
<b>Flower Overall length (m. m)</b>	3.0b ± .04	5.0a ± 0.06	3.5b ± 0.06	6.0a ± 0.24
<b>Leaf narrowness 4w (cm)</b>	3.6a ± 0.07	2.0c ± 0.06	3.2a ± 0.12	2.6b ± 0.08
<b>Leaf area LxW/2 (cm<sup>2</sup>)</b>	7.7b ± 0.22	11.4a ± 0.54	8.6b ± 0.38	11.7a ± 43

Values have the same latter in the same raw is not significant at  $P>0.05$

I: Form I found in the inundated area  
III: Form III found in the desert

II: Form II found in the inundated area  
IV: Form IV found in the desert

no. of mid rib vascular bundles, bundle sheath growth and sclerenchyma cells presence.

#### Micromorphological investigations

From thirty one anatomical characters, the four heteroforms of *Avicennia marina* differed in six characters (table 6 and figs. 3, 4&5). The main differed character is focused on stem outline. Forms I and III have circular outline while forms II and IV have angular outline. The other differed characters are thickness of cuticle layer of stem, petiole and leaf,

The anatomical data of the four heteroforms of *Avicennia marina*; stems, petioles, leaves, showed a significant difference between forms I&II as a group and Forms III&IV as other group in more than 44% of the studied characters (Table 7).

**Table (6): Micromorphological characters of the four heteroforms of *Avicenna marina***

			<b>I</b>	<b>II</b>	<b>III</b>	<b>IV</b>
<b>Stem</b>	<b>Outline</b>	Circular	1	0	1	0
		Angular	0	1	0	1
<b>Cuticle layer</b>	Thick	1	1	0	1	
	Thin	0	0	1	0	
<b>Epidermal cells</b>	Radial	1	1	1	1	
<b>Epidermal hairs types</b>	glandular non glandular hairs	1	1	1	1	
<b>Gortical cells types</b>	Parenchyma, Collenchyma, Sclerenchyma & air spaces	1	1	1	1	
<b>Pericycle</b>	Continuous ring of sclerenchyma	1	1	1	1	
<b>Cambium origin</b>	Inner most Cortical layers & pericycle	1	1	1	1	
<b>V. B. shape</b>	Angular	1	1	1	1	
<b>phloem</b>	Continuous layer of phloem & conjunctive Parenchyma	1	1	1	1	
<b>Hypoderms</b>	2-6 row	1	1	1	1	
<b>Xylem</b>	Continuous cylinder interfascicular rays	1	1	1	1	
<b>Secondary Thickening</b>	Anomalous	1	1	1	1	
<b>Pith cells Type</b>	Parenchyma & Sclerenchyma	1	1	1	1	
<b>Petiole</b>	<b>Out line shape</b>	Crescent with narrow groove	1	1	1	1
	<b>Cuticle</b>	Thick	1	0	0	1
		Thin	0	1	1	0
	<b>Epidermal cells</b>	Radial	1	1	1	1
	<b>Epidermal hairs</b>	Salt glands & non glandular septate uni seriate hairs	1	1	1	1
	<b>Ground tissue</b>	Collenchyma, sclerenchymas & airspaces	1	1	1	1
	<b>Main V. B. shape</b>	Arcshaped	1	1	1	1
	<b>No of additional small V. B.</b>	One pair	1	1	1	1
	<b>Small V. B. type</b>	Concentric & amphicribral	1	1	1	1
<b>Leaf</b>	<b>Cuticle layer</b>	Thick	1	0	1	1
		Thin	0	1	0	0
	<b>Epidermal cells</b>	Elongated	1	1	1	1
	<b>Epidermal hairs (U.S.)</b>	Salt glands	1	1	1	1
	<b>Epidermal hairs (L.S.)</b>	Salt glands & non glandular hairs	1	1	1	1
	<b>Hypodermal layer</b>	Several layers (6-8 rows)	1	1	1	1
	<b>Mesophyll type</b>	Dorsiventral	1	1	1	1
	<b>Mid rib V. B. No.</b>	One	1	1	1	0
		Two	0	0	0	1
	<b>Mid rib V. B. type</b>	Concentric & mphicribral	1	1	1	1
	<b>Bundle sheath</b>	Completed	0	1	1	1
		Uncompleted	1	0	0	0
	<b>Sclerenchyma cells presence</b>		1	1	0	0

U.S.: Upper surface L.S.: Lower surface

I: Form I found in the inundated area

III: Form III found in the desert

V. B.: Vascular bundle

II: Form II found in the inundated area

IV: Form IV found in the desert

**Table 7: Anatomical features of 3<sup>rd</sup> internode, leaf and petiole of 4 heteroforms of *A. marina* ( $\pm$  SD)**

	I	II	III	IV
<b>Internode</b>				
Diameter	2753.4b $\pm$ 158	2941.2b $\pm$ 164	3670.2a $\pm$ 171	3738.0a $\pm$ 176
Hair thickness	145.49a $\pm$ 6.2	104.8b $\pm$ 4.7	112.0b $\pm$ 5.1	141.8a $\pm$ 6.9
Cortex thickness	401.52b $\pm$ 15.3	402.2b $\pm$ 16.0	422.5a $\pm$ 17.6	411.9a $\pm$ 16.4
Cylindrical vascular diameter	1985.8b $\pm$ 141	2015.4b $\pm$ 150	2376.3a $\pm$ 164	2305.9a $\pm$ 157
Fiber layer thickness	54.3b $\pm$ 4.2	54.1b $\pm$ 4.2	102.3a $\pm$ 7.6	98.5a $\pm$ 6.8
Phloem thickness	136.4ab $\pm$ 7.5	119.4b $\pm$ 6.6	132.5a $\pm$ 8.2	142.4a $\pm$ 8.9
Xylem thickness	216.2b $\pm$ 7.6	242.5a $\pm$ 12.7	250.4a $\pm$ 12.5	253.0a $\pm$ 11.9
Pith diameter	786.5d $\pm$ 36.1	1011.8c $\pm$ 47.6	1755.2a $\pm$ 51.4	1354.1b $\pm$ 49.2
<b>Leaf</b>				
Mid rib thickness	1200.2b $\pm$ 96.8	1225.4b $\pm$ 102.4	1298.6a $\pm$ 110.3	1362.1a $\pm$ 112.5
Mid rib V. B. length	498.4c $\pm$ 21.5	589.8b $\pm$ 26.4	656.6a $\pm$ 28.2	678.1a $\pm$ 31.0
Mid rib V. B. width	895.7a $\pm$ 54.3	786.4b $\pm$ 43.2	912.2a $\pm$ 63.2	817.4b $\pm$ 57.6
Wing thickness	574c $\pm$ 30.5	647.8b $\pm$ 33.2	668.6b $\pm$ 36.4	773.0a $\pm$ 38.5
Hypodermis thickness	261.1c $\pm$ 10.1	274.2bc $\pm$ 10.8	282.6b $\pm$ 11.4	309.81a $\pm$ 13.5
Palisade thickness	223.5c $\pm$ 8.7	241.0b $\pm$ 12.4	231.2c $\pm$ 9.6	254.8a $\pm$ 12.6
Spongy thickness	142.9d $\pm$ 5.3	177.5c $\pm$ 6.4	193.4b $\pm$ 8.2	207.0a $\pm$ 8.7
Hair thickness	122.5c $\pm$ 4.2	140.5b $\pm$ 7.6	145.8b $\pm$ 6.2	195.2a $\pm$ 8.8
No of Xylem arches	45b $\pm$ 6	43b $\pm$ 7	55a $\pm$ 8	45b $\pm$ 6
<b>Petiole</b>				
Vertical thickness	1219.0b $\pm$ 86	1231.0b $\pm$ 95	1249.6a $\pm$ 99	1262.7a $\pm$ 83
Horizontal thickness	2066.2c $\pm$ 133	2280.4b $\pm$ 142	2291.1b $\pm$ 140	2382.0a $\pm$ 135
Hair thickness (outer)	112.8a $\pm$ 5.7	97.4b $\pm$ 6.2	90.7b $\pm$ 4.5	123.5a $\pm$ 5.8
Hair thickness (inter)	350.1c $\pm$ 4.9	426.7a $\pm$ 9.3	318.3b $\pm$ 7.5	445.0a $\pm$ 10.2
Xylem thickness	147.1d $\pm$ 5.4	184.0b $\pm$ 4.3	165.2c $\pm$ 6.3	216.1a $\pm$ 4.8
Phloem thickness	53.8d $\pm$ 3.4	76.6c $\pm$ 3.6	101.6b $\pm$ 5.3	135.4a $\pm$ 5.6
No of Xylem arches	52c $\pm$ 5	68a $\pm$ 4	62b $\pm$ 4	76a $\pm$ 5
No. of V. B.	3b $\pm$ 0	3b $\pm$ 0	5a $\pm$ 0	5a $\pm$ 0

Values have the same latter in the same raw is not significant at  $P>0.05$

I: Form I found in the inundated area

II: Form II found in the inundated area

III: Form III found in the desert

IV: Form IV found in the desert

#### Genetic characters based on RAPD analysis

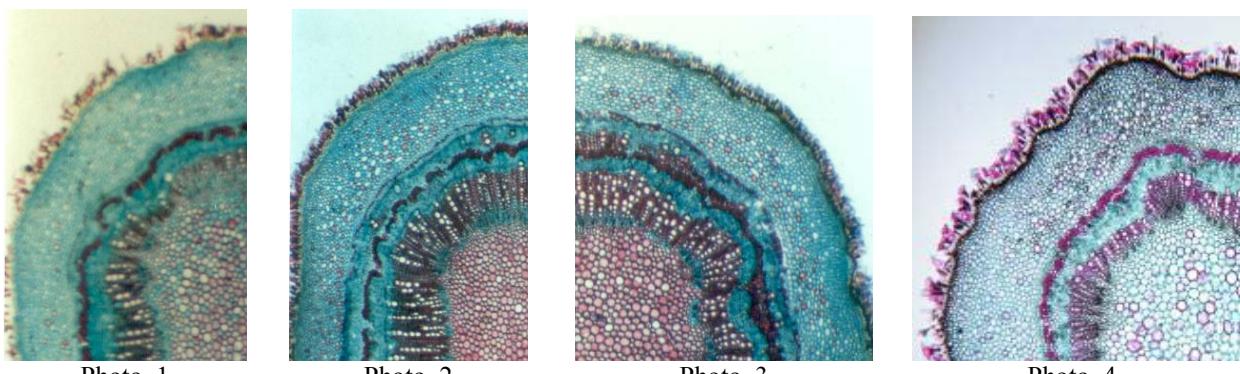
Ten-mer arbitrary oligonucleotide primers were initially used to establish RAPD- PCR fingerprints of the four forms *A. marina* plants, the results were demonstrated in table (8) and Fig (6).

A total number of 95 fragments were visualized across the four heteroforms. The number of bands was variable in each form as present or absent with a particular length in the RAPD patterns and change in the intensity of amplification of fragments with the same length. The primers produced band numbers ranging from 5 (primer OP-CO5) to 17 (primer OP-D-3) with size ranges between 100-1000 bp.

DNA polymorphism recorded 59 polymorphic bands with an average 5.9 polymorphic

fragments per primer, and the polymorphism percentage ranged from 42.857% (primer OPA-18) to 81.818% (primer OPB-17) with an average 61.73 %. On the other hand the highest monomorphic bands were 8 bands at primer OPD-3.

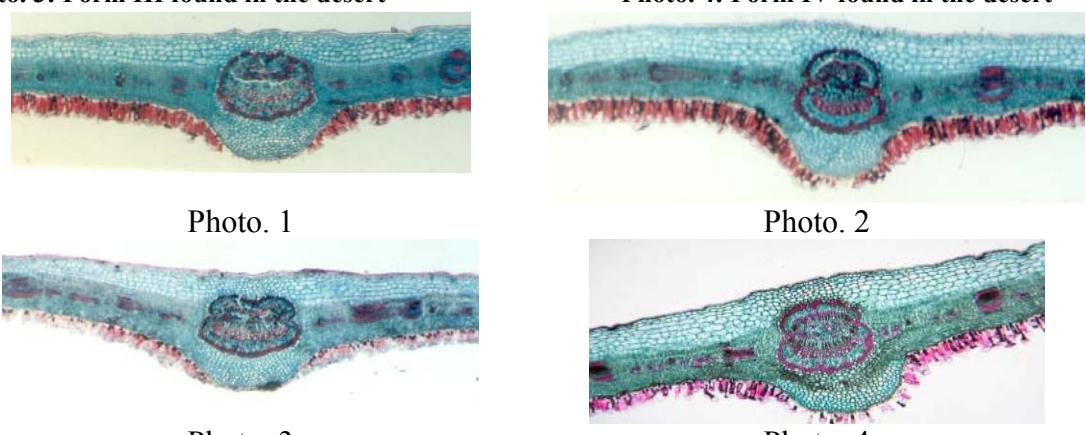
Table (9) and Fig (6) represent the distribution of molecular weight of unique bands. The maximum no. of the unique bands was observed in primer OP-A-7 and OP-Z-7 (6 and 7, respectively). The maximum no. of unique bands were revealed in form I (13 bands) and form III (10 bands) at primers OP-A3, OP A-7, OPA-18, OPA-19, OPB-15, OP B-17, OPCO-5, OP-D-3 and OP-Z-7. On the other hand, the minimum no. was revealed in forms II and IV (1 band for each form) at primers OP-D-3 and OP-Z-7, respectively.



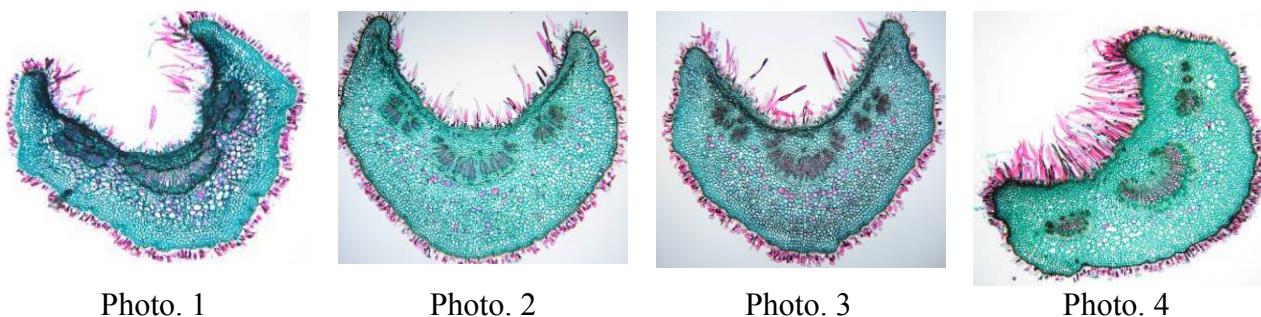
**Fig. 3: Transverse sections of the 3<sup>rd</sup> internodes of the four *A. marina* (X 160).**

**Photo. 1: Form I found in the inundated area**  
**Photo. 3: Form III found in the desert**

**Photo. 2: Form II found in the inundated area**  
**Photo. 4: Form IV found in the desert**



**Fig. 4: Transverse sections of the 3<sup>rd</sup> leaf of the four *A. marina* heteroforms (X 160).**



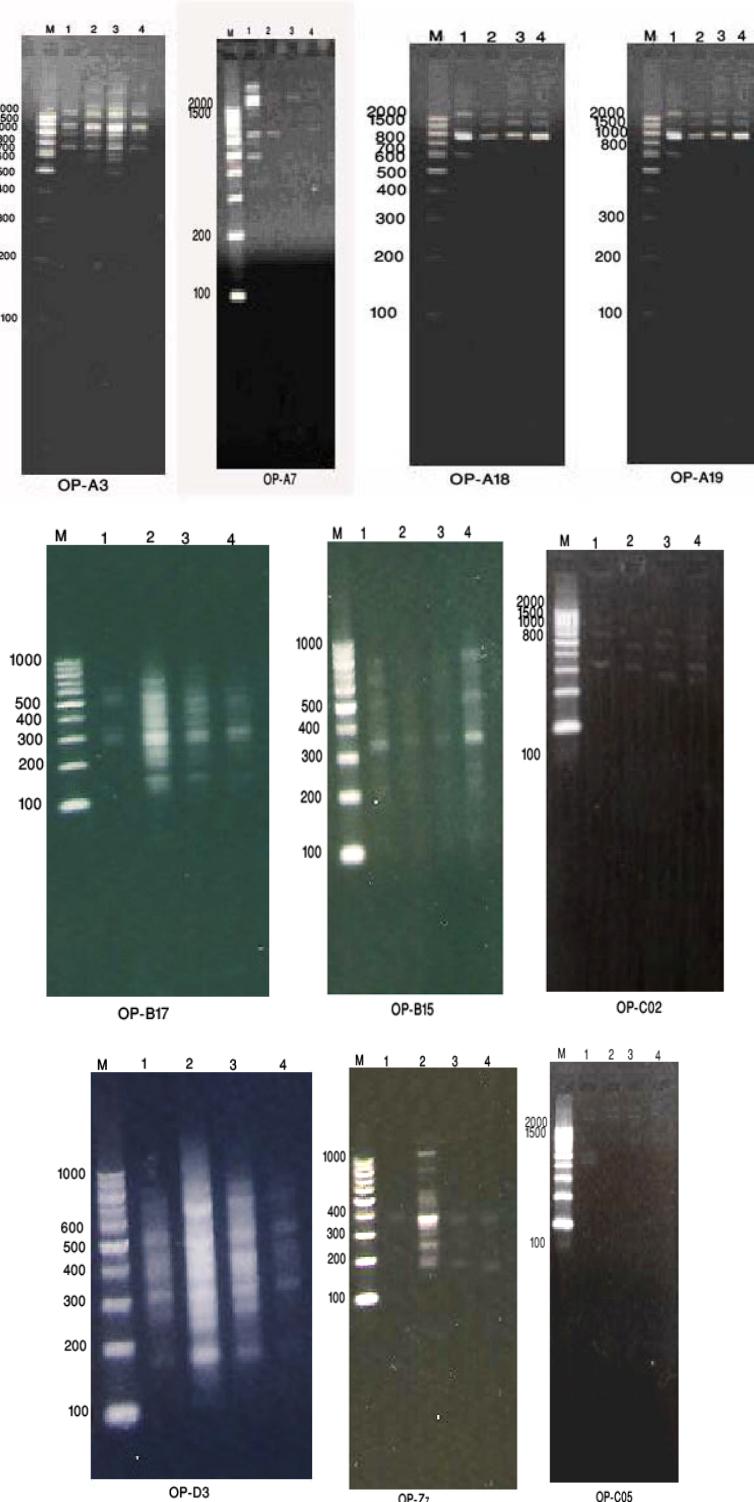
**Fig. 5: Transverse sections of the leaf petiole of the four *A. marina* heteroforms (X 160).**

**Photo. 1: Form I found in the inundated area**  
**Photo. 3: Form III found in the desert**

**Photo. 2: Form II found in the inundated area**  
**Photo. 4: Form IV found in the desert**

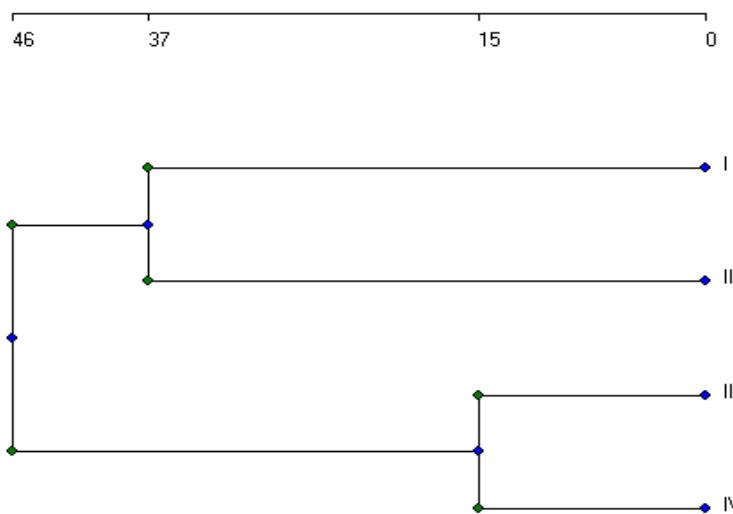
Clustering technique analysis based on morphological, anatomical and genetic evidences resulted a dendrogram, classified the four heteroforms of *Avicennia marina* to two groups. The first group contains form I and from III separated at

distance 37, while the second group contain form II and form IV separated at distance 15 (fig. 7).



**Fig 6: RAPD polymorphism of four heteroforms of *Avicennia marina* with ten random primers.**

**M: Marker    1: Form I found in the inundated area    2: Form II found in the inundated area  
3: Form III found in the desert    4: Form IV found in the desert**



**Fig. 7: Dendrogram of the four heteroforms of *A. marina* based on their morphological, anatomical and genetic evidences.**

**Table 8 Number of total bands, monomorphic (common) bands and polymorphic bands percentage of polymorphism revealed by the ten 10-mer primers in the four heteroforms of *Avicennia marina* by RAPD marker**

Primers	Total no. of bands	Monomorphic bands	Polymorphic bands		Polymorphism %
			Unique	Non unique	
<b>OP-A3</b>	10	5	4	1	50%
<b>OP-A-7</b>	11	4	1	6	63.636%
<b>OP-A-18</b>	7	4	1	2	42.857%
<b>OP-A-19</b>	9	2	3	4	77.778%
<b>OP-B-15</b>	9	4	4	1	55.556%
<b>OP-B-17</b>	11	2	8	1	81.818%
<b>OP-Co-2</b>	5	2	3	--	60%
<b>OP-Co-5</b>	5	2	2	1	60%
<b>OP-D-3</b>	17	8	7	2	52.941%
<b>OP-Z-7</b>	11	3	1	7	72.727%
<b>Total</b>	95	36	59		Average 61.73%

**Table 9: The distribution and molecular weight of unique bands (markers) revealed by RAPD among the examined samples of four heteroforms of *A. marina*.**

Primers	Samples		No. of unique band	MW (bp)
	Unique band number	Form		
<b>OP-A3</b>	(7)	Form (3)	1	856.962
<b>OP-A-7</b>	(1,3,5,6,9,10)	Form (1)	6	2080.949-1838.408-1495.348-1252.729-723.624-662.324
<b>OP-A-18</b>	(1,7)	Form (1)	2	1885.503-798.693
<b>OP-A-19</b>	(2,5) (4,9)	Form (1) Form (3)	2 2	2013.551-1702.76 1417.485-879.969
<b>OP-B-15</b>	(1)	Form (1)	1	1034.664
<b>OP-B-17</b>	(10)	Form (1)	1	166.516
<b>OP-Co-2</b>	-	-	-	-
<b>OP-Co-5</b>	(5)	Form (1)	1	601.872
<b>OP-D-3</b>	(9) (10)	Form (2) Form (3)	1 1	455.051 450.179
<b>OP-Z-7</b>	(1,2,4,5,7,8) (10)	Form (3) Form (4)	6 1	1144.343-900.085-612.982-482.142-349.262-306.582 247.851

#### 4. Discussion

Morphological, anatomical and genetic evidences of *Avicennia marina* four heteroforms in two different habitats at Al-Sharm Al-Bahari site, Al-Qussier region, Red Sea Coast, Egypt, were studied to reveal the taxonomic inter-specific relationships among them. Two forms of *Avicennia marina* (named form I and form II) are grown in marine aquatic habitat in the inundation area. The other two forms (named form III and form IV) are grown in the desert habitat. Significant difference recorded between the physico-chemical properties of the habitat's soil (texture, pH, EC, salt content and minerals content). The main difference between the two habitats focused on soil water content, the first habitat is waterlogged, while the second one have low water content and high aeration. The environmental differences in the two habitats according to many studies (Tomlinson, 1986; Duke, 1990; lakshmi *et al.*, 2000; Melville and Burchett, 2002; Melville, *et al.*, 2004; Chen, *et al.*, 2008; Deng *et al.*, 2009; Salas-Leiva *et al.*, 2009) have effects on the morphological, anatomical and genetic evidences of the four heteroforms.

Present study recorded high variations in the morphological characters between forms I & II (growing in aquatic habitat) and between forms III & IV (growing in the desert habitat). On the other hand, low variations between forms I & III and also between forms II & IV were recorded. Negative correlation has been recorded between habitat properties and morphological characters of stem, leaf and petiole. These results indicated that form I and form II in spite of they are growing in the same habitat, but they have low similarity index. On the other hand form I and form III while they are growing in two different habitats, but they have high similarity index.

Tomlinson (1979) reported that some morphological characters are stable in different habitats and they are genetically controlled as leaf apex, leaf shape, and stem surface.

Duke (1990) and Duke *et al.* (1998) found negative correlation between morphological criteria of *Avicennia marina* leaves and environmental conditions. In this respect, Melville and Burchett (2002) reported that, leaf morphology may be used as a genetic marker of population differentiation. The measurements included leaf area, length, average width, apex, thickness and succulence or water content.

According to Tomlinson (1986) and Melville and Burchett (2002), the morphological characters separated the four heteroforms to two grouped, the first group contains form I and form III, while the 2<sup>nd</sup> group contains form II and form IV.

The anatomical features of *Avicennia marina* four heteroforms in the present study are in agreement with description of Fahn and Shimony (1977), Metcalfe and Chalk (1979) and Tomlinson (1986). Anatomical sections of the four heteroforms have the same structure but with differed in the section layers thickness (cuticle, cortex, phloem, xylem, etc). The difference in layers thickness can be attributed to the environmental variations. This hypothesis is supported by the present findings, where as form I and form II found in the same environmental conditions (waterlogged habitat) and have significant differences in the morphological characters, but they differed in their stem, petiole and leaf dimensions. Stem outline of form I and III is circular while that of forms II and IV is angular. Tomlinson (1986) concluded that the inundated plants are more frequently by the saline tidal water than those of the ridge plants, the former group has to maintain large number of narrow vessels overcoming cultivation problem, density and diameter of vessels are influenced by environmental fluctuation. Recent anatomical data of the four heteroforms of *Avicennia marina* didn't show clear trend in forms classification.

Genetic diversity has been recorded in populations of mangrove species. Nettel and Dodd (2007) and Nettel *et al.* (2008) observed genetic diversity for *A. germinans* population along the Pacific coast of Central America. Results of Melville and Burchett (2002) indicate that the genetic differences among three estuaries populated by Australian *Avicennia marina* were not greatly influenced by sediment characteristics, but rather by geographic distance.

Random amplified polymorphic DNA (RAPD) marker was used to assess genetic diversity and inter-specific relationships among the four heteroforms of Egyptian *Avicennia marina*. The present results obtained from RAPD analysis revealed that low genetic variation between forms I & III and forms II & IV but high genetic variation were detected between forms I&II and forms III&IV. These results beside it can used as indicators in species taxonomy, Allphin *et al.* (1998), Hartl (1988) and Mitton, (1989) considered that the greater levels of genetic variation within species and populations are an advantage in the face of the environmental and anthropogenic challenges.

The data obtained from morphological, anatomical criteria and RAPD analysis suggested segregation of the four heteroforms of *Avicennia marina* into two groups, the first group contains Form I and form III, while form II and form IV represent the 2<sup>nd</sup> group.

Results of the present study on the four heteroforms provide evidence for one species, *A.*

*marina*, comprising two subspecies. According to Tomlinson (1986), Duke (1990), Duke *et al.* (1998) and Moldenke (1960 and 1967), forms I & III are *A. marina* (Forsk.) Vierh. variety *eucalyptifolia* and forms II & IV are *A. marina* (Forsk.)Vierh. variety *marina*.

According to the familiar formula of Stace (1980); Genotype + Environment → phenotype, it can be considered form III is a phenotype to form I and form IV is a phenotype to form II.

In summary, from the morphological, anatomical and molecular evidences, *Avicennia marina* population in Al-Sharm Al-Bahari site, Al-Qussier region, Red Sea Coast, Egypt, contain two subspecies; *A. marina* (Forsk.) Vierh. variety *eucalyptifolia* and *A. marina* (Forsk.)Vierh. variety *marina*. The two subspecies have distinct morphological characters and they grow in aquatic habitat. The environmental factors play a part in modifying the genotype to produce the phenotype. Both subspecies has phenotype grows in desert habitat.

#### Corresponding author

Nahla O. M. Ehsan\*

Botany Department, Women's College for Arts, Science, and Education, Ain Shams University.

[dr.nahla.osman@gmail.com](mailto:dr.nahla.osman@gmail.com)\*

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9/3/2010

# A High Temperature Sensitive Micro-Planes Damage Model for Plane Concrete

Mojtaba Labibzadeh<sup>1</sup>, Hamid Reza Ghafouri<sup>1</sup>, Ali Edalatbehbahani<sup>1</sup>

<sup>1</sup>Department of Civil Engineering, Faculty of Engineering, Shahid Chamran University, Ahvaz, Iran  
[Labibzadeh\\_m@scu.ac.ir](mailto:Labibzadeh_m@scu.ac.ir)

**Abstract:** A computational model simulating the behavior of the concrete subjected to the high temperature environment has been presented here by means of micro-planes framework. The constitutive equations using damage formulations developed earlier by (Labibzadeh and Sadrnejad, 2006) have been adapted here to account for the effects of elevated temperatures. These damage formulations have been founded upon five fundamental damage functions which are directly related to the loading history of each micro-plane. The characteristic features of the proposed model have been verified through making comparison with published experimental results for uniaxial compression and tension tests in the literatures. This suggested model could be easily implemented into a 3D finite elements code to detect damages of concrete structures subjected to fire events.

[Mojtaba Labibzadeh, Hamid Reza Ghafouri, Ali Edalatbehbahani,. A High Temperature Sensitive Micro-Planes Damage Model for Plane Concrete. Journal of American Science 2010;6(11):857-864]. (ISSN: 1545-1003).

**Keywords:** Constitutive equations, Micro-planes, Damage, High temperatures, Plane concrete

## 1. Introduction

Generally, most of concrete structures exposed to fire could be retrieved and returned to service even after severe fires. In this process the damaged structural members must reach to a minimum strength, ductility and stiffness which they have possessed before the fire. After heating concrete to the high temperatures, a series of physical and chemical reactions lead it to exhibit changes such as loss of moisture, decomposition of aggregate particles and dehydration of cement past. These changes could strongly affect the structure of concrete members by reducing mechanical properties of concrete, namely the decrease in both strength and stiffness of the concrete. Also variation of brittleness of the softening behavior is going to show more ductility with raising the temperature. To investigate and rehabilitate the mentioned structural members this point is crucial to have a good estimation on the effects of temperature on mechanical properties of concrete. This necessity rises when stress-strain relationships are required to predict the entire behavior for a further earthquake.

Many studies have been made to evaluate influence of the high temperature on physical properties of concrete (Malhotra, 1956; Abrams, 1971; Harda, et al., 1972). Consequently numerous models have been proposed to account high temperatures for concrete. Simo and Ju (1987) proposed continuous elasto-plastic damage models with considering strain and stress based dual framework. To propose a unified theory, Carol et al., (1994) merged elastic

degradation and damage theory. Another model based on viscosity plastic theory has been conducted by Faria et al., (1998) for massive concrete structures. Wu et al., (1999) tested 44 specimen exposed to the temperatures up to the 600 °C and extracted a stress-strain relation which is applicable for heated and unheated concrete. Thermo-chemo constitutive equations based on plasticity could be utilized to consider decohesion and the thermal damage (Ulm, et al., 1999). Also Hydro-thermo-mechanical analysis along with further development to account the damage at high temperatures has been considered for concrete (respectively Bagio et al., 1995 and Gawin, et al., 1999). Nechnech et al., (2002), proposed an elasto-plastic damage model for plane concrete subjected to the elevated temperature in which thermal damage has been defined via the variation of elastic modulus with the temperature.

Researches alluded above have been regarded concrete on the basis of plasticity, damage or a combination between both of them. Generally, continuous models are categorized into two main classes: **macroscopic models** which are presented by damage and plasticity theory or a combination between both and **mesoscopic models** such as multi-laminate or micro-plane models.

Through the mesoscopic notion a model presented a damage model for concrete on the basis of "micro-plane theory" which has employed experimentally damage functions for only mechanical loadings (Labibzadeh and Sadrnejad, 2006). In the present paper the previous model (Labibzadeh and Sadrnejad,

2006) is further developed to consider the effect of elevated temperatures. The new model has combined the mechanical damage with the thermal one which is defined with variation of elastic modulus.

## 2. Material and Methods

### 2.1 Micro-plane formulation based on kinematic constraint approach

At first, the slip theory developed based on the idea that constitutive behavior of material could be presented by the behavior of specified planes within the material. By applying this theory to account for continuum damage mechanics and cohesive frictional material, for the first time "slip or multi-laminate theory" changed its name to the "micro-plane theory".

Now we are presenting the applied formulation at the model which is a combination of micro-plane theory with assumption of kinematic constraint approach and damage theory. Models constrained with this approach are capable to depict softening behavior of plane concrete in a stable manner.

The unite sphere of micro-plane models includes 26 planes tangent to the sphere's surface (Figure 1). The position and orientation of each plane is specified with the unite normal to the plane with components of  $n_i, i = 1, 2, 3$  (any subscript refers to the components in Cartesian coordinate axis  $x_i$ ). Also to extract shear components on the micro-planes, we are required to define two extra coordinate directions  $M, L$  which represent two orthogonal unite coordinate vectors  $m_i, l_i$  respectively.

According to the kinematic constraint, at first macroscopic strain tensor is projected on the planes. This projection yields three components of micro-strains along the plane's triplet local directions which one is normal ( $\varepsilon_N$ ) and two of them are tangential ( $\varepsilon_M, \varepsilon_L$ ) (Figure 2). The following relations depict the projection process in mathematical form:

$$\varepsilon_N = N_{ij} \varepsilon_{ij}, N_{ij} = n_i n_j \quad (1)$$

$$\varepsilon_M = M_{ij} \varepsilon_{ij}, M_{ij} = (m_i n_j + m_j n_i) / 2 \quad (2)$$

$$\varepsilon_L = L_{ij} \varepsilon_{ij}, L_{ij} = (l_i n_j + l_j n_i) / 2 \quad (3)$$

Where repeated indices imply summation over  $i = 1, 2, 3$ .

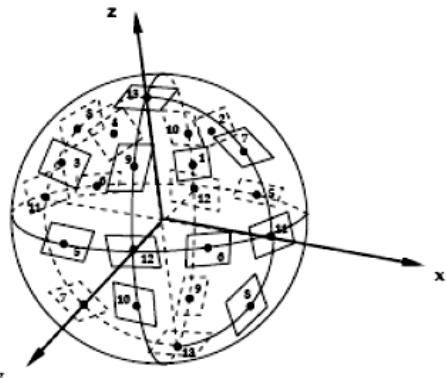


Figure 1. Position of integration points on the unite sphere surface.

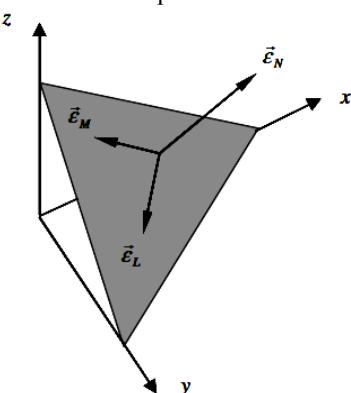


Figure 2. Strain components on a micro-plane.

Now with employing the appropriate constitutive laws at micro-plane level, micro-stresses are updatable through the executed strain components on the planes.

Totally, kinematic constraint models indefeasibly oblige the macroscopic stress tensor to have identical projection on the micro-planes with the stress components at the same planes. Thus these models are credible if and only if the constitutive laws at micro-level are particularly invented in a way that this condition could be satisfied. Due to the desired satisfaction through the analysis procedure is generally scared, the kinematically constrained models have been proceeded to fabricate a static equilibrium between plan's stress components and macro-level stress tensor. This equivalence could be supplied by means of the virtual work method. The equation (4) has equated the virtual work inside the unit sphere and on its surface:

$$\sigma_{ij} = \sigma_v \delta_{ij} + \frac{3}{2\pi} \int_{\Omega} [\sigma_D (N_{ij} - \frac{\delta_{ij}}{3}) + \sigma_L L_{ij} + \sigma_M M_{ij}] d\Omega \quad (4)$$

Where  $\Omega$  is the unit hemi-sphere surface,  $\sigma_L$  and  $\sigma_M$  are tangential stress components on each

plane,  $\sigma_v$  and  $\sigma_d$  are volumetric and deviatoric parts of normal micro-stress components which will be discussed later.

The introduced integration at equation (4) can be carried out by any numerical integration technique such as Gaussian integration. Here, an approximate formula with 26 integration points with a finite number of micro-planes for each point has been taken into account. The final portrait of the numerically performed equation (4) is:

$$D_{ijkl} = \frac{3}{4\pi} \int_{\Omega} \left( \frac{E}{1+v} \right) \left[ \left( N_y \frac{\delta_y}{3} \right) \left( N_u \frac{\delta_u}{3} \right) + M_y M_u + L_y L_u \right] d\Omega + \frac{E}{1-2v} \frac{\delta_u}{3} \delta_y \quad (5)$$

## 2.2 Elastic modulus at various temperatures

Generally with increasing temperature, concrete structures will be accompanied by changes such as reducing compressive (tensile) strength and increasing peak strain which means concrete is softening with raising temperature. For the same thermal condition, the reduction in compressive (tensile) strength is smaller than its counterpart in the elastic modulus. As shown in figures 4 and 5 the elastic modulus decreases with increasing temperature. Therefore to assess concrete structures damaged during severe fires, it is crucial to have precise visualize on the thermal effects over the concrete elastic modulus. For practical purposes at compressive state, the elastic modulus of heated concrete could be considered as secant modulus at 40% of experimental peak strain of compressive stress-strain curve. This percentage for the temperatures 200,400,600 is respectively reported as 80%, 40% and 6%.

## 2.3 Anisotropic damage model

Total deviatoric part of constitutive matrices is computed from superposition of its counterparts on the micro-planes which such counterparts in turn, are calculated based on the damage occurred on each plane depending on each specific loading condition (Labibzadeh and Sadrnejad, 2006). The prime skeleton of damage mechanism at the proposed model is based on five separate damage functions which one by one are constructed to cover one of the alluded loading situations. The five loading states are related as:

1. Hydrostatic compression
2. Hydrostatic extension
3. Pure shear

## 4. Shear + compression

## 5. Shear + extension

The damage evolution functions proposed by Labibzadeh and Sadrnejad, (2006) and here is adapted for thermal considerations are acquired through the authoritative laboratory tests which are carried on the concrete specimens under diverse compression and tension states of loading. Generally speaking, each function that is formulated for one of the five force conditions has been constructed upon two series of parameter. The first kind (parameter a to k) consists of 11 experimental parameter in a way that each of them has been allotted with considering both proportion of applied macro-level forces at loading directions and thermal condition. These parameters remain constant during loading procedure. Then, they only depend on the ratio of loading at applied directions while in the other hand should be allocated at different temperature ranges. The calibration process for them is a crucial step to satisfy both loading and thermal condition by implementing optimum values. The second type only consists of average strain parameters that are separately concluded from operative micro-strains on the planes. The proposed damage evolution functions are:

$$\omega_{HC} = 0.0 \quad (6)$$

$$\begin{cases} \omega_T = 0 & \text{if } \varepsilon_{eq} \leq a \\ \omega_T = 1 - \left( \frac{a}{\varepsilon_{eq}} \right) \times \exp\left[-\left(\frac{\varepsilon_{eq} - a}{c - a}\right)\right] & \text{if } \varepsilon_{eq} > a \end{cases} \quad (7)$$

$$\begin{cases} \omega_{ur} = 0 & \text{if } \varepsilon_{eq} \leq \sqrt{3}a \\ \omega_{ur} = 1.0 - \left( \frac{\sqrt{3}a}{\varepsilon_{eq}} \right) \times \exp\left[-\left(\frac{\varepsilon_{eq} - \sqrt{3}a}{b - \sqrt{3}a}\right)\right] & \text{if } \varepsilon_{eq} > \sqrt{3}a \end{cases} \quad (8)$$

$$\begin{cases} \omega_C = d \times \varepsilon_{eq} & \text{if } \varepsilon_{eq} \leq e \\ \omega_C = f(\varepsilon_{eq} - e)^2 + g(\varepsilon_{eq} - e) + h & \text{if } e < \varepsilon_{eq} < i \\ \omega_C = 1 - \left( \frac{j}{\varepsilon_{eq}} \right) \times \exp\left[-\left(\frac{\varepsilon_{eq} - j}{k - j}\right)\right] & \text{if } \varepsilon_{eq} > i \end{cases} \quad (9)$$

$$\omega_{sh} = 0.5 \times (\omega_C + \omega_T) \quad (10)$$

## 2.4 Damage evolution

In our formulation the thermal damage is defined by means of the variation of elastic modulus with temperature. The anisotropic damage model takes advantages of a total damage function ranging between zeros to one. Covering the mechanical and thermal effects, the value of zero expresses a micro-

plane surface with no crack or any crack initiation and the one is related to a totally damaged surface. The proposed Total Damage Function (TDF) is assembled for each plane through the aforementioned five damage function and also five new variables ( $HT, T, SH, C, HC$ ). These new ones are referred to the updated micro-stress components from projected strains on the micro-planes. This could be formulated as:

$$TDF(P) = HT(P) \times \omega_{HT}(P) + T(P) \times \omega_T(P) + SH(P) \times \omega_{SH}(P) + C(P) \times \omega_C(P) + HC(P) \times \omega_{HC}(P) \quad (11)$$

Where  $P$  indicates the micro-planes number 1 to 13. Planning procedure for micro-plane stress components employs macroscopic parameters,  $v, E$  in which contains thermal effects. Thus the Total Damage Formula is separately computed for each micro-plane on the basis of the variables affected by thermal and mechanical circumstances.

## 2.5 Model flowchart

Analysis procedure sequence followed by the model has been portrayed at figure 3.

## 3. Results

In this section validity of the proposed model has been examined by focusing on its capability to simulate constitutive responses of specimens submitted to the uniaxial compression and tension tests. The applied values for mechanical properties of concrete at different temperatures have been summarized through the Table 1 and Figure 4(a,b).

Table 1(Mechanical properties of concrete)

		At room temperature	Variation with temperature
Compression	$E_0$	29600 Mpa	Fig.4(a)
	$v$	0.2	constant
Tension	$E_0$	50000 Mpa	Fig.4(b)
	$v$	0.2	constant

|  
|

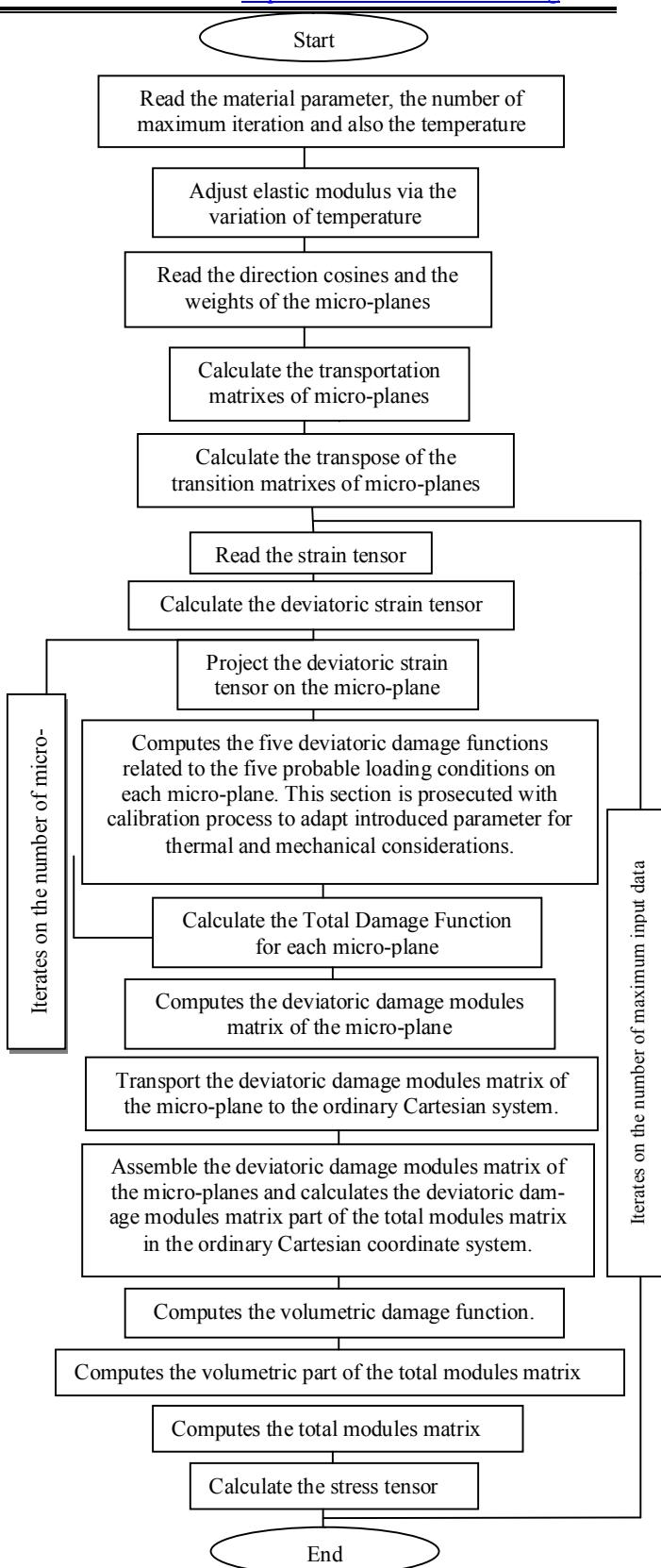


Figure 3. Corresponding model flowchart

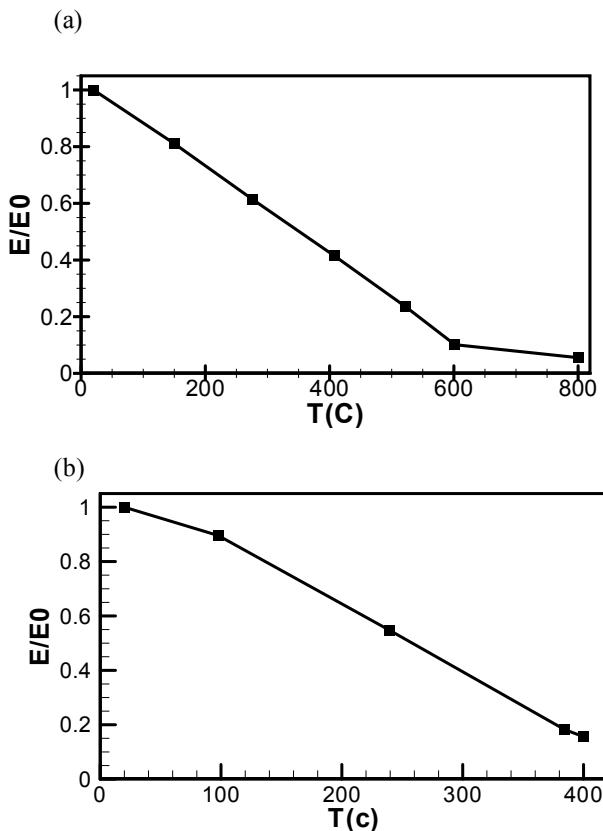


Figure 4(a,b). Variation of elastic modulus, (a) Compressive state (b) tensile state, with temperature.

### 3.1 Uniaxial compression (UC) test

The uniaxial compression tests on concrete performed by Chang et al., (2006) are conducted here, to assess coincidence between simulated responses of the proposed model and experimental stress-strain curves. The test instruction exposes concrete specimen to progressive temperature increase up to the desired level and then this is followed by natural cooling down. Afterward the specimen is conducted to the desired loading to get the stress-strain responses. Figure 5(a,b) shows a reasonable agreement between the results fulfilled by the proposed model and experimental stress-strain curves. These results declare that as temperature increases, both strength and material stiffness decline, concrete is softening and stress-strain curves become flatter. That's why the shape of the curves varies from unheated to the heated states and also between the ones at different temperatures.

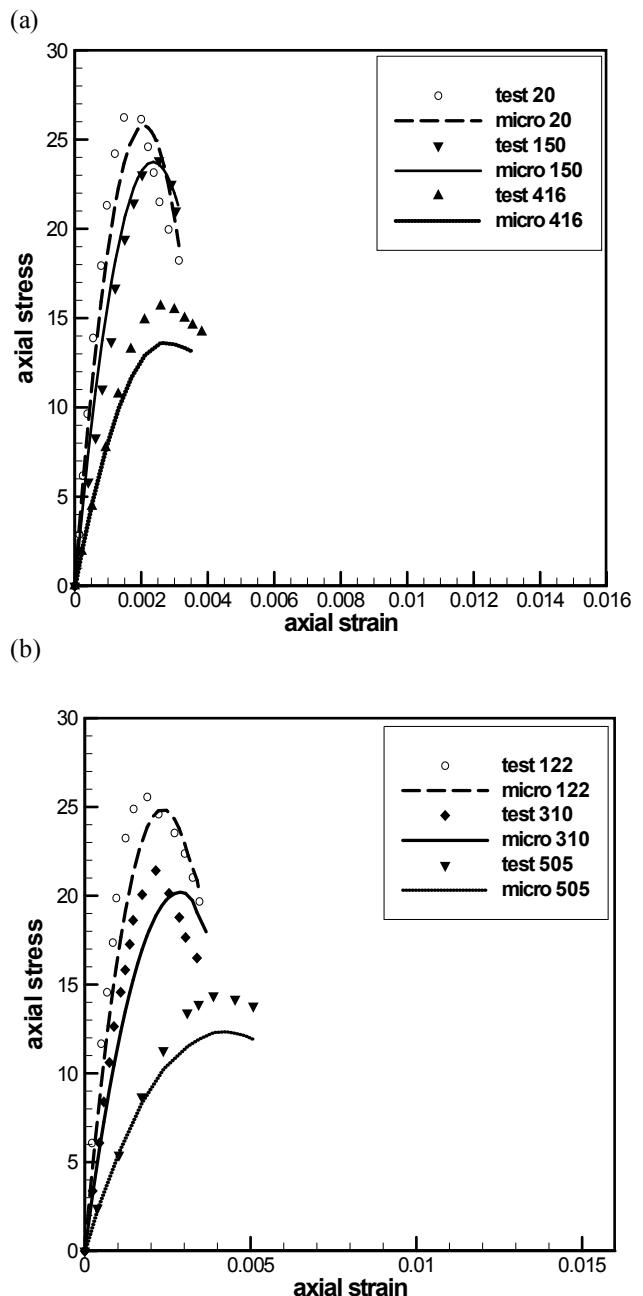


Figure 5(a,b). Comparison of proposed stress-strain curves with experimental results at UC test.

Now with applying the uniaxial compression load along the X-axis, the micro-plane 11 on the unite sphere is just loaded by compressive stress. Only tensile stress affects the planes number 9,10,11,12 situated normal to the loading direction and on the other eight micro-planes compression together with shear stresses operate with different ratios depending on geometric location of the plane. Figure 6 is

depicted damage evolution on the planes 5,6,7,8 during the test at different temperatures.

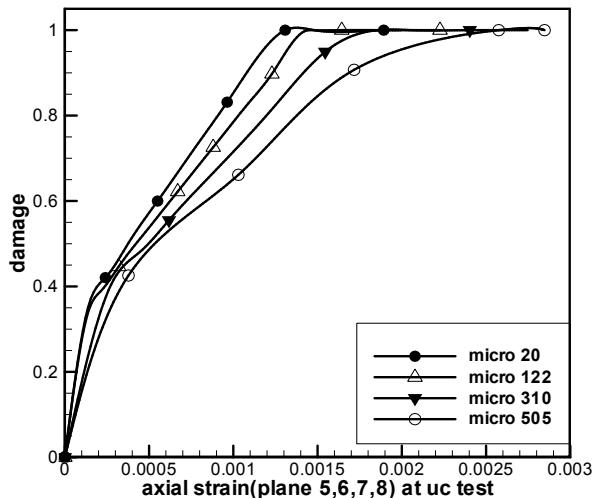


Figure 6. Damage evolution during uniaxial compression test

### 3.2 Uniaxial tension (UT) test

The tests performed by Felecitti and Gambarova , (1998) are considered here. The experimental stress strain responses of concrete specimen under uniaxial tensile loading at different temperatures (reported by the mentioned authors) in compare with simulated results of the proposed model are depicted at Figure 7.

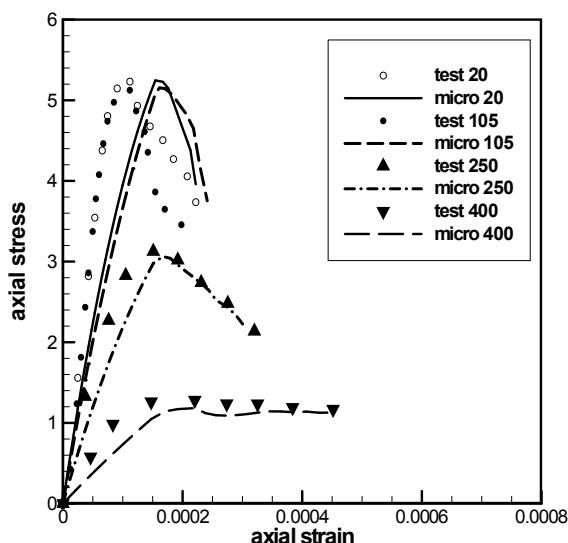


Figure 7. Comparison of proposed stress-strain curves with experimental results at UT test.

The damage values during the test are presented at Fig. 8. As we can see at the stress-strain curves, together with the raising temperature, the concrete softening behavior conveys the peak location in that its value increases in the strain axis. This event is clearly observable through the gradient of the damage-strain curves (Fig. 6, 8) in a way that with raising temperature the curves become less inclined to absorb higher strains.

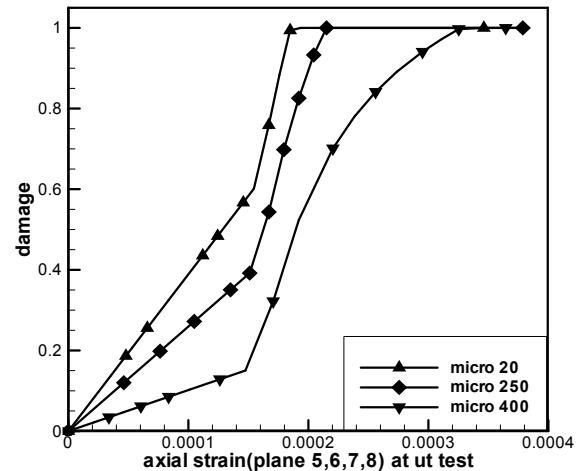
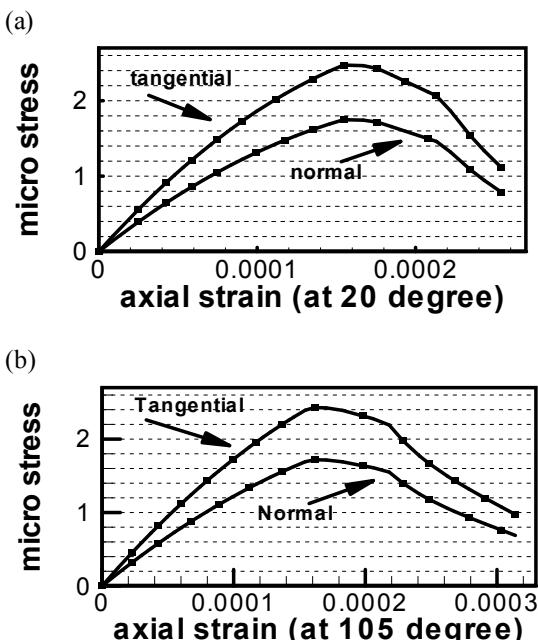


Figure 8. Damage evolution during uniaxial tensile test

Furthermore the micro-stress components fulfilled by the projection of macroscopic stress tensor, versus the axial strain are depicted at Figure 9 for the micro-planes number 1 to 4.



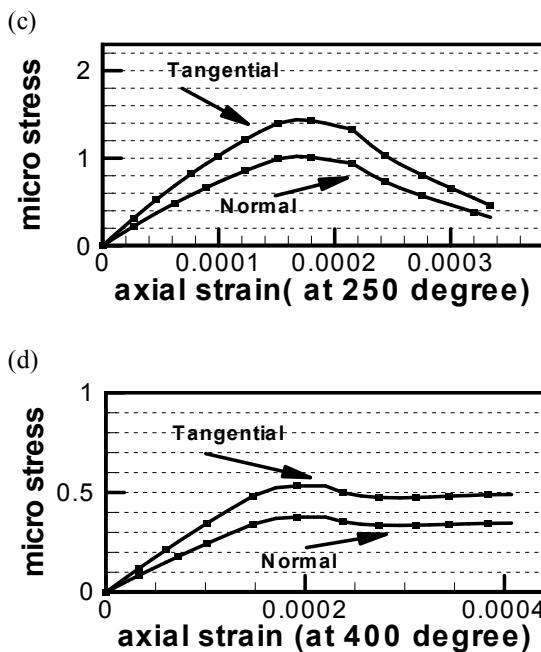


Figure 9. (a to d). Micro-stress-strain curves at various temperatures during UT test.

#### 4. Discussions

Because of demoting the mechanical properties of concrete such as elastic modulus and compressive (tensile) strength with elevating temperature, the proposed model has been defined the thermal damage via the variation of elastic modulus with temperature. The model implemented five separate damage functions to cover up the total damage included mechanical and thermal ones. The damage functions act via the projected strain components on the micro-planes and the parameters have been calibrated for thermal and mechanical effects by the published experimental results.

Raising temperature profoundly affects softening behavior and peak location of the stress-strain curves of plane concrete which the ascending part is turning to the linear state while descending curve becomes flatter.

Macroscopic strain tensor allocates three strain components, projected along the local coordinate axis, to each micro-plane (N- normal direction and M, L- tangential directions). This proficiency would capable the model to predict the direction of crack growth around a point.

#### Acknowledgements:

Authors are grateful to the Professor S. A. Sadrnejad and Dr. I. Rasoulan for the scientific cooperation to carry out this work.

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**Corresponding Author:**

Dr. Mojtaba Labibzadeh  
Head of Civil Department,  
Faculty of Engineering, Shahid Chamran University,  
Ahvaz, Iran.



9/6/2010

# Toxic Impact of Titanium Dioxide (TiO<sub>2</sub>) In Male Albino Rats with Special Reference to its Effect on Reproductive System

Nabela, I., EL- Sharkawy\*, Salah, M. Hamza and Ehsan, H., Abou-Zeid

Dept. of Forensic Medicine & Toxicology. Fac. of Vet. Med. Zagazig University, Zagazig, Egypt.  
[\\*nabelaimam@hotmail.com](mailto:nabelaimam@hotmail.com)

**Abstract:** The present study was directed to explore the toxic effects of orally administered TiO<sub>2</sub> in mature male albino rats. Eighteen mature male albino rats were classified into three equal groups. The first group was used as control and fed on TiO<sub>2</sub> free ration (C), the second and the third groups (T1) and (T2) were fed on ration containing 1% and 2% TiO<sub>2</sub> respectively for 65 days. The body weight of male albino rats fed 1% and 2% TiO<sub>2</sub> showed a significant decrease along the experimental period. Animals were scarified after termination of the experimental period. The sera were separated for estimation of nitric oxide and testosterone levels. Liver samples were preserved for antioxidants enzyme activities determination. Liver, testes and seminal vesicle samples were preserved in formalin for histopathological study. The results indicated that TiO<sub>2</sub> resulted in a significant decrease in body weight gain, sperm motility %, sperm cell concentration, sperm viability and serum testosterone level. While, a significant increase in sperm abnormalities, serum nitric oxide (NO), hepatic superoxide dismutase (SOD), glutathione reductase (GR) enzyme activities and malondialdehyde (MDA) concentration were recorded. Histopathological findings revealed reduction in the number and size of the epithelial lining of the tubuloalveolar gland and hyperplastic glandular epithelium of seminal vesicle. Testes showed mild spermatogenesis besides congested testicular blood vessels. Liver showing vacuolar, hydropic degeneration and cell death of some hepatic cells and steatosis. The present study concluded that, TiO<sub>2</sub> elicited a marked ruinous effect on male fertility and biochemical parameters as well as histopathological picture.

[Nabela, I., EL- Sharkawy, Salah, M. Hamza and Ehsan, H., Abou-Zeid. Toxic Impact of Titanium Dioxide (TiO<sub>2</sub>) In Male Albino Rats with Special Reference to its Effect on Reproductive System. Journal of American Science 2010;6(11):865-872]. (ISSN: 1545-1003).

**Keywords:** Toxic; Titanium Dioxide (TiO<sub>2</sub>); Rat; Reproductive System

## 1. Introduction:

Colors have been used to make food more attractive and appetizing for centuries. In Egyptian Tombs dating as far back as 1500 B. C depict the making of colored candy (Marmion, 1987). Several synthetic food colors used in food industry proved numerous side effects such as urticaria (Chafee and Settipane, 1967), genotoxic effects (Combes and Haveland-Smith, 1982), endocrinial disturbances (Jennings et al., 1990), behavioral disorders (Pollock and Warner, 1990), and neurological effects (Tanaka, 2001).

Titanium is one of the eight most abundant in the earth's crust and consequently enters the food chain to some degree. Human are estimated to consume approximately 300g/titanium/day in food (Dunford et al., 1997). Moreover, TiO<sub>2</sub> accounts for about 70% of the total volume of pigment production world wide (Bann et al., 2006).

The Federal Regulations of US Government limit usage of TiO<sub>2</sub> in food products to 1% by weight (Wang et al., 2007). Oral route is a potential exposure route for general population due to TiO<sub>2</sub> used as white pigment on tooth paste, drug capsule (Baan et

al., 2006), in tableted drug products (Ghoropade et al., 1995), in dairy based products as a whitener in manufacture of different types of cheese (Leone, 1973), dairy based drinks, chocolate, milk, coca, soybean products, milk powder, margarine, processed meat, table and soda water, sausage casing (JECFA, 2006), in bread flour and in the confectionary (Lorenz and Maga, 1973). Also, TiO<sub>2</sub> therapeutically used in sunscreens and cosmetic creams (Gelis et al., 2003). There have been a relatively few systematic studies that have employed pigmentary TiO<sub>2</sub> (Bermudez et al., 2002). Most studies on TiO<sub>2</sub> toxicity in mammals were focused on the pulmonary impact of inhaled or dermal exposure (Wang et al., 2007). Mahrousa (2004) reported that 4mg/kg body weight of TiO<sub>2</sub> for 90 days in rats resulted in non significant change in DNA, and RNA content in liver and testis. Schapiro et al., (1995) reported that there were numerous studies shown that TiO<sub>2</sub> significant increase the production of hydroxyl radicals. Guo et al. (2009) studied the effect of nanosized TiO<sub>2</sub> (200 and 500 mg/kg) in male ICR mice aged 6 weeks injected intraperitoneally ever other day for five times. One week after drug cessation, low dose group

showed non significant changes, while high dose group exhibited a significant increase in serum ALT, ALT/AST ration and BUN. Furthermore, reduction in sperm density, motility and increased sperm abnormalities with germ cell apoptosis concomitant with no obvious pathological changes in liver, kidney, spleen, testis and epididymis.

It is pertinent to record that paucity of information concerning the reproductive study on TiO<sub>2</sub>. Thus this study was carried out to investigation the oral toxic impact of titanium dioxide in male albino rats with special reference to its effect on fertility.

## 2. Materials and methods

### Experimental Animals

Eighteen mature male albino rats weighing 180- 200g were used. The animals were obtained from Faculty of Veterinary Medicine, Zagazig University (laboratory animal's housing unit). Animals were clinically healthy, kept under hygienic condition, housed in metal cages with hard wood shavings as bedding. They were maintained on basal ration and given water *ad-libitum* for two weeks of acclimatization before use.

### Chemical

Titanium dioxide (TiO<sub>2</sub>): It is manufactured by Riedel- deHaen and was obtained by Sigma-Aldrich Laborchemikalien GmbH. TiO<sub>2</sub> was added to the ration at level of 1% and 2% according to *Ghoropade et al.*, (1995).

### Description

It is a white odorless powder with molecular weight 79.88 g/mole

Solubility:-It is insoluble in water and other solvents. It is dissolved slowly in hydrofluoric acid and in hot concentrated sulphuric acid. Water soluble matter not more than 0.5%, some preparations can be made hydrophilic by suitable surface treatment.

### Synonyms

Titanium dioxide, titanium peroxide, Titania, anatase, cosmetic white, Tipaque, titanium oxide, pigment white 6, titanium white, E 171, Tania.

### Methods

Rats were randomly distributed into three groups each of six. The first group control group (C) fed on TiO<sub>2</sub> free ration. The second and third groups fed on ration containing 1% and 2% TiO<sub>2</sub> (T1&T2) respectively for 65 days according to (Wang et al., 2007). All rats were weighed before the start of the experiment (preliminary weights). Rats were scarified at the end of the experimental period. Serum samples

were collected and kept at -20°C for biochemical studies. For seminal picture; The cauda epididymis were minced in normal saline and a drop of this epididymal suspension was picked up for seminal analysis and recording the epididymal spermatozoal characters (Hafez, 1970), sperm motility (Slott et al., 1991), sperm cell concentration per ml of semen (Robb et al., 1987), sperm abnormalities and live % of spermatozoa (Filler, 1993). Serum testosterone was determined according to Wilson and Foster, (1992) using testosterone kit (Egyptian Co. of chemicals) which depend on the method of enzyme immunoassay. Serum concentrations of nitrite according to Torre et al., (1996) by Griess reaction, superoxide dismutase (SOD) activity was assayed by Niskikimi et al., (1972), glutathione reductase (GR) activity was determined by Beutler, (1975) and Malondialdehyde (MDA) concentration according to Draper and Hardly, (1990), using Shimadzu type spectrophotometer manufactured by Incorporation Kyoto, Japan. Testis, seminal vesicle and liver were fixed in 10% formalin for histopathological examination according to Bancroft et al., (1996). Statistical analysis of data was assessed according to SPSS, (1997).

## 3. Results

### Clinical signs

Clinical signs showed depression, anorexia and white feces among the different dose levels of TiO<sub>2</sub> treated male rats along the experimental period. Moreover, addition of TiO<sub>2</sub> either 1% or 2% for 65 days of feeding, significantly decrease ( $P < 0.05$ ) the body weight compared to the control (Table 1).

### Effects of TiO<sub>2</sub> on male fertility

Concerning the sperm motility; there was non significant decrease in sperm motility percentage of male albino rats fed on low level of TiO<sub>2</sub>, while a significant decrease ( $P < 0.05$ ) was recorded in high level fed group compared with the control. The mean values of sperm cell concentration recorded a significant decrease ( $P < 0.05$ ) in both treated groups comparing with the control. There was a significant increase in percentage of sperm abnormalities which was dose dependent and compared to the control one. Results are depicted in Table 2 (Fig 1-II, Fig 1-III A, B, C, D). Regarding sperm viability showed that 1% and 2% TiO<sub>2</sub> caused a significant decrease ( $P < 0.05$ ) comparing with the control group .The testosterone level recorded a significant decrease ( $P < 0.05$ ) in both TiO<sub>2</sub> treated groups compared to control (Table 2).

### Biochemical parameters

TiO<sub>2</sub> 1% and 2% resulted in a significant increase ( $P < 0.05$ ) in NO production, SOD and GR

enzyme activities compared with control. MDA showed non significant increase in TiO<sub>2</sub> treated groups (Table 3).

#### Post mortem changes

Macroscopically; seminal vesicles of TiO<sub>2</sub> treated male rats showed hypertrophy in low dose level, while atrophy in high level (Fig 2-A).

#### Histopathological findings

The epithelial lining of the seminal vesicle showed hyperplastic changes with little or absence of secretion beside edema of trabiculae observed in all rats fed 1% TiO<sub>2</sub> (Fig. 2-C), while there was

reduction in number and size of the epithelial cell lining of the tubuloalveolar glands in rats fed 2% TiO<sub>2</sub> (Fig 2-D).The seminiferous tubules revealed mild spermatogenesis with congested interstitial blood vessel with endotheliosis in T1 (Fig. 3-B). Thickened tunica albuginea with degenerated spermatogonial cell layers and spermatocytes with absence of spermatogenesis were also seen particularly in rats fed 2% TiO<sub>2</sub>. The hepatocytes suffered from various degenerative changes varied from vacuolar and hydropic degenerations to cell death in rats fed low dose of 1% TiO<sub>2</sub> (Fig 3-D). TiO<sub>2</sub> 2% resulted in more intense lesions mainly steatosis of the hepatic cells (Fig. 3-E).

**Table (1): Changes in mean body weight (g) of male albino rats fed on 1% and 2%TiO<sub>2</sub> containing rations for 65 days (Mean± S.E.).**

Group	Treatment	Mean of body weight at the beginning	65 days post- administration
C	Free diet	181.00±5.56 <sup>a</sup>	275.16±2.6 <sup>a</sup>
T 1	TiO <sub>2</sub> 1%	179.42±5.2 <sup>a</sup>	237.83±1.92 <sup>b</sup>
T 2	TiO <sub>2</sub> 2%	182.78±7.04 <sup>a</sup>	235.66±2.18 <sup>b</sup>

Means in the same row having different superscript were significantly different (P< 0.05)

**Table (2): Changes in epididymal sperm characters of male albino rats fed on TiO<sub>2</sub> 1% and 2% containing rations for 65 days and their serum testosterone level after 65 days. (Mean± S.E.)**

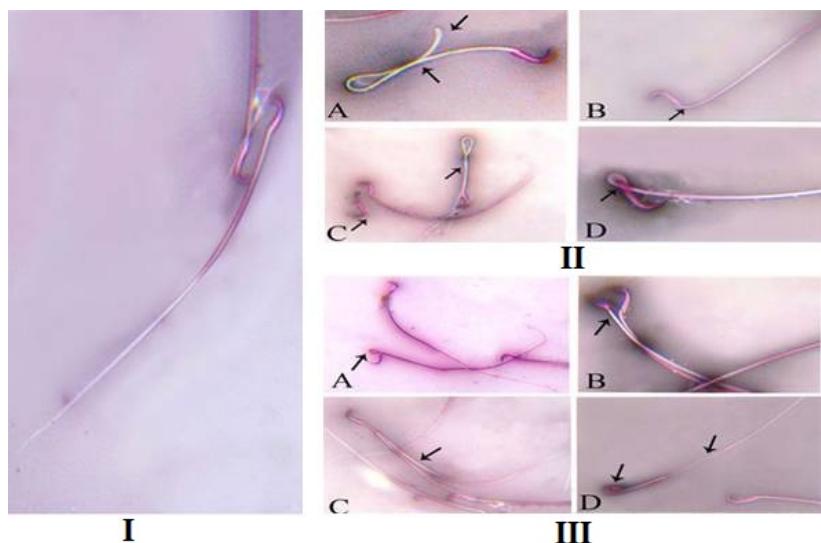
Treatment	Group	Motility %	Sp.C.C/ml x125x10 <sup>5</sup>	Abnormalities %	Live %	Testosterone (ng/ml)
Free diet	C	86.66±1.66 <sup>a</sup>	26.33±0.88 <sup>a</sup>	6.4±0.61 <sup>c</sup>	93.22±0.94 <sup>a</sup>	1.92±0.71 <sup>a</sup>
TiO <sub>2</sub> 1%	T 1	82.50±2.14 <sup>ab</sup>	18.5±2.10 <sup>b</sup>	14.95±1.65 <sup>b</sup>	86.84±3.71 <sup>b</sup>	0.622±0.21 <sup>b</sup>
TiO <sub>2</sub> 2%	T 2	72.50±3.81 <sup>b</sup>	13.83±1.35 <sup>b</sup>	28.15±1.90 <sup>a</sup>	76.32±1.89 <sup>b</sup>	0.573±0.33 <sup>b</sup>

Means in the same row having different superscript were significantly different (P< 0.05)

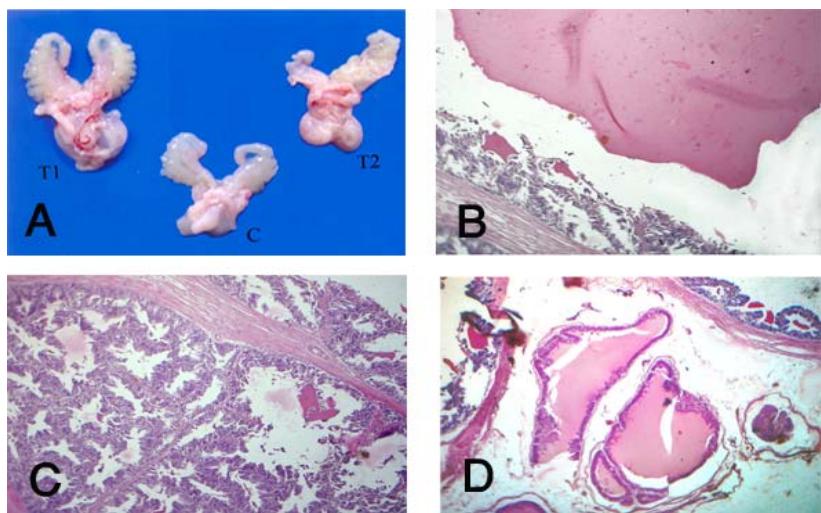
**Table (3): Changes in antioxidant enzymes activities in liver homogenate and serum nitric oxide level of male albino rats fed 1% and 2%TiO<sub>2</sub> containing rations for 65 days (Mean± S.E.)**

Duration	Group	SOD (u/gm tissue )	GR (u/gm tissue)	MDA (μmol/L homogenate)	Nitrite level (umol/L)
65 days	C	30.53±2.76 <sup>b</sup>	0.208±0.009 <sup>c</sup>	1.46±0.09 <sup>a</sup>	0.4623± 0.006 <sup>c</sup>
	T 1	71.98 ±5.6 <sup>a</sup>	0.412 ±0.07 <sup>b</sup>	1.87±0.03 <sup>a</sup>	1.414± 0.04 <sup>b</sup>
	T 2	78.33 ±6.3 <sup>a</sup>	0.899 ±0.27 <sup>a</sup>	1.93 ±0.05 <sup>a</sup>	1.6405±0.04 <sup>a</sup>

Means in the same row having different superscript were significantly different (P< 0.05)

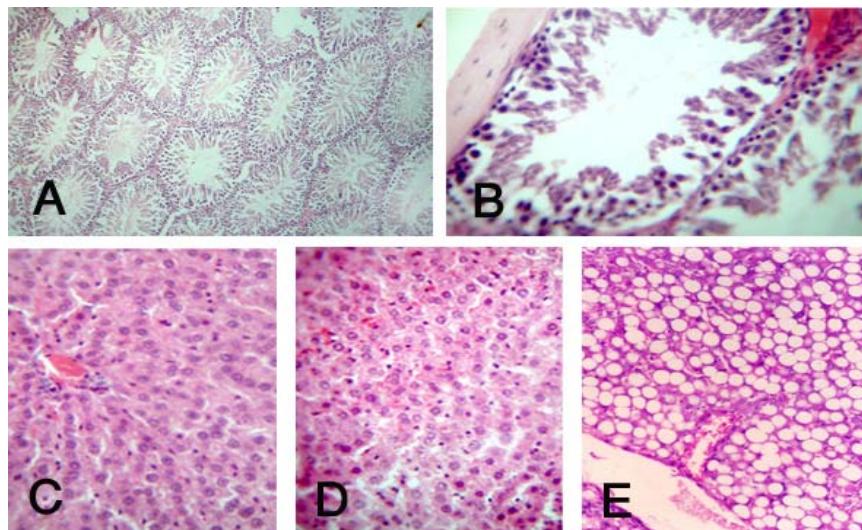


**Fig. (1): Spermatozoa of male albino rats fed on  $\text{NO}_2$  free ration (control, 1-I) showing normal hock shape spermatozoa. Spermatozoa of male albino rats fed on 1%  $\text{TiO}_2$  containing ration for 65 days showing abnormalities in the form of A) looped sperm, thickened tail. B) bent mid piece. C) Detached head and mid piece and looped sperm. D) Coiled mid piece (Fig 1-II). Spermatozoa of male albino rats fed on 2%  $\text{TiO}_2$  containing ration for 65 days showing abnormalities in the form of A) bent mid piece. B) Abnormal hock shape. C) Double tailed sperm. D) Denuded tail, deformed hock shape (Fig 1-III).**



**Fig. 2:**

- A : Gross picture of seminal vesicle of male albino rat fed  $\text{TiO}_2$  1% showing hypertrophy T1 and  $\text{TiO}_2$  2% showing atrophy T2 .
- B-Photomicrograph section of rat seminal vesicle control (H&E x 300).
- C- -Photomicrograph section of seminal vesicle of rat fed on  $\text{TiO}_2$  1% containing ration for 65 days showing hyperplastic glandular epithelium (H&E x 300).
- D--Photomicrograph section of seminal vesicle of rat fed on  $\text{TiO}_2$  2% containing ration for 65 days showing reduction in number and size of the epithelial lining of the tubuloalveolar gland (H&E x 300).

**Fig. 3:**

- A- Photomicrograph section of rat testes (control) showing normal testicular tissue (H&E x 300).**
- B- Photomicrograph of section of rat testes fed on TiO<sub>2</sub> 1% containing ration for 65 days showing mild spermatogenesis and congestion of blood vessel (H&E x 1200).**
- C- Photomicrograph section of rat liver (control) showing normal hepatic parenchyma (H&E x 1200).**
- D- Photomicrograph of section of rat liver fed on TiO<sub>2</sub> 1% containing ration for 65 days showing vacuolar and hydropic degeneration and cell death of some hepatic cells (H&E x 1200).**
- E- Photomicrograph section of rat liver fed on TiO<sub>2</sub> 2% containing ration for 65 days showing steatosis (fatty change) of hepatic cells (H&E x 1200).**

#### 4. Discussion

In the past, some food additives had been considered acceptable in the absence of adequate information. Safety data about TiO<sub>2</sub> is still limited and it has been needed to be evaluated repeatedly and determined its safety by Regulatory Agency in the country of use.

Regarding to the effect of TiO<sub>2</sub> on body weights, our results revealed that male albino rats fed on TiO<sub>2</sub> 1% and 2% had lowered mean value of body weights if compared with that of the control group. These results are consistent with those observed by Wang et al., (2007) who found that after acute oral administration of a single dose of TiO<sub>2</sub> (5g/kg body weight) decreased body weight of all treated mice , Mahrousa , (2004) who recorded that oral treatment of male rats with 4mg/kg body weight TiO<sub>2</sub> for 90 days resulted in a significant decrease in their body weight and Bermudez et al., (2002) who exposed six week old female mice, rats, and hamsters to 10, 50, or 250 mg/m<sup>3</sup> pigmentary TiO<sub>2</sub> for 6 hours per day and 5 days per week for 13 weeks. TiO<sub>2</sub> produced depression in the body weight in all species and in all groups.. The weight loss is paralleled with anorexia which was observed on the exposed animals in the present study and may be attributed to the disturbance in different metabolic systems which resulted from

feeding synthetic food colorants (Abdel- Rahim et al., 1989).

Regarding the effect of TiO<sub>2</sub> feeding on male fertility, the present study revealed that TiO<sub>2</sub> 1% and 2% feeding for 65 days demonstrated a significant dose dependent increase in sperm abnormalities % and significant decrease in sperm cell concentration. Sperm motility % was significantly decreased in TiO<sub>2</sub> 2% and non significantly decreased at 1% compared with the control group. Our results were in the same context with those previously reported (Guo et al., 2009). Changes in epididymal sperm characters obtained in our result may be postulated to the generated NO following TiO<sub>2</sub> which plays a role in sperm motility (Herrero et al., 1997). By the same way, excessive NO production in response to a variety of stressors, possibly reducing the survival rate and motility of sperm cells (Ozokutan et al., 2000). Serum testosterone level was lowered. The high level of NO may be responsible for reduction in testosterone secretion (Adams et al., 1994), which leads to hypospermatogenesis, testicular inflammation and disturbance of GnRH secretion (Ferrini et al., 2001) and supported our histopathological evidence in the present study. Degenerated spermatogonial cell layers may be attributed to decreased testosterone synthesis and disruption of normal androgen status (Xing-

Shou, 1983) or may be due to reduced serum cholesterol level which is the precursor of all the steroid hormones (Bush,1991). TiO<sub>2</sub> is one of ROS generators (Sayes,et al.,2006 and Gurr et al.,2005) and confirmed by elevated antioxidant enzyme activities , SOD and GR.The present study revealed that there was a significant increase in SOD and GR enzyme activities in male rats fed on 1% and 2% TiO<sub>2</sub>.Similar results obtained after ultra-fine TiO<sub>2</sub> intra tracheal exposure in alveolar macrophage and peripheral RBCs of treated rats (Afaaq et al., 1998) to face the high level of generated ROS mentioned .The present study showed a non significant increase MDA concentration in liver homogenate of TiO<sub>2</sub> treated rats. Our results are concordant with Maness et al., (1999) who mentioned that TiO<sub>2</sub> caused an exponential increase in the MDA production who explained an increase of lipids peroxidation due to excessive ROS generation. These results are in agreement with Gurr et al., (2005) and Olmedo et al., (2005) in human and rats bronchial epithelial cells. The pathological alterations induced by TiO<sub>2</sub> may be associated with the generation of ROS. Furthermore, hepatic lesions as a sequellae of accumulated TiO<sub>2</sub> particles which is difficultly cleared *in vivo* after oral ingestion (Wang et al., 2007).

**Acknowledgment** to Prof .Dr. Abd EL- Monem Ali Professor of Pathology Faculty of Veterinary Medicine for his help in histopathological examination.

#### Corresponding author

Nabela, I., EL- Sharkawy

Dept. of Forensic Medicine & Toxicology. Fac. of Vet. Med. Zagazig University, Zagazig, Egypt.

\*nabelaimam@hotmail.com

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9/28/2010

# Protective Effect of *Lepidium sativum L.* Seeds Powder and Extract on Hypercholesterolemic Rats

**Wafeka Abdulah Al Hamedan**

Department of Nutrition and Food Science, Home Economic, Collage, Princess Nora Bent abdul – rahman - University, Riyadh, Saud Arabia

**Abstract:** The present study was designed to investigate the effects of *Lepidium sativum L* (LS) on the nutritional status and some biochemical analyses of serum and liver in hypercholesterolemic rats. Forty-two adult albino male rats Sprague Dawley strain were classified into six groups. One was fed on standard diet and kept as control (-ve) group. The other five hypercholesterolemic rat groups were control (+ve), drug, LS extract, 5 % or 10 % LS powder rat groups. In comparison to control (- ve) group, the control (+ve) group showed a significant higher value of weight gain , feed efficiency ratio (FER), serum cholesterol, triglycerides , LDL-c ,VLDL-c, cholesterol/ HDL-c and LDL-c/ HDL-c, serum (AST& ALT) ,creatinine ,urea, liver cholesterol and total lipids but significant decrease in HDL-c, globulin and liver triglycerides .Also, LS extract and 5% LS powder rat groups showed a significant increase in weight gain, cholesterol/ HDL-c and LDL-c/ HDL-c, serum (AST& ALT) however, drug and 10% LS powder rat groups showed a significant increase in cholesterol/ HDL-c. On the other hand, The drug, LS extract , 5% and 10% LS powder rat groups showed a significant increase in serum cholesterol ,triglycerides VLDL-c , LDL-c ,serum creatinine and urea level when compared to control (- ve) group. In comparing with control (+ ve) group, The drug, LS extract , 5% and 10% LS powder rat groups showed a significant lower value of weight gain , feed efficiency ratio, serum cholesterol ,triglycerides VLDL-c , LDL-c level, cholesterol/ HDL-c , LDL-c/ HDL-c , serum (AST& ALT) ,serum creatinine, urea, liver cholesterol and total lipids with a significant increase in both serum globulin and liver triglycerides.

[Wafeka Abdulah Al Hamedan. Protective Effect of *Lepidium sativum L.* Seeds Powder and Extract on Hypercholesterolemic Rats. Journal of American Science 2010;6(11):873-879]. (ISSN: 1545-1003).

Key wards : *Lepidium sativum* – aqua extract- cholesterol and rats

## 1. Introduction:

Hypercholesterolemia refers to elevated serum LDL cholesterol or a combination of high levels of LDL cholesterol and triglycerides. Hypercholesterolemia, a significant cardiovascular risk factor, is one of the major oxidative stresses that generate excess of highly reactive free radicals. This exacerbates the development and progression of atherogenesis Hypercholesterolemia increased the risk of increased LDL-c or more accurately LDL-c/ HDL-c ratio. The atherogenic index decreased as a result of the reduction in LDL-c and increment in HDL-c (Durrington, 1995 and Abd El-Ghanny et al., 2007).

Plants still remain a major source for drug discovery in spite of the great development of synthetic molecules. Consequently, the uses of traditional plant extract in the treatment of various diseases have been flourished (Jouad et al., 2001).The garden cress seed oil, *Lepidium sativum L.*,(LS) is a fast growing annual herb belonging to the Brassicaceae family that is native to Egypt and west Asia The seeds are wildly consumed as salad and spice (Gokavi et al .,2004). Previous studies have demonstrated the protective action of LS against

carcinogenic compounds and growth inhibition of *Pseudomonas aeruginosa*, a bacteria strain with a potent antibiotic resistance (Abuja et al., 2001 and Kassie et al., 2003). LS recommended in the treatment of hypertension, diabetes and renal disease (Kirtkar and Basu 2005 and Tahraoui et al., 2007).

The present study was designed to investigate the effect of powder or aqua extract from *Lepidium sativum L.* seeds (LS) either on the nutritional status and some biochemical analyses of serum and liver in hypercholesterolemic rats.

## 2. Materials and methods

### I – Materials:

#### 1- Garden cress (*Lepidium sativum L.*) seeds:

*Lepidium sativum L.* seeds (LS) were purchased from Agricultural Research Center. Garden cress seeds were dried with hot air (40–60 °C) and grinded to powder. Garden cress seeds powder was used in preparation of aquatic extract and also added to the diet as 5 % and 10% of the constituent of fiber.

#### 2- Gemfibrozil capsules:

It was obtained from Amoun Pharmaceutical Industries Company. Each tablet contains 100 mg. It is lipid regulating agent which decrease lipid elevated serum lipids by lowering serum triglycerides and total cholesterol. Human therapeutic dose was 100 mg which converted to rat dose that was 9 mg/kg body weight daily which dissolved in distilled water and given to rats by oral intubations according to Paget and Barnes (1964).

### 3-Biochemical kits:

BioMerieux Kits were purchased from Alkan Co. for Chemicals and Biodiagnostics, Dokki,, Egypt.

### 4-Experimental animals:

A total of forty-two Sprague -Dawley adult male rats were purchased from the Agricultural Research Center, Giza, Egypt. The average weight was  $205 \pm 5$  g. The animals were kept under observation for five days before experiment and supplied with standard diet and water *ad libitum*. The standard diet comprised of casein (200g/kg), corn starch (497g/kg), sucrose (100g/kg) , cellulose (30 g/kg) ,corn oil (50g/kg), mineral mixture (100g/kg) , vitamin mixture (20g/kg) and DL-methionine (3g/kg).The standard diet was performed according to NRC (1995).

## II. -Methods:

### 1. Preparation of the garden cress aqueous extract:

The aqueous extract was prepared in a standardized manner by boiling 1 g of dried powdered seeds of LS in 100 ml of distilled water for 10 min and left for 15 min to infuse then cooled and filtered. The filtrate was lyophilized and the desired dose was then prepared and reconstituted in 10 ml of distilled water per kilogram body weight just before oral administration. The aqueous extract dose was 20 mg/kg body weight daily by oral intubations (Eddouks et al., 2005).

### 2- Grouping of rats and experimental design:

The experimental rats were divided into six groups (n= 7 rats). The first group which kept as normal control (-ve) group which fed on standard diet only. The rest of rats were fed on standard diet with 2 % cholesterol for 3 weeks to be hypercholesterolemic, then classified into 5 groups and remained fed on hypercholeslerolemic diet during the experimental period (8 weeks). One of them acted as control (+ve) and the other groups were drug, LS seeds extract, 5% and 10% LS powder.

### 3 –Calculation of some parameters:

Feeding and growth performance were carried out by determination of daily feed intake, body weight gain and feed efficiency ratio (FER) according to Chapman et al., (1950) using the following Formula

$$\text{FER} = \text{Body weight gain} / \text{Feed intake.}$$

### 5 –Collection of samples:

The rats were sacrificed at the end of the experiment (8 weeks). The collected blood samples were centrifuged at 3000 rpm/ 10 minutes to obtain serum. Livers of rats were also collected for some biochemical analysis.

### 5 -Biochemical analysis:

#### A- Serum analysis

Serum cholesterol, triglycerides (TG) and high density lipoprotein cholesterol (HDL-c) were determined by using enzymatic colorimetric methods (Allain et al., 1974, Buccolo and David (1973) and Kostener, 1977, respectively). Very low density lipoprotein cholesterol (VLDL-c) was calculated as TG/5 while low density lipoprotein cholesterol (LDL-c) was calculated as following [LDL-c= Total cholesterol -HDL-c -VLDL-c] according to Fruchart, (1982). Serum aspartate and alanine amino transferase (AST&ALT) enzymes, total protein, and albumin were estimated according to Reitman and Frankel (1957), Weichselbaum (1946) and Bartholomev and Delany (1966), respectively. Serum globulin (G) value was determined by subtracting the albumin from the total proteins according to Coles (1974). In addition, creatinine and urea were estimated according to Bonsens and Taussky, (1984), and Patton and Crouch, (1977) respectively. Atherogenic indexes (cholesterol/ HDL-c & LDL-c/ HDL-c) were calculated according to Castelli and levitar, (1977).

#### B- Liver analysis:

Livers of rats were rapidly removed and parts of them perfuse with 50 to 100 of ice cold 0.9%NaCL solution for estimation of cholesterol, triglycerides , and total lipids, according to Abell et al., (1952) , Seheletter and Nussel, (1975), Folch et al., (1957), respectively.

## III.- Statistical analysis:

Collected data were subjected to analysis according to SPSS Program Differences were considered significant at  $p < 0.05$  (Artimage and Berry, 1987).

## 3. Results:

Data recorded in table (1) showed that the control (+ve) group showed a significant higher value of weight gain and feed efficiency ratio (PER) at  $p<0.01$  while the LS extract, 5% and 10% LS powder rat groups showed a significant higher value of weight gain at  $p<0.05$  compared to control (- ve) group. The drug, LS extract , 5% and 10% LS powder rat groups showed a significant lower value of weight gain and feed efficiency ratio compared to control (+ ve) group.

Table (2) showed that the control (+ve) group showed a significant increase in serum cholesterol, triglycerides, LDL-c and VLDL-c ( $p<0.001$ ) but significant decrease in HDL-c ( $p<0.001$ ) in comparison with control (-ve). On the other hand, the drug, LS extract , 5% and 10% LS powder rat groups showed a significant increase in serum cholesterol ,triglycerides LDL-c and VLDL-c level ( $p<0.05\&0.01$ ) compared to control (- ve) group. The drug, LS extract , 5% and 10% LS powder rat groups showed a significant decrease in serum cholesterol ,triglycerides LDL-c and VLDL-c level in comparison with control (+ve).

Table (3) showed that, control (+ve), LS extract and 5% LS powder rat groups showed a significant increase in cholesterol/ HDL-c and LDL-c/ HDL-c ( $p<0.01\&0.05$ ) while drug and 10% LS powder rat groups showed a significant increase in cholesterol/ HDL-c ( $p<0.05$ ) compared to control (-ve) group. The drug, LS extract, 5% and 10% LS powder rat groups showed a significant decrease in value of cholesterol/ HDL-c and LDL-c/ HDL-c in comparison with control (+ve).

**Table (1): Mean values  $\pm$  SD of body weight gain, feed intake and feed efficiency ratio (FER) of experimental rat groups.**

Groups Variables	Control (-ve)	Hypercholesterolemic				
		Control (+ve)	Drug	LS		
				extract	5%	10%
Initial weight(g)	115.33 $\pm$ 3.41 <sup>a</sup>	116.25 $\pm$ 3.77 <sup>a</sup>	117.35 $\pm$ 4.12 <sup>a</sup>	117.50 $\pm$ 4.81 <sup>a</sup>	116.81 $\pm$ 4.99 <sup>a</sup>	116.75 $\pm$ 4.71 <sup>a</sup>
Final weight(g)	190.53 $\pm$ 11.22 <sup>a</sup>	217.42 $\pm$ 10.33 <sup>a</sup>	198.12 $\pm$ 11.44 <sup>a</sup>	210.64 $\pm$ 13.77 <sup>a</sup>	214.01 $\pm$ 13.25 <sup>a</sup>	211.78 $\pm$ 14.61 <sup>a</sup>
Weight gain(g)	75.20 $\pm$ 5.68 <sup>c</sup>	101.17 $\pm$ 8.24 <sup>a**</sup>	80.77 $\pm$ 7.69 <sup>bc</sup>	93.14 $\pm$ 8.21 <sup>b*</sup>	97.20 $\pm$ 9.11 <sup>b*</sup>	95.03 $\pm$ 8.61 <sup>b*</sup>
Feed intake(g/d)	15.11 $\pm$ 1.24 <sup>a</sup>	16.22 $\pm$ 1.18 <sup>a</sup>	15.75 $\pm$ 2.01 <sup>a</sup>	16.53 $\pm$ 2.11 <sup>a</sup>	16.31 $\pm$ 1.89 <sup>a</sup>	16.41 $\pm$ 1.49 <sup>a</sup>
FER	0.082 $\pm$ 0.003 <sup>b</sup>	0.103 $\pm$ 0.002 <sup>a**</sup>	0.085 $\pm$ 0.001 <sup>b</sup>	0.093 $\pm$ 0.001 <sup>b</sup>	0.099 $\pm$ 0.004 <sup>b</sup>	0.096 $\pm$ 0.005 <sup>b</sup>

Significant with control group \*  $P<0.05$  \*\*  $P<0.01$  \*\*\*  $P<0.001$

Mean values in each row having different superscript (a, b &c,) are significant.

From data presented in table (4), it could be noticed that control (+ve) ,drug, LS extract , and 5% LS powder rat groups showed a significant increase in serum alanine and aspartate aminotransferase (ALT &AST) at  $p<0.01$  compared to control (- ve) group. The drug, LS extract , 5% and 10% LS powder rat groups showed a significant decrease in serum alanine and aspartate aminotransferase enzymes in comparison with control (+ ve) group .

As shown in table (5), the control (+ve) showed a significant decrease in globuline ( $p<0.05$ ) but a significant increase in creatinine and urea ( $p<0.01$ ) compared to control (- ve) group. The drug, LS extract , 5% and 10% LS powder rat groups showed a significant increase in serum creatinine and urea ( $p<0.05$ ) compared to control (- ve) group. The drug, LS extract , 5% and 10% LS powder rat groups showed a significant increase in serum globulin and a significant decrease in serum creatinine and urea compared to control (+ ve) group.

As recorded in table (6) showed that control (+ve) group showed a significant increase in liver cholesterol and total lipids ( $p<0.01$ ) but significant decrease in liver triglycerides ( $p<0.05$ ) in comparison with control (- ve).The drug, LS extract, 5% and 10% LS powder rat groups showed a non significant difference in liver cholesterol, triglycerides and total lipids in comparison with control (- ve) group. The drug, LS extract , 5% and 10% LS powder rat groups showed a significant decrease in liver cholesterol and total lipids but significant increase in liver triglycerides in comparison with control (+ ve) group.

**Table (2) The Mean values ± SD of some serum lipid patterns of experimental rat groups.**

Groups Variables	Control (-ve)	Hypercholesterolemic				
		Control (+ve)	Drug	LS		
				extract	5%	10%
Cholesterol (mg/dl)	80.34± 7.88 <sup>c</sup>	199.77±11 .43 <sup>a***</sup>	106.78±10 .18 <sup>b*</sup>	110.01± 11.33 <sup>b*</sup>	113.24±12 .14 <sup>b*</sup>	105.35± 10.61 <sup>b*</sup>
Triglyceride (mg/dl)	70.31 ± 6.12 <sup>c</sup>	155.14 ± 18.48 <sup>a***</sup>	95.67 ± 10.11 <sup>b*</sup>	98.01 ± 11.31 <sup>b*</sup>	96.18 ± 10.15 <sup>b*</sup>	94.31 ± 9.96 <sup>b*</sup>
HDLc (mg/dl)	32.32 ± 3.47 <sup>a</sup>	20.11 ± 2.87 <sup>b***</sup>	29.75 ± 2.78 <sup>a</sup>	28.88 ± 3.11 <sup>a</sup>	31.14 ± 2.99 <sup>a</sup>	30.91 ± 3.03 <sup>a</sup>
LDLc (mg/dl)	33.06 ± 4.01 <sup>c</sup>	104.01 ± 10.22 <sup>a***</sup>	57.90 ± 6.08 <sup>b**</sup>	61.53 ± 7.18 <sup>b**</sup>	62.87 ± 7.33 <sup>b**</sup>	55.58 ± 6.14 <sup>b**</sup>
VLDLc (mg/dl)	14.01 ± 1.81 <sup>c</sup>	31.02 ± 3.17 <sup>a***</sup>	19.13 ± 2.15 <sup>b*</sup>	19.60 ± 2.11 <sup>b*</sup>	19.23 ± 2.16 <sup>b*</sup>	18.86 ± 2.22 <sup>b*</sup>

Significant with control group \* P&lt;0.05 \*\* P&lt;0.01 \*\*\* P&lt;0.001

Mean values in each row having different superscript (a, b &amp;c,) are significant.

**Table (3) The Mean values ± SD of atherogenic indexes of experimental rat groups.**

Groups Variables	Control (-ve)	Hypercholesterolemic				
		Control (+ve)	Drug	LS		
				extract	5%	10%
Cholesterol/H DLC	2.42± 0.38 <sup>c</sup>	9.93± 1.77 <sup>a***</sup>	3.58± 0.77 <sup>b*</sup>	3.80± 0.75 <sup>b*</sup>	3.63± 0.65 <sup>b*</sup>	3.40 ± 0.54 <sup>b*</sup>
LDLc/ HDLc	1.05± 0.16 <sup>c</sup>	5.17± 1.14 <sup>a***</sup>	1.94± 0.18 <sup>bc</sup>	2.13± 0.47 <sup>b*</sup>	2.01± 0.32 <sup>b*</sup>	1.79± 0.18 <sup>bc</sup>

Significant with control group \* P&lt;0.05 \*\* P&lt;0.01 \*\*\* P&lt;0.001

Mean values in each row having different superscript (a, b &amp;c,) are significant.

**Table (4) The Mean values ± SD of serum amino transferase (ALT & AST) enzymes of experimental rat groups.**

Groups Variables	Control (-ve)	Hypercholesterolemic				
		Control (+ve)	Drug	LS		
				extract	5%	10%
ALT (μ /ml)	27.69± 2.81 <sup>c</sup>	49.14± 4.31 <sup>a**</sup>	34.41± 3.27 <sup>b*</sup>	33.47± 3.61 <sup>b*</sup>	34.21± 3.11 <sup>b*</sup>	31.78± 3.18 <sup>bc</sup>
AST (μ /ml)	41.27± 4.22 <sup>C</sup>	67.71± 5.91 <sup>a**</sup>	51.17± 5.15 <sup>b*</sup>	49.39± 5.31 <sup>b*</sup>	48.11± 3.92 <sup>b*</sup>	40.31± 4.16 <sup>c</sup>

Significant with control group \* P&lt;0.05 \*\* P&lt;0.01 \*\*\* P&lt;0.001

Mean values in each row having different superscript (a, b &amp;c,) are significant.

**Table (5) The Mean values ± SD of serum total protein, albumin, globulin, creatinine and urea of experimental rat groups.**

Groups Variables	Control (-ve)	Hypercholesterolemic				
		Control (+ve)	Drug	LS		
				extract	5%	10%
T. protein (g/dl)	7.30± 1.21 <sup>a</sup>	6.11± 1.31 <sup>a</sup>	6.81± 1.30 <sup>a</sup>	6.87± 1.41 <sup>a</sup>	6.91± 1.35 <sup>a</sup>	6.90± 1.40 <sup>a</sup>
Albumin (g/dl)	3.71± 0.55 <sup>a</sup>	3.99± 0.45 <sup>a</sup>	3.59± 0.13 <sup>a</sup>	3.24± 0.19 <sup>a</sup>	3.49 ± 0.23 <sup>a</sup>	3.11± 0.16 <sup>a</sup>
Globulin (g/dl)	3.59± 0.22 <sup>a</sup>	2.12± 0.13 <sup>b*</sup>	3.22± 0.66 <sup>a</sup>	3.63± 0.45 <sup>a</sup>	3.42± 0.48 <sup>a</sup>	3.79± 0.55 <sup>a</sup>
Creatinine (mg/dl)	0.75± 0.01 <sup>c</sup>	1.01± 0.15 <sup>a**</sup>	0.98± 0.06 <sup>b*</sup>	0.97± 0.11 <sup>b*</sup>	0.95± 0.12 <sup>b*</sup>	0.92± 0.14 <sup>b*</sup>
Urea (mg/dl)	40.14± 4.77 <sup>c</sup>	55.79± 7.11 <sup>a**</sup>	50.17± 6.18 <sup>ab*</sup>	49.31± 5.11 <sup>b*</sup>	48.20± 4.25 <sup>b*</sup>	47.32± 4.60 <sup>b*</sup>

Significant with control group \* P&lt;0.05 \*\* P&lt;0.01 \*\*\* P&lt;0.001

Mean values in each row having different superscript (a, b &amp;c,) are significant.

**Table (6) The Mean values ± SD of liver cholesterol, total lipids and triglyceride of experimental rat groups.**

Groups Variables	Control (-ve)	Hypercholesterolemic				
		Control (+ve)	Drug	LS		
				extract	5%	10%
Cholesterol (mg/g)	3.66± 0.88 <sup>b</sup>	6.96± 1.41 <sup>a**</sup>	4.01± 0.89 <sup>b</sup>	4.35± 0.95 <sup>b</sup>	4.44± 0.58 <sup>b</sup>	4.25± 0.77 <sup>b</sup>
Total lipids (mg/g)	34.52± 3.21 <sup>b</sup>	48.99± 4.11 <sup>a**</sup>	36.11± 3.41 <sup>b</sup>	38.20± 2.71 <sup>b</sup>	38.77± 2.91 <sup>b</sup>	37.43± 3.11 <sup>b</sup>
Triglyceride (mg/g)	2.44± 0.11 <sup>a</sup>	1.65± 0.36 <sup>b*</sup>	2.11± 0.33 <sup>a</sup>	2.31± 0.35 <sup>a</sup>	2.01± 0.31 <sup>a</sup>	2.21± 0.30 <sup>a</sup>

Significant with control group \* P&lt;0.05 \*\* P&lt;0.01 \*\*\* P&lt;0.001

Mean values in each row having different superscript (a, b &amp;c,) are significant.

#### 4. Discussion:

Our investigation revealed that, consumption of *Lepidium sativum* L.(LS) seeds increase weight gain as LS seeds are found to contain 18–24% of fat which 34% of total fatty acids is alpha linolenic acid. It contains good amount of lignans and antioxidants, which can stabilize the n-3 polyunsaturated fatty acids in its seed oil. LS oil has alpha linoleic acid which could give it nutritional advantages (Gunstone, 2004 and Diwakara et al., 2008). The primary fatty acids in LS oil were oleic (30.6 wt %) and linolenic acids (29.3 wt %). LS seeds oil contained high concentrations of tocopherols. The primary phytosterols in L S were sitosterol and campesterol, with avenasterol (Bryan et al., 2009).

The results of lipid profile were agreed with results obtained by Das et al., (1997) who reported that the lipid profile of hypercholesterolemic animals were significantly higher than control rats for total lipid, total cholesterol , triglyceride, LDL-c and VLDL-c while only HDL-c was significantly lower in hypercholesterolemic rats than in control rats.

The increased plasma cholesterol, particularly LDL-c is one of the most important risk factor for coronary vascular disease. LDL-c particle are taken up by macrophage cells after oxidized or modified and then deposited in the arterial intima leading to formation of atheroma (Durrington, 1995). Low HDL-c levels are considered as a strong risk factor for coronary heart disease as HDL-c act as antioxidant and protect LDL-c from oxidation so that

reduce LDL-c from circulation (Boden and Pearson, 2000 and Glass and Witztum, 2001). Lepidium sativum L. seeds have revealed presence of glycoside, alkaloids, tannin (Phenolic compound), flavonoids, and amino acids like glutamine, cysteine, and glycine. The tannin and flavonoids may have antioxidant activity whenever glutamate, cysteine, glycine are intermediates for synthesis of the endogenous antioxidant glutathione. Diets rich in alpha linolenic acid have been associated with a reduced risk of fatal ischemic heart disease, a reduction in heart attacks and mortality from chronic vascular disease. Feeding alpha linolenic acid has also been shown to decrease platelet aggregation, total cholesterol, LDL cholesterol and triglycerides in humans and rats (Olsson, and Yuan 1996, Kirtkar and Basu 2005 and Hamer and Steptoe, 2006).

Protective and curative treatment of ethanolic extract of Lepidium sativum seeds L. in renal failure of rats significantly reduced the level of urea and creatinine that indicates increase glomerular filtration rate. Extract of Lepidium sativum L. seeds may be having nephroprotective and curative activity. Daily oral administration of aqueous LS extract for 3 weeks exhibited antihypertensive and diuretic activities. ALT and AST are closely correlated in most cases of liver diseases. Excessive storage of fat in the liver effects on liver functions and increases the susceptibility to free radical attack in hypcholesterolemic rats resulting in liver damage as described by Tahri et al .,(2000), Mhamed et al .,(2005) and Yadav et al .,(2009). Feeding rats with 10% Garden cress seed oil lowered hepatic cholesterol by 12.3% and serum triglycerides by 40.4% compared to SFO fed group. Very low density lipoprotein cholesterol (VLDL-C) and low density lipoprotein cholesterol (LDL-C) levels decreased by 9.45% in serum of 10% LS oil fed rats, while HDL remained unchanged among LS oil fed rats (Diwakar et al .,2008)

#### **Corresponding author**

Wafeqa Abdulah Al Hamedan  
Department of Nutrition and Food Science, Home Economic, Collage, Princess Nora Bent abdul - rahman -University, Riyadh, Saud Arabia

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9/29/2010

# Investigation of MLS<sub>B</sub> and tetracycline resistance in coagulase-negative staphylococci isolated from the skin of Egyptian acne patients and controls

**El-Mahdy, T.S.\*<sup>1,2</sup>; Abdalla, S.<sup>3</sup>; El-Domany, R.<sup>2</sup> and Snelling, A.M.<sup>1</sup>**

<sup>1</sup>Dept. of Biomedical Sciences, University of Bradford, UK <sup>2</sup>Faculty of Pharmacy, University of Helwan, Egypt

<sup>3</sup>Faculty of Pharmacy, University of Suez-Canal, Egypt

<sup>\*</sup>[Sata186@hotmail.com](mailto:Sata186@hotmail.com)

**Abstract:** A total of 335 antibiotic-resistant coagulase-negative staphylococci (CNS) were isolated from face of 53 Egyptian acne patients, 13 dermatology staff and 36 controls. Prevalence of tetracycline resistant CNS was the most common with a rate of 87.3% of total population sampled. Acne patients treated with antibiotics were found to have significant higher risk of carrying erythromycin and clindamycin resistant CNS than patients not under treatment. Staff group was the most common cohort to carry multi-resistant CNS strains with a prevalence of 81.2%. Four erythromycin-resistance genes were screened for 43 CNS strains from patients. The most widely distributed determinants were *msr(A)* alone (48.8%), followed by *erm(C)* alone (39.6% strains) while both determinants together were accounted in 11.6% of the isolates. In addition, 48 non-duplicate tetracycline resistant CNS strains from patients were screened for the presence of four tetracycline resistance genes. Forty-seven of the isolates (97.9%) had *tet(K)* gene. *Tet(L)* gene was only found in four isolates (8.3%), from which three isolates were found to carry also *tet(K)* gene. This study revealed that the high carriage rate of *msr(A)* in our isolates suggests the effective therapy with clindamycin for most of erythromycin resistant CNS infections. In addition, the mechanism of tetracycline resistance in our isolates is mainly by active efflux and we might expect the success of treatment with minocycline in most of tetracycline resistant CNS from Egypt.

[El-Mahdy, T.S.; Abdalla, S.; El-Domany, R. and Snelling, A.M. Investigation of MLS<sub>B</sub> and tetracycline resistance in coagulase-negative staphylococci isolated from the skin of Egyptian acne patients and controls. Journal of American Science 2010;6(11):880-888]. (ISSN: 1545-1003).

**Key words:** acne, coagulase-negative staphylococci, MLS<sub>B</sub>, resistance, tetracycline. *Running title:* Antibiotic resistance of Egyptian CNS.

## 1. Introduction:

Antibiotics remain the cornerstone of acne treatment. Whilst *Propionibacterium acnes* is being targeted, selective pressure is also exerted on other members of the commensal skin flora, including CNS. These bacteria may then act as reservoirs of resistance genes for more pathogenic strains or species. Several species of CNS are recognized as potential opportunistic pathogens, mainly causing nosocomial infections (Righter, 1987). CNS especially methicillin-resistant is one of the main causes of nosocomial blood stream infection in ICUs in Assiut University hospitals, Upper Egypt (Ahmed *et al.*, 2009)

Macrolide, lincosamide and streptogramin B (MLS<sub>B</sub>) antibiotics are chemically distinct but share a similar mode of action. Three mechanisms have been involved in staphylococcal resistance to macrolides; target-site modification [encoded by *erm* genes] (Skinner *et al.*, 1983), active efflux [encoded by *msr(A)*] (Ross *et al.*, 1989), and rarely by drug inactivation (Weisblum, 1998). CNS strains carrying *erm* genes are cross-resistant to all MLS<sub>B</sub>. However,

strains carrying *msr(A)* gene are only resistant to MS<sub>B</sub> antibiotics (Ross *et al.*, 1990).

Bacterial resistance to tetracycline was mediated by: efflux proteins [encoded by *tet(K)* and *tet(L)* genes], ribosomal protection proteins [encoded by *tet(M)* and *tet(O)* genes], enzymatic inactivation of tetracyclines and target modification (Roberts, 2005). *Tet(K)* and *tet(L)* genes confer high level resistance to tetracycline but not minocycline. In contrast, *tet(M)* and *tet(O)* genes confer resistance to minocycline (Chopra & Roberts, 2001).

Erythromycin, clindamycin and tetracycline are widely used in Egypt to treat acne, and available over the counter, but it is not known how this is affecting the commensal flora. This study sought to determine the prevalence of resistant CNS, and genes responsible, isolated from Egyptian acne patients attending dermatology clinics and controls.

## 2. Materials and methods

### Subjects

A total of 53 patients (23 male and 30 female) aged 15-29 years (average 20 years) attending two dermatology clinics in Cairo at:

Dermatology Clinic, Ain-Shams University Hospital and Cairo Dermatology Hospital, participated in this study. Thirteen dermatology staff (nurses and doctors) from the same dermatology clinics were also sampled. Patients were whether currently on or off treatment. Also 36 age-matched controls from the community were not suffering acne and no antibiotics taken in past six months. All patients, controls and dermatology staff were informed and gave their verbal and written informed consent to take part in this study. All participants in the study were asked to fill a questionnaire. The study was approved by the local Ethics Committees.

#### Sampling method

Cutaneous CNS isolates were collected from the face of Egyptian acne patients and controls according to the method applied by Ross *et al.* (2003). Applying firm pressure, the surface of the entire face was rubbed with a transport swab (Copan Italia, Brescia, Italy) moistened in sterile wash fluid (0.075 mol/L sodium phosphate buffer, pH 7.9) containing 0.1% Triton-X 100. Swabs were placed into tubes of Amies medium prior to transfer in the same day of sampling at 4°C to Bradford University, UK and arrived after two days by experienced courier on two shipments.

#### Isolation and identification of resistant CNS

Swabs were used to inoculate Muller-Hinton (MH) agar plates containing breakpoint concentrations according to CLSI guidelines of 4 mg/L erythromycin, 2 mg/L clindamycin or 8 mg/L tetracycline as well as antibiotic-free control plates, which were always inoculated last. After 48 hours aerobic incubation at 37°C, plates were inspected for growth. One representative isolate in colonies morphologically resembling staphylococci was chosen per plate but if more than one colony morphology was evident, a representative of each was selected for further study using Gram staining, coagulase test (Staphaurex, Remel, USA) and carbohydrate fermentation (method adopted from Kloos & Schleifer, 1975).

Selected coagulase-negative strains were also further identified using MASTRING *Staphylococcus* ID kit for identification of CNS (Mast, UK) as per manufacturer's protocol and PCR-ribotyping of staphylococci protocol. Eleven CNS reference strains were used, included *S. hominis* NCTC 11320; *S. warnari* NCTC 11044; *S. capitis* NCTC 11045; *S. epidermidis* NCTC 11047; *S. cohnii* NCTC 11041; *S. haemolyticus* NCTC 11042; *S. epidermidis* NCTC 2749; *S. aureus* NCTC 6571 (Oxford); *S. simulans* NCTC 11046; *S. xylosus*

NCTC 11043 and *S. hyicus* sub. *chromogenes* NCTC 11530.

#### Antibiotics

Antibiotics were purchased from Sigma (Poole, U.K.) and were dissolved in water with the exception of erythromycin, which was dissolved in absolute ethanol.

#### Determination of MICs

MICs for the three antibiotics were determined by agar dilution on MH agar as described by CLSI using multipoint inoculator (Denley, Tech Ltd, Bolney Sussex, U.K). Type strain *S. aureus* NCTC 6571 (Oxford) was included as a susceptible control.

#### DNA preparation

A. For PCR detection of tetracycline and erythromycin resistance genes in CNS

Lysostaphin (50µl at 2 mg/mL cells suspended in 1X TE buffer) was used to weaken the cell walls and incubating at 37°C for up to 1 h. Genomic DNA was extracted twice with phenol/chloroform and precipitated by absolute ethanol as described by Eady *et al.* (1993).

#### B. For PCR-ribotyping of CNS

Using NET (10 mM Tris, 1mM EDTA, 10 mM NaCl)/Achromopeptidase (stock solution 10 units/µl, Sigma, code A3547) solution as described by Kobayashi *et al.* (1994).

#### PCR-ribotyping for species identification of CNS

The PCR reaction was performed as mentioned before by Jensen *et al.* (1993). A pair of primers was used within the 16S-23S rRNA spacer region. The PCR-ribotyping amplification patterns of CNS isolates were visually compared with those obtained for the reference strains.

The electrophoresis of PCR-ribotyping of CNS was carried out using 1X TBE buffer (89 mM Tris, 89 mM borate, 2mM EDTA, pH 8.3) and the gel was run at 100V for three hours in a large gel tank. All obtained fragments were visualized by ethidium bromide (Sigma) staining after gel electrophoresis using 2% agarose gels. The sizes of the PCR products were determined by comparing them with the migration of 100-bp DNA ladder (Fermentas).

#### PCR to investigate tetracycline and erythromycin resistance genes in CNS

Table 1 gives the primer sequences and PCR reaction conditions for each target gene. The PCR reaction was performed in a 20 µl volume; containing 1 µl DNA extract, 2 µl of 10x thermopol buffer

containing 2mM MgSO<sub>4</sub> (New England Biolabs, Ipswich, UK), 2µl of PCR nucleotide mix including 2 mM each of dNTP (New England Biolabs, UK), 0.25 µl of 0.1nm/µl each primer (Sigma Genosys, Ltd, London, UK) and 0.1 µl of Taq DNA polymerase (New England Biolabs, NEB), the volume for each PCR reaction was completed to 20 µl by molecular

biology grade water (Eppendorf, Hamburg, Germany). All PCR reactions were started by an initial denaturation step at 94°C for 4 min, and ended by a final elongation step at 72°C for 5 min. All obtained fragments were visualized as mentioned before.

**Table 1: Primer sequences and PCR conditions used to detect tetracycline and erythromycin resistance determinants in CNS**

Resistance gene	PCR primer sequence 5'-3'	PCR reaction conditions	Amplicon Size (bp)	GenBank accession no.	Positive control/ Reference
<i>erm</i> (A)	<b>F</b> -GTTCAAGAACAAAT CAATACAGAG <b>R</b> -GGATCAGGAAAA GGACATTTCAC	30 cycles (30s at 94°C; 30s at 52°C; 1 min. at 72°C)	421	K02987	<i>S. aureus</i> CW9/pSES29/ Leclercq et al., 1989
<i>erm</i> (B)	<b>F</b> -CCGTTTACGAAAT TGGAACAGGTAAAGGGC <b>R</b> -GAATCGAGACTT GAGTGTGC	As <i>erm</i> (A)	359	U35228	<i>S. intermedius</i> / Trieu-Cuot et al., 1990
<i>erm</i> (C)	<b>F</b> -GCTAATATTGTTT AAATCGTCAATTCC <b>R</b> -GGATCAGGAAAA GGACATTTCAC	As <i>erm</i> (A)	572	X54338	<i>S. aureus</i> RN4220/pE194/ Leclercq et al., 1989
<i>msr</i> (A)	<b>F</b> -GGCACACAATAAGA GTGTTAAAGG <b>R</b> -AAGTTATATCATG AATAGATTGTCCTGTT	30 cycles (1 min at 94°C; 1 min at 50°C; 90s at 72°C)	940	X52085	<i>S. aureus</i> RN4220/pUL505 4/ Ross et al., 1990
<i>tet</i> (K)	<b>F</b> -GTAGCGACAATA GGTAAATAGT <b>R</b> -GTAGTGACAATA AACCTCCTA	30 cycles (30s at 94°C; 30s at 55°C; 30s at 72°C)	360	S67449	<i>S. aureus</i> RN4220/ PVPF5/ Guay et al., 1993
<i>tet</i> (L)	<b>F</b> -TCGTTAGCGTGCT GTCATTG <b>R</b> -GTATCCCACCAAT GTAGCCG	35 cycles (1 min at 94°C; 1 min at 58°C; 90s at 72°C)	267	U17153	<i>Bacillus cereus</i> VPC 1214/ Burdett et al., 1982
<i>tet</i> (M)	<b>F</b> -AGTTTAGCTCAT GTTGATG <b>R</b> -TCCGACTATTTAG ACGACGG	35 cycles (1 min at 95°C; 1 min at 50°C; 2 min at 72°C)	1862	M21136	<i>Enterococcus faecalis</i> fol / Nesin et al., 1990
<i>tet</i> (O)	<b>F</b> -AACTTAGGCATTC TGGCTCAC <b>R</b> -TCCCACTGTTCCA TATCGTCA	35 cycles (1 min at 94°C; 1 min at 50°C; 90s at 72°C)	515	Y07780	<i>Escherichia coli</i> DH5α/ Taylor et al., 1987

## Assay for resistance phenotype pattern in erythromycin resistant CNS

Selected CNS strains demonstrating erythromycin resistance were screened for the MLS<sub>B</sub> and MS<sub>B</sub> phenotype as described by CLSI guidelines, 2007. Flattening of the zone around the clindamycin disc indicated an inducible MLS<sub>B</sub> phenotype while constitutive MLS<sub>B</sub> phenotype shows no inhibition zone around both discs. In contrast, the MS<sub>B</sub> resistant isolates do not show flattening of the clindamycin zone next to the erythromycin disc.

### 3. Results and Discussion:

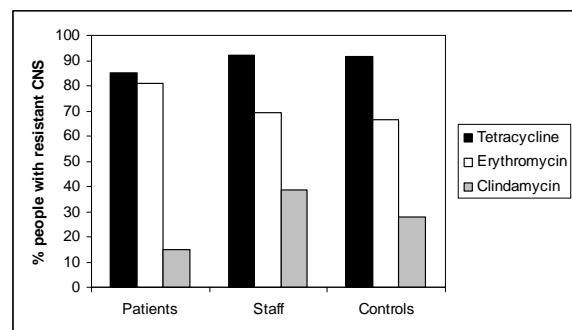
#### Demographics of study participants

More than half of the patients (62.2%) had acne from a period of 1-3 years. Twelve (22.6%) patients sampled had never used any specific acne treatment prior to the study. However, only 26.4% of patients were currently on any kind of acne therapy at the time of sampling. There was no significant difference between patient sex and severity of acne ( $P > 0.05$ ).

#### Prevalence of skin colonization with antibiotic resistant CNS

Figure 1 shows the % of people in each cohort carrying antibiotic resistant CNS amongst their skin flora, as determined from the primary selective plates. The prevalence of tetracycline resistance was the most common amongst antibiotics tested with a rate of 87.3% of all cohorts sampled. The difference in prevalence of tetracycline resistance between patients, clinic staff, and controls was not significant ( $P>0.05$ ). Patients on current or very recent antibiotic treatment were no more likely to carry tetracycline resistant strains (24 people of 45) than those using other or no medication (21 people of 45) ( $P>0.05$ ).

In keeping with the observations of Miller *et al.* (1996) where they studied the staphylococcal resistance on the skin of acne contacts and controls, a majority of our controls carried staphylococcal strains resistant to tetracycline (95.1% of their controls *vs* 91.4% of controls in the current study), erythromycin (70.7% *vs* 66.7%) and clindamycin (24.4% *vs* 27.8%). In addition, prevalence of bacterial resistance to erythromycin was 95% for *S. epidermidis* strains isolated from acne patients in a French study (Dreno *et al.*, 2001). These results are higher than those reported by Nishijima *et al.* (1994) in Japan and by Bouchami *et al.* (2007) in Tunisia with a percentage of 61% and 62%, respectively, but nearly similar to our current study (81.1%) in Egypt and to Forssman (1995) in Switzerland with a percentage of 100%.



**Figure 1: Prevalence of antibiotic resistant CNS amongst the cohorts tested**

Egyptian acne patients treated with antibiotics were found to have a higher risk of carrying erythromycin and clindamycin resistant CNS ( $P= 0.023$  and  $0.036$ , respectively) than patients not under treatment. On the contrary, Dreno *et al.* (2001) showed that the use of previous or current treatment with erythromycin does not influence the frequency of resistant strains of *S. epidermidis*. This contradiction may be explained by the extensive use of both antibiotics in treatment of acne in Egypt.

#### Phenotypic and MIC profiles of antibiotic-resistant CNS

The susceptibilities of the 335 resistant isolates to the three antibiotics were determined by agar dilution. Table 2 illustrates the number of antibiotic-resistant CNS within the different cohorts.

Forty-seven strains of 117 (40.2%) carrying erythromycin resistance in patients had high-level resistance ( $MIC > 1024$  mg/L). Eight CNS strains of 116 (6.9%) from patients were found to have high-level resistance to tetracycline ( $MIC \geq 256$  mg/L). Staff group was the most common cohort to carry multi-resistant CNS strains with a prevalence of 81.2% of total strains from staff having resistance to two or three antibiotics. The incidence of multi-resistant CNS amongst isolates retained from patients and controls were 75.5% and 71%, respectively. All clindamycin resistant strains were also resistant to erythromycin.

#### Genetic diversity of erythromycin -resistant CNS

A total of 43 erythromycin resistant strains were retained from the 43 patients who were colonized with erythromycin resistant-CNS and identified to the species level. The PCR-ribotyping amplification patterns of CNS isolates were visually compared with those obtained for the reference strains.

**Table 2: Number of antibiotic-resistant CNS strains and cross-resistance obtained from different cohorts**

Antibiotic	No. of CNS strains resistant to antibiotic(s) / %			
	All cohorts (Total=335)	Patients (Total=163)	Controls (Total=124)	Staff (Total=48)
Tet	238 / 71	116 / 71.2	82 / 66.1	40 / 83.3
Ery	221 / 66	117 / 71.8	71 / 57.2	33 / 68.8
Clin	59 / 17.6	21 / 12.9	28 / 22.6	10 / 20.8
Cross-resistance to two Abs	151 / 45.1	70 / 42.9	60 / 48.4	21 / 43.8
Cross-resistance to three Abs	99 / 29.6	53 / 32.5	28 / 22.6	18 / 37.5

High level resistance to erythromycin was seen in 51.2% of the tested isolates ( $MIC > 1024$  mg/L) and all of these isolates harbor *erm(C)* gene (Table 3). The most widely-distributed erythromycin resistance determinants was *msr(A)*. The expression of *erm(C)* was either inducible or constitutive. All CNS carrying only *msr(A)* were clindamycin susceptible and have low level resistance to

erythromycin ( $MIC \leq 128$  mg/L). All strains carrying both *erm(C)* and *msr(A)* genes phenotypically express the inducible or constitutive  $MLS_B$  pattern. *S. simulans* and *S. epidermidis* were the major CNS species with *erm* mechanism (81.8%, 18 of 22 strains), in contrast, *S. haemolyticus* and *S. hominis* were the predominant *msr(A)* carrying species (61.5%; 16 of 26 strains) (Table 3).

**Table 3: Genotypic and phenotypic distribution of erythromycin resistant CNS**

Ery resistance gene(s)	No. of strains (%) (n=43)	Ery MIC range mg/L (mode)	Phenotype	CNS species
<i>erm(A)</i>	0	0	0	0
<i>erm(B)</i>	0	0	0	0
<i>erm(C)</i>	17 (39.6)	>1024 (>1024)	13 i $MLS_B$ 4 c $MLS_B$	<sup>a</sup>
<i>msr(A)</i>	21 (48.8)	16-128 (32)	21 non-inducible MS <sub>B</sub>	<sup>b</sup>
<i>erm(C)/ msr(A)</i>	5 (11.6)	>1024 (>1024)	4 i $MLS_B$ 1 c $MLS_B$	<sup>c</sup>

i $MLS_B$ : inducible  $MLS_B$

c $MLS_B$ : constitutive  $MLS_B$

<sup>a</sup>7 *S. simulans*, 6 *S. epidermidis*, 2 *S. haemolyticus*, 1 *S. hominis*, and 1 *S. saprophyticus*.

<sup>b</sup>9 *S. haemolyticus*, 7 *S. hominis*, 2 *S. cohnii*, 1 *S. epidermidis*, 1 *S. saprophyticus*, and 1 *S. simulans*.

<sup>c</sup>3 *S. epidermidis*, and 2 *S. simulans*

The available data corresponding to this study was compared in Table 4. Lina *et al.* (1999) found that macrolide resistance due to *msr(A)* was more prevalent in CNS (14.6%) than in *S. aureus* (2.1%). This *msr(A)* ratio in CNS is much lower than our finding that 60.5% of selected erythromycin resistant strains of CNS from Egypt have *msr(A)* gene alone or in combination with *erm(C)*. Similarly to the current study, Bouchami *et al.* (2007) reported that the MIC of erythromycin varied between 32 and >1024 mg/L for isolates harboring *erm* genes and between 16 and 32 mg/L for those harboring *msr(A)*.

The present study extends the data from previous studies that  $MLS_B$  resistance in CNS was caused most often by *erm(C)*. Carriage of *msr(A)* is rarely seen in *S. aureus*, but seems to be more frequent in CNS (Lina *et al.*, 1999). However, one study from USA (Fiebelkorn *et al.*, 2003) reported that *msr(A)* gene was present in a high proportion of *S. aureus* isolates (36%), indicating that geographical differences may exist. It can be concluded from the present study that in our Egyptian CNS tested strains, clindamycin treatment should be considered as effective therapy due to the high carriage rate of the *msr(A)* gene by our isolates.

**Table 4: Comparison of relevant studies on distribution of resistance genes *erm(A)*, *erm(B)*, *erm(C)* and *msr(A)* among isolates of CNS**

Study	Location of isolates	Type of specimens	% of strains with					MLS <sub>B</sub> Phenotype
			<i>erm(A)</i>	<i>erm(B)</i>	<i>erm(C)</i>	<i>msr(A)</i>	<i>erm + msr(A)</i>	
Eady <i>et al.</i> , 1993	UK	Skin and clinical <sup>a</sup>	5.9	7.2	48	29.4	3.6	47% iMLS <sub>B</sub> 24% cMLS <sub>B</sub>
Lina <i>et al.</i> , 1999	France	Clinical	18	0.7	46.7	14.6	3.3	27.3% iMLS <sub>B</sub> 34.6% cMLS <sub>B</sub>
Novotna <i>et al.</i> , 2005	Czech Republic	Clinical	----	ND <sup>b</sup>	43 <sup>c</sup>	53	16.3	16% iMLS <sub>B</sub> 20% cMLS <sub>B</sub>
Martineau <i>et al.</i> , 2000	Canada, China and France	Clinical	6.3	0.7	87.4	5.6	0	ND <sup>b</sup>
Thakker-varia <i>et al.</i> , 1987	USA	Clinical	19	ND <sup>b</sup>	73.8	ND <sup>b</sup>	ND <sup>b</sup>	35.7% iMLS <sub>B</sub> 57.1% cMLS <sub>B</sub>
Gatermann <i>et al.</i> , 2007	Germany	Mostly clinical	5.3	2.3	65.6	23.6	2.4	25.6% iMLS <sub>B</sub> 51% cMLS <sub>B</sub>
Aktas <i>et al.</i> , 2007	Turkey	Clinical	8.9	6.4	78.2	11.5	3.8	20.6% iMLS <sub>B</sub> 57.8% cMLS <sub>B</sub>
Bouchami <i>et al.</i> , 2007	Tunisia	Clinical <sup>d</sup>	32	ND <sup>b</sup>	53	15	ND <sup>b</sup>	1% iMLS <sub>B</sub> 44% cMLS <sub>B</sub>
<b>Current study</b>	<b>Egypt</b>	<b>Skin</b>	<b>0</b>	<b>0</b>	<b>39.6</b>	<b>48.8</b>	<b>11.6</b>	<b>39.5% iMLS<sub>B</sub></b> <b>11.6% cMLS<sub>B</sub></b>

cMLS<sub>B</sub>: constitutive MLS<sub>B</sub>    iMLS<sub>B</sub>: inducible MLS<sub>B</sub>    <sup>a</sup> from human and animal<sup>b</sup> not determined<sup>c</sup> for *erm(C)* and *erm(A)*<sup>d</sup> from neutropenic patients

#### Genetic diversity of tetracycline-resistant CNS

A total of 48 non-duplicate tetracycline resistant CNS strains were chosen from patients and identified to the species level. Breakpoint of doxycycline and minocycline was 8 mg/L as set by CLSI. Forty-two strains (87.5%) had cross-resistance to doxycycline. The majority of isolates had doxycycline MICs in the range of 8-16 mg/L. None of the strains tested had resistance to minocycline.

All strains were screened for the presence of four tetracycline resistance genes; *tet(K)*, *tet(L)*, *tet(M)*, and *tet(O)*. Forty-seven of the isolates (97.9%) had *tet(K)* gene. *Tet(L)* gene was only found

in four isolates (8.3%), from which three isolates were found to also carry *tet(K)* gene (Table 5). Similarly, Bismuth *et al.* (1990) from France reported that 97.6% of tetracycline resistant CNS carry *tet(K)*, using DNA-DNA hybridization, which was detected in all of the species studied. Ardic *et al.* (2005) in Turkey reported that *tet(K)* genes were detected widely (42.9%) in CNS, whilst *tet(M)* genes were mainly seen in MRSA (50.0%). The frequency of *tet(K)* was much lower in Turkey than our study, but it is important to acknowledge that Ardic *et al.* (2005) had not selected the isolates on the basis of tetracycline resistance.

**Table 5: Distribution of *tet* gene classes among CNS**

<i>tet</i> resistance gene(s)	No. of strains (%) (n=48)	MIC range mg/L (mode)			CNS species
		Tetracycline	Doxycycline	Minocycline	
<i>tet(K)</i>	44 (91.7)	16->256 (64)	2-64 (8)	0.125-2 (0.25)	All <sup>a</sup>
<i>tet(L)</i>	1 (2.1)	64	16	0.25	<i>S. haemolyticus</i>
<i>tet(K) / tet(L)</i>	3 (6.2)	64-128 (128)	16	0.25-1 (0.5)	<i>S. haemolyticus</i> <i>S. epidermidis</i> <i>S. saprophyticus</i>
<i>tet(M)</i>	0	0	0	0	0
<i>tet(O)</i>	0	0	0	0	0

<sup>a</sup>13 *S. haemolyticus*, 11 *S. epidermidis*, 6 *S. hominis*, 4 *S. caprae*, 3 *S. cohnii*, 2 *S. saccharolyticus*, 2 *S. simulans*, 1 *S. saprophyticus*, 1 *S. lantus*, and 1 *S. capitis*.

The finding that 47 of our 48 isolates (97.9%) had *tet(K)* gene and all isolates were minocycline susceptible comes in agreement with the documented phenomenon that the efflux proteins don't confer resistance to minocycline. This might be because minocycline is a lipophilic tetracycline derivative, which readily crosses the cytoplasmic membrane of the bacteria, possibly at quicker rate than the efflux pumps encoded by *tet(K)* or *tet(L)* can remove it (Speer *et al.*, 1992).

In this study we illustrated that our CNS isolates from Egypt are resistant to tetracycline via the tetracycline efflux mechanism, and this is mainly due to the acquisition of the *tet(K)* gene and to a lower extent by *tet(L)*. The *tet(K)* determinant appears to be widespread amongst CNS isolates in a broad range of countries, regardless of whether antibiotics can be purchased over the counter or not. Fortunately, the efflux mechanism of resistance to tetracycline does not confer resistance to minocycline. Consequently we might expect the efficient treatment with minocycline for the most tetracycline-resistant CNS from Egypt.

#### **4. Conclusions and future work**

The almost universal carriage of tetracycline resistant strains by controls may reflect the extensive use of the tetracyclines in dermatology and general medicine. Also our observations confirm that CNS isolates show an important reservoir of multi-resistance to the standard antimicrobials used for acne therapy likely due to prolonged use of antibiotics for acne therapy (Eady, 1998). The hospital dermatology staff can be an important source of transmission of resistant CNS from patient to patient. Strategies for reducing antibiotic use remain the major means of controlling resistance.

Cross-resistance between erythromycin and tetracycline was common amongst the skin isolates. Further investigations for the mobile genetic elements carrying erythromycin and tetracycline resistance genes were needed. These will elucidate if the high carriage rate of CNS isolates having both erythromycin- and tetracycline-resistance from Egypt is due to that these resistance genes were carried on the same transposons or plasmids or not.

#### **Acknowledgements**

We thank the Egyptian Ministry of Higher Education for funding a channel scholarship for El-Mahdy, T.S.M. to study in the UK. We are indebted to Prof. Mahira El-Sayed, and to Dr. Mohamed Abd El-Aziz, for their dermatological professional guidance during skin swabs sampling.

#### **Corresponding author**

El-Mahdy, T.S.

<sup>1</sup>Dept. of Biomedical Sciences, University of Bradford, UK <sup>2</sup>Faculty of Pharmacy, University of Helwan, Egypt

\*[Sata186@hotmail.com](mailto:Sata186@hotmail.com)

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10/1/2010

## Evaluation of Rural Development in Guilan Province, Iran

Hamidreza Alipour<sup>1</sup> and Mohammad Sadegh Allahyari<sup>2</sup>

<sup>1,2</sup> Department of Agricultural Management, College of Agriculture, Islamic Azad University, Rasht Branch, Iran  
[Allahyari@iaurasht.ac.ir](mailto>Allahyari@iaurasht.ac.ir)

**Abstract:** The main purpose of this study was to measure the development level of Guilan rural districts based on Morris Inequality Index. The study employed a descriptive survey design. The statistical population of this study was all Guilan rural districts consisting of 109 rural districts in 2006. In order to investigate and to determine the key indexes of development or backwardness in each region, some variables in five groups (agricultural, health, infrastructure and social) had been used. For data analysis and assessment of development level, Morris Inequality Index was used. Findings revealed that out of the total Guilan rural districts in developmental situation, six rural districts were underdeveloped and more percent of villages were in less developed situation.

[Hamidreza Alipour and Mohammad Sadegh Allahyari. Evaluation of Rural Development in Guilan Province, Iran. Journal of American Science 2010;6(11):889-893]. (ISSN: 1545-1003).

**Keywords:** Development, Rural, coefficient of variation, Morris Inequality Index

### 1. Introduction

Development and Growth as an economic and social context, in one hand by economists and then by socialist and other researchers of some sciences such as geography had been paid attention and became as the base of planning. Permanent problems in study the economic development literature and social changes is to recognize the concept of development and growth (Ghadir Masoum and Habibi, 2004)

Development word has different definition and interpretation in view of development economists and researchers which including the increasing of production efficiency, promotion of life quality and quantity level, remove poverty and privation, promotion the health and therapy service level, removing unemployment problems and inflation and providing socio-economic requirements. In fact, development is a thing which influences our living. The ideal meaning of development is to improve all living quality (Khakpour, 2006). In other definition of development, we can consider it as an economic, social and political process which resulted from living standard and cause to improve the living level of increasing population. Development process has so importance that it must be observed parallel to population growth. The most important subject in definition of development is its attitude to humankind. One that is considering about development is its popularity, participation and endogenous. As we can say that, in fact, development is for human and about human and its final end is to reach human to satisfaction stage from his/her life (Eanali and Taherkhani, 2005).

During past decades, Iran, either before the revolution or after it had been had the witness of performing various development programs.

Development quality and its infrastructure had been created major problems in developmental trend of the country areas because of undesirable past national and focused planning. So, the subject of government investment between the economic area, sections and sub-sections always had been considered in order to justice distribution and to reduce unbalancing.

Various dimension and structure complexity of this subject is considering as one of the main constrain in provide suitable model to distribute the credits. In order to solve the problems result from regional unbalancing, the first step is to identify and determine the level of regions in fitness rate in socio-culture and etc area (Rezvani and Sahneh, 2005). Therefore, the study of socio-economic and regional-province unbalancing is one of the basic and necessary actions to planning and reform in order to provide economic growth along with social justice which can influence the resources allocation with the aim of remove the regional unbalancing (Ahanghari and Saadat Mehr, 2007)

Although it is possible to simply state that there is no rural district which is higher developed or higher back warded than the other rural district, but measuring development level is not a simple work. As we said, development word has very meaning. Whether the meaning of development is economic development, social, educational, cultural and health development or a combination of them? Even we define development in a more exact concept; its measurement is problematic (Khakpour, 2006) The main purpose of this study was to measure the development level of Guilan rural districts based on Morris Inequality Index.

### 2. Material and Methods

Guilan province has been located in the north of Iran in the area of  $36^{\circ}34'$  -  $38^{\circ}27'$  northern latitude and  $48^{\circ}53'$  -  $50^{\circ}34'$  eastern longitude from meridian as it is neighboring with Caspian sea and Azerbaijan by the north, and from the west by Ardabil province, and from the south by Qazvin and Zanjan province and from the east by Mazandaran province (figure 1).

The type of this study is applied and the research method is second analyzing attributive study of the existence information and documentary. Concerned indexes for each of the rural districts had been collected and dealing with ranking and leveling Guilan rural districts through suitable number taxonomy statistical methods. The indexes in this study had been collected by some organizations such as Iran Statistical Center, Health and Therapy centers, Education Department and Agricultural Organization by the separating of villages and rural districts. The statistical population of this study was all Guilan rural districts consisting of 109 rural districts in 2006. Rasht Township with the number of 18 rural districts and Bandar Anzali Township had been enjoyed from the most and least frequency, respectively. Data analyzing method was use of the indexes. Correlation rate, reducing the number of indexes into some general indexes and finally, grouping and ranking the rural districts in developing or deprivation point of view which performed using statistical software. In this research, in order to investigate and to determine the key indexes of development or backwardness in each region, some variables in five groups (agricultural, health, infrastructure and social) had been used.

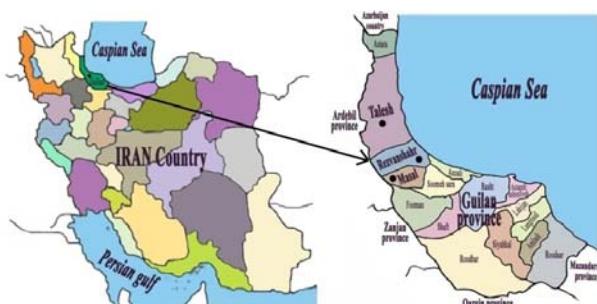


Figure 1, site of study

### The structure of Morris Inequality Index

United Nations Development Program (UNDP) had been applied a model to rank the areas in development point of view which it was both the most recent formal model used in global level and their extending and replacing capacity in the planned places are performable with various scales. This model is known as Morris Inequality Index. This model is important to determine establishing model

of settlement network, to determine rural system or rural development area. Morris Inequality Index identified the developmental place of each unit based on each one of selected indexes using accessing information for every settlement unit and finally, it had been determined the average of indexes using development index analyze simply but with great attention and then it deal with the ranking of settlements. Calculation way of this index is as follow:

$$Y_{ij} = \frac{X_{ij} - X_{ij}(\min)}{X_{ij}(\max) - X_{ij}(\min)} \times 100$$

$Y_{ij}$ : Inequality index to  $i_{th}$  variable in  $j_{th}$  unit

$X_{ij}$ :  $i_{th}$  variable in  $j_{th}$  unit

$X_{ij(\min)}$ : minimum rate of  $i_{th}$  variable

$X_{ij(\max)}$  : maximum rate of  $i_{th}$  variable

The most important point in this method is that the applied indexes must be monotonic or homo direction. In order to study the subject, all concerned indexes in the mentioned formula had been applied and finally, in order to find the main concerned development index for each unit, below equation was used.

$$D.I = \frac{\sum Y_{ij}}{n}$$

In this relation, n and D.I are consider as the number of studied indexes and the development main index of each unit, respectively. Morris development index coefficient is range between 0-100 where the closer to 100 the greater the development level (Allahyari, 2010).

In order to measure what extends of an index had been distributed imbalance among the rural district; Coefficient of Variation method (CV) was used. Coefficient of Variation is calculated using the following formula (Kalantary, 2001).

$$C.V = \sqrt{\frac{\sum_{i=1}^n (X_i - \bar{X})^2}{\sum_{i=1}^n X_i}}$$

In this method, the high number of (CV) indicating more inequality in the concerned index distribution.

C.V = Coefficient of Variation rate of an index

$X_1$  = an index rate in one region

$\bar{X}$  = The average of the same index

N = Number of regions (sector, rural district and village).

### 3. Results

Developmental coefficient of each one of Guilan rural districts had been calculated using the collected data in the form of health and therapy, agriculture, social and service indexes (18 indexes) and based on Morris Inequality Index. The results indicated that the developmental coefficient of rural districts was ranging between minimum of 4.51% to maximum of 75.2% as rural areas of *Yaylaghi* district in *Rezvanshahr* Township is enjoying the least developmental coefficient and at the other hand, rural area of *Licharegi* in *Bandar Anzali* Township is enjoying the highest development coefficient by 75.2% among the Guilan rural districts. In order to grouping rural area in Guilan province four categories were considered:

Under developed: 0- 24.99  
 Less developed: 25-49.99  
 Semi-developed: 50-74.99  
 Developed: 75-100

According to table 1, 53.2% (8 rural districts) from the studied rural districts are located in the less developed group and 40.4% (44 rural districts) are located in semi-developed group and only one rural district is in developed condition.

Table 1, grouping Guilan rural districts based on Morris Inequality Index

	Frequency	Percent	cumulative percent
Underdeveloped	6	5.5	5.5
Less developed	58	53.2	58.7
Semi developed	44	40.4	99.1
Developed	1	0.9	100

The results from the ranking of Guilan rural districts based on developmental coefficient and separate of each studied indexes indicated that in production indexes area (agriculture), this coefficient is ranging between 1.39% to 79.82% as *Lat Lil* rural district of *Langroud* Township and *Shirjou Posht* rural district of *Lahijan* Township had been enjoyed from the least agriculture developmental coefficient by 79.82% respectively.

Table 2, is indicating the grouping of Guilan rural districts based on agricultural development index. As you can see, more than half of Guilan rural districts (51.4%) are in underdeveloped level in agricultural development. Generally 94.5% of rural districts are in underdeveloped to less developed level.

Table 2, grouping of Guilan rural districts based on agricultural index

	Frequency	Percent	cumulative percent
Underdeveloped	56	51.4	51.4
Less developed	47	43.1	94.5
Semi developed	5	4.6	99.1
Developed	1	0.9	100

Also, in the health and therapy indexes area, developmental coefficient was ranging between 1.07% to 70.18%. The *Yaylaghi* district in *Rezvanshahr* Township and *Licharegi* rural district of *Bandar Anzali* are enjoying the least and the highest health and therapy developmental coefficient, respectively. According to performed grouping, it can see that none of the Guilan rural districts is located in developed health and therapy area as their 62 rural districts (%56.9) are in deprived level and also 36.7 are in less developed level.

Table 3, Grouping Guilan rural districts based on health and therapy index

	Frequency	Percent	cumulative percent
Underdeveloped	62	56.9	56.9
Less developed	40	36.7	93.6
Semi developed	7	6.4	100
Developed	0	0	-

In service and infrastructure indexes area, developmental coefficient is ranging between 5.36% to 64.27%. Out of this, The *Yaylaghi* district in *Rezvanshahr* Township is enjoying the least developmental coefficient of service and infrastructure and at the other hand *Saravan* rural district of *Rasht* Township is enjoying the highest developmental coefficient in service and infrastructure indexes. About 10 percent of Guilan townships are in less developed in services and infrastructure indexes and also 12.8% of Guilan rural districts are in semi-developed indexes.

Table 4, Grouping Guilan rural districts based of service and infra-structure index

	Frequency	Percent	cumulative percent
Underdeveloped	19	17.4	17.4
Less developed	76	69.7	87.2
Semi developed	14	12.8	100
Developed	0	0	-

In social indexes, developmental coefficient is ranging between 3.06% to 85.73%. out of Guilan rural district , the country- seat *Sayar Setagh* rural district of *Roudsar* Township and *Licharegi* rural district of *Bandar Anzali* Township were enjoy the least and highest developmental coefficient, respectively. 61.5% of Guilan rural district are in semi-developed level index and also 30 rural districts (27.5%) are in less developed condition (table 5)

Table 5, grouping Guilan rural district based on social index

	Frequency	Percent	cumulative percent
Underdeveloped	6	5.5	5.5
Less developed	30	27.5	33
Semi developed	67	61.5	94.5
Developed	6	5.5	100

In order to measure what extent of an index had been distributed among the rural districts, inbalance, coefficient of variation (CV) method was used.

In this method, the high rate of CV indicating more inequality in the concerned index distribution. As you can see in table 6, the highest rate of coefficient of variation is related to production and agricultural indexes (57%) which in distribution of this index among the Guilan rural districts.

The least coefficient of variation rate is finding among the social indexes (30%). With regard to this in measuring this index, women and man literacy variables had been investigated, it is indicating that most rural district have the same condition relatively low coefficient of variation is expectable (table 6).

Table 6, Investigation of variance coefficient about the studied indexes

	Social	Service	Health	Agriculture
Standard deviation	16.6	11.82	13.52	14.63
Mean	53.98	35.42	25.76	25.66
CV	0.3	0.33	0.52	0.57

#### 4. Discussions

Finding efficient and right method to measure development and then to provide service supply in rural area is very difficult. This is result from more frequency of rural, population dispersion, rural diversity and their distribution manner in the area, their communication situation, rural special characteristics, budget and developmental credit deficiency, expert personal deficiency and rural administrative management system deficiency and ignoring rural settlements in several past decades

(Rezvani and Sahneh, 2005). With regard to this issue that no program can without objective, so in planning stage, balancing developmental situation of rural districts and balancing in enjoying rate of various possibilities and services and to improve this indexes in order to provide community health are considered the key goals, goal which cause to social justice and provide sustainable development area (Khakpour, 2006).

The results indicate that out of the total Guilan rural districts in developmental situation, six rural districts were underdeveloped and more percent of villages were in less developed situation. One of the regional planning goals, is to balance develop of region and to prevent from generating under developed area. Use the results of this study to reach to above goals. As in allocation of improvement credits, allocation credits to each region can determine according to developmental degree and distance rate of each rural districts from ideal condition. So, developed rural district will receive less budget and underdeveloped rural districts will receive more budget.

#### Acknowledgements:

Authors are grateful to Islamic Azad University, Rasht Branch, Rasht, Iran for financial support to carry out this work.

#### Corresponding Author:

Dr. Mohammad Sadegh Allahayri  
Department Agricultural Management  
Islamic Azad University, Rasht Branch, Rasht, Iran  
E-mail: [allahyari@iaurasht.ac.ir](mailto:allahyari@iaurasht.ac.ir)

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10/10/2010

# Diagnosis and Epidemiological Studies of Bovine Trypanosomiasis in Kaliobia Governorate

**Mervat E.I. Radwan<sup>\*1</sup> and Reham El Madawy<sup>2</sup>**

Department of Infectious Diseases, Vet. Hospital<sup>1</sup>, Department of Parasitology<sup>2</sup>, Benha-University, Benha, Egypt  
[dr\\_mervat19@yahoo.com](mailto:dr_mervat19@yahoo.com)

**Abstract:** This investigation was performed on 131 animals (cattle and buffaloes) from farms located in different places in kaliobia aged from 1.5-5 years the samples were collected from clinically infected animals that suffer from "surra" disease and animals apparently healthy in contact with infected animals (subacute or chronic) Infected animals .This investigation reported that 51animals showed the Clinical signs of illness as pyrexia, parasitaemia,progressive emaciation, generalized edema and recurrent episodes of fever occur during course of disease .The microscopic examination of blood film revealed (*Trypanosoma evansi*) in 5 out of 80 apparently healthy animals (7.8%) while PCR examination found that 35 out of 75animals positive (46.7) so PCR is the most suitable diagnosis for early diagnosis and consequently controlling programs and consider the confirmatory test.

[Mervat E.I. Radwan and Reham El Madawy. Diagnosis and Epidemiological Studies of Bovine Trypanosomiasis in Kaliobia Governorate. Journal of American Science 2010;6(11):894-898]. (ISSN: 1545-1003).

**Keywords:** **Diagnosis; Epidemiological; Bovine Trypanosomiasis; Kaliobia Governorate**

## 1. Introduction:

Trypanosomiasis is a disease complex caused by several species of Flagellate protozoan parasites (Trypanosomes) that live in body fluids. It occurs through the tropical and subtropical regions of Africa. It affects cattle, buffalos and human. , the fact that the disease is transmitted mechanically as well as cyclinically has certainly expanded the disease distribution out of the tsetse belt area. Trypanosomiasis,also known as (surra ) is caused by *T.evansi* and is quite common among horse, cattle, buffaloes, and camels (Sehgal et. al.2006) in this study, trypanosomosis was examined in suspected to infected using PCRs confirmatory test for early diagnosis andconsequently controlling programs .The disease has economic important due to loss of condation,reduction in milk yield (Reghu Ravindran et.al.2008) decrease capacity of work and may affect on quality of semen in bulls and cause irregular estrus, abortion and stillbirth in cows ( El Sawlhy 1999). PCR is high sensitivety method that could detect *T.evasi* infection bovine three day earlier than miroscopically (Wasana et al. 2000). Prevalence of disease depends on rate of exposure, availability of infected animals, the insect reservoir and seasons (Mottib et al. 2005). The conventional parasitological methods lack sensitivity and serological techniques ,which detect antibodies or antigen lack specificity or sensitivity, respectively .Therefore molecular technique, especially (PCR) has been developed in order to overcome the problems faced with conventional and serological technique

.In addition ,it was reported that PCR is a reliable method for diagnosis and epidemiological studies (El-Metanawey et. al.2009)

## 2. Materials and methods

### Animals

Total number of animals\_131aged from 1.5-5years old from different location in Kalubaia was clinically examined with special attention to signs related to trypansoma evansi infection .Samples also collected from apparently healthy animals in contact to infective animals

### Samples

The blood samples were collected from jugular vein by sterile sharp needle with wide pore samples\_were collected in clean and dry test tube containing EDTA as anticoagulant for blood smear and PCR analysis

### Blood film:

Three thin blood film were prepared and left in air to dry and fixed in absolute methyl alcohol for 1-2min. Staining with freshly filtrated and diluted Giemsa stain for 30-45 min then washed with distal water to remove excess of stain after that the slides were left to dry, then put one drop of cider oil examined under oil immersion lens according to(Coles,1986) .

Examination of blood film for trypanosome:

1/4-1/2 inch from end of the film and transferred from one slide of film to other (cross-section method) to give constant and representative examination according to (Barrent 1965) animals be considered negative if the three slides were negative.

#### DNA extraction:

Two hundred and fifty microlitres of EDTA blood was mixed with 250 µl lysis buffer (0.32 M Sucrose, 0.01 M Tris, 5 mM MgCl<sub>2</sub>, 1% Triton X-100, pH 7.5). The mixture was centrifuged at 13,000 g for 25 sec. The supernatant was removed and the pellet washed with 500 µl lysis buffer. The centrifugation and washing were repeated twice. The final pellet was resuspended in 250 µl 1 x PCR buffer (10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.1 % Triton X- 100). 1.5 µl of proteinase-K (10 mg/ml) was added and vortexed. The samples were incubated at 56 °C for 1 hour and at 95°C for 10 min. (to inactivate the proteinase-K) and stored at -20°C until use. DNA was extracted as described by Higuchi (1989).

#### DNA amplification:

PCR were carried out in 25 µl reaction volumes containing 10 mM Tris-HCl (pH8.3)Polymerase chain reaction (PCR) assays were further performed to confirm the Trypanosomiasis in buffaloes were *T. evansi*. Forward 5-ACA TTC CAG CAG GAG TTG GAG-3 primer and reverse 5-CAC GTG AAT CCT CAA TTT TGT-3 primer, which are

trypanosome specific (Holland et al., 2001), were used for amplification of the 239-base pair (bp) fragment from *T. evansi* genomic DNA. Amplification was carried out in a thermal cycle .

The final reaction volume was 25 µl. For detection of amplified product, 5 µl of the PCR product was electrophoresed on a 1.5% agarose gel with Tris borate-EDTA as the running buffer (30 min at 50 V) and a 100-bp DNA. The gels were stained with ethidium bromide (5 µl/100 ml of gel) and analyzed on a UV transilluminator to visualize the expected size (239- bp) product.

### 3. Results

From table (1)showed that total number of 131 animals from cattle and buffaloes 1.5:5 years old were clinically examined with special attention to signs related to *T.evansi* infection as pyrexia, parasitaemia, progressive emaciated „, and recurrent episodes of fever occur during course of disease as shown in figure (2) , microscopic examination of blood film revealed that *T.evansi* is an extra cellular motile (rapid twisting motion)that stain bluish with red nucleus by Giemsa or leishman stain in 5 animals only as in figure(1) and the remains samples are negative in other hand when perform PCR for this animals found that 35 animals positive (infective) confirmed to be infected with *T.evansi* as in figure (3) where they lacking the clinical signs of Trypanosomiasis and microscopically negative .

**Table(1)Comparative study of techniques between infected animals with *T.evansi***

Total number of examined animals(cattle and buffaloes	Infected animals with clinical signs appearance		Animals apparently healthy in contact with infective animals (suspected infection)			
			Microscopic examination		PCR examination	
Examined animals	No.of +ve	Rate	No.of +ve	Rate	No.of +ve	Rate
131	51	38.9%	5	6.3%	35	46.7%

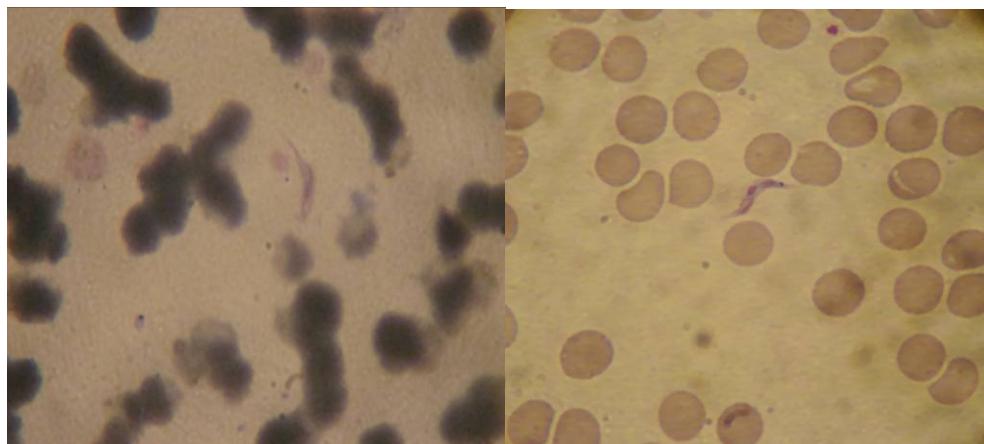


Figure (1) Giemsa stained blood film showed trypanosome bluish with red nucleus



Figure(2) showed clinical signs of trypanosomiasis :

1-emaciated ,pyrexia and anemia                  2-off food weakness and edema in different places

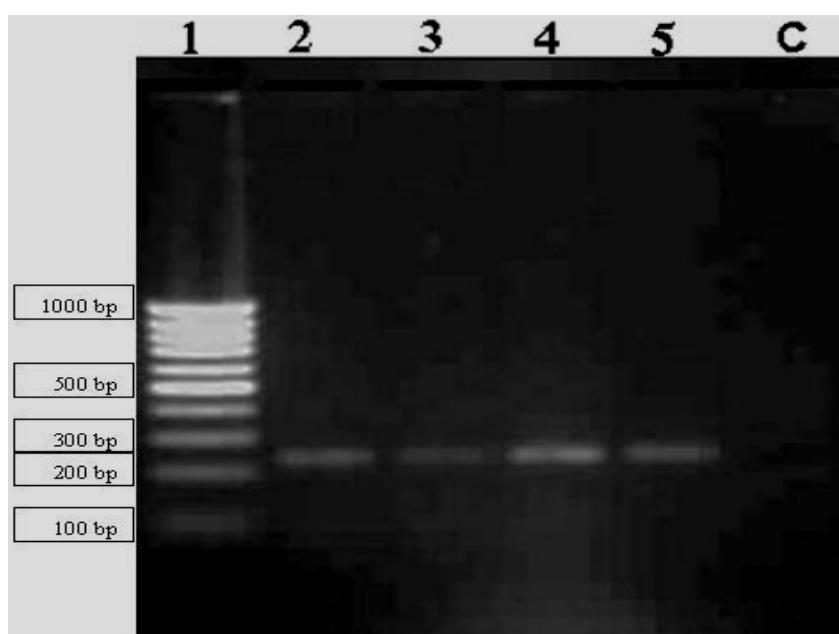


Figure (3) Electrophoresis gel(1.5% agarose,stained with ethidium bromide showing lanes from left to right 1.100-bp DNA ladder ; (2-5),Natural T.evansi infected blood of cattle(C), negative control .

#### **4. Discussion:**

Bovine trypanosomiasis is blood parasite disease that imposes large economic losses such as drop in milk yield, meat production, irregular estrous, abortion, stillbirth in cow and low quality semen in bulls (Wasana et.al.2000 and Reghu Ravindran et.al 2008)

Concerning the clinical signs, the affected cattle and buffaloes with *T.evansi* were suffered from pyrexia, parasitaemia, emaciation, generalized edema and recurrent episodes of fever occur during course of disease these results came agreement with (Ahmed El Sawalhy 1999)

The method of choice to detect *T.evansi* in blood of infected animals especially in acute cases was blood film examination In the present work, examination of Giemsa stained blood film revealed *T.evansi* belong to the subgenus trypanozoon, the organism is an extra cellular motile (rapid twisting motion)that stain bluish with red nucleus by Giemsa or leishman stain (Imadeldin E Aradaib and Ali A Majid )

Blood smear examination proved to be of limited value in diagnosis of subacute or chronic cases. In this study only 5 animals were positively identified by microscopic examination out of 80 samples were apparently healthy in contact with infected animals this result in agreement with Herbart W.J. and lumsden H.R.(1976) who found that when parasites number less than 2,500,000 parasites per ml present in blood samples ,microscopic detection is not feasible

The PCR technique as used in this study detected the low parasitaemia and suspected Trypanosomiasis infection using specific trypanosome primer (*T.evansi*) . PCR has some major advantages over the parasitological techniques; sample processing does not have to be done within minimum time after collection but can be delayed for some time after preservation at -20 °C. The PCR technique has been verified on blood samples of infected cattle, confirming its higher sensitivity and specificity when compared to parasitological techniques (Clausen, 1998). The PCR technique is accurate, more sensitive and specific method in diagnosis of trypanosomes infected cattle than other parasitological methods and overcome the problem of non specific reaction in case of serological tests; it can detect low parasitic cattle in the chronic cases. The strength of PCR was shown in detection of infection in a parasitaemic cattle showing clinical signs of diseases and was negative using parasitological tests.

The PCR test showed the best sensitivity compared with parasitological methods using classic investigation methods. Use of PCR as accurate and specific diagnostic technique, so treatment has to be carried out immediately in the field (Holland et.al.2004) facilitate control programming. PCR assays for diagnosis of trypanosome infection in cattle were evaluated for their ability to detect trypanosome DNA in blood spots samples collected from cattle in four different provinces from the Bolivian lowlands and the results compared with those obtained with standard parasitological Micro Haematocrit Centrifugation Technique (MHCT) and stained smears and serological methods (Card Agglutination Test for *T. evansi* and Antibody ELISAs for *T. vivax* and *T. congolense*). Kappa agreement analysis showed a significant agreement between PCR assays and results from parasitological methods. Results from PCR assays for *T. vivax* and *T. evansi* were combined with results from parasitological and serological assays to provide information on prevalence rates for the four provinces from where the samples were obtained. (Gonzales et al, 2003).

In this study out of 75 samples, PCR revealed that 35 individuals were confirmed to be infected with *T. evansi*, where they were lacking the clinical signs of Trypanosomiasis and negative by microscopic examination so this animal may be in early stage of subclinical and chronic infection.

#### **5. Conclusion:**

Early detection of *T.evansi* play an important role in epidemiology, control program of disease and treatment

#### **Corresponding author**

Mervat E.I. Radwan  
Department of Infectious Diseases, Vet. Hospital  
Benha, Egypt  
[dr\\_mervat19@yahoo.com](mailto:dr_mervat19@yahoo.com)

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10/9/2010

# Noise Prediction for Outdoor Cooling Systems; Case Study

Ahmed A. Medhat A.Fahim<sup>1</sup> and Hoda S. Seddeq<sup>\*\*</sup>

<sup>1</sup>HVAC Consultant & Fire Engineering Auditor in Electro-Mechanical Research Institute at Housing and Building National Research Center, HBRC, Cairo, Egypt, P.O.Box 1770 Giza,

<sup>2</sup> Acoustic laboratories in Building Physics research Institute at Housing and Building National Research Center, HBRC, Cairo, Egypt, P.O.Box 1770 Giza

[luukki@live.com](mailto:luukki@live.com) [hodasoliman@yahoo.com](mailto:hodasoliman@yahoo.com)

**Abstract:** Outdoor noise analyses are commonly required to estimate the sound levels at the property line of adjacent buildings. Outdoor cooling units, such as cooling towers, air-cooled chillers and rooftop units, all create noises at different levels that can disturb neighbors or occupants inside the building itself. Creating a comfortable acoustic environment in most of Heating, Ventilating and Air-Conditioning, HVAC, applications falls on the mechanical engineering disciplines because most background noise sources are generated by the mechanical apparatuses and cooling devices. This paper investigates the prediction of sound pressure levels emitted from outdoor HVAC systems. The sound level of outdoor units in various applications is dependent upon several significant factors. These factors include equipment location, directivity of the source, barrier shielding, sound path, and attenuation due to distance, atmospheric sound absorption and ground attenuation. A developed simplified model called "Outdoor Modeling Acoustic Code, OMAC" has been utilized taking into consideration the influences of previously mentioned parameters. This OMAC code has been used to analyze and predict the noise level emitted from roof-top air-cooled chillers located on office building as a case study. Predicted noise regimes were compared with the collected field measurements for the validation and verification purposes. Detailed analyses and comparisons between predicted and measured noise spectrums were carried out based on the local and international standards. These comparisons show a good agreement among predicted noise criterions, measured data and dedicated standard thresholds. It was concluded that it is mandatory to utilize such prediction "modeling" tool during the early stages of HVAC design process to allow the authority having jurisdictions to predict the impact outdoor noises within the new development urban.

[Ahmed A. Medhat A.Fahim and Hoda S. Seddeq. Noise Prediction for Outdoor Cooling Systems; Case Study. Journal of American Science 2010;6(11):898-905]. (ISSN: 1545-1003).

Key words: outdoor sound propagation, HVAC, sound power, directivity, barrier, atmospheric sound absorption, ground attenuation

## 1. Introduction

Noise is usually regarded as one of the factors threatening our living environments. Occupants are surrounded by numerous sources of noise that create pervasive environmental pollution and insufferable noise to the neighboring community. The major problem of the noise is not only that it is unwanted, but also that it negatively affects health and well-being<sup>1</sup>. Problems related to noise include hearing loss, stress, sleep loss, distraction, lost productivity, masking speeches and a general reduction in the quality of life and opportunities for tranquility. Humans can be both the cause and the victim of noise.

HVAC equipments are the dominating source of noise in buildings and it can even disturb people in the nearby surrounding, therefore it is importance to consider acoustic criterion and actions at the early stage of the design process. Accurate predictions for the sound pressure level emitted from HVAC equipments are needed to evaluate the noise reductions values required to meet local regulations; then the determination of each unit noise limits shall be obtained to select suitable noise controls method; and finally to confirm that the

plant will comply with applicable noise threshold as stated by Frank H. Brittain, Marlund E<sup>2</sup>.

Two quantities are needed to describe the strength of noise source, its sound power level and its directivity. The sound power level is a measure of total sound power radiated by the source in all directions. The directivity of a source is measure of the variation in its sound radiation with direction. Directivity is usually stated as a function of angular position around the acoustical center of the source and also as a function of frequency. Some sources radiate sound energy nearly uniformly in all directions. These are called nondirectional sources. Generally, such sources are small in size compared to the wave length of the sound radiated. Most practical sources are somewhat directional that is they radiate more sound in some directions than in others<sup>3,4</sup>.

Propagation of outdoors sound shall consider meteorological effects, the attenuation effects of ground coverings, atmospheric sound absorption, and the sound attenuation associated with barriers, and the effects of reflecting surfaces. Precious acoustical analyses associated with all of these can be rather complicated. However, the

accuracy is not often justified because the acoustical effects of the above factors are extremely fluctuated. Acoustical analysis presented herein will be simplified to give an estimate of outdoor sound pressure regimes and levels associated with HVAC equipment that are affected by spherical spreading, reflecting surfaces and barriers<sup>5</sup>.

The successful outdoor noise consultant needs five things:

- Measurement equipment (octave band sound level meter, perhaps a long term monitor)
- Noise control techniques (such as barriers, damping materials, enclosures, mufflers and silencers)
- A goal – what is a “good” level (from regulations, resident opinions, etc.)
- A good understanding of the physical mechanisms and a basic prediction model

## 2. Sound Propagation Outdoors

Normally if the equipment sound power level spectrum and ambient sound pressure level spectrum are known, the contribution of the equipment to the sound level at any location can be estimated by analyzing the sound transmission paths involved. When there are no intervening barriers, the principal factors outdoors are reflections from buildings near the equipment and the distance to the specific location. The following equation may be used to estimate the sound pressure level of the equipment at a distance from it and at any frequency when given the sound power level<sup>6</sup>

$$L_P = L_W + 10\log_{10} Q - 20\log d - 11 \quad (1)$$

where

$L_P$  = sound pressure level at distance  $d$  (m) from the acoustic center of the sound source, dB

$L_W$  = sound power level of sound source, dB

$Q$  = directivity factor associated with the way sound radiates from sound source (refer to figure 1)

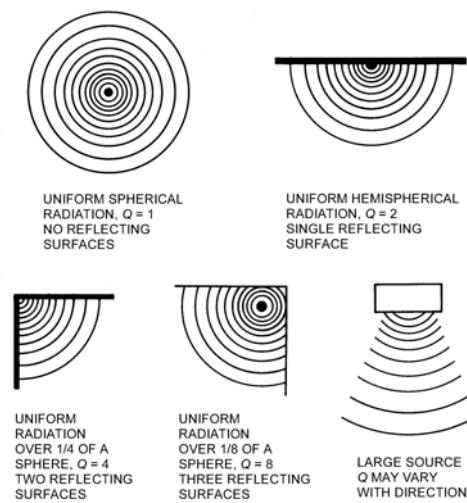
This equation does not apply where  $d$  is less than twice the maximum dimension of the sound source.  $L_P$ , may be low by up to 5 dB where  $d$  is between two and five times the maximum sound source dimension<sup>6</sup>.

If the source is directional, an additional term, the Directivity Index DI, is needed to account for the uneven distribution of the sound intensity as a function of direction. The Directivity Index is the difference between the actual sound pressure, and the sound pressure from a non-directional point source with the same total acoustic power. It can be determined experimentally, or calculated for a limited number of analytical cases, such as a piston in a baffle (a decent approximation of a loudspeaker), a piston in the end of a long tube (engine exhaust)<sup>4</sup>

$$D_I = 10\log_{10} Q \quad (2)$$

For an omni-directional source radiating into free space, DI = 0 dB. If that same source is

situated directly on a perfectly reflecting surface (hemispherical radiation), DI = 3 dB.



**Figure 1** Directivity factors for various radiation patterns

## 3. Excess Attenuation Model

Including absorption of sound in air, non-uniformity of the propagation medium due to meteorological conditions (refraction and turbulence), and interaction with an absorbing ground and solid obstacles (such as barriers). Equation 1 will be extended to account for atmospheric absorption and all other effects by introducing the concept of excess attenuation,  $A_E$  is defined as: - the total attenuation in addition to that due to spherical divergence and atmospheric absorption<sup>5,7</sup>, [ISO 9613 PART 2]. So equation (1) will be as follows:

$$L_P = L_W + 10\log_{10} Q - 20\log d - 11 - A_{abs} - A_E \text{ (dB)} \quad (3)$$

Where  $A_{abs}$  = atmospheric absorption,

$A_E$  = excess attenuation (dB)

The total excess attenuation  $A_E$  (dB) is a combination of all effects:

$$A_E = A_{Weather} + A_{ground} + A_{turbulence} + A_{barrier} + A_{vegetation} + \text{any other effects...} \quad (4)$$

where

Meteorological conditions attenuation –  $A_{weather}$

Attenuation due to ground interaction –  $A_{ground}$

Atmospheric turbulence attenuation –  $A_{turbulence}$

Attenuation due to vegetation –  $A_{vegetation}$

Attenuation due to barrier

Attenuation due to meteorological conditions  $A_{weather}$  such as wind, that can all bend sound waves and influence sound levels at large distances. Their effects are short term and generally not included in acoustic evaluations<sup>6,8</sup>.

Sound energy is dissipated in air by two major mechanisms<sup>9, 10</sup>

- Viscous losses due to friction between air molecules which results in heat generation (called “classical absorption”)
- Relaxation processes – sound energy is momentarily absorbed in the air molecules and causes the molecules to vibrate and rotate. These molecules can then re-radiate sound at a later instant (like small echo chambers) which can partially interfere with the incoming sound.

These mechanisms have been extensively studied, empirically quantified, and codified into an international standard for calculation: ANSI Standard S1-26:1995, or ISO 9613-1:1996. For a standard pressure of one atmosphere, the absorption coefficient  $\alpha$  (in dB/100m) can be calculated as a function of frequency  $f$ (Hz), temperature  $T$  (degrees Kelvin) and molar concentration of water vapor  $h$  (%) by:

$$\alpha = 869 \cdot f^2 \left\{ 1.84 \cdot 10^{11} \left[ \frac{T}{T_0} \right]^{1/2} + \left[ \frac{T}{T_0} \right]^{-5/2} \right. \\ \left. 0.01275 \frac{e^{-2239.1}}{F_{r,0} + f^2 / F_{r,0}} + 0.1068 \frac{e^{-3352/T}}{F_{r,N} + f^2 / F_{r,N}} \right\} \quad (5)$$

where

$$F_{r,0} = 24 + 4.04 \cdot 10^4 h \frac{0.02 + h}{0.391 + h} \text{ Oxygen relaxation frequency (Hz)} \quad (6)$$

$$F_{r,N} = \left[ \frac{T}{T_0} \right]^{-1/2} \left[ 9 + 280 h e^{\left\{ -417 \left[ \frac{T}{T_0} \right]^{-1/2} - 1 \right\}} \right] \text{ Nitrogen relaxation frequency (Hz)} \quad (7)$$

$$T_0 = 293.15^\circ K (20^\circ C) \quad (8)$$

To calculate the actual attenuation due to atmospheric absorption  $A_{abs}$  (dB) for a given propagation range for use in equation 3:

$$A_{abs} = \alpha r / 100 \quad (\text{dB}) \quad (9)$$

where :

$\alpha$  = absorption coefficients (dB/100m)

$r$  = range (meters)

#### 4. Estimation of Sound Pressure Levels

When investigating the propagation of sound outdoors, it is necessary to take into account the attenuation effects of ground coverings, atmospheric sound absorption, and the sound attenuation associated with barriers, and the effects of reflecting surfaces. Accurate acoustical analyses associated with all of these can be rather complicated. However, this accuracy often is not justified because the acoustical effects of the above factors are extremely variable.

The acoustical analysis presented in this section will be simplified to give an estimate of outdoor sound pressure levels associated with HVAC equipment that are affected by the directivity of a source , reflecting surfaces, barriers and air absorption. Air absorption is likely to be quite small, except for very high frequencies, Thus, for the case of construction projects in urban environments air absorption can be considered<sup>11</sup>. The simplified outdoor sound pressure levels can be estimate as follows:

$$L_P = L_W + 10 \log_{10} Q - 20 \log d - 11 - A_{ground} - A_{abs} \quad (\text{dB}) \quad (10)$$

Calculation of attenuation due to ground absorption<sup>1</sup>

$$A_{ground} = 4.8 - \left( \frac{2h_m}{r_2} \right) \left( 17 + \frac{300}{r_2} \right) \quad (11)$$

where

$A_{ground}$  - attenuation due to ground absorption

$h_m$  - mean height of the propagation path (meters)

$r_2$  - distance between the source and the receiving node (meters)

If the sound source is near a vertical reflecting surface, the sound pressure level at a distance  $r$  from the sound source is given by:

$$L_P = L_W + 10 \log_{10} Q - 20 \log d - 11 - A_{ground} - A_{abs} + \Delta L \quad (\text{dB}) \quad (12)$$

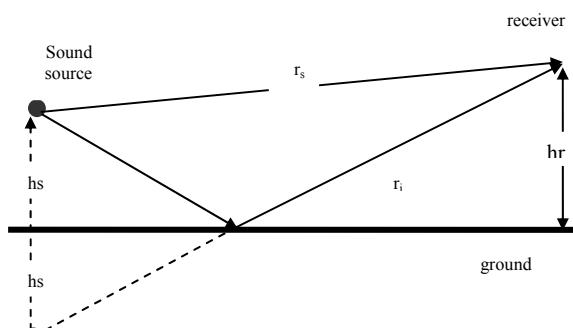
where

$\Delta L$  is the correction term associated with the vertical reflecting surface<sup>1</sup>.

Figure 2 shows a sound source near a hard reflecting surface. The effect of the reflecting surface can be modeled by placing an image sound source on the side of the reflecting surface opposite the sound source. As the figure indicates, these are two paths between the sound source and the receiver. One is the path of the directly radiated sound wave between the source and receiver. The distance of this path is  $r_s$ . The other is the path of the reflected sound wave between the sound source and the receiver. The distance of this path is  $r_i$  also is the distance between the image sound source and the receiver. If the reflecting surface is a vertical surface, the effects of this surface and the corresponding values of  $\Delta L$  are a function of the distance between the sound source and the reflecting surface and the distance between the sound source and the receiver. This relation is expressed by<sup>12</sup>

$$\Delta L = 3.00 - 9.29 \log_{10} \left[ \frac{r_i}{r_s} \right] + 10.13 \left[ \log_{10} \left[ \frac{r_i}{r_s} \right] \right]^2 - 3.84 \left[ \log_{10} \left[ \frac{r_i}{r_s} \right] \right]^3 \quad (13)$$

$$\Delta L = 0 \quad \text{for} \quad \frac{r_i}{r_s} > 10$$



**Figure 2 Sound paths between the sound source and the receiver**

### 5. Influence of Obstructions and Barriers

When the line of sight between a source and receiver is obstructed by a rigid, non-porous wall or building, appreciable noise reductions can occur. Sound waves must diffract around the obstacle in order to reach the receiver. This phenomena is used to great advantage in the attenuation of highway noise by barriers in congested urban areas. An observer in the vicinity of a rigid, infinitely long barrier (Figure 2), for sound from a point source, will experience an excess attenuation<sup>13</sup> of:

$$\Delta A = 20 \log \frac{\sqrt{2\pi N}}{\tan ch \sqrt{2\pi N}} + 5 \text{ (dB)} \quad \text{for } N \geq -0.2 \quad (14)$$

$$\Delta A = (0) \text{ otherwise}$$

$$\nabla A_{Total} = -10 \log \left[ \sum 10^{\nabla A_i / 10} \right] \quad (15)$$

$$N = \pm \frac{2}{\lambda} \partial \quad (16)$$

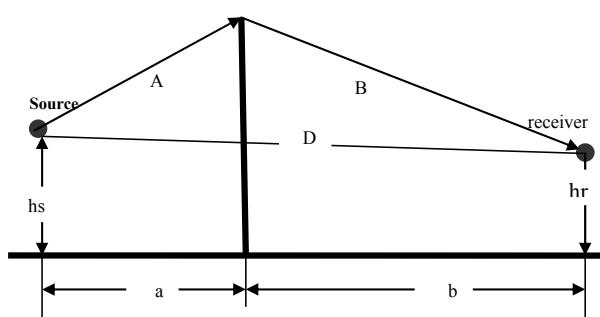
where

$\lambda$  is the wave length (m) that corresponds to the center of the frequency band being analyzed.  $\partial$  is called the path length difference (m) and is given by

$$\partial = A + B - D \quad (17)$$

where

A, B, C as shown in figure 3



**Figure 3 geometry of sound propagation path over or around a barrier**

Equation (16) is based on optical diffraction theory and was developed by Z. Maekawa<sup>14</sup>. The dimensionless quantity  $N$  is called

the Fresnel number and is a measure of how far below the line of sight (relative to a wavelength) the receiver lies. A negative sign for  $N$  indicates that the receiver can see the source, while a positive sign denotes that the receiver is in the shadow zone<sup>15</sup>. More complex models are needed to account for line sources, finite length and absorptive barriers. The barrier should be as tall as possible. The effectiveness of a barrier depends on how far below the line of sight the receiver lies.

When the excess attenuation for a barrier is taken into account equation (12) becomes

$$L_P = L_W + 10 \log_{10} Q - 20 \log d - 11 - A_{ground} - A_{abs} - \Delta A \text{ (dB)} \quad (18)$$

Many practical situations exist where barriers are of finite length. If the sound source is a point source, the sound level at the receiver location is obtained by first calculating the individual sound pressure levels for sound traveling over the barrier and around each end of the barrier. After the individual sound pressure levels at the receiver location have been calculated, the overall sound pressure level is obtained by adding the three individual sound pressure levels. For most situations where a barrier is used to attenuate noise from HVAC equipment, the barrier will enclose the equipment on three sides. When this is the case the barrier can be considered an infinite barrier with respect to barrier design, the following usually applies. Excess barrier attenuations of 10 dBA or less are easily attainable. Excess barrier attenuations up to 15 dBA are difficult to obtain. Excess barrier attenuations over 20 dBA are nearly impossible to obtain<sup>11,16</sup>.

### 6. Experimental Work

Outdoor Modeling Acoustic Code, OMAC has been created to estimate the sound pressure level emitted from outdoor HVAC equipments. This program based on the mathematical equation (3). Figure (4) shows the flow chart of OMAC to predict the outdoor sound pressure level at the receiver. Outdoor Modeling Acoustic Code, OMAC includes the following steps:

- Define the sound power from the catalogue of the equipment (measured by standard method)
- Calculate the directivity of the equipments.
- Calculate the attenuation factors.
- Calculate the effect of vertical surface.
- Calculate the effect of barrier.
- Calculate the combined sound pressure level from multiple sources.

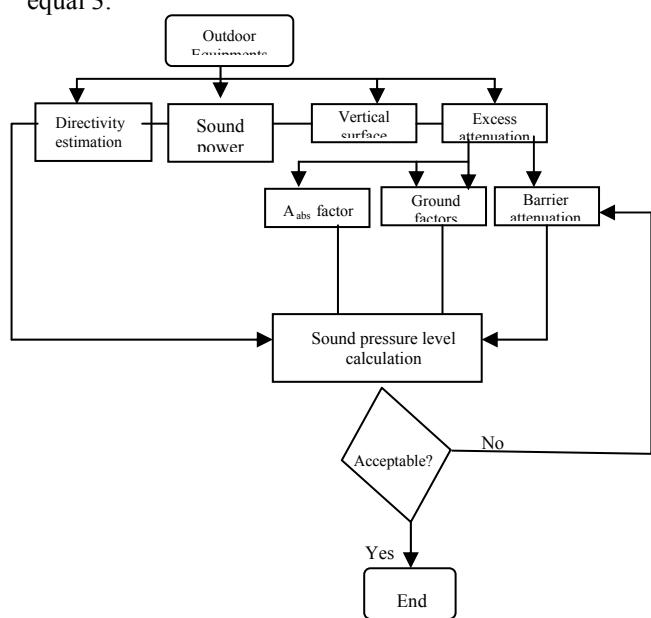
OMAC has been used to predict the sound pressure level emitted from 2 air-cooled scroll chillers that is installed on a roof at residential areas in which commercial building are located. The predicted sound pressure level has been compared to the field measured sound pressure levels at octave

band frequencies. The installed air-cooled scroll chillers have the specification as given in table (1):

**Table 1 - Specification of Outdoor Chiller**

Type	Capacity	Power	No of comp.	No of fans
Air-cooled scroll	60 Ton/ref	110 KW	2/each	6/each
<b>Sound power in dB</b>				
63		103		
125		103		
250		102		
500		99		
1000		99		
2000		97		
4000		90		
8000		84		

Outdoor sound analysis is closest to a free field analysis. The sound pressure level will depend on equipment location, directivity and the attenuation factors. For instance, an air-cooled chiller sends a significant amount of sound vertically from the condenser fans. The air-cooled scroll chiller is on the roof of a 4-story building. The horizontal distance from the chiller to the receiver was 10 m. The receiver measurement at point is 1.5 meter above grade. The source is directional and situated directly on a reflecting surface (hemispherical radiation) so the directivity index DI equal 3.



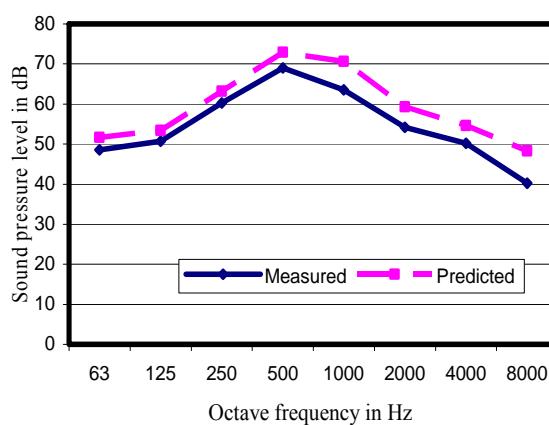
**Figure 4. Flow chart of OMAC Acoustic Code**

Figure 5 shows the comparison between the measured sound pressure levels with predicted sound pressure level at octave band without consider the absorption of the air.

It can be noticed that the difference between the measured and the predicted value increase at the high frequencies this may be due to fluctuation of atmospheric absorption of sound and its effect on sound propagation especially at high frequencies greater than 1000 Hz. The new ISO-DIS 9613 (parts 1 and 2) standard contains a detailed method for computing the sound propagation outdoors, taking into account the effects caused by the air absorption. So the absorption coefficient  $\alpha$  (in dB/100m) calculated again as a function of frequency  $f$  (Hz), temperature  $T$  (degrees Kelvin) and molar concentration of water vapor  $h$  (%) by equation 5, 6, 7, 8 and recalculated the outdoor sound pressure.

Figure 6 shows the comparison between the measured and predicted sound pressure level adding correction for atmospheric absorption.

It can be noticed that the predicted sound pressure level is higher than the permissible sound pressure limit according to the environmental low (table 3). So sound barriers can be installed to reduce the sound levels and hide equipment from view. The barrier creates an "acoustic shadow" that reduces sound pressure levels on the opposite side of the source. The reduction in sound level, or *Insertion Loss*, is based on the path length difference. The path length difference equals the path around the barrier minus direct path from the source to the receiver in feet (or meters). If nonporous barriers placed between the sound source and the receiver can result in significant excess attenuation of sound pressure level.



**Figure 5. Measured and Predicted Outdoor Chiller Sound Pressure Level**

**Table 2 - Measured and Predicted Outdoor Chiller Sound Pressure Level**

Octave frequency	Measured SPL (dB)	Predicted SPL (dB)
63	48.5	51.7
125	50.7	53.4
250	60.2	63.2
500	69	72.9
1000	63.5	70.6
2000	54.2	59.3
4000	50.2	54.6
8000	40.2	48.3
dBA	70.7	75.4

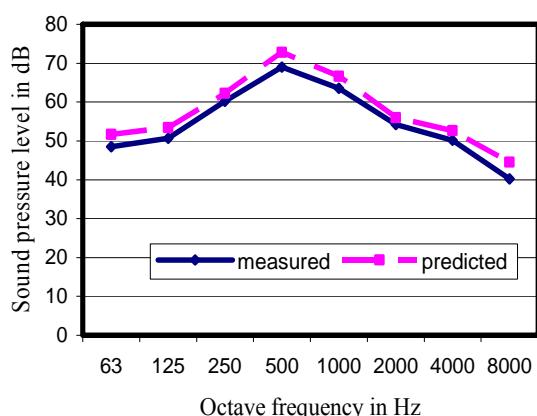


Figure 6. Measured and Predicted Outdoor Chiller Sound Pressure

**Table 4 predicted attenuation;  $\Delta A$  sound pressure level**

Octave frequency	Predicted $\Delta A$	Predicted SPL In dB
63	12.5	39.2
125	15.2	38.2
250	19.6	42.6
500	22.3	50.5
1000	21.5	45.1
2000	20.6	35.4
4000	23.1	29.5
8000	19.6	24.9
dB		52.6

The barrier TL (Transmission Loss) must be at least 10 dB greater than the insertion loss in each band<sup>6</sup>. Therefore the predicted transmission loss of the barrier must be at least as shown in the figure (6).

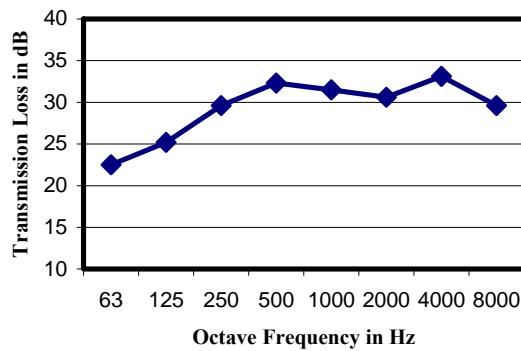


Figure 6. Transmission Loss of the Barrier

**Table 3 Comparing the measured SPL with Predicted SPL**

Octave frequency	Measured SPL	Predicted SPL
63	48.5	51.7
125	50.7	53.4
250	60.2	62.2
500	69	72.8
1000	63.5	66.6
2000	54.2	56
4000	50.2	52.6
8000	40.2	44.5
dBA	70.7	74

OMAC has been used to predict the sound pressure level for the same building. A barrier has been added at three sides around the chiller. It was 5 m from the chiller and 2 m taller than the chiller. The new sound pressure level in dBA has been predicted at point 1.5 meter above grade. Where the excess attenuation (*Insertion Loss*);  $\Delta A$ , is determined. Table (4) shows the predicted attenuation;  $\Delta A$

## 7. Conclusions

A developed simplified model called “Outdoor Modeling Acoustic Code, OMAC” has been utilized based on the measured sound power and taking into consideration the influences of equipment location, directivity of the source, barrier shielding, and attenuation due to distance, atmospheric sound absorption and ground attenuation. The OMAC code has been used to analyze and predict the noise level emitted from roof-top air-cooled chillers located on office building as a case study. The predicted sound pressure level has been compared to the field measured sound pressure levels at octave band frequencies. These comparisons show a good agreement among predicted noise criteria, measured data and dedicated standard thresholds. It was concluded that it is mandatory to utilize such prediction “modeling” tool during the early stages of HVAC design process to allow the authority having jurisdictions to predict the impact outdoor noises within the new development urban.

**Corresponding author**

Ahmed A.Medhat A.Fahim

HVAC Consultant & Fire Engineering Auditor in Electro-Mechanical Research Institute at Housing and Building National Research Center, HBRC, Cairo, Egypt, P.O.Box 1770 Giza,

[luukki@live.com](mailto:luukki@live.com)

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10/5/2010

## Seed Exomorphic Characters of some Taxa from Saudi Arabia

**Soliman, M.S.A.<sup>\*1</sup>, El-Tarras. A.S.<sup>2</sup> and El-Awady, M.A.<sup>2</sup>**

Biotech. & Genet. Eng. Res. Unit, Taif University, Taif, KSA

(Permanent address: Botany & Microbiology Dept., Fac. Sci., Helwan Uni.<sup>1</sup>and Genetics Dept., Fac. Agr., Cairo Uni.<sup>2</sup>, EGYPT.)

<sup>\*</sup>[prof.msoliman@yahoo.com](mailto:prof.msoliman@yahoo.com)

**Abstract:** Seed exomorphic characters of seven species collected from Taif province, Saudi Arabia, were investigated by the aid of Scanning Electron microscopy (SEM). The seed exomorphic characters that are diagnostic at the generic and specific level are seed shape, dimensions, epidermal cells, seed surface sculpture and aspects of anticlinal and periclinal walls. The seed coat of the studied taxa exhibit a wide range of morphological characters. Seed shapes varied from globoid, elliptic, oblong and kidney shaped. They showed either smooth or papilate surface. SEM investigation at higher magnifications revealed different types of seed surface pattern viz, is tuberculate, reticulate, scalariform and tenicostate. Seeds of *Cloeme droserifolia* and *Fagonia schweinfurthia* showed a deposition of wax on their surface. The present study is a modest contribution to previous studies on the flora of Saudi Arabia.

[Soliman, M.S.A. Al-Tarras. A. and Al-Awady, M. Seed Exomorphic Characters of some Taxa from Saudi Arabia. Journal of American Science 2010;6(11):906-910]. (ISSN: 1545-1003).

**Key words:** SEM, seed coat, exomorphic characters, *Cloeme droserifolia*, *Fagonia schweinfurthia*, flora, Saudi Arabia.

### 1. Introduction:

During the present decade, nature reserves were established in Arabia and Saudi Arabia becomes among the countries that are concerned with protection of nature and conservation of biodiversity. Saudi Arabia, has a diversified higher plant flora in its varied landscapes, with about 2243 species in 837 genera and 142 families (Collenette 1998 and 1999). The variety of wild plant species has a valuable economic importance due to its usage as pharmaceuticals, nutritional, fire wood suppliers for urban and rural populations as well as its use in popular remedy.

Aspects of the plant diversity of Saudi Arabia have been documented on phytogeographical bases by Mandaville (1990), Al-Farhan (1999), Chaudhary (1999), Chaudhary & Al-Jowaid (1999) and BaZaid and Mossallam (2000).

The importance of ultra-structural pattern analysis of the seed coat observed under the SEM; as a reliable approach for identifying the species and assessing taxonomic relationships; has been well recognized (Barthlott 1981, Koul *et al.* 2000 and Gamarra *et al.* 2007). Until now, the morphology of the seed coat sculpture under the SEM for the concerned taxa in the present study has not been studied except for *Aizone carsriense* L., by Kanwal, *et al.* (2009).

The aim of the present study is to illustrate the utility of seed coat micro-and macro-morphological characters of seven species using SEM in an attempt to provide some basic data for nature conservation and other applied programs as well as for wild plants taxonomy in Al-Taif province, Kingdom of Saudi Arabia. These taxa are: *Aizone carsriense* L., *Cloeme droserifolia* (Forssk.) Delile, *Fagonia schweinfurthia*, *Fosskaolea tenacissima*, *Peganum harmala*, *Resida lutea*, and *Zygophyllum simplex* L.

### 2. Materials and methods

The investigated taxa were collected during May-August 2009 from different localities (table1). Voucher herbarium specimens of the studied taxa are kept in BGERU (Biotechnology and Genetic Engineering Research Unit, Taif University, K.S.A.). The external macro-morphological aspects of the seeds of the studied taxa were investigated with the aid of scanning electron microscope (SEM). For SEM observations, dried mature seeds were mounted on brass stubs and coated with a thin layer of gold. Coated seeds were examined and photographed on a Joel JSM 6390LA, at the Electron Microscope Unit in Taif University. The terminology of Barthlott (1981 and 1984) and Stearn (1983) was adopted to describe the SEM aspects of seed coat.

**Table (1): List of the examined taxa and their position of collection.**

No .	Taxa examined	Family	Position		
			Alt.(fe.)	N.	E.
1	<i>Aizone carsriense</i> L.	Aizoaceae	4966 fe.	N 21° □□□26.045`	E □□□040°29.5 20`
2	<i>Cloeme droserifolia</i> (Forssk.) Delile.	Capparaceae	4966 fe.	N 21° □□□26.045`	E □□□040°29.5 20`
3	<i>Fagonia schweinfurthia</i>	Zygophyllaceae	4966 fe.	N 21° □□□26.045`	E □□□040°29.5 20`
4	<i>Fosskaolea tenacissima</i> L.	Urticaceae	4966 fe.	N 21° □□□26.045`	E □□□040°29.5 20`
5	<i>Peganum harmala</i> L.	Peganaceae	5222 fe.	□□□N 21° 20.074`	E 040° □□□27.447`
6	<i>Resida lutea</i> L.	Resedaceae	4966 fe.	N 21° □□□26.045`	E □□□040°29.5 20`
7	<i>Zygophyllum simplex</i> L.	Zygophyllaceae	4966 fe.	N 21° □□□26.045`	E 040°29.520`

### 3. Results and Discussion:

Ornamentation of seeds provide important characters to distinguish some taxa (Koul *et al.*, 2000), where, the microstructure details of the seed

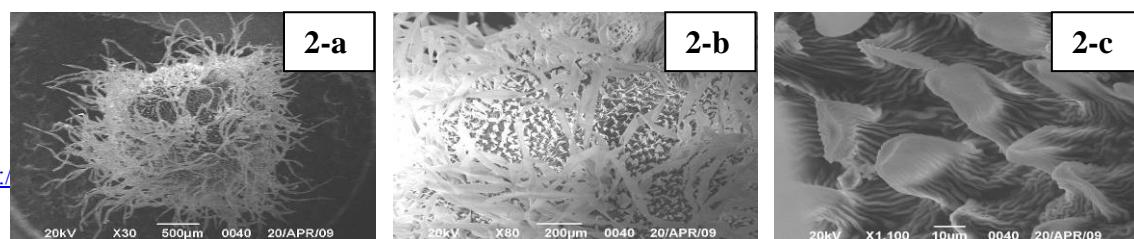
coat surface are strikingly constant from one sample to another in each species (Akbari and Azizian, 2006).



**Figure 1: SEM image for *Aizone carsriense* L. seed.**

As regards to the scanning electron microscopy for *Aizone carsriense* L., the seed shape as indicated in Figure (1), is kidney shaped with striated raised longitudinal and transverse striae, tenuicostate overall seed coat surface. Seed

dimensions (LxW) is (0.68x0.51 mm). The anticlinal cell wall level is raised with smooth texture, thin cell wall and Anticlinal cell wall relief is straight. Periclinal cell wall is flat and it's sculpture is nearly smooth.



**Figure 2: Different magnified SEM images for *Cloeme droserifolia* (Forssk.) Delile.**

Seed shape of *Cloeme droserifolia* (Forssk.) Delile is globular and covered with dense hairs, seed coat pattern is tuberculate, tubercles. Seed size (LxW) is (1.85x1.85). The seed surface shows

rugose-striate surface. Anticlinical walls sunken, straight while periclinical wall is convex, tuberculated and tubercles surface are striate-regate with striated strips, Figure 2 (a, b & c).

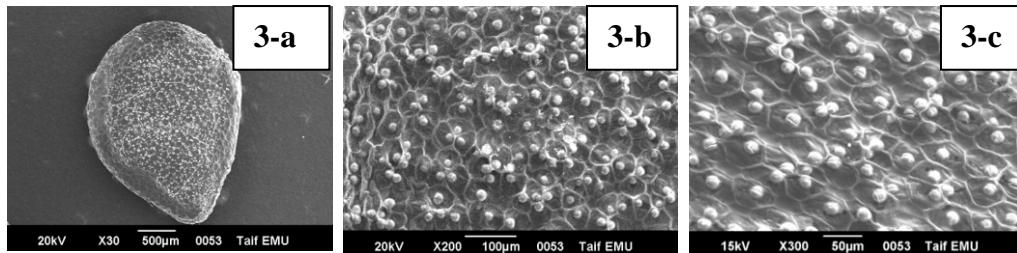
**Figure 3: Different magnified SEM images for *Fagonia schweinfurthia*.**

Figure 3 (a, b & c) shows that, *Fagonia schweinfurthia* seed is ovate has dimentions (LxW) of 2.66 x 2.51 mm and it's overall seed coat pattern is reticulate. The Anticlinal cell wall thickness is thin with smooth texture, has raised cell wall level and it's

relief is straight. The Periclinal cell wall sculpture is smooth to striated with flat cell wall level. Epicuticular wax depositions are noticed over the seed surface as globular particles.

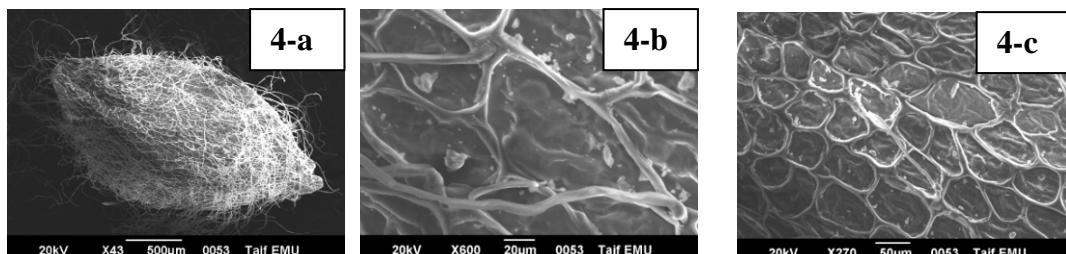
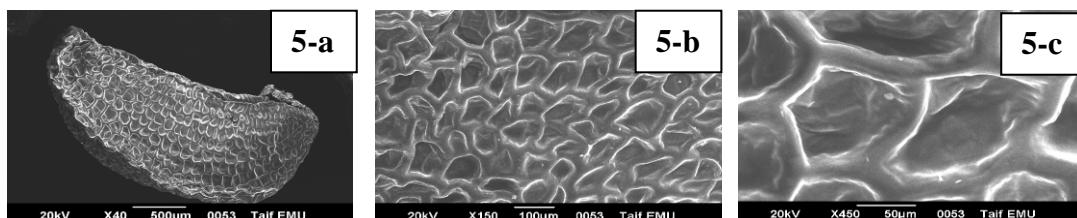
**Figure 4: Different magnified SEM images for *Fosskaolea tenacissima* L**

Figure 4 (a, b & c) shows that, seeds of *Fosskaolea tenacissima* L are elliptic in shape, covered with dense, long fine unicellular and uniseriate hairs. Their dimentions (LxW) is 2.61 x 1.33 mm. SEM investigation indicated that the seed surface sculpture

is typically reticulate. The anticlinal cell walls thickness are thin, straight, raised with smooth surface, whereas the periclinal walls are flat with ruminante surface. Epicuticular wax deposition is observed over the seed surface as irregular particles.



**Figure 5: Different magnified SEM image for *Peganum harmala L.***

Figure 5 (a, b & c) shows that, seeds of *Peganum harmala L.* are elliptic in shape, seed dimensions (LxW) is 2.622x1.786 mm. SEM investigation indicated that the seed surface sculpture is typically regular reticulate. The anticlinal cell walls

thickness are thick, straight, raised with finely striated texture, whereas the periclinal walls are concave with ruminate surface. Epicuticular wax deposition is absent.

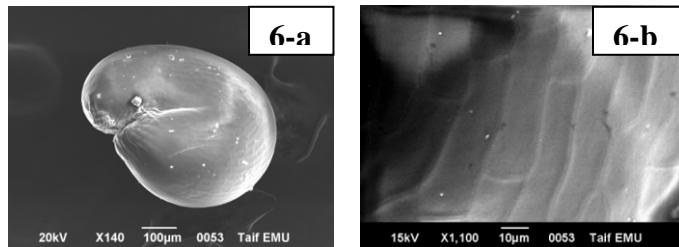
**Figure 6 (A & B): Different magnified SEM image for *Resida lutea L.***

Figure 6 (a & b) shows that, seeds of *Resida lutea L.* is kidney shaped with scalariform pattern. The seed dimensions (LxW) is 0.579 x 0.442 mm.

Anticlinical cell wall is raised and periclinical cell wall is flat with smooth texture.

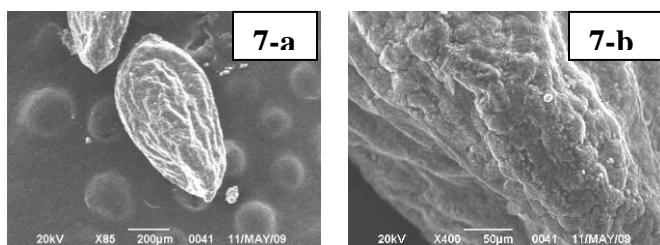
**Figure 7: Different magnified SEM images for *Zygophyllum simplex L.***

Figure 7 (a & b) shows that, seeds of *Zygophyllum simplex L.* is elliptic in shaped with irregularly granulated islands pattern. The seed dimensions (LxW) is 0.833 x 0.450 mm.

Biotech. & Genet. Eng. Res. Unit, Taif University, Taif, KSA.

[prof.msoliman@yahoo.com](mailto:prof.msoliman@yahoo.com)

#### Acknowledgements:

Authors gratefully acknowledge the support of Research Grants Programs, Taif University, Taif, KSA for funding the research project (No. 430-487-2).

We thank Prof. A. Shehata (Fac. of Sci., Taibah Univ.) for her guidance in seed-scanning description, Prof. Y. El-Sodany (Fac. of Sci., Taif Univ.) for his guidance in taxa identification, Prof. S. Ba-Zeid and Prof. A. Ashour (Fac. of Sci., Taif Univ.) for using the facilities of EM unit and

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#### Corresponding author

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10/2/2010

## Biomarkers Characteristics of Crude Oils from some Oilfields in the Gulf of Suez, Egypt.

**M. I. Roushdy, M. M. El Nady, Y. M. Mostafa, N.Sh. El Gendy and \*H. R. Ali**

Egyptian Petroleum Research Institute, Nasr City, Cairo, Egypt.

[\\*hugochem@yahoo.com](mailto:hugochem@yahoo.com)

**Abstract:** Seven representative crude oil samples from the Gulf of Suez were chosen for this study. The studied crude oils are Ras Badran, Belayim marine, Belayim Land, Rahmi, West Bakr, Esh El Mellaha and Geisum. The oils were fractionated by medium pressure liquid chromatography into saturated hydrocarbons, aromatic hydrocarbons and polar compounds. The saturated hydrocarbons were determined by gas chromatography and gas chromatography/mass spectrometry (GC/MS). Ratios of certain biomarkers, (Pristane/phytane, isoprenoids/n-alkanes, CPI, Homohopane, Diasteranes, Gammacerane index, C<sub>29</sub> 20S/20S+20R, C<sub>29</sub>/C<sub>30</sub> hopanes and Ts/Tm) referred to as source correlation indices, are sensitive to the geological source of oil. The results of evaluation suggest that two types of oils could be recognized as marine oils. These oils are characterized by high level of maturation and sourced mainly from source rocks rich in marine organic matters with few inputs from terrestrial origin.

[M. I. Roushdy, M. M. El Nady, Y. M. Mostafa, N.Sh. El Gendy and \*H. R. Ali. Biomarkers Characteristics of Crude Oils from some Oilfields in the Gulf of Suez, Egypt. Journal of American Science 2010;6(11):911-925]. (ISSN: 1545-1003).

**Key words:** Egypt, Gulf of Suez, Homohopanes, Diasteranes, Gammacerane index, C<sub>29</sub> 20S/20S+20R, C<sub>29</sub>/C<sub>30</sub> hopanes and Ts/Tm, Crude oils.

### 1. Introduction:

The biological marker patterns of crude oils are commonly used for oil/oil and oil/source rock correlations and to assess such source rock attributes as lithology, depositional environment, kerogen type and maturity (Waples and Machihara, 1991; Peters and Moldowan, 1993). Exploration applications of biomarkers rest on the pattern of the oil imprinted by the source rock. For example, bulk geochemical evidence and biological markers distributions enable the characterization and distinction of ancient marine and non marine petroleum source rocks (Peters et al., 1986). However, the source-related biomarkers pattern of an oil may have been altered by a number of processes after generation and primary migration (expulsion) from the source rock.

The Gulf of Suez province is highly faulted and corresponds to a continental rift, which started in evolution at the beginning of the Miocene times, consisting of an elongated graben about 300 km long and 30 km wide between the Sinai Peninsula and the Eastern Desert of Egypt (Fig. 1). Various putative source rocks deposited in distinct, well-defined environments exist within the Gulf of Suez basin (Fig. 2).

Source rocks and sandy reservoirs are abundant in both the pre-rift and the post rift sedimentary rocks (Nagaty, 1992 and Shahin and Shehab, 1984). Most accumulations of crude oils in Gulf of Suez are found in different reservoirs and pay zones, ranging in age from Paleozoic to Middle

Miocene (Fig. 2). These accumulations were occurred in structural fault blocks formed at the time of rifting (Chowdhary and Taha, 1987). Since the beginning of this century, the Gulf of Suez has been highly prospective and has drawn the attention of many explorationists.

### Previous Work

The geochemical characteristics of crude oils discussed by Abdel Azim (1970) showed, through statistical comparison of specific gravities of the Gulf of Suez crud oils, that the API gravity and paraffinic content increase with the depth. Zein El Din et al. (1981) reported that the oils produced from some fields in the central Gulf of Suez are derived from similar marine source rocks. Roharback (1983) suggested that the oil samples of the major horizon in the Gulf of Suez have the same genetic family, highly similar in their source rocks, as they reflect a marine origin and show no biodegradation. Shaltut et al. (1985) divided the oils of the central Gulf of Suez into two groups based on porphyrins distribution; the first group is characterized by the increase in vanadyl porphyrins distribution with the depth, while the second group shows inverse relation. Mostafa and Khaled (1988) concluded that the reservoir depth of the oils in the Gulf of Suez is not correlated with the oil maturity, based on sulfur percent, specific gravity, NSO compounds, asphaltenes and carbon isotope ratios. Mostafa and Ganze (1990) revealed that the crude oils of the Rudeis oil field are similar in their

origin; also they concluded that the Eocene Thebes Formation and the Upper Senonian Brown limestone may act as source for the Abu Rudeis oil. Barakat et al. (1996) divided the oils of the southern part of the Gulf of Suez into three types. Type I is sourced from carbonate source rocks (mainly of marine origin), type II appears to be originated from Tertiary source rocks with contribution from high land plants, and type III is a mixture of type I and type II. Sharaf (1998) recognized two oil groups for the oils from October and Abu Rudeis fields. Group "1" heavy oil, is derived mainly from hypersaline reducing environment and group "2" moderate to light oils, is formed under low salinity environment with minor contribution from terrigenous organic matter. Hammad and Barakat (2000) concluded that the oils from some oil fields in the Gulf of Suez were generated from mixed and algal organic matter deposited in transitional and reducing environments. El Nady (2001) recognized that the crude oils of the Gulf of Suez are sourced mainly from marine organic matters. Barakat et al. (2002) stated that there is a close genetic relation between the oils in the southern part of Gulf of Suez. El Nady and Harb (2005) recognized that the crude oils in the north Gulf of Suez are mature and derived mainly from mixed organic sources (mainly terrestrial with marine input) under transitional environments. El Nady (2006) reported that the crude oils in the south Gulf of Suez are mature, originated mainly from marine sources and show good correlation with the Lower Miocene source rocks in the southern part of Gulf of Suez. El Nady et al., (2007) divided the crude oils in the central part of Gulf of Suez into tow groups: The first group includes moderately mature oil from the N. October, Belayim marine, Belayim Land and July fields which are typical of crude oils generated in a strongly reducing (marine). The second group comprises mature oils generated from source rocks deposited in lacustrine palaeoenvironmental conditions and includes oils from Issaran, E. Kareem, El-Khaligue and El Ayun fields. Harb and El Nady (2010) divided crude oils in the Gulf of Suez into 1. Heavy oils characterized by low maturation and originated mainly from terrestrial organic sources. 2. Light oils of high maturity level originated mainly from marine organic sources. Faramawy et al., (2010) classified the crudes in central Gulf of Suez, as aromatic intermediate oils heavy oils of low wax content characterized by high maturity level and derived from mixed organic sources (mainly marine with few inputs from terrestrial origin) belong to the carbonate type, deposited in transitional environments under reducing-oxidizing conditions.

### Aim of the Study

The present work attempts to evaluate the geochemical relationships between the oils recovered from some oil fields within the Gulf of Suez to assess and investigate oil characterization, maturation, source depositional environments and oil families. This target was achieved through analytical results of gas chromatography and gas chromatography-mass spectrometry analysis (GC-MS) for "7" crude oil samples collected from seven oilfields namely: Ras Badran, Belayim marine, Belayim Land, Rahmi, West Bakr, Esh El Mellaha and Geisum distributed within the Gulf of Suez (Fig. 1). These samples are representative for the producing horizon zones (Belayim, Rudies and Nuhkul formations.) of Upper-Lower Miocene age (Figure 2) characterized by limestone facies with depths ranging from 2250 to 8286 ft (Table 1). The oil samples were kindly supplied by (EGPC) from Gulf of Suez and Belayim.

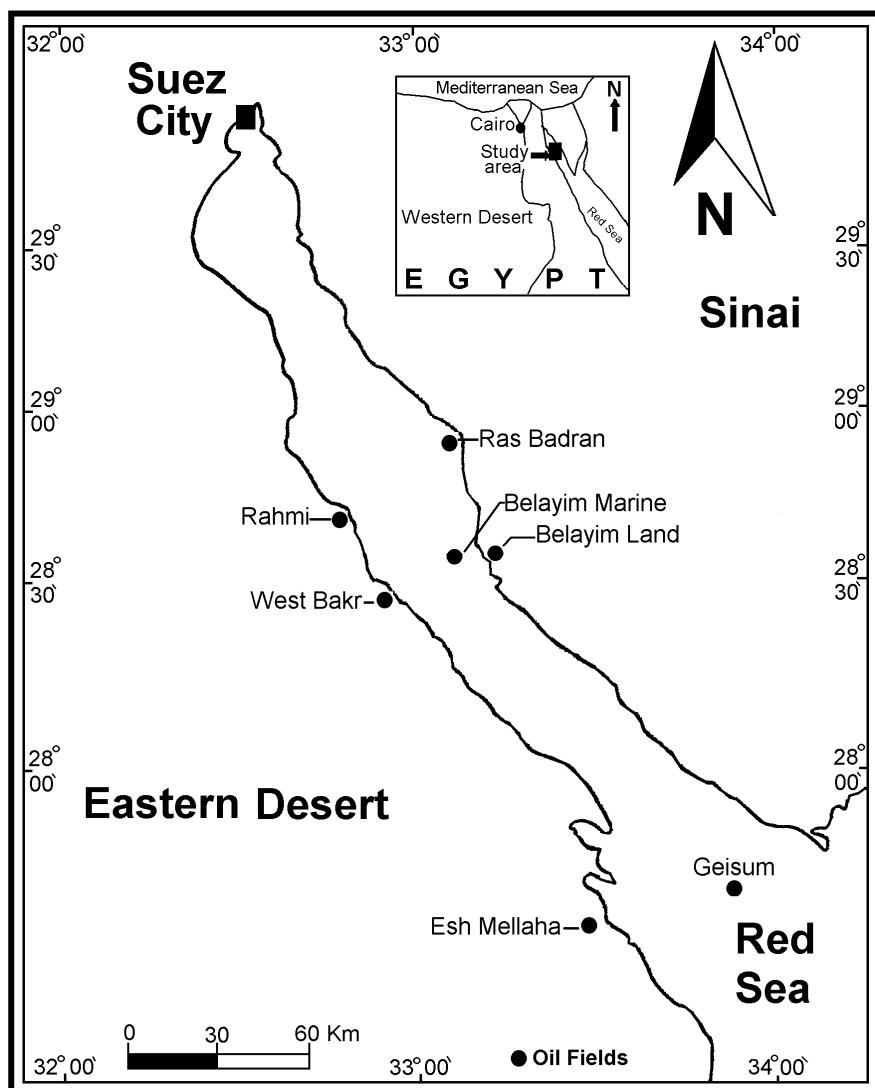
### 2.Experimental

1. The crude oil samples were fractionated by medium pressure liquid chromatography into saturated hydrocarbons, aromatic hydrocarbons and polar compounds. The saturated hydrocarbons were analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS).
2. Gas chromatographic analysis of the saturated hydrocarbon fractions was achieved by Perkin Elmer Instrument Model 8700, provided with a flame ionization detector (FID). Oven temperature programmed for 100 to 320°C at 3°C/min. and final time 20 min. SPB-1 capillary column of 60 m. in length and 0.53 i.d. Nitrogen was used as carrier gas, the optimum flow rate was 6 ml min.
3. Gas chromatography-mass spectrometry used a 50m x 0.25mm fused silica capillary column of bonded SE 54 installed with a finnigan MAT TSQ-70 combined gas chromatography/quadrupol mass spectrometer. The column oven was programmed from 100 to 310°C at 4°C/min. These analyses were done in the laboratories of the Egyptian Petroleum Research Institute (EPRI).

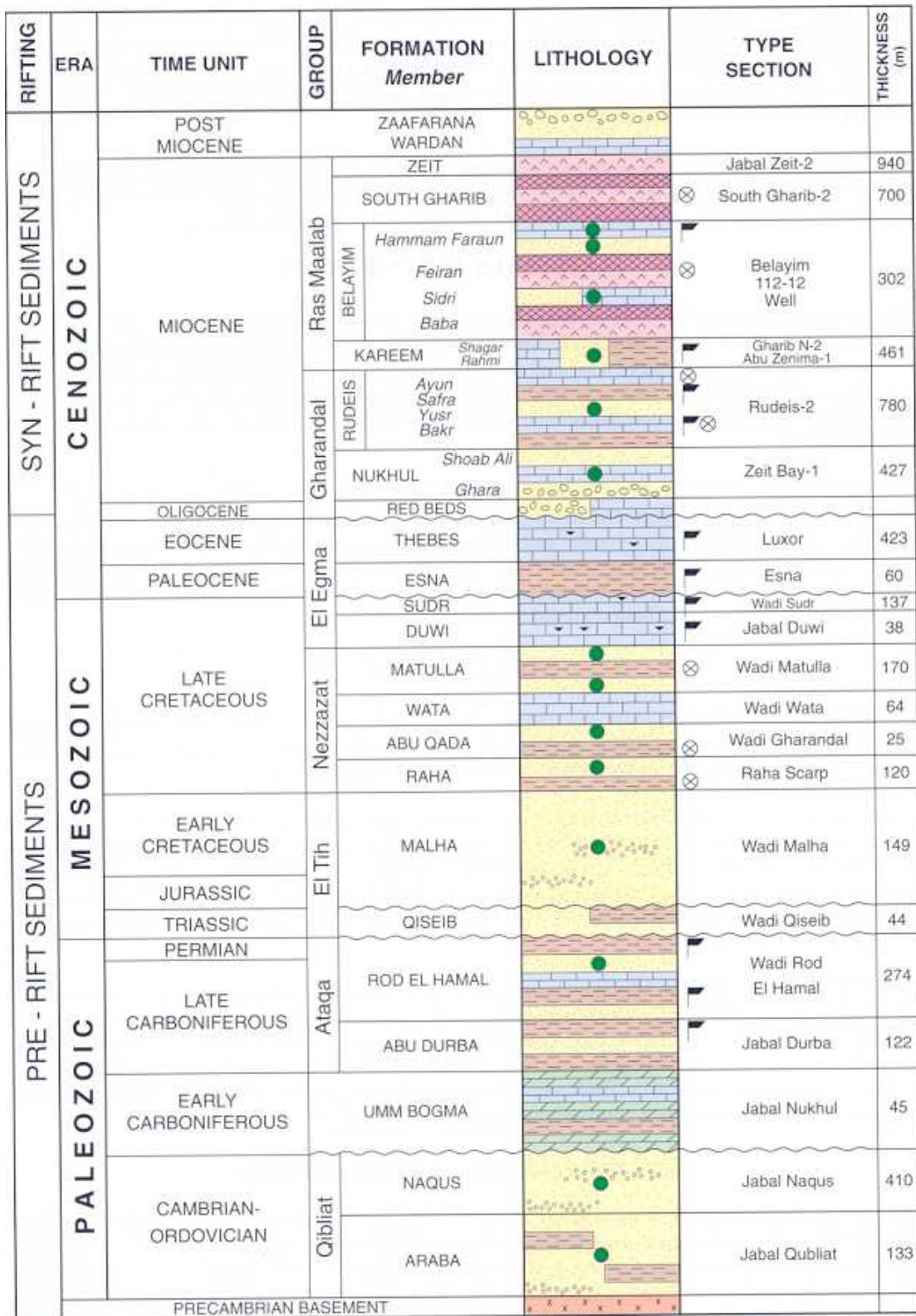
### 3. Results and Discussion

#### Normal -alkanes Characteristics

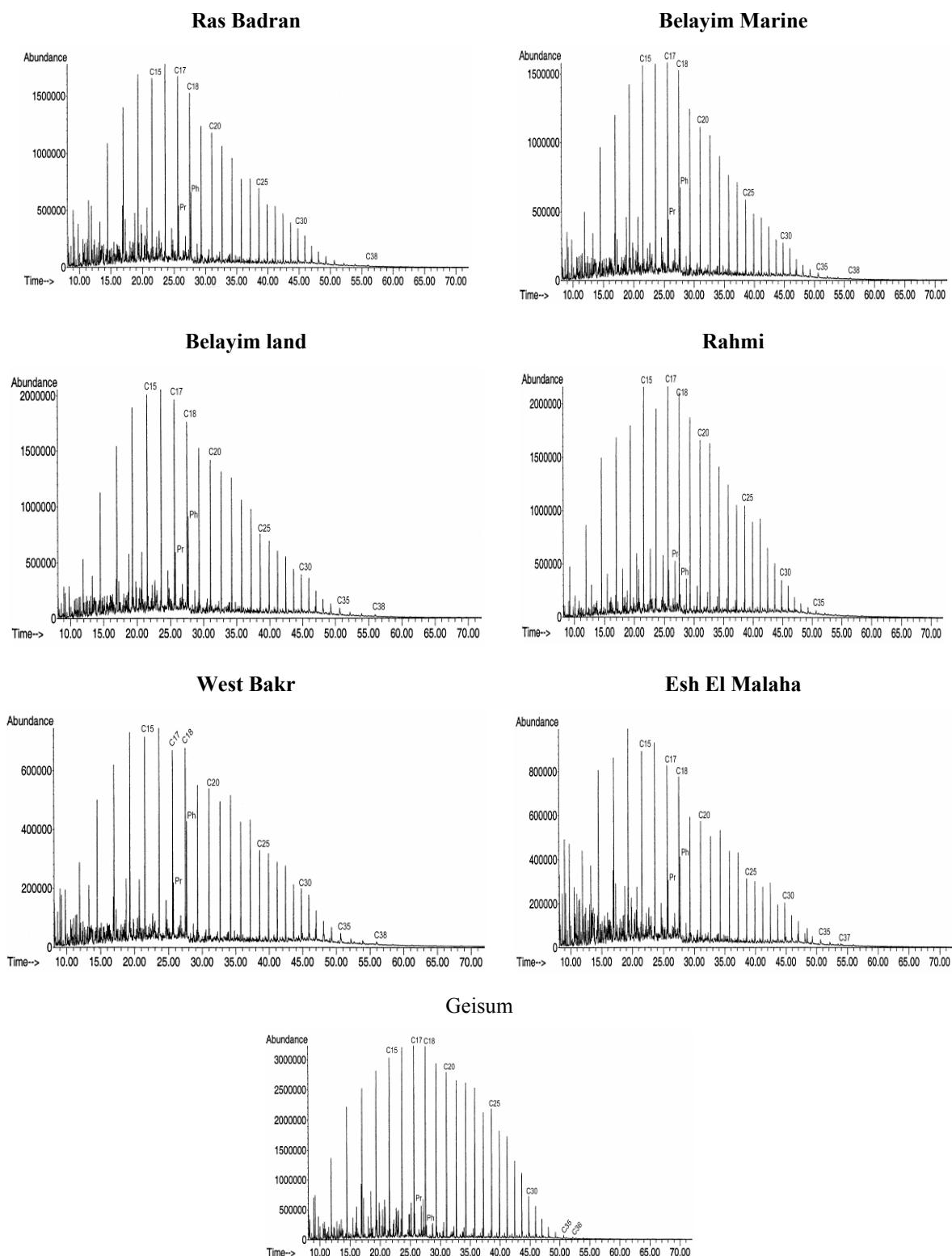
The distribution of n-alkanes in crude oils can be used to indicate the organic matter source (Duan and Ma, 2001). Figure 3 shows the fingerprints of gas chromatography for the saturated hydrocarbons of the



**Fig.1. Location map of the studied oilfields in the Gulf of Suez, Egypt.**



**Fig. 2: Stratigraphic column of the Gulf of Suez (Alsharhan and Salah, 1997). Oil reservoirs are indicated by green circles (●), source rocks as flags (■) and seals (⊗)**



**Fig. 3. Gas chromatograms of saturated hydrocarbons of the studied oil samples in the Gulf of Suez, Egypt**

studied crude oil samples. These fingerprints show that the studied oils appear to be mature, based on the abundance of n-alkanes in the range n-C<sub>15</sub> to n-C<sub>20</sub>, slightly even carbon preference and moderately to low concentration of heavy normal alkanes. The increase in the n-C<sub>15</sub> to n-C<sub>20</sub>, suggests marine organic matters with contribution to the biomass from algae and plankton (Peters and Moldowan, 1993). The striking molecular feature of oils is that all are characterized by uniformity in n-alkanes distribution patterns, suggesting that they are related and have undergone similar histories, with no signs of water washing or biodegradation ( Ficken et al. 2000 and Duan and Ma, 2001). The carbon preference index (CPI) of the studied oils ranging from 1.00 to 1.1 (Table 1) generally shows no even or odd carbon preference, indicate mature samples (Tissot and Welte, 1984).

#### Degree of Waxiness

**Table 1 Geochemical parameters derived from GC and GC-MS analyses of studied oilfields in the central Gulf of Suez, Egypt**

	Oilfields						
	Ras Badran	Belayim marine	Belayim Land	Rahmi	West Bakr	Esh El Mellaha	Geisum
Depth(ft)	2250	8286	7786	2600	2268	3254	2897
Reservoirs	L. Rudies	Belayim	Belayim	Up. Rudies	L. Rudies	Rudies	Nuhkul
Age	L. Miocene	U. Miocene	U. Miocene	L. Miocene	L. Miocene	Miocene	L.Miocene
Lithology	Sandstone	Limestone	Sandstone	Sandstone	Sandstone	Sandstone	Sandstone
Pristane/phytane	0.32	0.26	0.14	0.30	0.31	0.36	0.20
Pristane/n-C <sub>17</sub>	0.32	0.26	0.14	0.30	0.31	0.36	0.20
Phytane/n-C <sub>18</sub>	0.42	0.43	0.17	0.51	0.63	0.54	0.19
CPI	1.003	1.054	0.997	1.009	1.012	0.992	1.046
ΣC <sub>21</sub> -C <sub>31</sub> /ΣC <sub>15</sub> -C <sub>20</sub>	0.75	0.68	0.79	0.83	0.83	0.81	0.94
C <sub>27</sub> Steranes (%)	32.24	24.04	25.83	17.14	44.76	30.00	13.18
C <sub>28</sub> Steranes (%)	27.03	35.24	27.97	29.27	30.01	30.00	38.45
C <sub>29</sub> Steranes (%)	40.74	40.72	46.37	53.58	25.23	40.00	48.37
Homohopane index <sup>a</sup>	0.56	0.74	0.98	0.79	0.58	0.21	0.94
Diasteranes index <sup>b</sup>	0.11	0.11	0.11	0.11	0.10	0.08	0.11
Gammacerane index <sup>c</sup>	0.44	0.47	0.45	0.44	0.49	0.43	0.46
C <sub>29</sub> 20S/20S+20R <sup>d</sup>	0.11	0.12	0.09	0.10	0.10	0.08	0.09
C <sub>29</sub> /C <sub>30</sub> hopane <sup>e</sup>	0.04	0.04	0.05	0.05	0.07	0.05	0.04
Steranes/hopanes <sup>f</sup>	01.25	01.28	01.30	01.28	02.00	00.57	00.82
	0.54	0.53	0.60	0.58	0.62	0.60	0.65
<i>Ts/Tm<sup>g</sup></i>							

CPI: Σodd/Σeven carbon numbers,

a; Homohopane index: (C<sub>35</sub> homohopane S + R)/(C<sub>31</sub> + C<sub>32</sub> + C<sub>33</sub> + C<sub>34</sub> + C<sub>35</sub> homohopanes S + R).

b: Diasteranes index: (C<sub>27</sub> diasteranes S + R)/[(C<sub>27</sub> diasteranes + R) + C<sub>29</sub> steranes S + R].

c: Gammacerane index: gammacerane/(gammacerane + C<sub>30</sub> hopane).

d: C<sub>29</sub> 20S/20S+20R,

e:C<sub>29</sub>/C<sub>30</sub> hopane,

f: Steranes/17α (H)-hopanes ratio

g: Ts/Tm: Trisnorhopanes/Trisnorhopanes ratios.

#### Isoprenoids

#### Pristane/Phytane

The pristane/phytane (Pr/Ph) ratio is one of the most commonly used geochemical parameters and

The standard method of categorizing the amount of land-derived organic material in an oil is to determine its degree of waxiness. This method assumes that terrigenous material contributes a high molecular-weight normal paraffin components to the oil (Köket al., 1997). Thus, recent studies about oil classification by source input have relied heavily on waxiness as an environmental source input parameter (Connan and Cassou, 1980). The degree of waxiness in this study is expressed by the ΣC<sub>21</sub>-C<sub>31</sub>/ΣC<sub>15</sub>-C<sub>20</sub>, ratios (Table 1).

The studied oils are characterized by high abundance of of n-C<sub>15</sub>to n-C<sub>20</sub> n-alkanes in the saturate fractions reflecting low waxy (Moldowan et al., 1994). The dege of waxness (ΣC<sub>21</sub>-C<sub>31</sub>/ΣC<sub>15</sub>-C<sub>20</sub> ratios)ranging from 0.68 to 0.94 (Table 1) confirms low waxy nature and suggests marine organic sources (Peters and Moldowan, 1993).mainly of higher plants, deposited under reducing condition.

has been used as an indicator of depositional environment with low specificity due to the interferences by thermal maturity and source inputs (Didyk et al., 1978, Peters et al., 2005). ten Haven et

al. (1987) stressed that high Pr/Ph (>3.0) indicates terrigenous input under oxic conditions and low Pr/Ph (<0.8) indicates anoxic/hypersaline or carbonate environments. According to Lijmbach (1975) low values (Pr/Ph<2) indicate aquatic depositional environments including marine, fresh and brackish water (reducing conditions), intermediate values (2–4) indicate fluviomarine and coastal swamp environments, whereas high values (up to 10) are related to peat swamp depositional environments (oxidizing conditions).

The studied oil samples are characterized by pristane/phytane ratios (0.14 to 0.36) and waxiness values (0.68 to 0.94, Table 1), confirming that these oils have been originated from marine organic source deposited under suboxic conditions. Figure 4 exhibiting the relationship between Pr/Ph and waxiness confirms this conclusion. Furthermore, the cross plot of Pr/Ph versus CPI (Figure 5) shows that, the oils fall in the field of more reducing zone of thermal maturation level.

#### Isoprenoides/n-alkanes ratios

In crude oil studies, the ratios of isoprenoids to n-alkanes are widely used since they provide informations on maturation and biodegradation as well as source (Ficken et al., 2002). Consequently, a plot of Pr/n-C<sub>17</sub> versus Ph/n-C<sub>18</sub> in Figure 6 as originally proposed by Lijmbach (1975). The isoprenoids/n-alkanes ratios (pr/n-C<sub>17</sub> and ph/n-C<sub>18</sub>, shown in Table 1), suggest marine organic matters source (mainly algae) deposited under reducing environment. It also indicates a genetic close relation between the studied oils.

#### Steranes (m/z 217) Distribution

The distribution of steranes is best studied on GC/MS by monitoring the ion m/z=217 which is a characteristic fragment in the sterane series. The resulting mass chromatograms for the representative samples are shown in Fig. (7) and the labeled peaks are summarized in Table (2). The compounds were identified in their key fragmentograms based on the relative retention times and by comparison of their mass spectra with the published data. It is agreed that the relative amounts of C<sub>27</sub>-C<sub>29</sub> steranes can be used to give indication of source differences (Lijmbach, 1975)

The studied crude oils of Ras Badran, Belayim, West Bakr and Esh Elmalaha are characterized by predominance of C<sub>28</sub>, C<sub>29</sub> and C<sub>30</sub> steranes (20S and 20R, Fig. 7, see peak identifications in Table 2)

which indicate an origin of the oils derived mainly from mixed terrestrial and marine organic sources. While oil samples from Rahmi and Geisum (Fig. 7) show slightly low abundance of C<sub>28</sub> and C<sub>29</sub>, and relatively higher concentrations of C<sub>27</sub>steranes (20S and 20R, see peak identifications in Table 2) which indicate more input of marine organic source. The steranes diagram (Fig. 8) confirms these assumptions.

The amount of C<sub>27</sub> diasteranes, diasteranes index and concentration of (20S) and (20R) isomers influences the maturity level of crude oils. Peters and Moldowan (1993); Petersen et al., (2000) and Andrew et al. (2001) recognized that the maturity level of oils increases with the increase of these parameters (i.e. high concentration of C<sub>27</sub> diasteranes, > 0.1 diasteranes index, and > 0.5 C<sub>29</sub> 20S/20S+20R). Thus, it is obvious that the studied oils have slightly high concentration of C<sub>27</sub> diasteranes (20S and 20R) peaks A, B and C with diasteranes index range from 0.10 to 0.11 and C<sub>29</sub> 20S/20S+20R from 0.1 to 0.12 (Table 1). These data reveal that the studied oils are characterized by high maturity level.

#### Triterpanes (m/z 191) Distribution

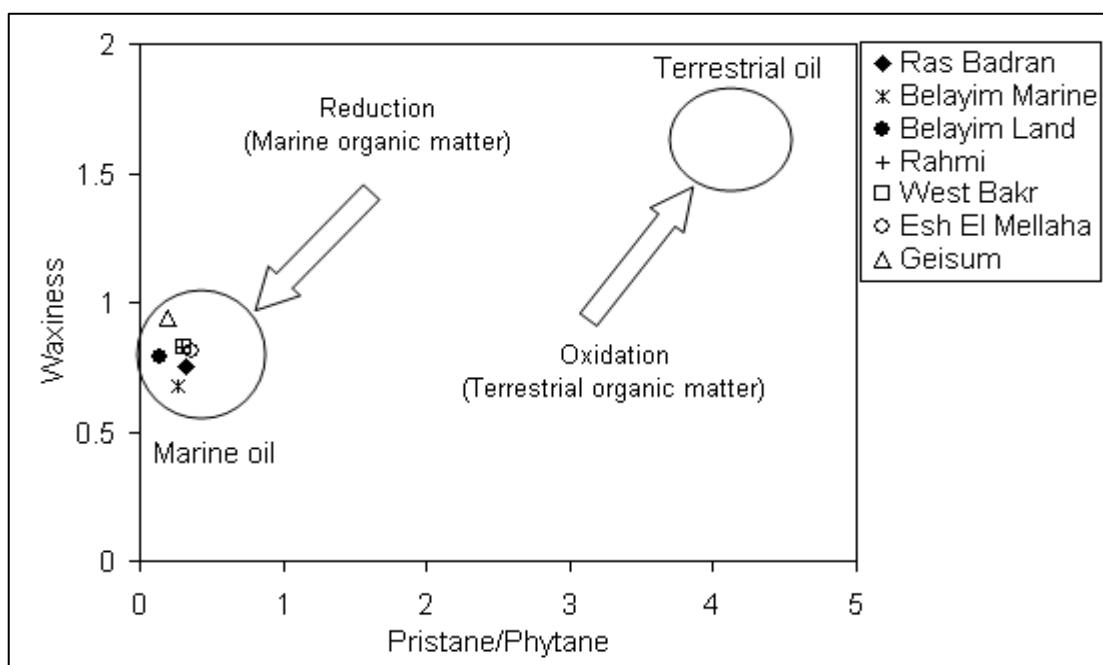
Together with steranes, triterpanes belong to the most important petroleum hydrocarbons that retain the characteristic structure of the original biological compounds. Tricyclic, tetracyclics, hopanes, and other compounds contribute to the terpane fingerprint mass chromatogram (m/z=191) are commonly used to relate oils and source rocks Hunt, 1996). Mass fragmentogram at m/z=191 was used to detect the presence of triterpanes in the saturate hydrocarbon fraction of the studied oils (Fig. 9). The assignment of the peaks labeled in fig. (9) are listed in Table (2). the most distinct features are Tricyclic Terpanes, Ts/Tm and C<sub>29</sub>/C<sub>30</sub> hopane ratios

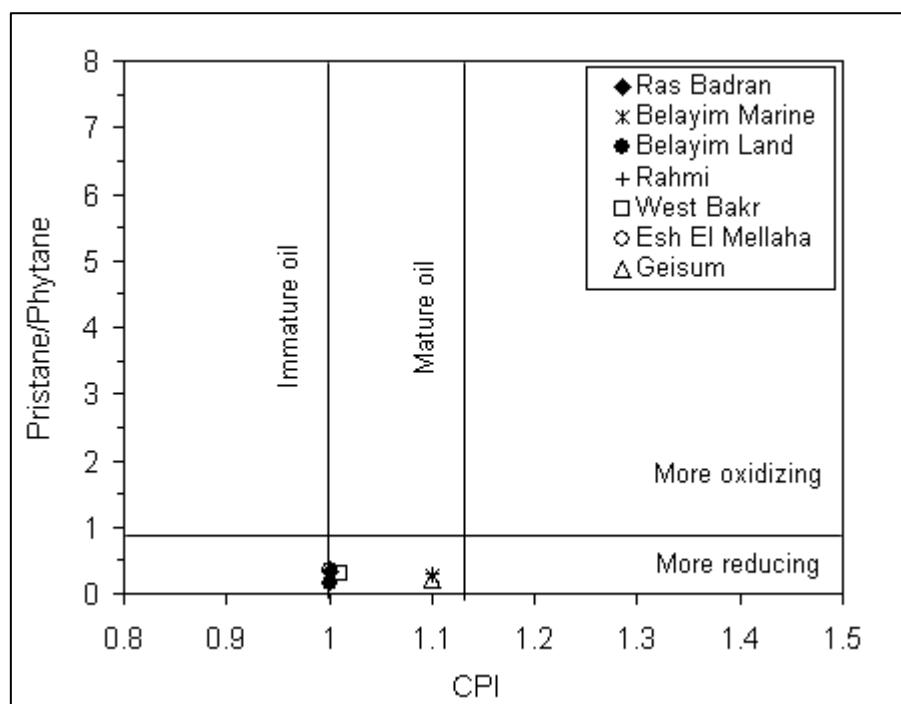
#### Tricyclic Terpanes

The concentration of tricyclic terpanes in crude oils is more sensitive to the specific paleoenvironments (Waples and Machihara, 1992 and Andrew et al., 2001). In addition it has been used as a qualitative indicator of maturity (Van Grass, 1990). In high mature oils, the tricyclic terpanes is dominated more than in low mature oils (Hunt, 1996). Aquino et al. (1983) indicated that tricyclic terpanes are normally associated with marine source. Our study reveals that the concentration of C<sub>22</sub> tricyclic terpanes peak B in the studied oil samples (Fig. 9, see peaks identifications in Table 2) is higher which support the idea that the oils are more

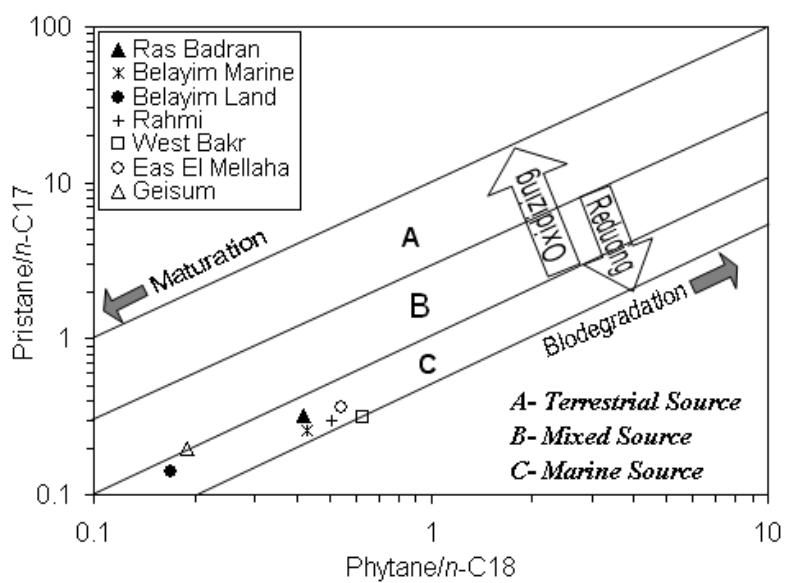
Table 2. Identification of peaks in triterpanes ( $m/z$  191) and steranes ( $m/z$  217) mass fragmentograms.

Triterpanes ( $m/z$ 191)		Steranes ( $m/z$ 217)	
Peaks	Compounds Name	Peaks	Compounds Name
A	C <sub>21</sub> Tricyclic terpane	A	C <sub>27</sub> αβ diasterane (20S)
B	C <sub>22</sub> Tricyclic terpane	B	C <sub>28</sub> βα diasterane (20S)
C	C <sub>23</sub> Tricyclic terpane	C	C <sub>28</sub> βadiasterane (20R)
D	C <sub>24</sub> Tricyclic terpane	D	C <sub>27</sub> ααα sterane (20S)
E	C <sub>25</sub> Tricyclic terpane (22R)	E	C <sub>27</sub> αββ sterane (20S)
F	C <sub>24</sub> Tricyclic terpane	F	C <sub>27</sub> αααsterane (20R)
G	C <sub>28</sub> Tricyclic terpane (22R)	G	C <sub>29</sub> βα diasterane (20R)
H	C <sub>28</sub> Tricyclic terpane (22S)	H	C <sub>29</sub> βα diasterane (20S)
I	C <sub>28</sub> Tricyclic terpane (22S)	I	C <sub>28</sub> ααα sterane (20S)
J	C <sub>27</sub> 18αH-Trisnorneohopane (Ts)	J	C <sub>28</sub> αββ sterane (20R)
K	C <sub>27</sub> 17αH-Trisnorhopane (Tm)	K	C <sub>28</sub> αββ sterane (20S)
L	C <sub>28</sub> Bisonorhopans	L	C <sub>28</sub> ααα sterane (20R)
M	C <sub>29</sub> 17αH,21βH-Norhopanes	M	C <sub>29</sub> ααα sterane (20S)
N	C <sub>29</sub> 17βH,21αH-Normoretane	N	C <sub>29</sub> αββ sterane (20R)
O	C <sub>30</sub> 17αH,21βH-Hopane	O	C <sub>29</sub> αββ sterane (20S)
P	C <sub>29</sub> 17βH,21αH-Moretane	P	C <sub>29</sub> ααα sterane (20R)
Q	C <sub>31</sub> 17αH,21βH-Homohopane (22R)	Q	C <sub>30</sub> αββ steranes (20R)
R	C <sub>30</sub> Gammacerance	R	C <sub>30</sub> αββsteranes (20S)
S	C <sub>32</sub> 17αH,21βH--Homohopane (22R)	S	C <sub>30</sub> ααα steranes (20R)
T	C <sub>33</sub> 17αH,21βH--Homohopane (22R)		
U	C <sub>34</sub> 17αH,21βH--Homohopane (22S)		
V	C <sub>35</sub> 17αH,21βH--Homohopane (22R)		

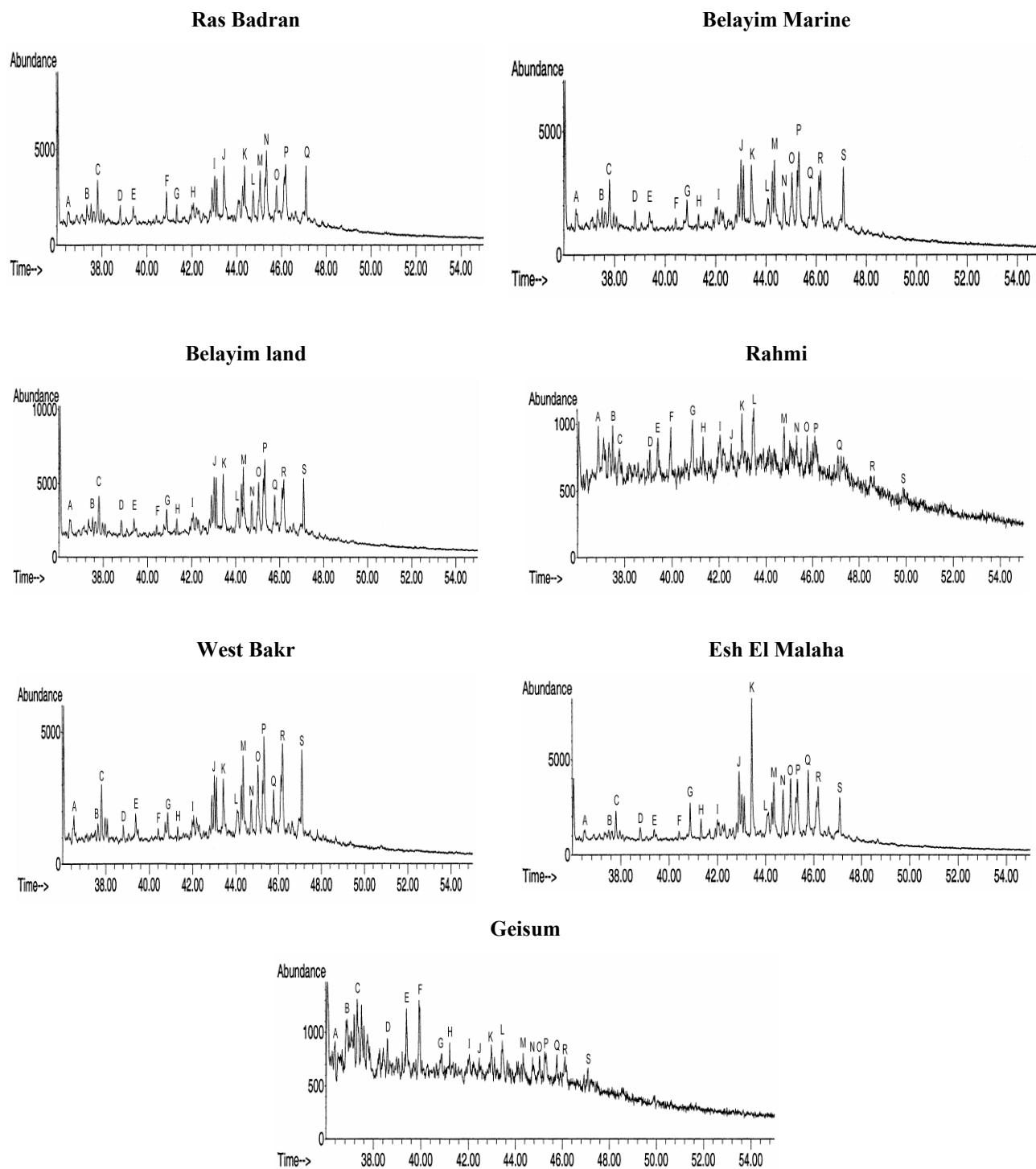
Fig. 4: Cross plots of Pr/Ph versus waxiness  $\sum(n\text{-C}21\text{-n-C}31)/\sum(n\text{-C}15\text{-C}21)$  for the studied samples.



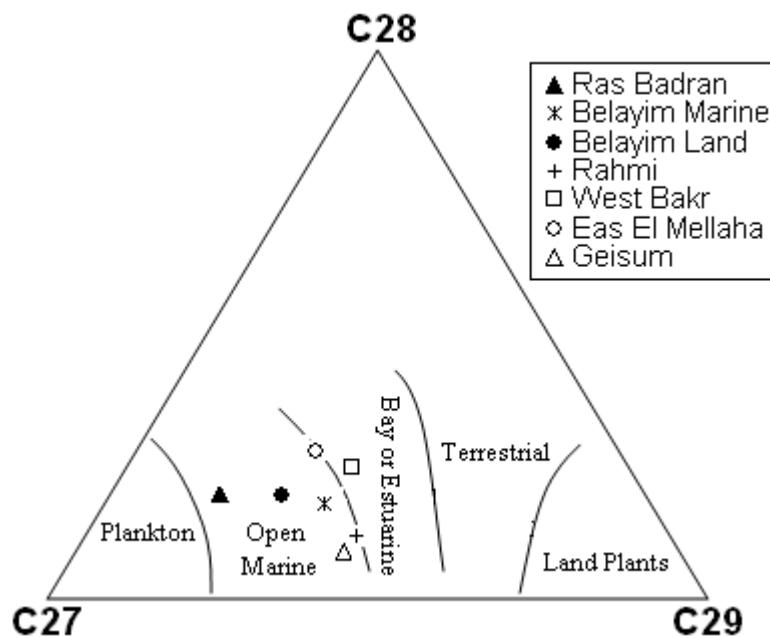
**Fig. 5: Pristane/Phytane versus CPI showing the sources of studied crude oils (Akinula et al., 2007).**



**Fig. 6. Plot of pristane/n-C<sub>17</sub> versus phytane/n-C<sub>18</sub> (Shanmugam, 1985), showing the organic sources and maturation of studied oil samples.**



**Fig. 7. Ion fragmentgrams ( $m/z$  217) steranes of saturated hydrocarbons of the studied oil samples in the Gulf of Suez, Egypt**



**Fig. 8. Distribution of C<sub>27</sub>, C<sub>28</sub>, and C<sub>29</sub> regular steranes (Huang and Meinschein, 1979), showing organic facies of the crude oils in the Gulf of Suez, Egypt.**

mature and sourced mainly from carbonate source rocks. On the other hand, C<sub>21</sub>, C<sub>23</sub> to C<sub>28</sub> tricyclic terpanes peaks A, C to I are generally of low detection levels in the studied oils indicating that these oils have some inputs derived from terrestrial organic material (Hunt, 1996). This confirms with the conclusion of steranes biomarkers.

#### Homohopanes

The homohopanes (C<sub>31</sub> to C<sub>34</sub>) are believed to be derived from bacteriopolyhopanol of prokaryotic cell membrane. C<sub>35</sub> homohopane may be related to extensive bacterial activity in the depositional environment (Ourisson et al., 1984). The distribution of 17 $\alpha$ ,21 $\beta$ (H)-29-homohopanes 22R+22S C<sub>35</sub>/(C<sub>31</sub>-C<sub>35</sub>) (or simply homohopane index) in crude oils can be used as an indicator of the associated organic matter type, as it can also be used to evaluate the oxic/anoxic conditions of source during and immediately after deposition of the source sediments (Peters and Moldowan, 1991). Low C<sub>35</sub> homohopanes is an indicator of highly reducing marine conditions during deposition, whereas high C<sub>35</sub> homohopane concentrations are generally observed in oxidizing water conditions during deposition, consistent with the oxic conditions as suggested by high pristane/phytane ratios (Peters and Moldowan, 1991).

The studied crude oils have low concentrations of C<sub>31</sub>-C<sub>35</sub> homohopanes (20S and 20R) (peaks S to V Fig. 8, Table 2) which are more significant to hypersaline marine oils. The homohopane indices

values 0.21 to 0.98 (Table 1) confirms the above conclusion.

#### Gammacerane

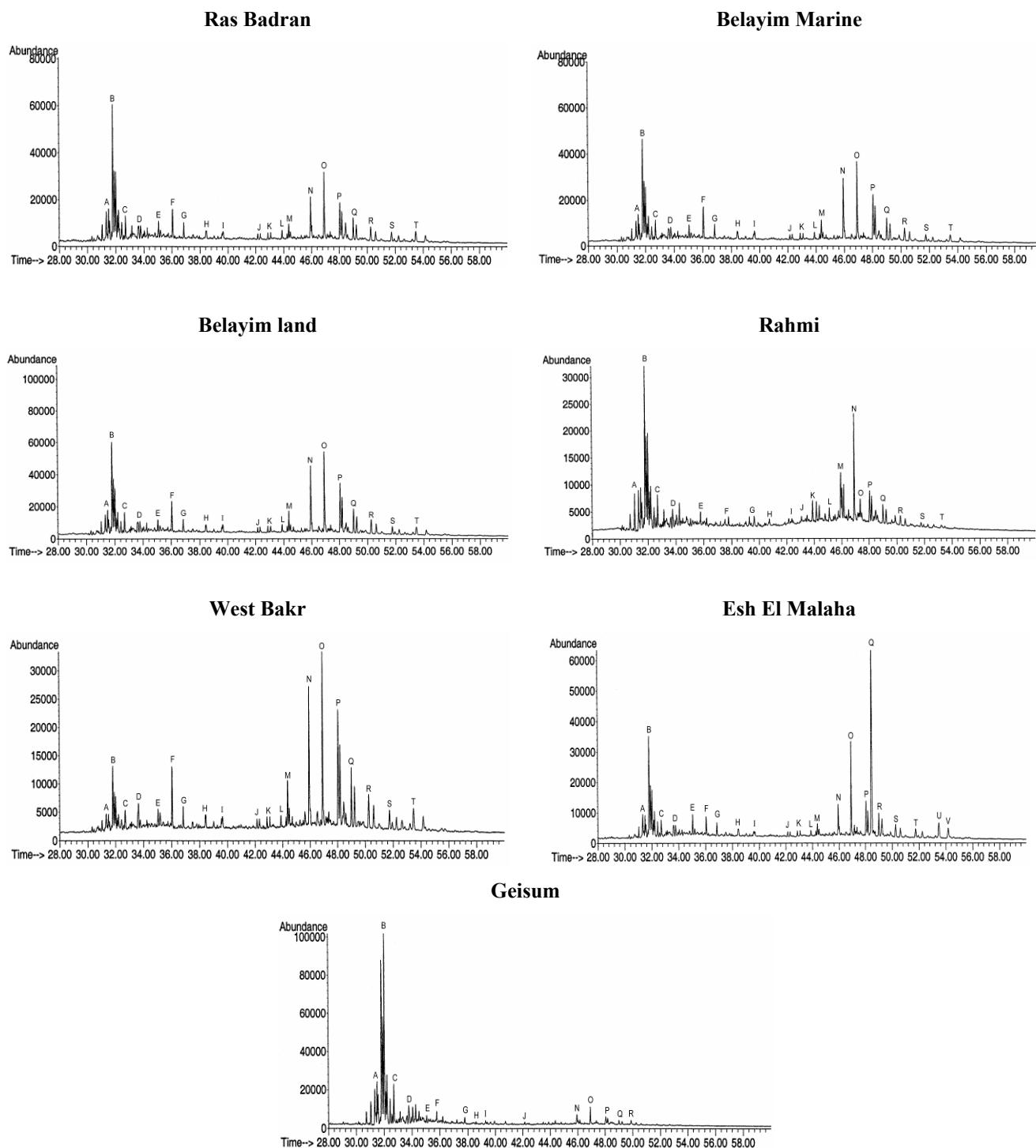
Gammacerane, is associated with environments of increasing salinity, both marine and lacustrine (Waples and Machihara, 1991; and Peters and Moldowan, 1993). Gammacerane (peak R, see peaks identifications in Table 2, Fig. 8) is only detected in relatively low amount in the studied oils indicating input of marine organic matter in different saline environments, e.g. lakes, lacustrine, ponds, ... etc .

#### Ts/Tm

The ratio of Ts (trisnorneohopane) to Tm (trisnorhopane) more than (0.5) increases as the portion of shale in calcareous facies increases (Hunt, 1996). Also, this ratio was proved to be useful in paleofacies predictions, though not as decisive as maturity parameters. Van Grass (1990) stated that Ts/Tm ratios begin to decrease quite late during maturation, but Waples and Machihara, (1991) reported that Ts/Tm ratio does not appear to be appropriate for quantitative estimation of maturity. In the present study we show that the Ts/Tm ratios range from 0.53 to 0.65 for the studied oils (Table 1). These relatively high ratios suggest that the studied oils were generated mainly from calcareous facies.

#### C<sub>29</sub>/C<sub>30</sub> hopanes ratios

C<sub>29</sub>/C<sub>30</sub> hopanes ratios are generally high (>1) in oils generated from organic rich carbonates and evaporates (Connan et al., 1986). The majority



**Fig. 9. Ion fragmentgrams ( $m/z$  191) triterpanes of saturated hydrocarbons of the studied oil samples in the Gulf of Suez, Egypt**

studied oil samples, having higher concentrations of C<sub>29</sub> than C<sub>30</sub> hopane, peaks O and Q respectively with high C<sub>29</sub>/C<sub>30</sub> hopane ratios range from 1.01 to 1.40 (Table 1), except oils from wells Esh El Mellaha and Geisum which have lower concentrations of C<sub>29</sub> than C<sub>30</sub> hopane (Fig. 8), with low values of C<sub>29</sub>/C<sub>30</sub> hopanes (0.04 to 0.07, Table 1). These data illustrate that the oil samples might be sourced from source rocks rich in carbonaceous organic matters. This assumption is confirmed by the low abundance of C<sub>29</sub> moretane (peak P), gammacerane (peak R) and slightly higher concentration of C<sub>28</sub> bisnorhopanes (peak L), Philp (1985); Riediger et al. (1990) and Waples and Machihara, (1991).

#### Steranes/17 $\alpha$ (H)-hopanes ratio

The regular steranes /17 $\alpha$ (H)-hopanes ratio reflects input of eukaryotic (mainly algae and higher plants) versus prokaryotic (bacteria) organisms to the source rock (Noriyuki et al,1996). The sterane/hopane ratio is relatively high in marine organic matter, with values generally approaching unity or even higher. In contrast, low steranes and sterane/hopane ratios are more indicative of terrigenous and/or microbially reworked organic matter (Noriyuki et al,1996). From steranes/hopanes ratio of the studied crude oils range 1.25 to 2.00 of wells Ras Badran, Belayim marine, Belayim Land, Rahmi, and West Bakr and 0.57 and 0.82 Esh El Mellaha and Geisum crude oils (Table 1). This indicates that the majority of the studied crude oils can be considered generated from high marine organic matter source (marine and lacustrine algae). These results show an agreement with the data obtained from the relationship of Pr/nC<sub>17</sub> versus Ph/nC<sub>18</sub> (Fig. 6).

#### 4. Conclusions

Utilizing the GC and GC/MS technique made it possible to arrive at a clear characterization and classification of crude oils according to their sources. This has been achieved from the acyclic isoprenoids, steranes and terpanes biomarkers. Geochemical parameters based upon these components coupled with bulk geochemical parameters did indicate whether the crude oils are of marine, terrestrial or mixed marine-terrestrial origin.

Biomarkers analyses of crude oils from the Gulf of Suez, suggest the following: oils are more mature and derived mainly from mixed organic sources from terrestrial and marine inputs contribution to the biomass from algae and plankton in different saline environments.

The few discrepancies that appear between the results obtained by using the different parameters can be related to the alteration caused by the number

of processes (physical, chemical and/or biological) affecting part of the source related biomarkers pattern of the oil after generation and/or primary migration from the source rock.

#### Corresponding author

H. R. Ali

Egyptian Petroleum Research Institute, Nasr City,  
Cairo, Egypt.

[\\*hugochem@yahoo.com](mailto:hugochem@yahoo.com)

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10/10/2010

## Drug resistance and recent therapeutic measures in controlling of fascioliasis

A. Z. Mahmoud<sup>1</sup>; Mokhtar M. Taha<sup>1</sup>; Salah M. H. Afifi<sup>1</sup>; Khaled M. A. Hassanein<sup>1</sup>  
and Amal Mohamed Abdo Elmatary<sup>2</sup>

Department of Pathology & Clinical Pathology, Faculty of Veterinary Medicine, \* Parasitology department, Faculty of Medicine, Assiut University, Assiut, Egypt.

\* [amatalmatary@yahoo.com](mailto:amatalmatary@yahoo.com)

**Abstract:** Fascioliasis is a widely distributed disease affecting herbivorous animals. As a result of drug resistance a mixture of two antifasciola drugs (Triclobendazole and Superivomec) was used in trial to overcome this drug resistance. Twenty eight newly weaned white Boskat rabbit aging 1.5 month were divided into 7 groups, six of them were experimentally infected with metacercaria of *Fasciola gigantica* and one kept as -ve control group. Faecal egg count during the clinical course of the disease, counting the worm and its morphological studies and lesion score after postmortem examination were the parameters used to evaluate the effect of different drug mixtures. It had been concluded that the mixture of triclabendazole and superivomec was the mixture of choice.

[A. Z. Mahmoud; Mokhtar M. Taha; Salah M. H. Afifi; Khaled M. A. Hassanein and Amal Mohamed Abdo. Drug resistance and recent therapeutic measures in controlling of fascioliasis. Journal of American Science 2010;6(11):926-933]. (ISSN: 1545-1003).

**Key words:** Fascioliasis, Metacercaria, Triclabendazole, Rabbit and Superivomec.

### 1. Introduction:

Fascioliasis or liver flukes is a disease affecting herbivorous animal and caused by *Fasciola hepatica* and *Fasciola gigantica*. It has a worldwide distribution in a large variety of grass-grazing animals as sheep, goats, cattle, buffaloes, horses and rabbits. In Egypt, donkeys and camels as well, are hosts for *Fasciola gigantica*. Fascioliasis may occasionally affect man (Haseeb et al., 2002; Sanad and Al-Megrin, 2005).

Fascioliasis gives rise to important economic losses such as great expenses with anthelmintics, liver condemnation, production loss due to mortality, lower production of meat, milk and wool; reduced weight gain, and impaired fertility (Parr and Gray, 2000; Marques and Scroferneker, 2003).

Two clinical stages are recognized in fascioliasis. An acute stage coincides with the larval migration and worm maturation in the hepatic tissue, and a chronic stage coincides with the persistence of *Fasciola* worms in the bile ducts (Haseeb et al., 2002).

Fascioliasis is controlled by a combination of anthelmintic therapy and management measures. These methods are costly and may lead not only to anthelmintic resistance, but also to undesirable residues in food or the environment (Pérez et al., 2002). Consequently, the control of fascioliasis is one of important aims in our work.

These studies were designed to select an effective treatment against different stages of *Fasciola gigantica* were including immature worm, adult worm, and eggs using mixture of drugs to avoid development of drug resistance.

### 2. Materials and methods

#### I. Materials

##### Experimental animals:

A total number of 28 newly weaned white Boskat rabbit aging 1.5 months (obtained from faculty of agriculture farm, Assiut University) were divided into 7 groups (4 rabbits each).

Group (1): (Control +ve group): Rabbits were infected with 30 metacercaria/ rabbit orally by stomach tube. Rabbit were slaughtered 11 weeks post infection (PI).

Group (2): Flubendazole treated group: Rabbits were infected with 30 metacercaria/ rabbit orally and treated orally with flubendazole at 6 weeks (PI) (100 mg/kg b.wt).

Group (3): Flubendazole and superivomec treated group: Rabbits were infected with 30 metacercaria/ rabbit and treated orally with flubendazole (50 mg/kg b.wt.) plus superivomec (ivermectin 100 µg and clorsulon 1 mg/ kg b.wt).

Group (4): Superivomec treated group: Rabbits were infected with 30 metacercaria/ rabbit orally and treated S/C with superivomec at 6 weeks (PI) (ivermectin 200 µg and clorsulon 2 mg /kg b.wt).

Group (5): Triclabendazole treated group: Rabbits were infected with 30 metacercaria/ rabbit orally and treated orally with triclabendazole (10 mg/kg b.wt).

Group (6): Triclabendazole and superivomec treated group: Rabbits were infected with 30 metacercaria/ rabbit orally and treated orally with triclabendazole (5 mg/kg b.wt) plus superivomec (ivermectin 100 µg and clorsulon 1 mg per kg b.wt).

Group (7): (Control -ve group): Rabbits were slaughtered 11 weeks (PI).

## II. Methods

### A) Parasitological examination:

#### Faecal samples:

A piece of faecal sample was dispersed and thoroughly mixed with about 10 parts by volume of saline solution. Examination of faecal samples was carried out according to Melvin and Brooke, (1982)

#### Fluke samples:

Samples of flukes were taken to study changes in morphology by carmine stain and scanning electron microscopy.

Methods used to study the effect of the drug on the worm:

- Egg count at different periods of infection during the experiment.
- The number of worms were counted and measuring the length of the flukes.
- The morphology of adult flukes was studied by carmine stain and Scanning Electron Microscope (S.E.M).

### B) Pathological examination:

Tissue samples: Samples from liver were taken for histopathological examination.

Gross pathology: Rabbits were dissected for the presence/absence of any gross lesions in the liver.

Histopathological examination: Routine review histopathological picture were carried on formalin fixed paraffin embedded sections from the liver and stained by H & E (Bancroft et al., 1994).

### C) Statistical analysis:

The data were statistically analyzed using general linear model (G. L. M) procedure of SAS (1996). The significance differences between treatment means were tested by Duncan multiple range test (Steel and Torrie, 1982).

## 3. Results

The clinical signs were observed 4 weeks post infection with *Fasciola gigantica*. The signs in the form of dullness, rough hair coat. At 9 weeks (PI), loss of hair coat, anorexia, paleness of the mucous membranes and emaciation were observed

especially in control +ve group and flubendazole treated group when compared with normal rabbits (Figs. 1A,B).

Faecal examination revealed the presence of eggs of *Fasciola gigantica* as early as 40 days (PI) in all infected groups. The eggs appeared oval, operculated, delicate light yellow in color (Fig. 1C).

Statistical analysis of the egg counts from 7 to 11 weeks (PI) revealed significant increase in eggs in group 1 and 2 when compared with other groups. In group 3 and 4, highly significant decrease of egg count was observed when compared with group 1 and 2. Statistical investigation of egg count in the group of rabbits treated with triclabendazole either alone or in combination with superivomec revealed highly significant decrease in the egg count during the 7<sup>th</sup> week (PI) when compared with group 1. Complete cessation of the eggs was observed in both groups during the 8<sup>th</sup> weeks (PI) (Table 1).

Gross examination of the liver in group 1,2 and 3 showed enlargement, congestion and multiple necrotic foci on the liver surface. In addition, multiple migratory tracts were observed. Perihepatitis was observed. Normalization of the liver with no migratory tracts and liver flukes were seen in group 4, 5 and 6.

Histopathological examination of the liver in group 1 and 2 showed congestion, thrombosis, vasculitis and perivasculitis of hepatic vasculatures. Bile duct proliferation, hyperplasia, cholangitis and pericholangitis were observed. The bile duct changes in group 2 were more severe than group 1. The histopathological changes of the hepatic cells were ranged from degenerative changes (vacuolar degeneration and glycogen infiltration), necrosis (coagulative and lytic) and hepatitis either focal or diffuse (Figs. 1D-G).

The histopathology of the liver in group 3 showed the same pathological changes as group 2 but less severe. The histopathological changes of liver in group 4 were quite similar to those observed in the previous groups. While triclabendazole treated group revealed normal appearance of the different hepatic structures except a minor changes expressed by congestion and thrombosis of some hepatic vasculatures. The bile ducts showed bile duct proliferation and hyperplasia, cholangitis and pericholangitis. The hepatic parenchyma showed focal granular degeneration. In superivomec + triclabendazole treated group, most of the hepatic tissues appeared more or less normal except minor pathological alterations.

As shown in Table (2) the lesion scores of histopathological results of group 1 revealed significant increase in the bile duct lesions, portal lesions, migratory tracts and focal liver lesions in

group 1 when compared with other groups except group 2. In group 3, the lesion score demonstrated that the bile duct lesion and portal lesion were significantly decreased when compared with group 1 and 2. In group 4, minimal lesion scores were observed. In group 5, the lesion scores revealed absence of migratory tracts and diffuse liver lesion. The lesion scores of bile duct and portal lesions were significantly decreased. In group 6, lesion scores obtained after treatment with the mixture revealed significant decrease in all parameter when compared with other groups.

Careful counting of the liver flukes and statistical analysis revealed significant increased of flukes numbers in group 1 when compared with other groups (Table. 3). *Fasciola gigantica* stained with carmine revealed maturity of the worm while in flubendazole treated group it was decreased than control +ve group. The flukes stained with carmine showed that the mixture of flubendazole and superivomec had a prominent effect on the maturation of the fluke especially those of the sexual organs. Significant decrease in the number and the length of flukes in superivomec treated group when

compared with other groups was seen. Also, it has a prominent effect on maturation of sexual organs and to lesser extend on the digestive organs (Table. 3) & (Figs.2A-D).

Scanning electron microscopy of the liver flukes in control +ve group revealed rough tegumental surface of the adult *Fasciola gigantica*. The surface covered with posterior directed spines and transverse folds. The anterior ventral portion showed oral and ventral suckers and gonoopore between them (Fig. 3). In flubendazole treated group minor changes in the form of shrunken oral sucker and dilated ventral sucker. The spines surrounded the oral sucker and mid-lateral aspect of the ventral surface of the flukes showed shortening of the scale-like spines. The ventral surface of the flukes showed large area of sloughed tegument which filled with debris while the dorsal surface showed disruption in the form of furrowing of the tegumental surface (Fig. 4). Scanning electron microscopy to the liver flukes of flubendazole + superivomec treated group showed more prominent changes especially in the anterior half of the fluke (Figs. 5).

**Table (1): Mean values of numbers of eggs at 7 weeks, 8 weeks, 9 weeks, 10 weeks and 11 weeks post infection in different groups.**

	7 wks.	8 wks.	9 wks.	10 wks.	11 wks.	Average
<b>Control + ve group</b>	14.00±1.75 <sup>a</sup>	20.57±1.91 <sup>a</sup>	53.71±6.44 <sup>a</sup>	101.43±5.24 <sup>a</sup>	194.14±15.48 <sup>a</sup>	76.77±11.86 <sup>a</sup>
Flubendazole	13.71±1.25 <sup>a</sup>	17.57±3.25 <sup>ab</sup>	42.71±4.97 <sup>a</sup>	61.86±5.26 <sup>b</sup>	111.14±9.05 <sup>b</sup>	49.40±6.50 <sup>a</sup>
Flu + Super	8.29±0.75 <sup>b</sup>	10.57±2.44 <sup>b</sup>	22.00±2.90 <sup>b</sup>	10.67±2.23 <sup>c</sup>	17.00±2.65 <sup>c</sup>	13.79±1.32 <sup>b</sup>
Superivomec	8.14±1.10 <sup>b</sup>	13.00±1.09 <sup>ab</sup>	28.86±3.18 <sup>b</sup>	8.00±1.14 <sup>c</sup>	-	15.00±1.96 <sup>b</sup>
Triclabendazole	5.00±1.33 <sup>b</sup>	-	-	-	-	5.00±1.33 <sup>b</sup>
Super + Tric	5.57±1.07 <sup>b</sup>	-	-	-	-	5.57±1.07 <sup>b</sup>

a, b and c means in the same column differ at (p<0.05)

**Table (2): Mean values of lesion scores in different groups.**

	Control + ve	Flubendazole	Flub + Super	Superivomec	Triclabenda-zole	Sup + Tric
<b>Bile duct lesions</b>	11.80±1.03 <sup>b</sup>	13.80±0.92 <sup>a</sup>	9.00±0.77 <sup>c</sup>	7.60±0.58 <sup>c</sup>	4.90±0.48 <sup>d</sup>	2.10 ±0.31 <sup>e</sup>
<b>Portal lesions</b>	11.90±1.05 <sup>a</sup>	11.90±1.03 <sup>a</sup>	9.50±0.79 <sup>b</sup>	7.40±0.45 <sup>bc</sup>	6.30±0.93 <sup>c</sup>	2.40±0.45 <sup>d</sup>
<b>Migratory tracts</b>	6.70±1.00 <sup>a</sup>	5.10±0.85 <sup>ab</sup>	4.00±0.71 <sup>ab</sup>	3.00±0.55 <sup>b</sup>	-	-
<b>Focal liver lesions</b>	9.33 ±2.19 <sup>a</sup>	4.67± 0.50 <sup>bc</sup>	3.78±1.10 <sup>bc</sup>	7.50±1.43 <sup>ab</sup>	2.00±0.00 <sup>c</sup>	1.75±0.25 <sup>c</sup>
<b>Diffuse liver lesions</b>	6.00±1.08 <sup>a</sup>	5.70±1.07 <sup>a</sup>	4.25±0.98 <sup>ab</sup>	1.33±0.33 <sup>c</sup>	-	1.33±0.33 <sup>c</sup>

a, b, c and d means in the same column differ at (p<0.05)

**Table (3): Mean values of liver flukes numbers in different groups.**

Groups	Numbers of liver flukes
<b>Control +ve group</b>	13.90±1.38 <sup>a</sup>
<b>Flubendazole</b>	8.60±1.53 <sup>b</sup>
<b>Flub + Superivomec</b>	5.60±0.93 <sup>bc</sup>
<b>Superivomec</b>	-
<b>Triclabendazole</b>	-
<b>Super + Tricla</b>	-

**Table (4): Mean values of the length of liver flukes in different groups.**

Groups	Length of liver flukes
<b>Control +ve group</b>	4.35±0.26 <sup>a</sup>
<b>Flubendazole</b>	3.73±0.16 <sup>b</sup>
<b>Flub + Superivomec</b>	2.33±0.18 <sup>bc</sup>
<b>Superivomec</b>	-

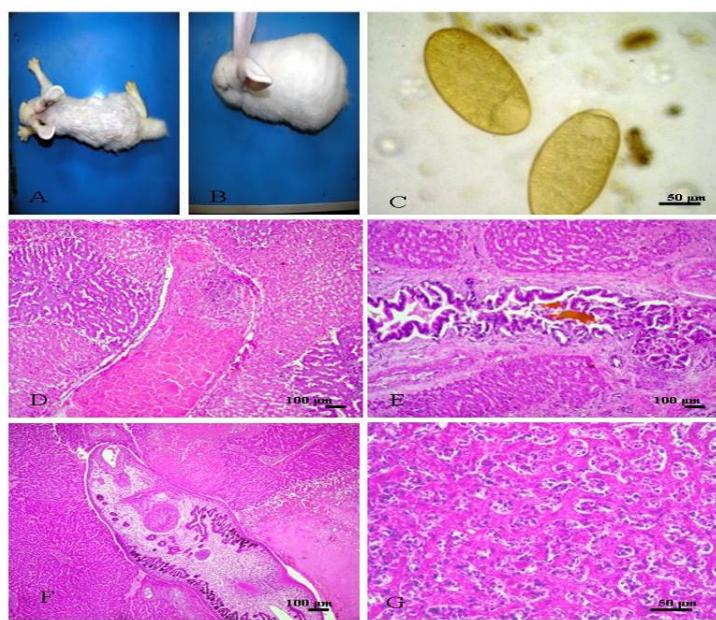


Fig. 1

**Fig. (1):** A. Rabbit showing emaciation, loss of hair coat. Control +ve group. B. Normal rabbit. C. Eggs of *Fasciola gigantica*. Faecal smear. X 40. D. Mixed thrombus in a large blood vessels. E. Cholangitis of bile duct with bile thrombi (arrow). F. Liver showing migratory tract containing mature fluke. G. Liver showing hepatitis demonstrated by necrosed hepatocytes and impaction of sinusoids with dead eosinophils. Control +ve group. H&E.

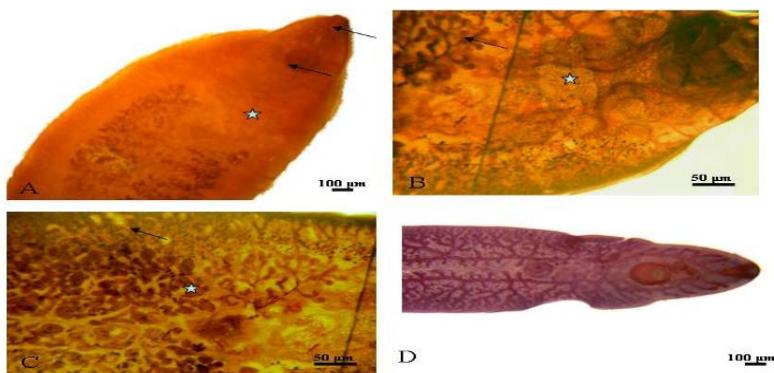
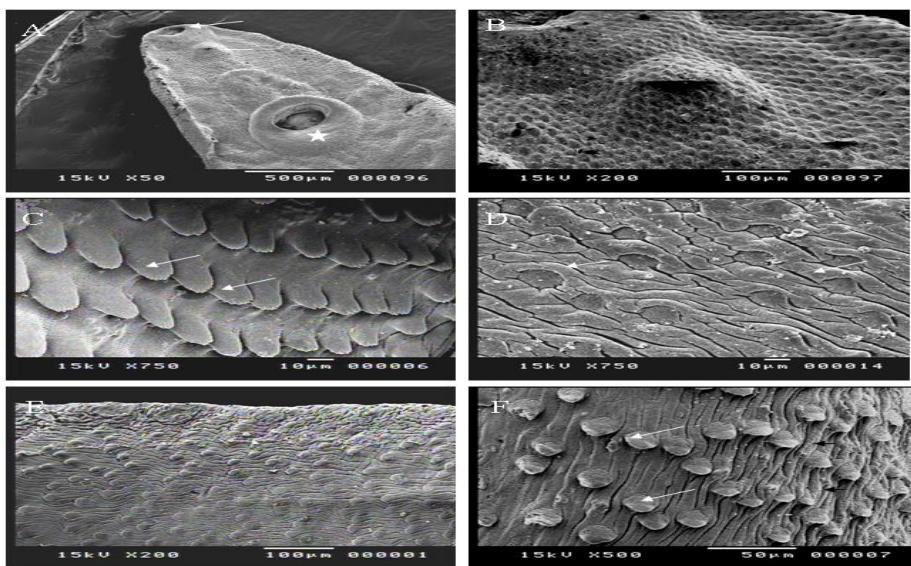


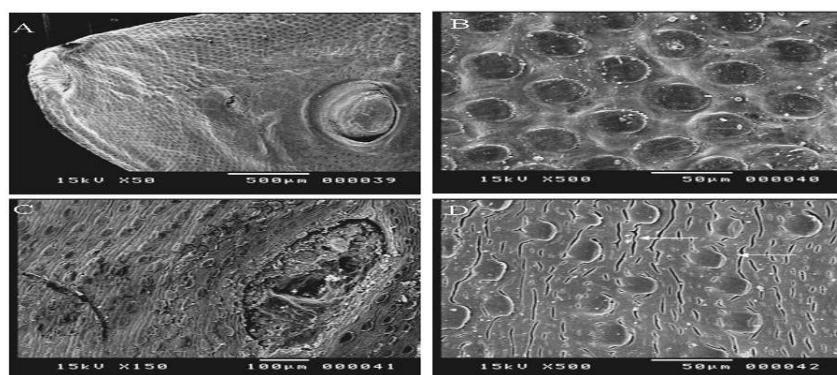
Fig. 2

**Fig. (2):** A. Anterior portion of *Fasciola gigantica* showing oral and ventral suckers (arrows) well developed uterus (star). B. Anterior portion of *Fasciola gigantica* showing well developed uterus (star) and well developed intestinal ceca (arrow). C. Posterior portion of *Fasciola gigantica* showing well developed testis (star) and vitelline glands (arrow). Control +ve group. Carmine stain X10 D. Anterior portion of *Fasciola gigantica* showing immature sexual organs and stunted growth. Carmine stain X10.

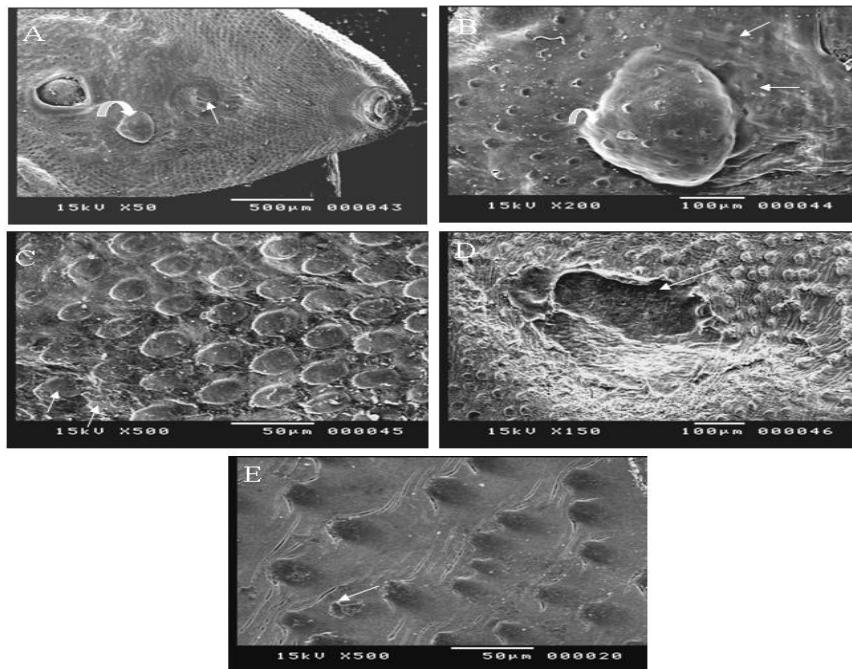
**Fig. 3**

**Fig. (3): Scanning electron micrograph (SEMs) of *Fasciola gigantica*. Control +ve group.**

- A. Oral (arrow), ventral (star) suckers and gonopore (line) of the liver fluke. X 50.
- B. The oral sucker and gonopore surrounded with spines. X 200.
- C. The spines posteriorly directed with serrated margin (arrows). X 750.
- D. The posterior ventral surface showing transverse folds (arrows) and grooves in between with less and depressed spines. X 750.
- E. The dorsal surface showing tegument with spines. X 200.
- F. Higher power showing less number of spines (arrows) and transverse folds (arrowheads) X 500.

**Fig. 4**

**Fig. (4): Scanning electron micrograph (SEMs) of *Fasciola gigantica*. Flubendazole treated group.** A. Anterior portion showing shrunken oral sucker. X 50. B. The spines of the anterior portion showing shortening and compression. X 500. C. Ventral surface posterior to the ventral sucker showing large area of tegumental sloughing. X 150. D. Dorsal surface showing furrowing of the tegument (arrows). X 500.

**Fig. 5**

**Fig. (5):** Scanning electron micrograph (SEMs) of *Fasciola gigantica*. Flubendazole + superivomec treated group. A. Anterior portion showing sloughing to the gonopore between oral and ventral sucker (arrow) and swelling (curved arrow). X 50. B. Higher power showing large swelling (curved arrow) and swelling of adjacent area (arrows) with no spines around it. X 500. C. The spines surrounded the oral sucker and mid-lateral aspect showing destruction (arrows). X 500. D. The middle portion of the ventral surface showing sloughing of the tegument (arrow). X 150. E. Dorsal surface showing furrowing of the tegument with destructed spines (arrow). X 500.

#### 4. Discussion:

*Fasciola hepatica* remains one of the single most important helminth parasites of many countries in the world. Its tropical counterpart is *Fasciola gigantica*. There are effective strategies for the control of fascioliasis, based largely on drug (fasciolicide) use but allied to epidemiological data (Boray, 1997 and Malone, 1997). While most of the experimental work has been conducted with *Fasciola hepatica*, the problems associated with fascioliasis and its control is similar in large parts of the world where only *Fasciola gigantica* is present. All drugs effective against one of the species are equally effective against the other (Boray, 1986).

In the present study, the histopathological changes in the form of vascular changes, bile duct lesions, portal lesions, migratory tracts and hepatocellular changes were reported by many authors (Farha, 1993; Yoshida et al., 1996; Pérez et al., 1999 and Adedokun and Fagbemi, 2001).

The eggs of *Fasciola gigantica* were appeared in the faeces at 40 days post infection as oval, operculated and delicate yellow in color. This finding was in agreement with the studies of many authors (Haseeb et al., 2002; Lotfy and Hillyer, 2003). Statistical analysis of the egg counts from 7 to 11 weeks (PI) revealed significant increase in eggs in control +ve group when compared with other groups.

The egg counts increased gradually from 7 to 11 weeks. These results are in harmony with the studies of Sherif et al., (2001) who reported that the egg counts increased gradually from 10 weeks to 16 weeks (PI). Also, Sewell, (1966) mentioned that egg counts of *Fasciola* increased gradually up to a peak 108 days (PI).

In the present work, counting of the liver flukes and statistical analysis revealed significant increase of fluke's numbers in control +ve group ( $13.90 \pm 1.38$ ) when compared with other groups. These results were obtained by Schillhorn Van Veen et al., (1980) and Adedokun and Fagbemi, (2001) who reported that the pathological findings were related to the number of the flukes recovered at post mortem.

*Fasciola gigantica* stained with carmine revealed maturity of the worm with eggs in the uterus and well developed intestinal ceca and testes at 9 and 11 weeks post infection. Similarly, Olaechea et al., (1991) found that there was individual variation in numbers of flukes per animal and the mean percentage of mature worm with eggs in uterus at 10 weeks post infection. Kolodziejczyk et al., (2006) said that the juvenile form of the flukes in the liver tissue and mature forms in the bile ducts of rats were seen at 7 weeks (PI).

Scanning electron microscopy to the liver flukes in control +ve group revealed rough tegumental surface of the adult *Fasciola gigantica*. The surface covered with posterior directed spines and transverse folds. These results obtained by many investigators (Meany et al., 2006 and Mahmoud and Hegazi, 2007). This can be explained by the increasing efficacy of absorption and exchange of materials by the tegument, such features are also observed in other trematodes (Jinxin and Yixun, 1981). The anterior and middle regions tend to have more developed spines or folds than the posterior region. These results were agreed with the studies of Mahmoud and Hegazi, (2007) who suggested different capabilities of absorption in various regions of tegument. The presence of numerous spines, covering the body surface may facilitate movement of *Fasciola* in the bile ducts of the liver. In the flubendazole treated group, minor changes of the flukes in the form of shrunken oral sucker and dilated ventral sucker, shortening of the scale-like spines and sloughed tegument which filled with debris. These findings coincide with those described by Omran et al., (2006) who studied the effect of flubendazole on *Schistosoma mansoni*.

In superivomec treated group, there was significant decrease in the number and the length of flukes when compared with other groups. Also, it has a prominent effect on maturation of sexual organs

and to lesser extend on the digestive organs. These results were in agreement with the findings of Vera-Montenegro et al., (2003) who reported that clorsulon causing stunted growth of the fluke and egg production was markedly decreased and the findings of Fetterer et al., (1985) and Sundlof et al., (1991) who reported that clorsulon was highly effective in reducing worm burden.

It had been concluded that experimental fascioliasis in rabbits resulted in severe deleterious effect on the liver tissue. The flubendazole was not effective against fascioliasis. The uses of triclabendazole in the treatment of fascioliasis eliminate the gross lesions and liver fluke and minimize the histopathological alterations. The results produced by the mixture of triclabendazole and superivomec may be due to its effectiveness in overcoming the eggs as early as one week of treatment and its effectiveness against the fluke. So we advise using this mixture in the treatment of *Fasciola gigantica* in the field (mixture of choice).

#### Corresponding author

Amal Mohamed Abdo

Parasitology department, Faculty of Medicine, Assiut University, Assiut, Egypt.

[amalmatary@yahoo.com](mailto:amalmatary@yahoo.com)

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10/11/2010

# The Effect of Green, Roasted and Decaffeinated Coffee on Serum Glucose, Insulin and Serum Lipid Profile in Diabetic Experimental Animals

Eman A.Sadeek<sup>\*1</sup>, Hala, A. Abd El;-Rahman<sup>2</sup> and Waffa, Sh. Ali<sup>3</sup>

<sup>1</sup>Department of Biochemistry & Nutrition -Women's College –Ain –Shams University. <sup>2</sup> Food Tech. Res. Ins. Agric. Res. Center. <sup>3</sup>College of Home Economics, Helwan University. Cairo, Egypt

<sup>\*</sup>[dr\\_emansadeek@yahoo.com](mailto:dr_emansadeek@yahoo.com)

**Abstract:** Aim of the work: Assessing the Effect of green, roasted and decaffeinated coffee on serum glucose, insulin and serum lipid profile in diabetic rat models. Methods: Design of the Study: Thirty female wistar rats weighing  $124.5 \pm 5.41$ g (mean  $\pm$ S.D) were divided into 5 groups. The first group served as a control and consumed a standard diet according to (AIN – 93). The other 4 groups were injected intraperitoneally with 105 mg / kg body weight of alloxan . One group was kept without further treatment and served as a positive diabetic control. Groups 3, 4, 5 consumed 5% green, roasted and decaffeinated coffee in drinking water, respectively. The feeding trial continued for four weeks. At the end of the experiments, the animals were sacrificed, blood samples were collected, and the liver, kidney ,spleen and heart were separated, washed ,dried and weighed. Laboratory investigations Consisted of serum glucose, insulin, calcium, phosphorus and complete lipid profile was determined to test the magnitude of antioxidant potential green, roasted and decaffeinated coffee. Results: The present study show a significant difference ( $p < 0.05$ ) in body weight gain and food intake between all treatment groups , with non significant difference in water intake , relative weight of organs including liver , kidney , spleen and heart . the study also shows significant elevation ( $p < 0.05$ ) in serum glucose and insulin in diabetic control group as compared to normal control group. This indicates uncontrolled hyperglycemia in alloxan diabetic rats. While consumption of green, roasted and decaffeinated coffee resulted in a decrease in serum glucose and insulin ( $p < 0.05$ ) .There is a significant decrease ( $p < 0.05$ ) in serum calcium and serum phosphorus in groups 3,4 and 5 fed green, roasted and decaffeinated coffee respectively indicating an association between coffee consumption and bone health. our results also shows that alloxan injection produced a significant increase( $p < 0.05$ ) in serum total- cholesterol(TC); triacylglycerol(TAG); LDL-C ; VLDL-C and in LDL\ HDL ratio and TC \ HDL ratio however a significant decrease ( $p < 0.05$ ) in serum HDL-C is observed ; In diabetic rats compared to normal control .green, roasted and decaffeinated coffee resulted in a significant decrease ( $p < 0.05$ ) in triacylglycerol (TAG); LDL-C ; VLDL-C and in LDL\ HDL ratio and TC \ HDL ratio .on the other hand a significant increase ( $p < 0.05$ ) in serum HDL-C is observed in green, roasted and decaffeinated coffee groups compared to diabetic rats compared to normal control with the highest value for green coffee .Non significant effect on serum total- cholesterol(TC) reported in this study . Conclusion: The observed improvement in glucose, insulin profile, triacylglycerol and HDL-C confirm the potent biological action of green, roasted and decaffeinated coffee and suggest that chlorogenic acid (a component in coffee) might have an antagonistic effect on glucose transport. Suggesting a novel function of coffee on lowering the risk factors of diabetes and delaying the progress of diabetes complications as well.

[Eman A.Sadeek, Hala, A. Abd El;-Rahman and Waffa, Sh. Ali. The Effect of Green, Roasted and Decaffeinated Coffee on Serum Glucose, Insulin and Serum Lipid Profile in Diabetic Experimental Animals. Journal of American Science 2010;6(11):934-943]. (ISSN: 1545-1003).

**Key words:** Green, roasted, decaffeinated coffee, glucose, insulin and lipid profile.

## 1. Introduction:

Type 2 diabetes is a chronic disease associated with high rates of morbidity and premature mortality(Nathan , 1993)1 An alarming increase in the prevalence of type 2 diabetes is expected,( Wild et al ., 2004 ) and the need for preventive action is widely acknowledged. While increased physical activity and restriction of energy intake can

substantially reduce the incidence of type 2 diabetes (Tuomilehto et al., 2001 Knowler et al., 2002) , insight into the role of other lifestyle factors may contribute to additional prevention strategies for type 2 diabetes.

Coffee is considered one of the most popular beverages consumed in the world due to its pleasant flavor and pharmacological properties(DÓREA and

COSTA ., 2005). Prospective and epidemiologic studies of green and especially of roasted coffee consumption has been carried out to investigate its biological effects on lipids, blood pressure and glycaemia(CORTI et al ., 2002 ; DAGLIA et al ., 2000 and ROBINSON et al .,2004) . Scientific evidences have demonstrated that green and regular coffee beverages present high antioxidant properties in vivo and in vitro (KARAKAWA, 2004 and SOMOZA et al ., 2003). Few recent studies have indicated that soluble extracts of green coffee were effective against the high blood pressure in mice(SUZUKI, A. et al .,2000) and in human(KOZUM. et al ., 2005 and OCHIAI, . et al. 2004). It is possible that its antihypertensive action be related to vasoreactive factors produced and released from the vascular endothelium (OCHIAI, R. et al . 2004).

The roasting process causes a loss of water from the green bean and degradation of many of the compounds including the antioxidant polyphenols; however, there is very little difference in total antioxidants between the different roasts of a bean (Daglia et al., 2000).

There are three main methods of coffee preparation; boiled unfiltered coffee, filtered coffee, and decaffeinated coffee, the latter primarily consumed as instant coffee.

There are over a thousand compounds, many formed during the roasting process, which produce the unique taste and smell of coffee(Parliament et al ., 2005). However, from the point of view of concentration in coffee, prior detection of the parent compound or metabolites in the body, and physiological effects, there are essentially only three ingredients that are important; caffeine, the diterpene alcohols cafestol and kahweol, and chlorogenic acid and other polyphenols. In specialty coffees consumed outside the home the range is 18–80 mg/cup and decaffeinated coffees averaged 5 mg/cup(McCusker et al ., 2003). Coffee is an important source of caffeine; it provides 71% of the caffeine in the US diet (Frary et al., 2005). The diterpenoid alcohols are the oils in coffee and their concentration depends on the how the coffee is prepared. Filtered coffee has less than 0.1 mg/100 ml, i.e. essentially none, and unfiltered coffee can have between 0.2 and 18 mg/100 ml depending on the method.

High consumption of unfiltered types of coffee, such as French press and boiled coffee has been shown to increase low-density-lipoprotein-cholesterol concentrations. In addition, limiting caffeinated coffee intake during pregnancy seems a prudent choice. However, evidence has been accumulating that frequent consumption of coffee

may reduce risk of type 2 diabetes and liver cancer(van Dam ., 2008).

Higher habitual coffee consumption was associated with higher insulin sensitivity (Arnlöv et al ., 2004) and a lower risk for type 2 diabetes(van Dam et al ., 2002 ; Rosengren et al ., 2004 ; Salazar-Martinez et al ., 2004 ; Tuomilehto et al ., 2004 and Carlsson et al ., 2004) in diverse populations. In contrast, short-term metabolic studies showed that caffeine intake can acutely lower insulin sensitivity (Keijzers et al ., 2002 and Thong et al ., 2002 ) and increase glucose concentrations(Mougiakos et al ., 2003 and Lane et al ., 2004 )

Tunnicliffe and Shearer, 2008 found that Coffee consumption may also mediate levels of gut peptides (glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1), hormones intimately involved in the regulation of satiety and insulin secretion. Finally, coffee may have prebiotic-like properties, altering gut flora and ultimately digestion.

It has been reported that Coffee intake might have an antiatherogenic property by increasing of ATP-binding cassette transporter (ABC)G1 and scavenger receptor class B type I (SR-BI) expression and enhancing HDL-mediated cholesterol efflux from the macrophages via its plasma phenolic acids(Uto-Kondo et al ., 2010).

## 2. Materials and methods

Materials :

Chemicals:

All chemicals including alloxan were fine grade, chemicals purchased from local distributor (Sigma chemical) Cairo.Egypt.

Green, roasted and decaffeinated coffee where purchased from a local market ,Cairo, Egypt and was added to drinking water at a concentration of 5 g% after following preparation : 5g of green ,roasted and decaffeinated coffee dissolved in 100 ml boiled water for 10 minutes .

The basal standard diet was prepared in accordance with AIN-93 formulation (Reeves et al., 1993).

Composition of diet (g/100g)

Corn starch 62.07;casein 14 ;sucrose 10 ;cellulose 5 ;corn oil 4 ;salt mixture 3.5 ; vitamin mixture 1;L-cystine0.18; choline bitartrate 0.25 and tert.butylhydroxy quinine 0.008.

Animals

In the present study 30 female rats of wistar strain weighing ( 124.50 ±5.41 g) obtained from Institute of Ophthalmology(Cairo, Egypt) were used in this study . The rats were maintained under

standard laboratory conditions in an air conditioned room and housed in stainless steel cages one per cage at temperature  $22\pm3$  °C and relative humidity 30-70 %. The animal diet was given ad libitum . Animals were acclimatized for one week prior to experiment. Thirty rats were divided into 6 groups each of 6 rats. Group 1(G1): Served as normal control and received standard diet.

Group 2(G2): Diabetic control group. Green, roasted and decaffeinated coffee

Group 3(G3): Diabetic group which received 5 % green coffee in drinking water.

Group 4(G4): Diabetic group which received 5 % roasted coffee in drinking water.

Group 5(G5) Diabetic group which received 5 % decaffeinated coffee in drinking water.

The experiment lasted for 4 weeks.

#### Assays:

At the end of experimental period, all rats were fasted overnight and then anesthetized by ether and sacrificed. Blood was collected and allowed to clot; serum was separated by centrifugation at 3000 rpm for 15 minutes serum was then transferred into properly labeled sterile vials and stored at -20° C till the performance of Laboratory analysis.

liver , kidney and spleen and heart were excised, rinsed in chilled saline solution and then blotted on filter paper ,weighed separately to calculate the relative weight.

$$\text{The relative weight of organ} = \frac{\text{absolute weight of organ}}{\text{Final body weight of rat}} \times 100$$

Serum was used for determination of serum glucose according to Barham and Trinder , (1972) . Serum insulin was determined according to Vuppugalla et al., (2003). Serum total cholesterol was assayed by the method of Richmond , (1973) , serum triacylglycerol according to Fossati and Prencipe , (1982) , serum HDL by the method of Steele et al ., (1976 ) while serum LDL-cholesterol by the use of the equation of Friedewald et al ., (1972) .

#### Statistical analysis:

Statistical analysis: were performed using SPSS for Windows 10.0(SPSS Inc,Chicago.IL.USA). Data were expressed as mean  $\pm$  S.D. One way analysis of variance (ANOVA) at (  $p < 0.05$  ) was used to compare mean values of continuous variable in cases and control.

### 3. Results

The present study show a significant difference ( $p <0.05$ ) in body weight gain and food intake between all treatment groups, with non significant difference in water intake, relative weight

of organs including liver , kidney , spleen and heart .these data suggesting that green, roasted and decaffeinated coffee did not influence the relative organ weight and caused the reduction in food intake and gain weight in diabetic rats as compared to normal control group.(Table 1).

Table 2 shows significant elevation ( $p < 0.05$ ) in serum glucose and insulin in diabetic control group as compared to normal control group at the end of experiment. This indicates uncontrolled hyperglycemia in alloxan diabetic rats. While consumption of green, roasted and decaffeinated coffee resulted in a decrease in serum glucose and insulin ( $p <0.05$ ) with the lowest glucose value observed with green coffee and with decaffeinated coffee for insulin.

There is a significant decrease ( $p < 0.05$ ) in serum calcium and serum phosphorus in groups 3,4 and 5 fed green and roasted coffee respectively indicating an association between caffeine consumption and bone health.(Table 3)

Table (4) shows that alloxan injection produced a significant increase( $p < 0.05$ ) in serum total- cholesterol(TC);triacylglycerol(TAG); LDL-C ; VLDL-C and in LDL\HDL ratio and TC \ HDL ratio however a significant decrease ( $p < 0.05$ ) in serum HDL-C is observed ; In diabetic rats compared to normal control .

Green, roasted and decaffeinated coffee resulted in a significant decrease ( $p <0.05$ ) in triacylglycerol(TAG); LDL-C ; VLDL-C and in LDL\ HDL ratio and TC \ HDL ratio .on the other hand a significant increase ( $p < 0.05$ ) in serum HDL-C is observed in green, roasted and decaffeinated coffee groups compared to diabetic rats with the highest value for green coffee .Non significant effect on serum total- cholesterol(TC) reported in this study .

### 4. Discussion:

In this study the observed decrease in body weight is in agreement with animal studies and the prospective epidemiologic studies on weight loss (Muroyama et al ., 2003 and van Dam et al., 2006 ) suggest that long-term caffeine and coffee consumption could decrease body weight in humans.

Shimod et al., (2006) showed that consumption of green coffee bean extract (GCBE) for 14 days caused a suppressive effect on weight gain and visceral fat accumulation in mice. GCBE contains 10% caffeine and 27% chlorogenic acid as the principal constituents, and these constituents showed a tendency to suppress body weight gain and visceral fat accumulation. Thus, these constituents are suggested to be partially involved in the suppressive effect of GCBE on body weight gain and visceral fat

accumulation. Caffeine is known to be a lipolytic

compound. On the other hand, the effect of

**Table (1): Effect of green, roasted and decaffeinated coffee on weight gain , food intake and water intake/day and relative weights of different organs (liver , kidney& spleen and heart ) In diabetic rats (Mean ± S.D.).**

Parameters \ Groups	Group (1) Normal control	Group (2) Diabetic	Group (3) Diabetic + Green coffee	Group (4) Diabetic + Roasted coffee	Group (5) Diabetic + Decaffeinated coffee
Weight gain(g)	45.00 ± 4.98	35.83 ± 2.99 <sup>a</sup>	36.00 ± 4.98 <sup>a</sup>	36.17 ± 3.60 <sup>a</sup>	36.50 ± 2.81 <sup>a</sup>
Food intake(g/day)	18.10 ± 0.85	17.82 ± 0.96	15.75 ± 0.62 <sup>a,b</sup>	15.67 ± 1.18 <sup>a,b</sup>	15.05 ± 0.80 <sup>a,b</sup>
Water intake (ml/day)	14.25 ± 1.44	14.67 ± 1.13	14.42 ± 1.06	14.83 ± 1.37	14.42 ± 1.66
Relative weight of liver (g%)	2.62 ± 0.33	2.63 ± 0.19	2.61 ± 0.11	2.43 ± 0.29	2.58 ± 0.25
Relative weight of kidney (g%)	0.57 ± 0.08	0.53 ± 0.11	0.59 ± 0.09	0.57 ± 0.09	0.64 ± 0.05
Relative weight of spleen (g%)	0.16 ± 0.03	0.15 ± 0.04	0.17 ± 0.03	0.16 ± 0.02	0.16 ± 0.03
Relative weight of heart (g%)	0.24 ± 0.03	0.25 ± 0.04	0.25 ± 0.05	0.26 ± 0.03	0.24 ± 0.05

Significant difference (P < 0.05): (a) compared to group 1, (b): to group 2, (c): to group 3, (d): to group 4, (e): to group 5.

**Table (2) : ) Effect of green, roasted and decaffeinated coffee on serum glucose and insulin In diabetic rats (Mean ± S.D.).**

Parameters \ Groups	Group (1) Normal control	Group (2) Diabetic	Group (3) Diabetic + Green coffee	Group (4) Diabetic + Roasted coffee	Group (5) Diabetic + Decaffeinated coffee
Glucose (mg/dl)	96.40 ± 0.42	183.03 ± 2.18 <sup>a</sup>	97.77 ± 1.06 <sup>b</sup>	101.40 ± 0.72 <sup>a,b,c,e</sup>	98.58 ± 1.35 <sup>a,b,d</sup>
Insulin ( $\mu$ ml)	35.67 ± 0.43	42.18 ± 1.71 <sup>a</sup>	36.33 ± 0.64 <sup>b</sup>	37.37 ± 0.84 <sup>a,b</sup>	36.13 ± 1.18 <sup>b</sup>

Significant difference (P < 0.05): (a) compared to group 1, (b): to group 2, (c): to group 3, (d): to group 4, (e): to group 5.

**Table (3) : ) Effect of green, roasted and decaffeinated coffee on serum calcium and phosphorus In diabetic rats (Mean ± S.D.).**

Parameters \ Groups	Group (1) Normal control	Group (2) Diabetic	Group (3) Diabetic + Green coffee	Group (4) Diabetic + Roasted coffee	Group (5) Diabetic + Decaffeinated coffee
Calcium (mg/dl)	7.75 ± 0.23	7.48 ± 0.29	6.53 ± 0.22 <sup>a,b,e</sup>	6.57 ± 0.18 <sup>a,b,e</sup>	7.43 ± 0.46 <sup>c,d</sup>
phosphorus (mg/dl)	3.53 ± 0.15	3.62 ± 0.22	2.75 ± 0.15 <sup>a,b</sup>	2.74 ± 0.13 <sup>a,b</sup>	3.29 ± 0.47 <sup>c,d</sup>

Significant difference (P < 0.05): (a) compared to group 1, (b): to group 2, (c): to group 3, (d): to group 4, (e): to group 5.

**Table (4) : ) Effect of green, roasted and decaffeinated coffee on serum total- cholesterol(TC); triacylglycerol(TAG); LDL-C ; HDL-C ;VLDL-C and on LDL/HDL ratio and TC /HDL ratio In diabetic rats (Mean ± S.D.).**

Parameters \ Groups	Group (1) Normal control	Group (2) Diabetic	Group (3) Diabetic + Green coffee	Group (4) Diabetic + Roasted coffee	Group (5) Diabetic + Decaffeinated coffee
(TC ,mg/dl)	91.75 ± 0.73	a 132.65 ± 0.70	a 131.78 ± 1.15	a 132.50 ± 0.75	a 131.83 ± 0.98
(TAG,mg/dl)	79.30 ± 0.89	a 161.95 ± 1.59	a,b 146.75 ± 1.36	a,b 145.77 ± 1.84	a,b 146.53 ± 1.79
(HDL-C ,mg/dl)	28.95 ± 0.69	a 19.37 ± 0.79	a,b 27.13 ± 0.63	a,b,c 26.13 ± 0.89	a,b 26.75 ± 0.58
(LDL-C,mg/dl)	46.94 ± 1.17	a 80.89 ± 1.21	a,b 75.30 ± 1.14	a,b,c 77.21 ± 0.95	a,b 75.78 ± 1.54
(VLDL-C, mg/dl)	15.86 ± 0.17	a 32.39 ± 0.32	a,b 29.35 ± 0.27	a,b 29.15 ± 0.37	a,b 29.31 ± 0.36
<b>LDL/HDL ratio</b>	1.62 ± 0.07	a 4.18 ± 0.22	a,b 2.78 ± 0.082	a,b,c 2.96 ± 0.13	a,b 2.83 ± 0.11
<b>TC /HDL ratio</b>	3.17 ± 0.09	a 6.86 ± 0.28	a,b 4.86 ± 0.097	a,b,c 5.07 ± 0.16	a,b 4.93 ± 0.13

Significant difference ( $P < 0.05$ ): (a) compared to group 1, (b): to group 2, (c): to group 3, (d): to group 4, (e): to group 5.

chlorogenic acid on body weight gain has not yet been established.

Elevated serum glucose and insulin in diabetic control group as compared to normal control group confirm uncontrolled hyperglycemia, whereas green, roasted and decaffeinated coffee decreased serum glucose and insulin ( $p < 0.05$ ) with the lowest glucose value observed with green coffee and with decaffeinated coffee for insulin.

These results are with the line of van Dam., (2008) who found that frequent consumption of coffee may reduce risk of type 2 diabetes and liver cancer.

Several plausible mechanisms for a beneficial effect of coffee on glucose metabolism exist. Coffee has been shown to be a major contributor to the total in vitro antioxidant capacity of the diet (Pulido et al ., 2003) which may be relevant as oxidative stress can contribute to the development of type 2 diabetes. Coffee is the major source of the phenol chlorogenic acid.( Clifford 2000) Intake of chlorogenic acid has been shown to reduce glucose concentrations in rats(Andrade-Cetto and Wiedenfeld ., 2001 and Rodriguez de Sotillo and Hadley 2002 and intake of quinides, degradation products of chlorogenic acids, increased insulin sensitivity in rats.( Shearer et al ., 2003) Chlorogenic

acid contributes to the antioxidant effects of coffee, (Clifford 2000) may reduce hepatic glucose output through inhibition of glucose-6-phosphatase, (Arion et al ., 1997 )and may improve tissue mineral distribution through its action as a metal chelator. (Rodriguez de Sotillo and Hadley 2002). In addition, chlorogenic acid acts as a competitive inhibitor of glucose absorption in the intestine. ( Clifford 2000)Indeed, decaffeinated coffee seemed to delay intestinal absorption of glucose and increased glucagon-like peptide-1 concentrations in an intervention study in humans.( Johnston et al ., 2003) Glucagon-like peptide-1 is well known for its beneficial effects on glucose-induced insulin secretion and insulin action.( Drucker 1998) This effect may explain the observation that higher coffee consumption was associated with lower postload, rather than fasting, glucose concentrations.( Yamaji et al ., 2004 and )

Caffeine ingestion can acutely reduce glucose storage, but beneficial effects of caffeine on lipid oxidation and uncoupling protein-3 expression have also been suggested.( Yoshioka et al ., 2004) In US studies, decaffeinated coffee consumption was inversely associated with risk of type 2 diabetes.( Salazar-Martinez et al ., 2004) In addition, in a Japanese study, the inverse association with hyperglycemia was stronger for coffee than for

caffeine.( Isogawa et al ., 2003) These observations suggest that coffee components other than caffeine may have beneficial effects on risk of type 2 diabetes. Coffee also contains substantial amounts of magnesium, which has been linked to better insulin sensitivity and insulin secretion.( de Valk 1999) However, adjustment for magnesium intake did not explain the association between coffee consumption and risk of type 2 diabetes(Salazar-Martinez et al., 2004)

As the beneficial effects of coffee consumption exist for both decaffeinated and caffeinated coffee, a component of coffee other than caffeine must be responsible. Tunnicliffe and Shearer2008 reported that, being plant-derived; coffee contains many beneficial compounds found in fruits and vegetables, including antioxidants. In fact, coffee is the largest source of dietary antioxidants in industrialized nations. When green coffee is roasted at high temperatures, Maillard reactions create a number of unique compounds. Roasting causes a portion of the antioxidant, chlorogenic acid, to be transformed into quinides.

Decreased serum insulin in this study is in agreement with The decreased insulin secretion reported by Tianying et al., (2005) is consistent with the increased insulin sensitivity observed by Arnlov et al.,(2004). In contrast, Arnlov et al., 2004 did not observe a decrease in insulin secretion as assessed by early insulin response under glucose stimulation. However, C-peptide has a longer half-life than insulin and thus may better represent insulin secretion than insulin levels do (Chen et al., 1999).The independent association between decaffeinated coffee and C-peptide indicates active ingredients other than caffeine. Antioxidants may improve insulin sensitivity Bruce et al., 2003 (in type 2 diabetes and decrease insulin levels in rats (Thirunavukkarasu., 2004).

Tianying et al., (2005) concluded caffeinated and decaffeinated coffee consumption might prove to be an effective strategy for reducing insulin resistance, especially in overweight women. Oka, 2007 demonstrated that the prophylactic effects of coffee on diabetes involve pleiotropy of plural components in accordance to the degree of the roasting. A new concept of nutritional blended coffee may be important to optimize the prophylactic effects of coffee on lowering the risk factors of diabetes and delaying the progress of diabetes complications as well.

On the other hand Contrary to our study Kempf et al., 2010 demonstrated that coffee consumption led to an increase in coffee-derived compounds, mainly serum caffeine, chlorogenic acid, and cafffeic acid metabolites. , Whereas no changes

were seen for markers of glucose metabolism in an oral-glucose-tolerance test.

On the other hand Robinson et al ., 2004 found evidence of a non significant caffeine-induced increase in insulin secretion in men with type 2 diabetes, and Petrie et al ., 2004 found no increase in such insulin secretion in obese men.

In this study the significant decrease in serum calcium and serum phosphorus in groups 3, 4 and 5 fed green, roasted coffee respectively is in agreement with the finding of Barrett-Connor et al., (1994) who reported that caffeinated coffee intake equivalent to two cups per day is associated with decreased bone density in older women who do not drink milk on a daily basis.

Also are in agreement with those of Rapuri, et al ., (2001) who reported that Intakes of caffeine in amounts >300 mg/d ( $\approx$ 514 g, or 18 oz, brewed coffee) accelerate bone loss at the spine in elderly postmenopausal women. They found a significant negative correlation between caffeine intake and calcium intake and suggested that high caffeine consumption per se has a negative effect on bone mineral density (BMD), which may be further accentuated by low calcium intakes. However, they could not gain insight into the mechanism of how caffeine exerts its negative effect because we found no significant changes in any of the biochemical indexes measured.

The decrease in serum calcium may be due to the effect of coffee consumption which caused an increase in endogenous fecal calcium and urinary calcium excretion.

our results on the other hand disagree with those of Sakamoto et al ., (2001) reported that strongly indicates that coffee does not stimulate bone loss in rats. They clarify the relationship between coffee consumption and bone metabolism using male Wistar rats. assigned to three treatment groups including a control-diet group , a 0.62% coffee-diet group, and a 1.36% coffee-diet group. They indicated no significant differences in body weight change, serum and urinary biochemical markers of bone metabolism, and bone histomorphometry were found between the coffee-diet groups and the control-diet group, except that urinary phosphorus excretion after 140 days of both coffee diets was significantly increased compared with controls ( $p < 0.05$ ). In addition, the coffee diets were not associated with differences in tumor necrosis factor- $\alpha$  and interleukin-6, which have been implicated in the pathogenesis of bone loss together with interleukin-1 $\beta$ .

Green, roasted and decaffeinated coffee resulted in a significant decrease ( $p < 0.05$ ) in triacylglycerol (TAG); LDL-C; VLDL-C and in LDL\ HDL ratio and TC \ HDL ratio. on the other hand a significant increase ( $p < 0.05$ ) in serum HDL-C is observed in green, roasted and decaffeinated coffee groups compared to diabetic rats compared to normal control with the highest value for green coffee ,with non significant effect on serum total- cholesterol(TC) reported in this study .

Our results are in agreement with those of Kempf et al., 2010 who reported that coffee consumption led to an increase in coffee-derived compounds, mainly serum caffeine, chlorogenic acid, and cafffeic acid metabolites. Significant changes were also observed for serum concentrations of interleukin-18, 8-isoprostanate, and adiponectin (8 compared with 0 cups coffee/d). Serum concentrations of total cholesterol, HDL cholesterol, and apolipoprotein A-I increased significantly by 1, whereas the ratios of LDL to HDL cholesterol and of apolipoprotein B to apolipoprotein A-I decreased significantly by 8% and 9%, respectively (8 compared with 0 cups coffee/d), this indicate that coffee consumption appears to have beneficial effects on subclinical inflammation and HDL cholesterol.

In accordance to our study Shimod et al.,(2006) reported that serum and hepatic TG levels were lowered with intravenous administration of chlorogenic acid in Zucker fa/fa rats. However, the TG level in the adipose tissue was not lowered. Therefore, chlorogenic acid is suspected to be effective on hepatic TG, and not adipose TG. Chlorogenic acid is also a dietary polyphenolic compound with antioxidative activity. Thus, it is suggested that caffeine, chlorogenic acid and other polyphenolic compounds in GCBE act synergistically to suppress body weight gain and visceral fat accumulation in mice.

Uto-Kondo et al. ( 2010) hypothesized that coffee may enhance reverse cholesterol transport (RCT) as the antiatherogenic properties of high-density lipoprotein (HDL). Caffeic acid and ferulic acid, the major phenolic acids of coffee, enhanced cholesterol efflux from THP-1 macrophages mediated by HDL, but not apoA-I. Furthermore, they concluded that Coffee intake might have an antiatherogenic property by increasing of ATP-binding cassette transporter (ABC)G1 and scavenger receptor class B type I (SR-BI) expression and enhancing HDL-mediated cholesterol efflux from the macrophages via its plasma phenolic acids.

Lee ., 2009 demonstrated that coffee may guard against Alzheimer's disease and other forms of dementia and somehow soften the blow of a heart attack.

Ozercana et al. ( 2006) found that lipid peroxidation products that increased in the plasma and liver tissue of the CCl4 group decreased by (instant coffee) IC administration. There was an increase in the measured antioxidant parameters, which were total antioxidant capacity (TAOC), sulphhydryl (SH) and ceruloplasmin levels. They concluded that IC had a protective role in acute liver injury induced by CCl4, but did not affect steatosis.Lopez-Garcia et al., (2006) reported that there is no evidence that coffee consumption increases the risk of CHD.

Our results on the other hand disagree with the fnding of Rodrigues and Klein. (2006) who found that Caffeine is the most widely consumed psychostimulant drug in the world that mostly is consumed in the form of coffee. They examined the effects of caffeine intake, both alone and via coffee consumption, on key blood markers of CVD risk: lipoproteins (cholesterol, triglycerides), fibrinogen (a biomarker of blood clotting) and C-reactive protein (CRP; a biomarker of inflammation). They indicated a strong relationship between boiled, unfiltered coffee consumption and elevated cholesterol levels. Also disagree with those of Ricketts et al. (1993) who suggest that caffeine consumption is associated with increased serum cholesterol and/or low density lipoprotein cholesterol. They confirmed that when consumption of caffeine reaches 200 mg or more total cholesterol significantly increased in males. Low density lipoprotein cholesterol concentrations were somewhat increased in males who consumed 200 mg or more. In women, triglyceride levels significantly increased when dietary caffeine intake was 200 mg or higher. Dietary caffeine intake may be a factor to consider when evaluating serum lipid levels.

## 5. Conclusion

The observed improvement in glucose, insulin profile, triacylglycerol and HDL-C confirm the potent biological action of green, roasted and decaffeinated coffee and suggest that chlorogenic acid (a component in coffee) might have an antagonistic effect on glucose transport. Suggesting a novel function of coffee on lowering the risk factors of diabetes and delaying the progress of diabetes complications as well.

## Corresponding author

Eman A.Sadeek

Department of Biochemistry & Nutrition -Women's College –Ain –Shams University, Cairo, Egypt

\*[dr.emansadeek@yahoo.com](mailto:dr.emansadeek@yahoo.com)

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10/2/2010

# The Impact of Obesity and Weight Reduction Program with Xenical Drug Treatment on Health Status of Obese Adolescent Girls in Saudi Arabia

**Hend Hassan Ali Ganbi**

Nutrition and Food Science Dept., Faculty of Education for Home Economics and Art Education , King Abd – Elaziz University, Jeddah , Saudi Arabia  
Dr.gamal rawayshed@yahoo.com

**Abstract:** The present investigation aimed to assess the health problems and diseases associated with obesity in Saudi Arabian adolescent girls, and to evaluate the impact of obesity and weight reduction program with xenical (Orlistat) drug treatment on health status of tested obese adolescent patients. This study was performed on a group of 160 obese adolescent girls, aged 15 – 20 years, attending Physical Rehabilitation and Obesity Treatment Centers at Jeddah, Saudi Arabia. The obese patients group, under investigation, was selected from the adolescent girls who want in the treatment of obesity after obtaining their consent to participate in this study. The present results revealed that most tested obese adolescent girls suffered with a lot of health problems and diseases. The obesity was associated with deterioration of health status for obese subjects. Also, the weight reduction program with xenical (Orlistat) drug treatment caused a significant loss ( $\leq 0.01$ ) in body weight of obese adolescent girls by 7.42 and 11.35 % after 30 and 60 days respectively. In addition that tested weight reduction program with orlistat treatment exhibited a significant enhancing impact ( $\leq 0.01$ ) on all tested health status parameters of obese patients ; especially when obese patients engaged with tested weight – reduction program based upon being on a nutritionally balanced , reduced – calorie dietary regimen and practicing the physical activities regularly at least 6 hours a day , as showing by its enhancing effect on liver functions, serum lipid profile , liver and renal functions and by its lowering effect on blood glucose , insulin and LDL - cholesterol levels ; within the reference reported range of all health status items for health individual adolescent girls. Therefore, it is recommended that the obese adolescent girls and women should be orally treated with xenical drug capsules with their obligation by being on a dietary regimen ; a nutritionally balanced , reduced – calorie diet (800 – 1200 calorie) , and practicing the physical activities regularly at least 6 hours a day.

[Hend Hassan Ali Ganbi. The Impact of Obesity and Weight Reduction Program with Xenical Drug Treatment on Health Status of Obese Adolescent Girls in Saudi Arabia. Journal of American Science 2010;6(11):944-958]. (ISSN: 1545-1003).

**Key words :** Obesity ; Weight reduction program ; Obese adolescent girls ; Liver functions ; lipid profile ; Renal functions ; Health status ; Xenical drug ; Physical activity ; Dietary regimen.

## Introduction :

Obesity is defined as the accumulation of fatty tissue to such a level that overall health might be adversely affected and it reflects an imbalance between energy uptake and expenditure that is mediated by behavior (Neeley and Gonzales , 2007 and Knecht *et al.*, 2008). The corpulence is not only a disease itself , but the harbinger of others , recognizing that obesity is a medical disorder that leads to many comorbidities (Lavie and Milani , 2003). Obesity is associated with physical and endocrine changes of the body. Adipose tissue synthesizes and releases into the bloodstream peptides and nonpeptide compounds. Like in other organs with endocrine function , disequilibrium o the released hormones affects the homeostasis of other systems throughout the body (Schindler *et al.*, 2006 ; Knecht *et al.*, 2008 and Nejat *et al.*, 2010). Environmental factors including sedentary lifestyle and the

consumption of high-energy foods and drinks are thought to play key roles in the development of obesity ( Baur & O'Connor , 2004 and Neeley & Gonzales , 2007). Television viewing has been particularly implicated (Robinson , 2001). Dietary intake is also important. High-fat foods and sugar-containing soft drinks have been associated with increases in obesity (Astrup , 2001 and Ludwig *et al.*, 2001) , as well as more high fructose corn syrup foods and drinks (Bray *et al.*, 2004 and Elliott *et al.*, 2002). Lack of physical activity was shown to be associated with obesity is a risk factor (Whitaker *et al.*, 1997 and Neeley & Gonzales , 2007).

Overweight and obesity in children and adolescents are a serious issue with many health and social consequences that often continuo into adulthood (Foxhall , 2006 and Rimmer *et al.*, 2007). The world wide epidemic of obesity that has emerged with the dawning of the 21<sup>st</sup> century are a major public health problem , having struck developed

countries as well as those still developing (Wang *et al.*, 2005). Globally , at the turn of the century more than 300 million persons were considered obese. This increased prevalence of obesity , in part driven by over-nutrition and physical inactivity , leads to many public health problems (WHO , 2005 and Engelgau *et al.*, 2007). The global obesity epidemic is advancing at an ever-accelerating pace with the United States taking an embarrassing lead (NCHS., 2006). Currently , 32% of U.S. adults are obese as defined by a body mass index (BMI) of greater than 30 Kg/m<sup>2</sup> , according to the latest National Health and Nutrition Examination Survey (Ogden *et al.*, 2006). It is estimated that by 2015 , 75% of U.S. adults will be overweight or obese (BMI) of greater than 25 Kg/m<sup>2</sup> and 41% will be obese (Wang and Beydoun , 2007). Excess weight has emerged as one of the leading factors in predicting chronic disease and even death in later life (Strauss & Pollack , 2001 and Dietz & Robinson , 2005). Accruing data identify detrimental consequences of early obesity on life time health ; BMI greater than 25 Kg/m<sup>2</sup> at age 18 was associated with an increased risk of premature death in a large cohort from the Nurses' Health Study (Van-Dam *et al.*, 2006). National Health and Nutrition Examination Survey (NHANES) studies have been undertaken in the United States since the early 1960s and evaluate many parameters of health and growth. In these studies , children aged 6 to 11 had a prevalence of overweight of only 4.2 % in 1965 compared with 15.8 % in 2002. Those aged 12 to 19 experienced a similar change in prevalence. The prevalence of obesity in adults increased from 13.3 % to 31.3 % , and the prevalence of overweight increased from 45 % to 65.2 % in that same time period (Wang and Beydoun , 2007). In the last two decades , an alarming increase in the prevalence of overweight and obesity has been reported and become a serious public health problem affecting different social and economic classes as well as different age-groups in Saudi Arabia and other Asian countries (Abahussain *et al.*, 1999 ; Al-Mousa and Parkash , 2000 ; Sakamoto *et al.*, 2001 ; Ramachandran *et al.*, 2002 ; Al-Almaie , 2005 and Khalid , 2008)

The primary concern with obesity is the health risks that it imparts on the afflicted person. It has been reported that obesity and overweight have been associated with an increased risk of many diseases and complications include non-insulin-dependent diabetes mellitus (Wang *et al.*, 2002 ; Foxhall , 2006 and Brennan *et al.*, 2009) , gastro – intestinal problems ( Kaats *et al.*, 1996 and Knecht *et al.*, 2008) , hypertension and hyperlipidemia ( Anon , 1998 ; Sanchez – Castillo *et al.*, 2005 ; Poirier *et al.*, 2006 and Knecht *et al.*, 2008) , stroke disease ( Suk *et al.*, 2003 and Thomas *et al.*, 2005) , cerebro – and

cardiovascular diseases (Berenson , 2001 and Wilson *et al.*, 2002 ) , dementia ( Yaffe *et al.*, 2004 and Whitmer *et al.*, 2005) , sleep disorders ( O'Donnell *et al.*, 2000 and Knecht *et al.*, 2008) , depression (Faith *et al.*, 2002 and Knecht *et al.*, 2008) ; cholelithiasis , particularly in women ( Bellentani *et al.*, 2000 and Knecht *et al.*, 2008) , pulmonary and renal diseases ( Bray , 2004 and Nejat *et al.*, 2010) , psychosocial problems (Everson *et al.*, 2002 , Knecht *et al.*, 2008 and Brennan *et al.*, 2009) , musculoskeletal disorders (Cicuttini *et al.*, 2002 and Brennan *et al.*, 2009) ; alteration of the endocrine and immune systems ( Heber *et al.*, 2000 and Knecht *et al.*, 2008) , and various cancers including breast , cervical , ovarian , gall bladder , prostate , stomach and colon cancer (Must *et al.*, 1999 ; Van den – Brandt *et al.*, 2002 ; Call *et al.*, 2003 ; Schouten *et al.*, 2004 ; Olsen *et al.*, 2007 ; Renehan *et al.*, 2008 ; Fader *et al.*, 2009 and Nejat *et al.*, 2010).

Orlistat is approved for weight reduction. It is a hydrogenated derivative of a bacterial lipase inhibitor that blocks pancreatic lipase , thus decreasing intestinal digestion of fat and increasing fecal fat loss. Its efficiency in weight reduction depends on the dietary fat content. It can reduce digestion of up to 30 % of orally ingested triglyceride from a diet containing 30 % fat. The drug was reported to produce a weight loss of about 9 – 10% (Bray and Tartaglia , 2000 and Knecht *et al.*, 2008). In secondary prevention studies for weight maintenance , orlistat also attenuated the regain of weight. (Li *et al.*, 2005)

The immobile life style and consumption of high – calorie food are the most remarkable risk – factors of the obesity. It is well known that moderate weight loss (5 – 10 %) in overweight and obesity is clinically beneficial in reduction the risk of obesity – related diseases and health hazards , as well as in improving quality of life (Toplak *et al.*, 2005 and Knecht *et al.*, 2008).

Therefore , this work was performed to assess the obesity – associated diseases and health problems , as well as to determine the impact of obesity and tested weight – reduction program with orally treatment of xenical drug on body weight loss and health status of obese adolescent girls in Saudi Arabia.

## **Material and Methods :**

### **Xenical drug capsules:**

Xenical capsules contained 120 mg of the active ingredient ; orlistat , for each produced on August 2009 by Roch Pharmaceutical Industries Co., Jeddah , Saudi Arabia.

## **Subjects :**

This study was performed on 160 obese adolescent girls , aged 15 – 20 years , which were chosen from whom attending the physical rehabilitation and obesity treatment centers at Jeddah , Saudi Arabia on August 2009. The obese patients , under investigation , were selected from the adolescent girls having a desire to treatment of obesity after obtained their consent to participate in this study. The body mass index (BMI) of selected obese patients was ranged from  $\geq 30$  to  $< 40$  Kg / m<sup>2</sup>. At the beginning of study , the selected obese patients were kept to weight – reduction program based upon there obligation with being on a dietary regimen ; feeding on a nutritionally balanced , low – calorie diet (800 – 1200 calorie) , and in practicing the physical activities regularly at least 6 hours per day. Whereas , the selected obese patients were divided randomly into two equal main groups composed of 80 obese female patients for each. The first group was not treated with xenical drug capsules , while all individual subjects of the second were orally given one xenical capsule 3 times a day with each main meal ; during the 60 days of studying period. Each main group of the selected obese adolescent girls was divided into 4 unequal subgroups according to their obligation and regularity on the tested weight – reduction program throughout the two experiment periods (30 and 60 days) as follows:

**Group 1 :** The obese individual patients group was neither being on the tested dietary regimen program nor practicing the physical activities regularly at least 6 hours a day.

**Group 2 :** The obese individual patients group was being obligation on the tested dietary regimen with no practicing the physical activities regularly at least 6 hours a day.

**Group 3 :** The obese individual patients group was not being obligation on the tested dietary regimen with practicing the physical activities regularly at least 6 hours a day.

**Group 4 :** The obese individual patients group was being obligation on the tested dietary regimen with practicing the physical activities regularly at least 6 hours a day.

## **Lifestyle exposures :**

All measures of dietary intake were self – reported using a validated food – frequency questionnaire (Cancer Council of Victoria , 2005 and Brennan *et al.*, 2009). The nutritive value and the energy value (calories) of the daily consumed food were calculated by using food composition tables of WHO (1992). The obligation of individuals obese patients was self – reported using a special lifestyle questionnaire which was included information about

Leisure – Time Physical Activity (LTPA) , Occupational activity (OA), afternoon siesta, sleeping hours, means of transportation are used for even short – distance, availability of domestic help, and thereupon practicing hours of the physical activities a day were calculated (Cancer Council of Victoria , 2005).

## **Assessment of health problems and diseases associated with obesity :**

The information about health problems and diseases associated with obesity for each patient was collected from the records of the Physical Rehabilitation and Obesity Treatment Centers depending upon the clinical examination and from interviewing the obese patient and his parents about what are the health problems and diseases which is he suffering from?.

## **Anthropometric measurements :**

Body weight of obese girls was measured and recorded using an Avery Beam weighing scale (SECA , Hamburg , Germany) to the nearest 0.1 kg. Standing height of patients was measured and recorded to the nearest 0.1 cm with a stadiometer (SECA , Hamburg , Germany) without shoes. The body mass index (BMI) of obese patients was calculated as weight / height squared (kg / m<sup>2</sup>) and categorized as being normal  $< 25$  (kg / m<sup>2</sup>) , overweight 25.0 – 29.9 (kg / m<sup>2</sup>) or obese  $\geq 30$  (kg / m<sup>2</sup>) ; as reported by NHMRC. (2003).

## **Biochemical analysis :**

Biochemical analysis methods were carried out on all selected obese adolescent girls orally treated or not with xenical drug capsules at the beginning of experiment and after 30 and 60 days (the half and the end of this study period).

Serum total lipids , triglyceride (TG) , total cholesterol and high density lipoprotein cholesterol (HDL) levels by using enzymatic colorimetric methods of Knight *et al.* (1972) ; Foster and Dunn (1973) ; Hewitt and Pardue (1973) and Lopes – Virella *et al.* (1997) , respectively. Serum low density lipoprotein cholesterol (LDL) and very low density lipoprotein cholesterol (VLDL) levels were calculated by using the following equations of Lee and Nieman (1996) as follows:

$$\text{VLDL} = \text{Triglycerides (TG) level} / 5$$

$$\text{LDL} = \text{Total cholesterol} - (\text{HDL} + \text{VLDL})$$

Atherogenic indices were calculated as reported by Aviram & Fuhrman (1998) and Hollander *et al.* (1998) as : (1). Total cholesterol / HDL. (2). LDL / HDL. (3). (LDL + VLDL) / HDL.

Serum aspartate and alanine aminotransaminase (AST; ALT) and alkaline phosphatase (ALP)

activities were determined by using enzymatic colorimetric methods of Reitman and Frankel (1957) and Haussement (1977) , Respectively.

Serum creatinine , urea , uric acid and glucose levels were estimated by enzymatic colorimetric methods reported by Schirmeister (1964) ; Patton and Crouch (1977) ; While *et al.* (1970) and Trinder (1969) , respectively. Serum insulin level was determined by radioimmunoassay reported by Wilson and Miles (1977).

#### **Statistical analysis:**

Statistical analysis for the obtained data was carried out using IBM-PC computer and SAS program as the procedure of ANOVA and Duncan's Multiple Range according to Helwing (1983).

#### **Results and Discussion :**

##### **Distribution of the subjects among obesity associated health problems and diseases :-**

Obesity is a serious health hazard. It puts extra strain on heart , lungs , muscles , bones and joints , and it increases the susceptibility to diabetes mellitus and hypertension. It increases surgical risks , shortens the life-span , causes psychosocial problems , and is associated with a lot of health problems and diseases.

The obtained data (Table 1) illustrated that the most evident health problem among the obese adolescent girls was osteoarthritis ; bone and joint diseases , which represented 48.1 % of obese subjects , this is possibly as the result of the excess weight on the joints. As also shown in Table (1) , the second predominant health compliant , linked to obesity was pulmonary problems which found in 39.4 % of obese subjects. The present results ( Table 1) also showed that the third order of obesity – associated health complaints was gastrointestinal problems , which were exhibited in 31.9 % of selected obese adolescent girls , including gallbladder disease , gallstones , constipation , flatulence , irritable colon , sensation of bloating , anorexia , heart burn , nausea , vomiting , dyspepsia and gastric , and peptic ulcers. In addition , the obtained results ( Table 1 ) also showed that neuropsychiatric problems , cardiovascular disease , hypercholesterolemia , hypertension , dyslipidemia , diabetes , reproductive problems and cancer disease were present in 22.5 , 20 , 18.8 , 16.9 , 21.3 , 17.5 , 14.4 and 10.6 % of the selected obese adolescent girls, respectively. These results are in accordance with those reported by Knecht *et al.* (2008) ; Brennan *et al.* (2009) and Nejat *et al.* (2010).

**Table (1) : Distribution of the subjects among obesity - associated health problems and diseases**

Health problem and disease	Distribution of obese subjects	
	Number	%
Osteoarthritis	77	48.1
Pulmonary problems	63	39.4
Gastro - Intestinal problems	51	31.9
Neuropsychiatric problems	36	22.5
Cardiovascular (CV) disease	32	20.0
Hypercholesterolemia	30	18.8
Dyslipidemia	27	16.9
Hypertension	34	21.3
Diabetes	28	17.5
Reproductive problems	23	14.4
Cancer diseases	17	10.6

##### **Impact of obesity and weight – reduction program on tested anthropometric measurements of obese subjects :**

As shown in Table (2) , the body weight and body mass index (BMI) of untreated obese adolescent girls with xenical drug were increased significantly (at  $\leq 0.01$ ) throughout the experiment period ( 60 days) at different rates affecting by their obligation on tested weight – reduction program based upon being on a dietary regimen and practicing the physical activities regularly at least 6 hours a day. The increment rate was significantly decreased (at  $\leq 0.01$ ) with the engagement by the tested former weight reduction program.

The obtained results ( Table 2) also illustrated that the body weight and the BMI of obese subjects orally treated with xenical drug ; as a source of orlistat , were significantly decreased ( at  $\leq 0.01$  ) during the experiment period ; especially in obese subjects group which engaged with weight – reduction program (G4). Whereas , the body weight of the fourth group (G4) of treated subjects with orlistat were decreased by 7.42 ; 11.35 % and its BMI was reduced from 35.1 kg/m<sup>2</sup> at the beginning of experiment to 31.7 and 29.6 kg/m<sup>2</sup> ; after 30 and 60 days , respectively.

On the other hand , the height ( 161.9 – 165.5 m) of all tested obese adolescent girls treated or not with orlistat was not , somewhat , significantly changed during the experiment period. In this respect , Carolynn *et al.* (2000) and Knecht *et al.* (2008) noted that there is no magic way of losing weight and maintaining the reduced weight , but there is a key to it. That key is changing eating habits and doing physical activities regularly at least 3 to 5 times a week for 30 minutes. These results are in accordance with those obtained by Bray and Tartaglia (2000) ;

Toplak *et al.* (2005) ; Garcia *et al.* (2006) ; Totoian *et al.* (2006) and Knecht *et al.* (2008) whom reported that orlistat is an anti – obesity agent , especially for treatment of obese woman. It blocks pancreatic lipase , thus decreasing intestinal digestion and absorption of dietary fat by approximately 30 % and increasing fecal fat loss , and thereupon promotes weight loss. Furthermore, Li *et al.* (2005) and Zanella *et al.* (2006)

that orlistat treatment of obese subjects attenuated the regain of body weight and long – term orlistat therapy helped to reduce and maintain a lower body weight. Furthermore , Hutton and Fergusson (2004) suggested that the orally ingestion of 120 mg orlistat 3 times a day is effective for improving the weight loss , health status and quality of life , as well as maintaining the body weight in obese patient.

**Table (2): The most important anthropometric measurements of different tested obese adolescent girls.**

Variables	Anthropometric Measurement ( $M \pm SE^*$ )								
	Initial	After 30 days				After 60 days			
		G1	G2	G3	G4	G1	G2	G3	G4
<b>Untreated with Orlistat Drug:-</b>									
Weight (kg)	92.1 <sup>a</sup> ± 3.84	96.9 <sup>bc</sup> ± 3.9	93.8 <sup>ab</sup> ± 4.26	95.3 <sup>b</sup> ± 3.13	93.7 <sup>ab</sup> ± 3.97	98.2 <sup>c</sup> ± 5.18	95.6 <sup>b</sup> ± 4.41	97.7 <sup>c</sup> ± 5.05	96.1 <sup>bc</sup> ± 4.89
Height (cm)	161.9 <sup>ab</sup> ± 7.07	160.3 <sup>a</sup> ± 6.61	160.9 <sup>a</sup> ± 8.14	161.7 <sup>ab</sup> ± 7.56	162.2 <sup>ab</sup> ± 7.99	160.6 <sup>a</sup> ± 6.73	161.3 <sup>ab</sup> ± 9.86	162.3 <sup>ab</sup> ± 7.90	162.8 <sup>b</sup> ± 9.05
BMI (kg/m <sup>2</sup> )	35.1 <sup>a</sup> ± 2.19	37.7 <sup>b</sup> ± 2.63	36.2 <sup>ab</sup> ± 1.99	36.9 <sup>ab</sup> ± 2.01	35.6 <sup>a</sup> ± 2.73	38.1 <sup>b</sup> ± 3.07	36.7 <sup>b</sup> ± 1.81	37.1 <sup>ab</sup> ± 3.28	36.3 <sup>ab</sup> ± 2.97
<b>Treated with Orlistat Drug:-</b>									
Weight (kg)	92.1 <sup>cd</sup> ± 3.84	94.2 <sup>d</sup> ± 3.19	89.5 <sup>c</sup> ± 2.76	93.6 <sup>d</sup> ± 3.93	84.8 <sup>b</sup> ± 2.60	97.9 <sup>c</sup> ± 4.06	86.1 <sup>b</sup> ± 2.99	91.7 <sup>cd</sup> ± 3.56	81.2 <sup>a</sup> ± 2.81
Height (cm)	161.9 <sup>a</sup> ± 7.07	162.4 <sup>a</sup> ± 5.98	162.8 <sup>ab</sup> ± 6.21	163.1 <sup>ab</sup> ± 6.67	163.5 <sup>ab</sup> ± 7.39	162.7 <sup>a</sup> ± 6.71	163.5 <sup>ab</sup> ± 7.83	163.9 <sup>ab</sup> ± 5.95	165.5 <sup>b</sup> ± 7.70
BMI (kg/m <sup>2</sup> )	35.1 <sup>d</sup> 2.19	35.7 <sup>de</sup> ± 2.43	33.8 <sup>c</sup> ± 1.77	35.2 <sup>d</sup> ± 2.09	31.7 <sup>b</sup> ± 1.81	37.0 <sup>e</sup> ± 2.63	32.2 <sup>bc</sup> ± 1.86	34.1 <sup>cd</sup> ± 2.09	29.6 <sup>a</sup> ± 1.83

**$M \pm SE^*$ :** Mean ± Standard error of each anthropometric measurement of obese patients' group throughout experiment period ( in the same row ) having different superscripts are significantly varied.      **G1:** Patients group was neither being on the tested dietary regimen nor practicing physical activities regularly at least 6 hours a day.    **G2:** Patients group was being on the tested dietary regimen with no practicing physical activities regularly at least 6 hours a day.    **G3:** Patients group was not being on the tested dietary regimen with practicing physical activities regularly at least 6 hours a day.    **G4:** Patients group was being on the tested dietary regimen with practicing physical activities regularly at least 6 hours a day.

#### **Impact of obesity and tested weight – reduction program with Xenical drug treatment on serum lipid profile fractions of obese subjects :**

From the obtained results (Table 3) , it could be noticed that serum lipids profile fractions for untreated obese adolescent girls with xenical drug treatment were increased significantly ( $\leq 0.01$ ) during the experimental period (60 days) , with the exception of high density lipoprotein cholesterol (HDL) which was exhibited a significant reduction ( $\leq 0.05$ ) throughout the same periods. The engagement

with both being on tested dietary regimen and values of serum lipids profile fractions of obese subjects were much higher than the normal ranges of practicing physical activity regularly caused a marked improvement in serum lipids profile of obese subjects as illustrated in subgroups No.4 (G4). The mean these fractions for health individual adolescent girls reported by Murray *et al.* (1993) and Anon (1998) , except the high density lipoprotein cholesterol (HDL) values which were within the normal values (70 – 170 mg/dL).

**Table (3): Serum lipids profile fractions (mg / dL) of different tested obese adolescent girls.**

Variables	Serum lipid profile fractions ( $M \pm SE^*$ ) as mg/dL								NR •	
	Initial	After 30 days				After 60 days				
		G1	G2	G3	G4	G1	G2	G3		
<b>Untreated with Orlistat Drug:-</b>										
Total lipids	832.6 <sup>a</sup> ± 31.3	996.8 <sup>e</sup> ± 37.1	917.3 <sup>c</sup> ± 39.9	982.9 <sup>de</sup> ± 27.3	832.5 <sup>a</sup> ± 30.1	1064.7 <sup>f</sup> ± 42.3	954.2 <sup>d</sup> ± 37.4	1020.8 <sup>ef</sup> ± 41.6	873.1 <sup>b</sup> ± 33.5	
Triglycerides	201.8 <sup>b</sup> ± 7.72	264.5 <sup>d</sup> ± 8.99	232.0 <sup>c</sup> ± 7.87	258.5 <sup>d</sup> ± 8.30	190.9 <sup>a</sup> ± 6.85	297.0 <sup>f</sup> ± 9.16	269.7 <sup>d</sup> ± 8.33	283.5 <sup>e</sup> ± 9.01	226.3 <sup>c</sup> ± 7.80	
Total Cholesterol	322.8 <sup>b</sup> ± 11.6	363.5 <sup>e</sup> ± 13.3	346.1 <sup>d</sup> ± 12.5	360.7 <sup>e</sup> ± 10.9	327.3 <sup>bc</sup> ± 10.6	362.4 <sup>e</sup> ± 9.98	331.9 <sup>c</sup> ± 9.24	359.4 <sup>e</sup> ± 10.2	311.7 <sup>a</sup> ± 8.85	
HDL - Cholesterol	102.5 <sup>g</sup> ± 4.71	83.9 <sup>d</sup> ± 3.83	92.2 <sup>f</sup> ± 4.09	88.1 <sup>e</sup> ± 3.94	93.8 <sup>f</sup> ± 5.10	70.1 <sup>a</sup> ± 3.63	77.5 <sup>bc</sup> ± 4.09	74.6 <sup>b</sup> ± 5.19	79.9 <sup>c</sup> ± 4.31	
LDL - Cholesterol	179.9 <sup>a</sup> ± 6.83	226.7 <sup>e</sup> ± 7.40	207.5 <sup>d</sup> ± 5.31	220.9 <sup>f</sup> ± 7.25	195.3 <sup>e</sup> ± 6.98	232.9 <sup>e</sup> ± 8.16	200.5 <sup>cd</sup> ± 6.32	228.1 <sup>e</sup> ± 7.05	186.5 <sup>b</sup> ± 6.09	
VLDL - Cholesterol	40.4 <sup>b</sup> ± 1.79	52.9 <sup>de</sup> ± 2.31	46.4 <sup>c</sup> ± 1.96	51.7 <sup>d</sup> ± 2.44	38.2 <sup>a</sup> ± 1.86	59.4 <sup>g</sup> ± 2.71	53.9 <sup>e</sup> ± 1.83	56.7 <sup>f</sup> ± 2.09	45.3 <sup>c</sup> ± 1.77	
<b>Treated with Orlistat Drug:-</b>										
Total lipids	832.6 <sup>e</sup> ± 31.3	809.2 <sup>e</sup> ± 40.8	721.7 <sup>c</sup> ± 27.2	769.3 <sup>d</sup> ± 31.8	691.9 <sup>bc</sup> ± 29.4	743.1 <sup>cd</sup> ± 41.0	636.5 <sup>a</sup> ± 29.8	701.2 <sup>bc</sup> ± 34.1	693.6 <sup>b</sup> ± 37.8	
Triglycerides	201.8 <sup>a</sup> ± 7.72	173.5 <sup>e</sup> ± 9.07	136.5 <sup>d</sup> ± 6.31	164.0 <sup>f</sup> ± 7.55	126.1 <sup>c</sup> ± 7.08	188.5 <sup>f</sup> ± 9.12	112.0 <sup>b</sup> ± 5.98	159.5 <sup>e</sup> ± 6.70	108.0 <sup>ab</sup> ± 5.49	
Total Cholesterol	322.8 <sup>d</sup> ± 11.6	256.5 <sup>bc</sup> ± 12.9	249.2 <sup>b</sup> ± 10.7	258.8 <sup>c</sup> ± 10.3	219.2 <sup>a</sup> ± 9.48	316 <sup>f</sup> ± 11.4	270.6 <sup>d</sup> ± 12.2	281.9 <sup>e</sup> ± 11.64	258.7 <sup>c</sup> ± 10.1	
HDL - Cholesterol	102.5 <sup>b</sup> ± 4.71	94.7 <sup>a</sup> ± 4.39	119.0 <sup>de</sup> ± 5.64	107.6 <sup>c</sup> ± 3.99	125.3 <sup>f</sup> ± 4.72	117.9 <sup>d</sup> ± 3.66	130.3 <sup>g</sup> ± 6.01	121.7 <sup>e</sup> ± 5.28	146.3 <sup>h</sup> ± 6.75	
LDL - Cholesterol	179.9 <sup>g</sup> ± 6.83	127.1 <sup>e</sup> ± 5.20	102.9 <sup>c</sup> ± 5.16	118.4 <sup>d</sup> ± 6.07	68.7 <sup>a</sup> ± 3.99	160.4 <sup>f</sup> ± 5.21	117.9 <sup>d</sup> ± 4.87	128.3 <sup>e</sup> ± 5.11	90.8 <sup>b</sup> ± 3.93	
VLDL - Cholesterol	40.4 <sup>g</sup> ± 1.79	34.7 <sup>e</sup> ± 2.31	27.3 <sup>c</sup> ± 1.29	32.8 <sup>de</sup> ± 1.95	25.2 <sup>b</sup> ± 1.36	37.7 <sup>f</sup> ± 2.03	22.4 <sup>a</sup> ± 1.61	31.9 <sup>d</sup> ± 2.04	21.6 <sup>a</sup> ± 1.38	

**M±SE\*:** Mean ± Standard error of each biological parameter in serum of obese patients' group throughout experiment period ( in the same row ) having different superscripts are significantly varied.

**G1:** Patients group was neither being on the tested dietary regimen nor practicing physical activities regularly at least 6 hours a day.

**G2:** Patients group was being on the tested dietary regimen with no practicing physical activities regularly at least 6 hours a day.

**G3:** Patients group was not being on the tested dietary regimen with practicing physical activities regularly at least 6 hours a day.

**G4:** Patients group was being on the tested dietary regimen with practicing physical activities regularly at least 6 hours a day.

**NR •:** Normal Range (mg/dL) for health adolescent girls reported by Murray et al. (1991) and Anon (1998).

With regards orally treated obese adolescent girls with orlistat, as a source of orlistat , as shown in Table (3) , serum lipid profile of treated – obese subjects with orlistat was improved significantly ( $\leq 0.01$ ) ; especially in subgroup No.4 (G4) which obligated with the tested weight – reduction program based on being a dietary regimen and practicing the physical activities regularly. As illustrated in Table (3) , serum total lipids , triglyceride , total cholesterol , low density lipoprotein cholesterol (LDL) , very low density lipoprotein cholesterol (VLDL) values of the

obese adolescent girls were decreased significantly ( $\leq 0.01$ ) during the experiment period (60 days) , compared to the corresponding untreated – obese subjects. On the contrary , the HDL – cholesterol values of obese patients were increased significantly ( $\leq 0.05$ ). The alteration rates in serum lipid fractions of obese subjects treated with orlistat were varied depending upon their engagement with the tested weight – reduction program , lipid fraction itself and the period of experiment. It is worth to note that the mean values of serum lipid fractions for treated –

obese subjects with orlistat after 30 and 60 days of treatment were within the normal values reported for health individual adolescent girls. These finding are in agreement with those found by Sanchez – Castillo *et al.* (2005) and Knecht *et al.* (2008) who reported that overweight and obesity are associated with elevation the serum total lipids , triglyceride and the LDL concentrations values , as well as with lowering the HDL ; especially in adolescent girls. Also , these results are in a quite accordance with those obtained by Bray and Tartagila (2000) ; Bettina *et al.* (2001) ; Mulls *et al.* (2001) and Knecht *et al.* (2008) who

reported that orlistat is a bacterial lipase inhibitor that blocks pancreatic lipase , thus decreased intestinal digestion and absorption of fat and increasing fecal fat derivatives from a diet containing 30 % fat. Therefore, orlistat therapy is associated with improving lipid loss. It can decrease the digestion and absorption of up to 30 of orally ingested triglyceride and cholesterol metabolism processes and serum lipid profile in obese patients as shown by a greater decline in serum total lipids and cholesterol, triglyceride and LDL levels.

**Table (4): Atherogenic indices of different tested obese adolescent girls.**

Variables	Atherogenic Index (M±SE*)									Risk ratio*	
	Initial	After 30 days				After 60 days					
		G1	G2	G3	G4	G1	G2	G3	G4		
<b>Untreated with Orlistat Drug:-</b>											
Total Cholesterol / HDL	3.15 <sup>a</sup>	4.33 <sup>e</sup>	3.75 <sup>c</sup>	4.09 <sup>d</sup>	3.49 <sup>b</sup>	5.17 <sup>g</sup>	4.28 <sup>de</sup>	4.82 <sup>f</sup>	3.90 <sup>cd</sup>	3.5 : 5.5	
	± 0.29	± 0.36	± 0.31	± 0.27	± 0.23	± 0.40	± 0.34	± 0.37	± 0.31		
LDL / HDL	1.76 <sup>a</sup>	2.70 <sup>e</sup>	2.25 <sup>c</sup>	2.51 <sup>d</sup>	2.08 <sup>b</sup>	3.32 <sup>g</sup>	2.59 <sup>e</sup>	3.06 <sup>f</sup>	2.33 <sup>cd</sup>	1.4 : 3.5	
	± 0.18	± 0.23	± 0.18	± 0.20	± 0.16	± 0.29	± 0.26	± 0.29	± 0.21		
(LDL + VLDL) / HDL	2.15 <sup>a</sup>	3.33 <sup>e</sup>	2.75 <sup>c</sup>	3.09 <sup>d</sup>	2.49 <sup>b</sup>	4.17 <sup>g</sup>	3.28 <sup>e</sup>	3.82 <sup>f</sup>	2.90 <sup>c</sup>	-	
	± 0.21	± 0.27	± 0.21	± 0.26	± 0.18	± 0.31	± 0.23	± 0.30	± 0.19		
<b>Treated with Orlistat Drug:-</b>											
Total Cholesterol / HDL	3.15 <sup>e</sup>	2.71 <sup>d</sup>	2.09 <sup>b</sup>	2.41 <sup>c</sup>	1.75 <sup>a</sup>	2.86 <sup>d</sup>	2.08 <sup>b</sup>	2.32 <sup>c</sup>	1.77 <sup>a</sup>	3.5 : 5.5	
	± 0.29	± 0.23	± 0.17	± 0.21	± 0.18	± 0.25	± 0.17	± 0.23	± 0.19		
LDL / HDL	1.76 <sup>e</sup>	1.34 <sup>d</sup>	0.86 <sup>b</sup>	1.10 <sup>c</sup>	0.54 <sup>a</sup>	1.36 <sup>d</sup>	0.90 <sup>b</sup>	1.05 <sup>c</sup>	0.62 <sup>a</sup>	1.4 : 3.5	
	± 0.18	± 0.13	± 0.08	± 0.12	± 0.07	± 0.14	± 0.07	± 0.11	± 0.05		
(LDL + VLDL) / HDL	2.15 <sup>f</sup>	1.71 <sup>e</sup>	1.09 <sup>d</sup>	1.41 <sup>c</sup>	0.75 <sup>a</sup>	1.68 <sup>d</sup>	1.08 <sup>b</sup>	1.32 <sup>c</sup>	0.77 <sup>a</sup>	-	
	± 0.21	± 0.16	± 0.12	± 0.15	± 0.09	± 0.17	± 0.11	± 0.13	± 0.09		

**M±SE\*:** Mean ± Standard error of each atherogenic index of obese patients' group throughout experiment period ( in the same row ) having different superscripts are significantly varied.

**G1:** Patients group was neither being on the tested

dietary regimen nor practicing physical activities regularly at least 6 hours a day. **G2:** Patients group was being on the tested dietary regimen with no practicing physical activities regularly at least 6 hours a day. **G3:** Patients group was not being on the tested dietary regimen with practicing physical activities regularly at least 6 hours a day.

**G4:** Patients group was being on the tested dietary regimen with practicing physical activities regularly at least 6 hours a day. **Risk ratio •:** Risk ratio of atherogenic index for obesity associated diseases and health problems reported by Vazquez – Freire *et al.* (1996) and Hollander *et al.* (1998)

From the results represented in Table (4), it could be showed that all determined atherogenic indices; including total cholesterol / HDL, LDL / HDL and (LDL + VLDL) / HDL , for untreated obese adolescent girls with orlistat were increased significantly ( $\leq 0.01$ ) with prolonging the tested period of experiment. The atherogenic indices' values were within the risk ratios for obesity – associated diseases and health hazards. The increment rates of atherogenic indices in untreated patients by orlistat

were decreased with their obligation and attendance by the tested weight – reduction program as shown in subjects' subgroups No.4 (G4).

Therefore , the risk of obesity – associated decreases and health hazards were elevated during the span – life of obese subjects ; especially with no obligation by being on tested dietary regimen and practicing physical activities regularly ( Lavie and Milani , 2003 ; Knecht *et al.*, 2008 and Brennan *et al.*, 2009).

Concerning orlistat – treated obese patients as illustrated in Table (4) , the orally orlistat treatment caused a significant reduction and improvement ( $\leq 0.01$ ) in the values of all atherogenic indices for the risk factors of obesity – associated diseases and health problems in obese adolescent girls. The highest reduction rates in the values of atherogenic indices were observed in the individuals obese patients engaged by the tested weight – reduction program as evident in obese patients' subgroup No.4 (G4). Whereas , all atherogenic indices of different orlistat – treated obese patients' groups were much lower than those causing the increased risk of obesity – associated diseases and health problems. Therefore , the orally treatment of obese patients with orlistat (120 mg – capsule 3 times a day with the main diets) with attendance by their being on tested weight – reduction program caused a greater reduction in the risk of many diseases and health problems associated with obesity in adolescent girls and therefore improved their health status , and the quality of life in obese patients. These results are in agreement with those found by Vazquez – Freire *et al.* (1996) ; Hollander *et al.* (1998) ; Bray (2004) ; Hutton and Fergusson (2004) ; Shaheen (2007) and Knecht *et al.* (2008) who reported that orlistat treatment for overweight and obese patients caused a significant reduction ( $\leq 0.01$ ) in atherogenic indices and therefore a greater improvement in the risk factors' numbers of coronary heart disease (CHD) and other diseases , and health problems associated with overweight and obesity.

#### **Impact of obesity and tested weight – reduction program with Xenical drug treatment on liver and renal functions in the serum of obese subjects :**

The liver functional enzymes (AST , ALT and ALP) activity in serum are most frequently measure for diagnosis of liver diseases particularly infective hepatitis , alcoholic cirrhosis , biliary obstruction , toxic hepatitis and liver cancer. The former liver functional enzymes are not secreted into the blood , therefore any elevation in their values in blood is resulted from leakage of liver damage cells and from the disturbance and dysfunctions in liver functional enzymes activity. In addition, the liver functional enzymes (AST, ALT and ALP) activity in human and experimental animals are considered the excellent markers of liver dysfunctions and damages that probably associated with overweight and obesity or caused by exposure to the toxic substances or some drugs and therapeutic substances. Therefore, they are considered the good successful criterion for health status of obese adolescent girls (Murray *et al.*, 1993 and Sabuncu *et al.*, 2003).

As shown in Table (5), the obesity was associated with hyper activity of liver functional enzymes; aspartate and alanine amino transferases (AST; ALT) and alkaline phosphatase (ALP), during the tested experimental period (60 days), as illustrated in untreated obese adolescent girls with orlistat. The hyperactivity extent of liver functional enzymes in the serum of untreated subjects with orlistat was less with their obligation by tested weight – reduction program based upon being on tested dietary regimen and practicing physical activities regularly at least 6 hours a day. Whereas , the liver functional enzymes' activities in obese patients' serum were much higher throughout the period of experiment than the normal ranges for healthy individuals adolescent human females. This finding is in accordance with those reported by Hickman *et al.* (2004) and Knecht *et al.* (2008) who mentioned that overweight and obesity were associated with an exceptional elevation of liver functional enzymes activity in serum of adult women.

The obtained results (Table 5) also exhibited that the orally orlistat drug treatment caused a high significant improvement ( $\leq 0.01$ ) in liver functional enzymes (AST , ALT and ALP) activity in serum of obese patients ; especially with their engagement by attending the tested weight – reduction program , as shown in the fourth group of obese adolescent subjects. Where ; AST , ALT and ALP activities were reduced from 42.8 , 36.2 and 97.7 Unit / L at the beginning of experiment period to 21.6 , 17.8 and 51.2 Unit / L at the end of experiment period (after 60 days) for the orlistat – treated subgroup (G4) which engaged by the attendance with the tested weight – reduction program ; respectively. The functional liver enzymes ' activities for orlistat – treated obese patients , which engaged with the tested weight – reduction program , were within the normal values range for health individuals adolescent human females reported by Murray *et al.* (1993) and Anon (1998). The improvement effect of orlistat drug treatment on liver functions of obese subjects may be due to the protection effect of orlistat against the oxidation of lipids ; especially the LDL – cholesterol in liver and plasma , and to its reduction effect on the heart disease risk by improving blood profile of lipid constituents with decreasing the LDL – cholesterol level and lowering the blood pressure (Gacob , 1994 ; Sabuncu *et al.*, 2003 ; Hickman *et al.*, 2004 and Knecht *et al.*, 2008) , as well as to its enhancement effect on metabolic processes (Demidova *et al.*, 2006).

With regards the renal functions in the serum of obese patients as affected by obesity and tested weight – reduction program , it worth to mention that the normal levels' range of kidney functions in the serum of health individuals adolescent girls are 2.6 –

6.0, 10 – 50 and 0.6 – 1.1 mg / dL for uric acid , urea and creatinine , respectively (Murray *et al.*, 1993 and Anon , 1998). The exceptional elevation of the former levels into two times or more in mammalian blood is resulted from kidney damage cells, disturbance and dysfunctions (Murray *et al.*, 1993 and Jacob, 1994).

From the data presented in Table (6), it could be observed that the renal functions; serum uric acid, urea and creatinine values of untreated obese patients with orlistat were elevated exceptionally during the experiment period ( 60 days ). The highest

elevation rate was observed in the individual obese patients of subgroup No.1 (G1) whom was neither being on the tested dietary regimen nor practicing the physical activity regularly at least for 6 hours a day. The renal function values for all untreated obese subjects with orlistat drug were much higher than the normal values for health adolescent human female. These data are in accordance with those found by Vasanthi *et al.* (2003) ; Shaheen (2007) and Knecht *et al.* (2008).

**Table (5): Liver functions (Unit / L Serum) of different tested obese adolescent girls.**

Variables	Liver function (M±SE*) as (Unit / L Serum)								• N R		
	Initial	After 30 days				After 60 days					
		G1	G2	G3	G4	G1	G2	G3			
<b>Untreated with Orlistat Drug:-</b>											
AST	42.8 <sup>a</sup>	54.3 <sup>e</sup>	48.7 <sup>c</sup>	51.5 <sup>d</sup>	45.9 <sup>b</sup>	68.2 <sup>g</sup>	52.8 <sup>de</sup>	56.6 <sup>f</sup>	47.9 <sup>bc</sup>	0 : 30	
	± 3.35	± 4.57	± 3.90	± 4.73	± 3.48	± 5.15	± 4.92	± 5.04	± 3.62		
ALT	36.2 <sup>a</sup>	46.9 <sup>e</sup>	41.4 <sup>c</sup>	43.1 <sup>cd</sup>	38.6 <sup>bc</sup>	61.8 <sup>f</sup>	44.3 <sup>d</sup>	60.7 <sup>f</sup>	39.5 <sup>b</sup>	0 : 34	
	± 2.19	± 3.44	± 2.78	± 2.96	± 3.01	± 4.99	± 3.70	± 4.12	± 2.88		
ALP	97.7 <sup>a</sup>	129.2 <sup>e</sup>	106.5 <sup>b</sup>	120.8 <sup>d</sup>	112.3 <sup>c</sup>	143.1 <sup>g</sup>	128.0 <sup>e</sup>	135.9 <sup>f</sup>	121.1 <sup>d</sup>	24 : 90	
	± 6.02	± 7.87	± 5.92	± 7.76	± 6.20	± 8.04	± 6.96	± 7.58	± 6.09		
<b>Treated with Orlistat Drug:-</b>											
AST	42.8 <sup>g</sup>	40.5 <sup>f</sup>	31.9 <sup>cd</sup>	36.3 <sup>e</sup>	27.8 <sup>b</sup>	39.4 <sup>f</sup>	30.7 <sup>c</sup>	33.2 <sup>d</sup>	21.6 <sup>a</sup>	0 : 30	
	± 3.35	± 3.91	± 2.67	± 3.09	± 1.66	± 3.72	± 2.80	± 2.69	± 1.33		
ALT	36.2 <sup>e</sup>	33.9 <sup>h</sup>	26.1 <sup>d</sup>	30.6 <sup>f</sup>	21.4 <sup>b</sup>	32.7 <sup>g</sup>	23.9 <sup>c</sup>	28.5 <sup>e</sup>	17.8 <sup>a</sup>	0 : 34	
	± 2.19	± 2.74	± 1.87	± 1.99	± 1.23	± 2.18	± 1.53	± 1.91	± 1.26		
ALP	97.7 <sup>h</sup>	81.1 <sup>f</sup>	69.6 <sup>d</sup>	75.2 <sup>e</sup>	60.7 <sup>c</sup>	87.3 <sup>g</sup>	56.5 <sup>b</sup>	62.9 <sup>c</sup>	51.2 <sup>a</sup>	24 : 90	
	± 6.02	± 6.38	± 5.10	± 5.64	± 4.79	± 5.90	± 4.82	± 5.07	± 4.54		

M±SE\*: Mean ± Standard error of each liver function in serum of obese patients' group throughout experiment period ( in the same row ) having different superscripts are significantly varied. G1: Patients group was neither being on the tested dietary regimen nor practicing physical activities regularly at least 6 hours a day. G2: Patients group was being on the tested dietary regimen with no practicing physical activities regularly at least 6 hours a day. G3: Patients group was not being on the tested dietary regimen with practicing physical activities regularly at least 6 hours a day.

G4: Patients group was being on tested dietary regimen with practicing physical activities regularly at least 6 hours a day.  
N R •: Normal Range (Unit / L Serum) for health adolescent girls reported by Murray *et al.* (1991) and Anon (1998).

Concerning orlistat – treated obese subjects , as evident from the results recorded in Table (6) , the tested renal functions for orlistat – treated adolescent girls were improved significantly ( $\leq 0.01$ ) as shown from a high decrement of the values of these functions , when compared with those of the corresponding subgroups of untreated obese patients with orlistat. The highest improvement in renal functions was found in the individuals obese subjects 'subgroup No.4 (G4) which engaged by their attendance with being on the tested weight – reduction program. Whereas , serum uric acid , urea

and creatinine levels for orlistat – treated obese patients were decreased from 7.23 , 61.6 and 1.32 mg / dL at the beginning of experiment to 4.83 , 27.5 and 0.73 mg / dL at the end of experiment (after 60 days) , within the normal levels of the health individuals adolescent girls , respectively.

These results are in agreement with those reported by Demidova *et al.* (2006) and Shaheen (2007) whom reported that xenical ; orlistat , treatment was beneficial for patients to correct obesity because it improved metabolic processes and therefore kidney and liver functions.

**Table (6): Renal functions (mg / dL) of different tested obese adolescent girls.**

Variables	Initial	Renal function (M±SE*) as (mg/dL)								NR •	
		After 30 days				After 60 days					
		G1	G2	G3	G4	G1	G2	G3	G4		
<b>Untreated with Orlistat Drug:-</b>											
Uric acid	7.23 <sup>a</sup>	8.86 <sup>c</sup>	7.91 <sup>b</sup>	8.29 <sup>bc</sup>	7.65 <sup>ab</sup>	9.72 <sup>d</sup>	8.54 <sup>bc</sup>	8.80 <sup>c</sup>	7.97 <sup>b</sup>	2.6: 6.0	
	± 0.38	± 0.51	± 0.43	± 0.36	± 0.42	± 0.54	± 0.47	± 0.39	± 0.31		
Urea	61.6 <sup>a</sup>	79.2 <sup>de</sup>	71.5 <sup>c</sup>	73.5 <sup>c</sup>	68.1 <sup>b</sup>	87.6 <sup>f</sup>	76.9 <sup>d</sup>	81.2 <sup>e</sup>	72.4 <sup>c</sup>	10 : 50	
	± 4.10	± 5.69	± 4.81	± 5.74	± 4.99	± 6.07	± 5.18	± 6.64	± 5.06		
Creatinine	1.32 <sup>a</sup>	1.77 <sup>f</sup>	1.60 <sup>d</sup>	1.67 <sup>de</sup>	1.43 <sup>b</sup>	1.90 <sup>g</sup>	1.72 <sup>e</sup>	1.85 <sup>fg</sup>	1.59 <sup>c</sup>	0.6 : 1.1	
	± 0.14	± 0.18	± 0.15	± 0.12	± 0.16	± 0.18	± 0.15	± 0.13	± 0.11		
<b>Treated with Orlistat Drug:-</b>											
Uric acid	7.23 <sup>e</sup>	7.05 <sup>de</sup>	5.97 <sup>bc</sup>	6.82 <sup>d</sup>	5.30 <sup>ab</sup>	6.71 <sup>d</sup>	5.40 <sup>b</sup>	6.29 <sup>c</sup>	4.83 <sup>a</sup>	2.6: 6.0	
	± 0.38	± 0.42	± 0.29	± 0.33	± 0.25	± 0.28	± 0.21	± 0.30	± 0.19		
Urea	61.6 <sup>g</sup>	54.9 <sup>f</sup>	41.5 <sup>d</sup>	48.6 <sup>e</sup>	32.1 <sup>b</sup>	46.8 <sup>e</sup>	34.3 <sup>c</sup>	43.1 <sup>d</sup>	27.5 <sup>a</sup>	10 : 50	
	± 4.10	± 3.76	± 3.87	± 4.01	± 2.63	± 3.96	± 2.80	± 4.06	± 2.18		
Creatinine	1.32 <sup>f</sup>	1.19 <sup>e</sup>	0.92 <sup>c</sup>	1.08 <sup>d</sup>	0.86 <sup>bc</sup>	1.02 <sup>d</sup>	0.84 <sup>b</sup>	0.90 <sup>bc</sup>	0.73 <sup>a</sup>	0.6 : 1.1	
	± 0.14	± 0.12	± 0.09	± 0.11	± 0.09	± 0.07	± 0.08	± 0.07	± 0.06		

**M±SE\*:** Mean ± Standard error of each renal function in serum of obese patients' group throughout experiment period (in the same row ) having different superscripts are significantly varied. **G1:** Patients group was neither being on the tested dietary regimen nor practicing physical activities regularly at least 6 hours a day. **G2:** Patients group was being on the tested dietary regimen with no practicing physical activities regularly at least 6 hours a day. **G3:** Patients group was not being on the tested dietary regimen with practicing physical activities regularly at least 6 hours a day. **G4:** Patients group was being on the tested dietary regimen with practicing physical activities regularly at least 6 hours a day. **NR •:** Normal Range (mg / dL) for health adolescent girls reported by Murray *et al.* (1991) and Anon (1998).

#### Impact of obesity and tested weight – reduction program with Xenical drug treatment on serum glucose and insulin levels of obese subjects :

As shown in Table (7) , there was a significant increase ( $\leq 0.01$ ) in serum glucose level of obese adolescent girls throughout the experiment period (60 days) at different rates affecting by their obligation with tested weight – reduction program based upon being on the tested dietary regimen and practicing the physical activities regularly at least 6 hours a day. The lowest increment rate in serum glucose level was observed in subgroup of obese individual patients No.4 (G4) which engaged by their attendance with selected weight – reduction program. Serum glucose level for untreated obese patients with orlistat ranged from 154.2 to 194.5 mg / dL ; that much higher than the normal levels for healthy obese individuals adolescent girls which ranged from 70 to 140 mg / dL ( Murray *et al.*, 1993 and Anon , 1998). These results are well in line with those obtained by Foxhall (2006) and Knecht *et al.* (2008) whom reported that obesity is associated with much elevation in serum glucose level in obese adult human

as well as with non – insulin dependent diabetes mellitus disease.

The obtained data (Table 7) also illustrated that orlistat orally treatment of obese adolescent girls caused a significant reduction ( $\leq 0.01$ ) in their serum glucose level during the experiment period (60 days) when compared to patients no treated with orlistat , especially in those engaged by tested weight – reduction program as shown in subgroup No.4 (G4). The serum glucose level for orlistat – treated obese subjects was ranged from 86.1 to 124.5 mg / dL throughout the experiment period (60 days) ; within the normal value (70 – 140 mg / dL) for health individuals adolescent girls reported by Murray *et al.* (1993) and Anon (1998). This observation is in agreement with those of Hollander *et al.* (1998) ; Demidova *et al.* (2006) and Shaheen (2007).

With regards serum insulin level of obese subjects , as illustrated in Table (7) , the insulin level in serum of untreated obese patients with orlistat was increased significantly ( $\leq 0.01$ ) during the experiment period (60 days) from 11.8 mg / dL at the beginning of experiment to 12.7 – 17.8 mg / dL , affecting by

**Table(7): Serum glucose and insulin levels (mg/dL) of different tested obese adolescent girls.**

Variables	Tested Variable level (M±SE*) as (mg/dL)								N R •		
	Initial	After 30 days				After 60 days					
		G1	G2	G3	G4	G1	G2	G3			
<b>Untreated with Orlistat Drug:-</b>											
Glucose	154.2 <sup>a</sup>	186.8 <sup>e</sup>	174.2 <sup>d</sup>	167.7 <sup>bc</sup>	161.9 <sup>b</sup>	194.5 <sup>f</sup>	191.8 <sup>f</sup>	182.3 <sup>e</sup>	169.5 <sup>c</sup>	70 : 140	
	± 9.65	± 11.21	± 10.69	± 8.43	± 9.10	± 11.38	± 10.53	± 9.10	± 8.92		
Insulin	11.8 <sup>a</sup>	14.2 <sup>d</sup>	12.7 <sup>b</sup>	13.5 <sup>c</sup>	16.3 <sup>f</sup>	15.9 <sup>ef</sup>	13.6 <sup>c</sup>	15.4 <sup>e</sup>	17.8 <sup>g</sup>	9 : 12	
	± 0.51	± 0.76	± 0.59	± 0.72	± 0.87	± 0.69	± 0.62	± 0.78	± 0.63		
<b>Treated with Orlistat Drug:-</b>											
Glucose	154.2 <sup>g</sup>	124.5 <sup>f</sup>	112.1 <sup>d</sup>	98.8 <sup>c</sup>	90.4 <sup>b</sup>	116.2 <sup>e</sup>	101.5 <sup>e</sup>	93.7 <sup>b</sup>	86.1 <sup>a</sup>	70 : 140	
	± 9.65	± 8.08	± 6.44	± 5.10	± 4.82	± 7.19	± 5.74	± 6.01	± 5.86		
Insulin	11.8 <sup>f</sup>	11.2 <sup>e</sup>	10.3 <sup>d</sup>	10.6 <sup>d</sup>	9.1 <sup>bc</sup>	10.6 <sup>d</sup>	8.9 <sup>b</sup>	9.5 <sup>c</sup>	8.3 <sup>a</sup>	9 : 12	
	± 0.51	± 0.47	± 0.36	± 0.41	± 0.29	± 0.43	± 0.27	± 0.36	± 0.29		

**M±SE\*:** Mean ± Standard error of either glucose level or insulin level in serum of obese patients' group throughout experiment period ( in the same row ) having different superscripts are significantly varied.

**G1:** Patients group was neither being on the tested dietary regimen nor practicing physical activities regularly at least 6 hours a day.   **G2:** Patients group was being on the tested dietary regimen with no practicing physical activities regularly at least 6 hours a day.

**G3:** Patients group was not being on the tested dietary regimen with practicing physical activities regularly at least 6 hours a day.   **G4:** Patients group was being on the tested dietary regimen with practicing physical activities regularly at least 6 hours a day.   **N R •:** Normal Range (mg / dL) for health adolescent girls reported by Murray et al. (1991) and Anon (1998).

the experimental period and the obligation of patients with their being on tested weight – reduction program. Serum insulin levels for obese patients were much higher than the normal values for health individuals adolescent girls (9 – 12 mg / dL) reported by Murray *et al.* (1993) and Anon (1998). These results are in accordance with those reported by Hickman *et al.* (2004) and Heilbronn *et al.* (2006).

The results presented in Table (7) also showed that serum insulin levels for orlistat – treated obese patients were reduced significantly ( $\leq 0.05$ ) during the experiment period , on the contrary to untreated ones. The highest reduction in or controlling on insulin secretion into the blood of obese subjects was observed throughout tested experimental period in obese patients' subgroups No.4 (G4) which engaged by their obligation and attendance with the tested weight – reduction program. Whereas , the serum insulin level for orlistat – treated subjects was ranged from 8.3 to 11.2 mg / dL ; within the normal values (9 – 12 mg / dL) reported for health individuals adolescent girls. These observations are in agreement with those noticed by Hickman *et al.* (2004) and Heilbronn *et al.* (2006) who reported that weight – reduction program with orlistat treatment caused a significant decrease in serum insulin of obese patients and other benefits included improvement in quality of life , amelioration of dyspnea and chest pain , and

reduction in number of risk leave days. While , these results are in a disagreement with those obtained by Shaheen (2007) who reported that the serum insulin levels in obese experimental animals did not significantly affected by orlistat treatment.

### Conclusion and Recommendation :

It can be concluded that over consumption of high calorie – foods and the lack of practicing the physical activity regularly at least 6 hours a day were the two important factors in the development of obesity among the adolescent girls. Furthermore , the attendance of obese patients by the tested weight – reduction program with the orally treatment of xenical drug (orlistat) for 60 days caused the loss of body weight of obese adolescent girls by about 11.35 % with high enhancement the health status parameters such as serum lipid profile , renal and liver functions and glucose , and insulin levels.

Therefore , it is recommended that : (1) – Women whom are mothers of today need to be targeted for health education programs related to an awareness of appropriate body weight , healthy life style and obesity control. (2) – Adequate facilities for healthy foods teaching , and physical activity programs must be introduced by the different specific institutions. (3) – The obese adolescent girls and women should be orally treated with xenical drug capsules with their obligation and attendance by being

on a dietary regimen ; a nutritionally balanced , reduced – calorie diet (800 – 1200 calorie) , and practicing the physical activities regularly at least 6 hours a day.

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# Molecular Characterization of Egyptian Isolates of *Lactobacillus* and *Bifidobacterium*

Hashem S.<sup>1</sup>; H. H. Sabit<sup>2</sup>; M. Amin<sup>3</sup>; W. Tawakkol<sup>4</sup>; and A. F. Shamseldin<sup>4</sup>

<sup>1</sup>Microbiology Dept., College of Medicine, Assiut University, Assiut, Egypt

<sup>2</sup>Microbial Genetics Dept., College of Biotechnology, Misr University for Science and Technology, Cairo, Egypt

<sup>3</sup>Microbiology Dept., College of Pharmacy, Cairo University, Cairo, Egypt

<sup>4</sup>Microbiology Dept., College of Pharmacy, Misr University for Science and Technology, Cairo, Egypt

**Abstract:** Strains of *Lactobacillus* and *Bifidobacterium* were isolated from processed milk collected in Cairo, Egypt. *Lactobacilli* was isolated on Acetate media (SL) of Rogosa and Mitchell-Weisman. While *Bifidobacterium* was isolated on DSM medium (Difco Sporulation Medium). The isolates were characterized microscopically, morphologically and by some biochemical tests. DNA was extracted from the specified isolates using (Qiagen, Germany. Cat #51306) and species-specific primers for *Lactobacillus* and *Bifidobacterium* were designed to amplify the 16s rDNA gene as a conserved region in the bacterial DNA. Elution of the target band from the gel was performed efficiently and the 16S rDNA region was subjected to sequencing using Sequencer ABI PRISM 3730XL Analyzer. The sequencing data obtained suggested that the two studied isolates were (at the genus level) designated as *Lactobacillus* and uncultured *Bifidobacterium*. When the sequencing data was aligned on <http://www.ncbi.nlm.nih.gov>, it shows 88% homology and expected value of 7e-164 to *Lactobacillus kiranofaceins* but dendogram tree shows more homology to *Lactobacillus plantarum* family. While the other sample showed 91% homology and expected value of 3e-113 with Uncultured *Bifidobacterium* Clone R333 16S rRNA gene. [Hashem S.; H. H. Sabit; M. Amin; W. Tawakkol; and A. F. Shamseldin. Molecular Characterization of Egyptian Isolates of Lactobacillus and Bifidobacterium. Journal of American Science 2010;6(11):959-964]. (ISSN: 1545-1003).

Keywords: Molecular Characterization of Egyptian Isolates of *Lactobacillus* and *Bifidobacterium*

## 1. Introduction:

Probiotics are defined as live microbial food ingredients that have a beneficial effect on human health. The concept of probiotics evolved at the turn of the 20th century from a hypothesis first proposed by Nobel Prize winning Russian scientist Elie Metchnikoff (Bibl, 1988). He suggested that the long, healthy life of Bulgarian peasants resulted from their consumption of fermented milk products. He believed that when consumed, the fermenting bacillus (*Lactobacillus*) positively influenced the microflora of the colon, decreasing toxic microbial activities. For human adult use, this includes fermented milk products as well as over-the-counter preparations that contain lyophilized bacteria. The microorganisms involved are usually lactic acid producers such as *lactobacilli* and *bifidobacteria*. An effective probiotic should exert a beneficial effect on the host, be nonpathogenic and nontoxic, contain a large number of viable cells, it should be capable of surviving and metabolizing in the gut, also remain viable during storage and use, having good sensory properties, and finally be isolated from the same species as its intended host (Gonzalez *et al.*, 1995).

Much attention has focused on decreasing colon cancer risk through increasing intake of dietary fiber; recently, this has included interest in the consumption of prebiotics and probiotics (Brady *et al*, 2000). Furthermore, (Balish *et al*, 1997) reported that the probiotic bacteria manifested different capacities to adhere to epithelial surfaces, disseminate to internal organs, affect the body weight of adult mice and the growth of neonatal mice, and stimulate immune responses. Although the probiotic species were innocuous for adults, his results suggest that caution and further studies to assess the safety of probiotic bacteria for immunodeficient hosts, especially neonates, are required.

## 2. Materials and methods

### 2.1 Isolation

Milk samples were collected from Cows at Six October governorate, Egypt. Serial dilution for the samples was carried out under aseptic conditions, 100 µl of 10<sup>-7</sup> dilution from each sample was transferred to a Petri dish. Warm Acetate media (SL) of Rogosa and Mitchell-Weisman agar medium and DSM agar medium were poured on each plate for *Lactobacillus* and *Bifidobacterium* respectively. The

plates were then incubated under anaerobic conditions at 37 °C for 24 hours. Single colonies were examined morphologically and microscopically using gram stain. The *Lactobacillus* colonies appeared as white small colonies around 2 mm in diameter with entire margin, while the *Bifidobacterium* were punctiform cream colonies with 0.5 mm in diameter. Both isolates were able to ferment lactose, glucose and sucrose but not mannitol. Furthermore, *Bifidobacterium* was able to ferment hexose by fructose-6-phosphate phosphoketolase (F6PPK) shunt. In addition, both isolates were subjected for catalase and indol test, and negative reaction were appeared in both samples.

## 2.2 DNA Extraction

MRS and DSM broth media were inoculated with *Lactobacillus* and *Bifidobacterium* respectively. 1.5 ml of the overnight culture was transferred to each eppendorf tube and were centrifuged at 8000 rpm for 1 min at 4 °C. Supernatant was discarded and 1 ml of washing buffer SET (20% sucrose, 50 mM Tris-HCL and 50 mM EDTA, pH 7.6) was added to each eppendorf. Cells were re-suspended and centrifuged and the supernatant was discarded again. 100 µl of SL (SET + 25 mg/ml Lysosyme) and 10 µl of Proteinase K (10 mg/ml) was added to the cells which is then was re-suspended by the aid of vortex. Cells were incubated in water bath at 37 °C for 2 hours. 70 µl of 10% SDS was added and re-incubated in the water bath for 10 minutes. 500 µl of TE buffer (10 mM Tris HCl and 0.2 mM EDTA) was added to each eppendorf, followed by the addition of 600 µl of Phenol:Chloroform:Isoamly mixture (25:24:1 respectively). The tubes were mixed gently, and then centrifuged at 12000 rpm for 15 minutes at 4 °C. The upper aqueous phase was transferred to a clean eppendorf tube, and equal volume of cooled Isopropanol was added and re-centrifuged for 10 minutes at 12000 rpm. Supernatant was discarded and purification was applied by addition of double volume of 70% cooled Ethanol, samples were centrifuged for more 10 minutes, and then pellet was re-suspended in 100 µl of water after removing the supernatant.

## 2.3 Polymerase Chain Reaction (PCR)

Extracted DNA was electrophoresed in a 1.5% agarose gel (Fisher Scientific) and was subsequently visualized with UV illumination after staining with ethidium bromide. DNA concentration was measured using Spectrophotometer apparatus Biometra then DNA was diluted to 50 ng/ml. The oligonucleotide primers used in this study were purchased from LabTechnology (Promega Corp.). Primer PAF [5' AGA GTT TGA TCC TGG CTC AG 3'] position 8-27 (using the *Escherichia coli* numbering system) and 536R [5' GTA TTA CCG CGG CTG CTG 3'] position 519- 536 were used to amplify the 5' region of the 16S rDNA gene (Yeung *et al.*, 2002). PCR was performed in Biometra PCR System. For each reaction, a 50-µl reaction mixture was prepared. The reaction mixture contained 1× buffer without MgCl<sub>2</sub> (Promega Corp., Madison WI), 1.5 mM MgCl<sub>2</sub>, 20 µM dNTP, 0.1 µM primers PAF and 536R, 1.5 U Taq Polymerase (Promega Corp.), and 3 µl of DNA template. The amplification program was as follows: preheating at 94°C for 2 min, followed by 40 cycles at: 94°C for 45 s, 55°C for 45 s, and 72°C for 60 s. After these cycles, the reaction was maintained at 72°C for 7 min and then cooled to 4°C. Five microliters of the PCR products were electrophoresed in a 1.5% agarose gel and were subsequently visualized by UV illumination after ethidium bromide staining.

3' position 8-27 (using the *Escherichia coli* numbering system) and 536R [5' GTA TTA CCG CGG CTG CTG 3'] position 519- 536 were used to amplify the 5' region of the 16S rDNA gene (Yeung *et al.*, 2002). PCR was performed in Biometra PCR System. For each reaction, a 50-µl reaction mixture was prepared. The reaction mixture contained 1× buffer without MgCl<sub>2</sub> (Promega Corp., Madison WI), 1.5 mM MgCl<sub>2</sub>, 20 µM dNTP, 0.1 µM primers PAF and 536R, 1.5 U Taq Polymerase (Promega Corp.), and 3 µl of DNA template. The amplification program was as follows: preheating at 94°C for 2 min, followed by 40 cycles at: 94°C for 45 s, 55°C for 45 s, and 72°C for 60 s. After these cycles, the reaction was maintained at 72°C for 7 min and then cooled to 4°C. Five microliters of the PCR products were electrophoresed in a 1.5% agarose gel and were subsequently visualized by UV illumination after ethidium bromide staining.

## 2.4 Sequencing of 16S rDNA gene

Sequencing reactions were performed in a MJ Research PTC-225 Peltier Thermal Cycler using a ABI PRISM BigDyeTM Terminator Cycle Sequencing Kits with AmpliTaq DNA polymerase (FS enzyme) (Applied Biosystems), following the protocols supplied by the manufacturer. Single-pass sequencing was performed on each template using PAF Primer [5' AGA GTT TGA TCC TGG CTC AG 3'] position 8-27 and 536R [5' GTA TTA CCG CGG CTG CTG 3'] primer. The fluorescent-labeled fragments were purified from the unincorporated terminators with an ethanol precipitation protocol. The samples were resuspended in distilled water and subjected to electrophoresis in an ABI 3730xl sequencer (Applied Biosystems).

## 3. Results and Discussion:

In the present study, molecular identification of the isolates under study (*Bifidobacterium* and *Lactobacillus*) was performed. Significant value of these strains as an immunomodulator and stimulator of immune responses and also for the fact that they are opportunistic pathogens, especially in immunodeficient hosts as Probiotics appear to be innocuous for immunocompetent hosts and bacteria closely related to probiotic species have been associated with infections in patients. For example, *Streptococcus* spp. and *Lactobacillus* spp. have been isolated from patients with heart valve replacements who have endocarditis (Balish *et al.*, 1997). Lactic acid bacteria and bifidobacteria are increasingly being administered to pregnant women and infants with the intention of improving health. Although these organisms have a long record of safe use (Morgan *et al.*, 2010).

Amplification of conserved region 16S rDNA using PAF and 536-R primers is shown in figure (1), both the target bands for *Bifidobacterium* and *Lactobacillus* were appeared around 500 bp which was in agreement with data indicated by (Yeung *et al.*, 2002) who used the same primers with 26 different strain of *Lactobacillus* and *Bifidobacterium*. On the other hand, The PCR sequencing of a 470-bp fragment of the 16S rRNA gene, using primers plb16 and mlb16 (positions 8 to 27 and 507 to 526 in the 16S rRNA gene sequence of *Escherichia coli*, respectively) was used to identify the bifidobacteria at the species level (Rodríguez *et al.*, 2009).

Sequencing result in Figure (2) of the 518 bp DNA segment shows a high GC content in the *bifidobacterium* sample which might be studied in future for possible immune-regulation activity in mammals through out the CpG island of the foreign bacterial DNA. This might be the case as many studies (Koo and Rao, 1991) examined the effects of administration of both *bifidobacteria* (*B. pseudolongum*) and 5% neosugar [fructooligosaccharide (FOS)] to female mice given DMH resulted in 50% as many AC as in control animals at 18 and 38 weeks.

Furthermore, Figure 4 shows alignment of the base sequence “FASTA format” using the blasting tool on <http://www.ncbi.nlm.nih.gov> shows 91% homology, a high score of 407 and expected value 3e-113 with Uncultured *Bifidobacterium* sp. Clone R333 16S ribosomal DNA gene. Figure 5 demonstrates the genetic relationship between the Egyptian *Bifidobacterium* isolate with other control

samples in a dendrogram which relies on the results of the partial 16S rDNA analysis.

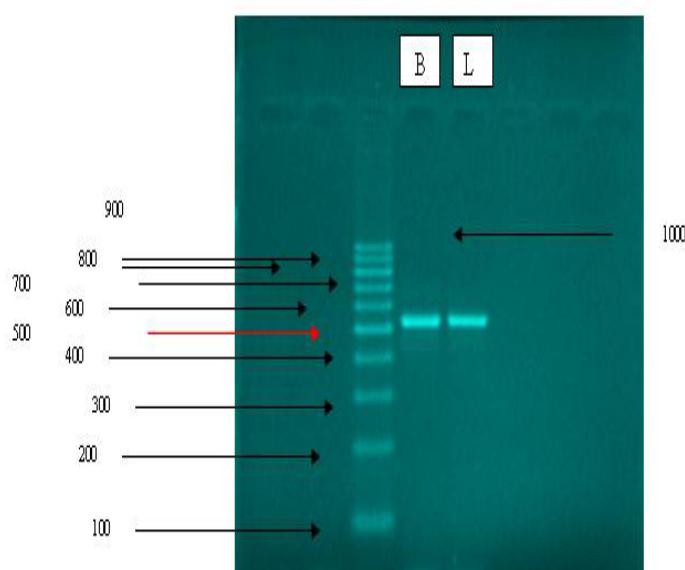
On the other hand, the sequence of the partial 16S rDNA gene 673 bp for the isolated *Lactobacillus* is illustrated in Figure 3. *Lactobacillus* sequence results were aligned using BLAST tool which is illustrated in Figure 5, result shows homology of 88%, 577 score and expected value 7e-164 with *Lactobacillus kefiransaceins* and is illustrated by a dendrogram in Figure 6.

#### 4. Conclusion

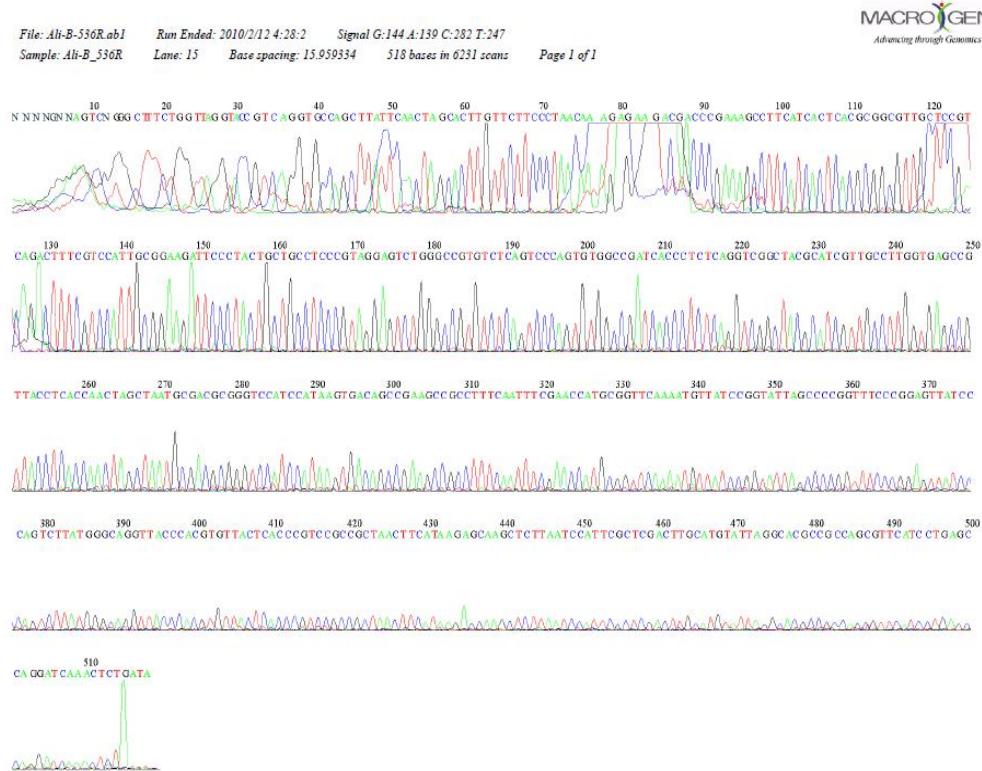
The study describes the molecular identification of two Egyptian isolates *Bifidobacterium* and *Lactobacillus* using species specific primers for amplification of 16S rDNA and comparing them with standard strains after alignment using blast tool on <http://www.ncbi.nlm.nih.gov>, bioinformatics analysis and dendrogram study of partial 16S rDNA gene showed a homology of 91% for the *Bifidobacterium* samle with Uncultured *Bifidobacterium* sp. Clone R333 and 88% homology with *Lactobacillus kiransaceins* for the *Lactobacillus* isolate.

#### Acknowledgment

The research was supported by the College of Biotechnology, College of Pharmacy, Misr University for Science and Technology (MUST) together with Faculty of Medicine, Assiut University, Egypt. Moreover, the bioinformatics analysis was carried out by the help of Mohammed Ezz, research assistant at College of Biotechnology, MUST.



**Figure 1: 16S rDNA amplification using F-PAF and 536-R primers, 16S rDNA is appeared at 518 bp and 534 bp for *Bifidobacterium* (B) and *Lactobacillus* (L) respectively.**



**Figure 2:** Sequencing of partial 16S rDNA gene of *Bifidobacterium* isolate.



**Figure 3:** Sequencing of partial 16S rDNA gene of *Lactobacillus* isolate.

**Sequences producing significant alignments:**  
(Click headers to sort columns)

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
AF429517.1	Lactobacillus kefiranofaciens ATCC 43761 16S ribosomal RNA gene	577	577	70%	7e-164	88%
DQ298155.1	Lactobacillus sp. SWM_Isolation_3 16S ribosomal RNA gene	555	555	74%	3e-157	86%
DQ141558.2	Lactobacillus plantarum strain HDRS1 16S ribosomal RNA gene	468	468	67%	4e-131	85%
DQ486145.1	Lactobacillus plantarum strain SFCB2-7c 16S ribosomal RNA gene	468	468	67%	4e-131	85%
AY230227.1	Lactobacillus plantarum strain K14 16S ribosomal RNA gene	468	468	67%	4e-131	85%
AY230221.1	Lactobacillus plantarum strain H19 16S ribosomal RNA gene	468	468	67%	4e-131	85%
AY230220.1	Lactobacillus plantarum strain H 16S ribosomal RNA gene	468	468	67%	4e-131	85%
AB365977.1	Lactobacillus capillatus gene for 16S rRNA, partial sequence	466	466	74%	1e-130	83%
AB112083.1	Lactobacillus plantarum gene for 16S ribosomal RNA, partial sequence	466	466	67%	1e-130	85%
AY230225.1	Lactobacillus plantarum strain H8 16S ribosomal RNA gene	464	464	67%	5e-130	85%

**Figure 4: Alignment of partial 16S rDNA gene using blast for Lactobacillus isolate.**

**Sequences producing significant alignments:**  
(Click headers to sort columns)

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
FJ518641.1	Uncultured Bifidobacterium sp. clone R333 16S ribosomal RNA gene	407	407	57%	3e-113	91%
FJ518676.1	Uncultured Bifidobacterium sp. clone R330 16S ribosomal RNA gene	287	287	44%	3e-77	88%
AB437351.1	Bifidobacterium psychraerophilum gene for 16S ribosomal RNA gene	265	265	81%	2e-70	78%
NR_029065.1	Bifidobacterium psychraerophilum strain T16 16S ribosomal RNA gene	250	250	79%	5e-66	78%
AB507085.1	Bifidobacterium breve gene for 16S rRNA, partial sequence	246	246	86%	6e-65	77%
FJ518643.1	Uncultured Bifidobacterium sp. clone R339 16S ribosomal RNA gene	246	246	43%	6e-65	86%
AY735402.1	Bifidobacterium breve strain BR2 16S ribosomal RNA gene	246	246	86%	6e-65	77%
AB507110.1	Bifidobacterium sp. JCM 7013 gene for 16S rRNA, partial sequence	241	241	86%	3e-63	77%
EF589112.1	Bifidobacterium longum strain IDCC 4101 16S ribosomal RNA gene	241	241	82%	3e-63	77%
AY850360.1	Bifidobacterium breve strain BGM6 16S ribosomal RNA gene	241	241	86%	3e-63	77%

**Figure 5: Alignment of partial 16S rDNA gene using blast for Bifidobacterium isolate.**



**Figure 6: Dendogram analysis for partial 16S rDNA gene of Bifidobacterium isolate, main tree.**

**Corresponding author**

Hashem S.

<sup>1</sup>Microbiology Dept., College of Medicine, Assiut University, Assiut, Egypt**5. References:**

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9/3/2010

## Maturation and Histological characteristics of ovaries in Mudskipper, *Periophthalmus papilio* from Lagos lagoon, Nigeria.

LAWSON, Emmanuel Olugbenga

Department of Fisheries, Faculty of Science, Lagos State University, Ojo.  
P.O. Box 001, LASU Post Office Box, Lagos, Nigeria.  
[ollulawson@yahoo.com](mailto:ollulawson@yahoo.com).

**Abstract:** Maturation and histological characteristics of female gonads in mudskipper, *Periophthalmus papilio* from Lagos lagoon, Nigeria were investigated between July 2004 and July 2006. This species is found in abundance in the mud flats of the mangrove swamps of Lagos lagoon where it forms part of its fisheries. Its importance lies on its availability as food for man and as baits for both artisanal and offshore fisheries. Diurnal collections were made with non return valve traps. Biometric data were recorded and sexes separated. Ovaries were carefully removed from 1390 individual specimens that were with no abnormalities or pathological changes. The histological structure of the ovaries was based on a temporal scale after intensive sampling. The ovaries were observed macroscopically and processed by standard histological technique. ICES, BITS and IBTS scales and Bucholtz manuals were employed in the classifications of its maturity and gonadal stages. Seven stages of maturity which included: immature (stage I), immature and developing (stage II), ripening (stage III), ripe (stage IV), ripe running (Stage V), spent (stage VI) and recovering-spent (stage VII) were observed among the specimens. These constituted 1.15, 47.99, 15.32, 9.86, 19.50, 4.68 and 1.51% of the specimens examined in the study respectively. The pre-spawning phase was represented by stages I, II and III; the spawning by IV and V; and post-spawning by VI and VII. Histological development of the species indicated six (6) developmental stages of oocytes development viz: oogonium, primary oocyte, primary, secondary, and tertiary vitellogenic and hyaline oocytes. Specimens were found with oocytes which had developed over the migratory nucleus stage, indicating maturation can still proceed in the fish on the mudflats before migrating to spawning nests in the burrows. Stages V and VI ovaries contained all stages of oocyte. The GSI of the species increased at initial phase and then became stable at the later period. The species was a multiple and synchronous spawner, spawning in February, March, and October. The mean GSI varied from  $1.03 \pm 0.09\%$  in May to  $8.40 \pm 1.67\%$  in February 2006. Less than  $8.40 \pm 1.67\%$  % of the body biomass was converted by the species to development of ovaries. The minimum size of spawning females was 110 mm TL. Therefore, this study provides the necessary information on maturation and histological development of oocytes as an appropriate strategy for optimum utilization and conservation of this commercially valued fish species in Lagos lagoon, Nigeria.

[LAWSON, Emmanuel Olugbenga. Maturation and Histological characteristics of ovaries in Mudskipper, *Periophthalmus papilio* from Lagos lagoon, Nigeria. Journal of American Science 2010;6(11):965-976]. (ISSN: 1545-1003).

**Key words:** Chromatin, zona radiata interna, externa, maturation, nucleolar, vitellogenic.

### 1. Introduction

Mudskipper, *Periophthalmus papilio* (Bloch and Schneider 1801) is a member of the family Periophthalmidae. It is the only reported species of the family in the Gulf of Guinea, which includes Lagos lagoon in Nigeria (FAO, 1990; Lawson, 1998), where it has been reported in large number (Etim *et al.* 1996; King and Udo, 1996; Udo 2002). Irvine (1947) grouped *Periophthalmus* into indigenous or permanent element of the brackish waters of estuaries and lagoons. Other related species found in other parts of the world include: *P. chrysospilos* in Singapore (Ip *et. al.* 1990), and *P. koelreuteri* in East Africa. *Boleophthalmus boddaerti* and *B. woberi* are found inhabiting estuary of Pasir Ris in Singapore. Importance of this fish lies on its commercial value as food especially in Niger Delta region and as bait in

artisanal and offshore fisheries. It is reported to cost as high as \$20/kg in Taiwan and Japan (Khaironizam and Norma-Rashid (2002). Reviews on the *P. papilio* include that of King and Udo (1996) on its length-weight relationships; Etim *et al* (1996) gave a report on its population dynamics in Eastern Nigeria; and Lawson (1998) documented the aspects of its bioecology; its distribution, age determination, and growth patterns (Lawson, 2004a); its salinity tolerance and preference (Lawson, 2004b); and its blood osmolality contents (Lawson, 2004c). Aspects of its food and feeding habits (Lawson, 2004d); length-weight relationships and fecundity estimates (Lawson, 2011) were also investigated in Lagos lagoon, Nigeria. Several reviews on the maturation, histological and ultrastructural characteristics of non related species include that of Marcus (1982) on

Clupeid, *Ilisha africana*; and Ugwumba (1984) on the ten pounder, *Elops lacerta* off Nigerian coasts. Reviews from other parts of the world include that of Washio *et al* (1993) on Mudskipper, *Boleophthalmus pectinirostris*; Assem (2000) on Carangid, *Caranx cryos*; Grier (2000) on Common snook, *Centropomus undecimalis*; Srijuungam and Wattanasirmkit (2001) on Nile tilapia, *Oreochromis niloticus*; Assem (2003) on *Pagellus erythrinus*, Okuthe *et al.* (2004) on freshwater shrimp, *Caridina nilotica*; Valdés (2004) on Common pandora, *Pagellus erythrinus*, Ito (2005) on Pejerrey, *Odontesthes bonariensis*; Garcia-Diaz *et al* (2006) on Black comber, *Serranus atricauda*; Ortiz-Ordóñez (2006) on the butterfly goodeid, *America splendens*; Honji *et al* (2006) on Argentine hak, *Merluccius hubbsi*, Koç (2007) on Chub, *Leuciscus cephalus*; Bucholtz *et al* (2008) on Baltic herring, *Clupea harengus*; Lawson and Jimoh (2010) on Grey mullet, *Mugil cephalus*, Mohamed (2010) on Gadidae fish, *Merluccius merluccius*, and Saeed (2010) on Kutum, *Rutilus frisii kutum*. Guraya (2000) reported biology of gonad development, sex differentiation and maturation, and sex reversal in fish at cellular, molecular and endocrinological levels. Several studies from other teleosts showed that histological analysis of gonadal development is the most accurate methodology to determine the individual stage of sexual maturation, exhibiting more consistent results than visual staging of reproductive organs (Murua and Motos, 1998; Saborido-Rey and Junquera, 1998; Kjesbu *et al.*, 2003; Tomkiewicz *et al.*, 2003).

Histological study of this species though very strenuous is very essential especially in reproductive system. It is the most accurate method to determine the reproductive state of female fish (West, 1990). Therefore, the study on histology of ovaries of fish will provide a basic knowledge of reproductive system of fish and will be a useful tool for further applications in other species. This study has sought to investigate maturation and characterize the histology and ultrastructure of the ovary in mudskipper, *P. papilio* from the mangrove swamps of Lagos Lagoon, Nigeria.

## 2.0 Materials and Methods

### 2.1 Collection of specimens:

1390 female individuals of mudskipper, *Periophthalmus papilio* were caught from the mudflats of Lagos lagoon (longitude: 3°20'-3°50'W and latitude: 6°24'-6°36'N) between July 2004 and July 2006. The diurnal collections were carried out with non return valve traps. Services of artisanal fishermen were employed.

### 2.2. Laboratory procedures and data collections:

In the laboratory, collections of biometric data such as sex, total length (TL) and body weight (BW) measurements were carried out, TL to the nearest 1 mm and BW to the nearest 0.1 g. The specimens were examined for abnormality or pathological changes and were cut opened through the ventral position. Sexes and gonad maturity stages were ascertained by naked eye examination of the gonads and were confirmed under the light microscope. Ovaries were removed from the specimens considered to be females, the paired ovaries were weighed (GW) to the nearest 0.1 g. The ovaries were fixed in Bouin's fluid. Sections were taken from the middle part of each ovarian lobe, dehydrated in alcohol, cleared in xylene, and impregnated in paraffin wax between 52-60 °C melting points. They were embedded in paraffin wax and sectioned at 6 µm thick. The sections were stained in Ehrlich haematoxylin and Eosin (H&E) following Belelander and Ramaley (1979). Microscopic observations of the ovaries were done under binocular microscope that was mounted with camera and photographs taken.

To determine the individual stage of sexual maturation, visual staging of reproductive organs was applied. The description of macroscopic criteria was developed by comparing the histological results with the photographic records of the ovaries. Maturity stages were evaluated using scales from which each gonad was judged by visual analysis of external features. Sexual maturity of each specimen was classified according to macroscopic scales used in the IBTS (International Bottom Trawl Survey), BITS (Baltic International Trawl Survey), ICES (International Council for Exploration of the Sea of 1963, 1999) and recently, Bucholtz *et al* (2008) manual, and as well using a microscopic scale, based on histological analysis (Vitale *et al.*, 2005). The microscopic criteria applied in the classification of ovarian development were based on oocyte characteristics such as the formation of cortical alveoli, degree of yolk accumulation and nuclear migration. This microscopic classification underlines the importance of the passage from endogenous to exogenous vitellogenesis, which coincides with the beginning of yolk production in the oocytes.

The gonadosomatic index (GSI) of the fish was calculated by dividing the ovaries weight by the whole body weight and multiply by 100. Thus:

$$\text{GSI} = \frac{\text{GW} \times 100\%}{\text{BW}}$$

### 3.0 Results

#### 3.1 The structure of ovary in *P. papilio*.

The morphology of ovaries in different developmental stages is presented in Figure 1. Ovary of *P. papilio* was observed to be a paired, elongated bodies situated in the posterior half of the body cavity and suspended from the body wall by the mesovarium. Anteriorly, the two lobes were free but posteriorly they bent downwards and inwardly to form a short oviduct leading to the genital pore. The length, width, and colour of ovaries were seen changing as maturity progressed due heavy vascularization. The colour turned yellow on

maturity and reddish when the fish were ready to spawn (in stages IV and V). Stage I ovaries were not represented because they were not discernible enough to be classified as males or females

#### 3.2. Macroscopic characteristics of ovaries.

Macroscopically ovaries in *P. papilio* were classified into seven (7) developmental stages (Table 1). The stages were classified as Immature (Stage I), Immature and Developing (Stage II), Ripening (Stage III), Ripe (Stage IV), Ripe running (Stage V), Spent (Stage VI), and Recovering-spent (Stage VII).

Table 1: Macroscopic characteristics of ovaries in *P. papilio*

Maturity stage	Degree of maturation	External or macroscopic appearance of females
Stage I	Immature	The external examination did not show sexual differentiation, the gonads were rudimentarily developed and could not be differentiated as males or females. Hence the specimens were classified as immature. 16 specimens were observed as immature.
Stage II	Immature and developing	The ovaries were small, rounded with a rough surface and soft texture. They were pinkish in colour, translucent with blood vessels forming internally, and occupying between 1/8 <sup>th</sup> (12.5%) and 1/4 <sup>th</sup> (25.0%) of the length of the abdominal cavity. None of the oocytes were visible.
Stage III	Ripening	The ovaries were swollen and lobed. A heavy network of vessels appeared externally on the surface of the ovarian wall. Yellowish oocytes were visible to naked eye through the ovarian wall. The gonad extended for about 60 – 70% of the abdominal cavity.
Stage IV	Ripe	Ovaries at this stage were almost filling the body cavity occupying 80 – 90% of abdominal cavity. They were orange yellowish in colour. The shedding of eggs has not commenced and otherwise soft. The eggs were rounded with a rough granular surface given a hollow sac like appearance. Blood vessels coalesced to form larger ones on the external surface of the ovary wall. Yellowish colour was possible due to the large yellow oocytes that were visible through ovary wall.
Stage V	Ripe running	The eggs flowed from the vent on slight pressure and the ovary occupied 99% of the abdominal cavity and rendered alimentary canal and gut almost inconspicuous.
Stage VI	Spent	The red ovaries were flaccid and vascularized with reduced size, the ovarian wall was tough and smooth with no granulation. The residual eggs were visible through the flabby wall. The ovary length: width ratio was 4.5 and the gonads occupied 50% of the abdominal cavity. There were large numbers of surface blood vessels.
Stage VII	Recovering-spent	Externally, ovaries were firmer than spent stage but mainly red in colour. It occupied 60% of the body cavity and none of the residual oocytes were visible through the ovary wall.

#### 3.3. Comparison of present study with other maturity scales.

Table 2 describes the conversion of the scale developed in this study to the scales of Bucholtz et al (2008), and ICES (1963) and as well as the scales used for the BITS and IBTS surveys. The ICES scale is commonly used in most laboratories, the BITS and IBTS scales were similar. Also similar were Bucholtz et al (2008) and ICES (1963) scales except the addition

of abnormal stage in former covering a stage of reproductive malfunction (stage VII). However, these scales were modified and simplified in this study for better understanding of the histology of this species and other teleosts. Common to all these scales were a recovering-spent stage which encompassed the final recovery of the spent gonad as well as the beginning of a new maturation cycle.



**Figure 1:** Morphology and gonadal stages (II-VII) in female *P. papilio* from Lagos lagoon, Nigeria.

II, immature and developing; III, ripening; IV, ripe;  
V, ripe running; VI, spent; VII, recovering-spent.

Table 2. Comparison of the present scale with other maturity scales currently in use.

Scale generated from the present study	Current maturity scales in use			
	Bucholtz et al 2008	ICES	BITS	IBTS
I. Immature	I. Juvenile	I. Virgin	I. Virgin	I. Immature
II. Immature and Developing	II. Early maturation	II. Virgin maturing VII. Recovering- spent		
III. Ripening	III. Mid maturation	III. Maturing		
IV. Ripe	IV. Late maturation	IV. Maturing	II. Maturing	II. Maturing
V. Ripe running	V. Spawning capable	V. Maturing		
VI. Spent	VI. Spawning	VI. Spawning	III. Spawning	III. Spawning
VII. Recovering- spent	VII. Spent-recovery VIII. Abnormal	VII. Spent	IV. Spent V. Resting	IV. Spent

ICES, International Council for Exploration of the sea; BITS, Baltic International Trawl Survey; IBTS, International Bottom Trawl survey.

### 3.4. Histological characteristics of Ovaries in *P. Papilio*.

The histological characteristics of the ovaries of this species in their different developmental stages are represented with photomicrographs in Figure 2A-F.

#### 3.4.1 Immature and developing stage:

Histological appearance (Figure 2A) of the ovary was characterized by the presence of many oocytes between 0.025 and 0.05 mm. The larger oocytes were seen with cytoplasm vacuoles and were irregularly shaped but few were rounded. The thickness of the ovarian wall was 50 µm and folded. Empty follicles and space were visible.

667 specimens belong to this category.

#### 3.4.2 Ripening:

The histological observation of the ovaries at this stage showed that many oocytes between 0.1 and 0.2 mm were present. Larger oocytes with cytoplasmic vacuoles were very few and had small yolk droplets (Figure 2B). The primary and secondary vitellogenic oocytes dominated while tertiary vitellogenic oocytes were represented in the gonad. The cytoplasm of larger oocytes was filled with densely staining yolk granules.

The ovarian wall was 70 µm thick. N=213.

#### 3.4.3. Ripe:

The histological observation of the gonads showed that the secondary and tertiary vitellogenic oocytes dominated the gonad with very few primary oocytes (Figure 2C). The *theca externa* were prominent. The hyaline oocytes were present but

usually collapsed by histological processing. Ovary wall was 90  $\mu\text{m}$  thick; many oocytes were between 0.2 to 0.5 mm in diameter and usually 0.35 mm in size. Many oocytes were at stages II and III. There were blood vessels internally but some of the yolk oocytes were atretic. N=137.

#### 3.4.4. Ripe running:

Oocytes looked exactly like those in the ripe stage and were laid singly with space (septa) in between as shown in Figure 2D; most of the oocytes were in their tertiary vitellogenic stage. N=271.

#### 3.4.5. Spent:

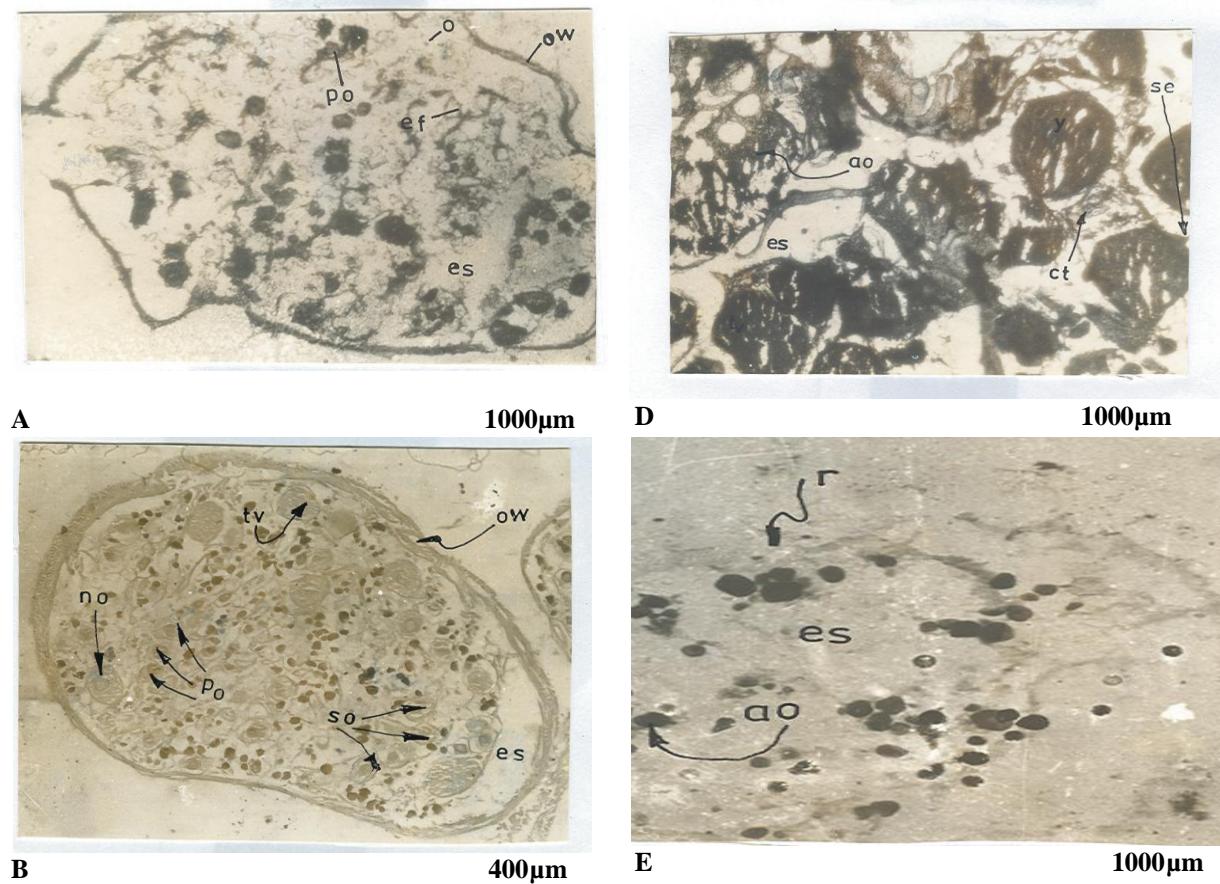
A few atretic residual oocytes were seen, the invasion of oocytes by follicular cells was noted (Figure 2E). High level of oocyte atresia was noted.

There was disorganization of septum, no empty follicular coat. The ovarian wall was 300  $\mu\text{m}$  thick while the lumen contained debris of the residual cells N=65.

#### 3.4.6. Recovering and resting:

The residual atretic oocytes were present but the septum was not very organized (Figure 2F). Reorganization of ovigerous lamellae started. A few reabsorbing oocytes were also present. N=21.

The vascularized and ripe stage oocytes showing different developmental characteristics are present in Figure 3. The six (6) oocyte developmental stages in this study included: oogonium, primary oocyte, primary, secondary, tertiary vitellogenic and hyaline oocytes.



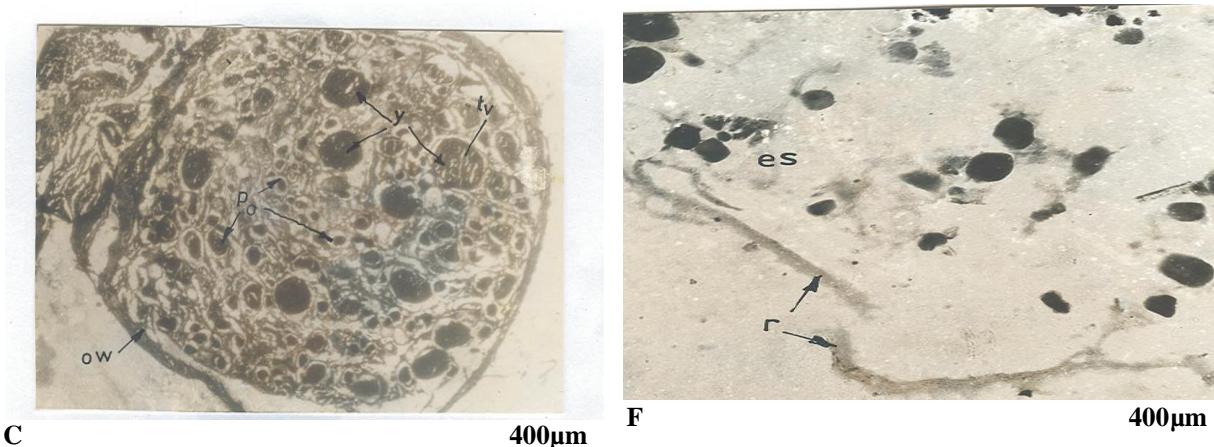


Figure 2. Photomicrographs of ovaries in their various maturation stages in *P. papilio* from Lagos lagoon, Nigeria. A: A section through an ovary in immature and developing; B: An ovary in ripening stage; C: A section through a ripe stage ovary; D: An ovary showing tertiary vitellogenic or ripe oocytes in ripe running stage; E: A section through an ovary in spent stage; F: An ovary in recovering-spent stage.

o, oogonium; po, primary oocyte; es, empty space; ef, empty follicle; ow, thick ovarian wall; s<sup>o</sup>, secondary oocyte; no, nucleolus; y, yolk; pv, primary vitellogenic oocyte; tv, tertiary vitellogenic oocyte; ga, gap between ovigerous fold; of, ovigerous fold; bv, blood vessel; ha, haline oocyte; ct, connective tissue; se, septum; ao, atretic oocyte; r, rupture ovarian wall.

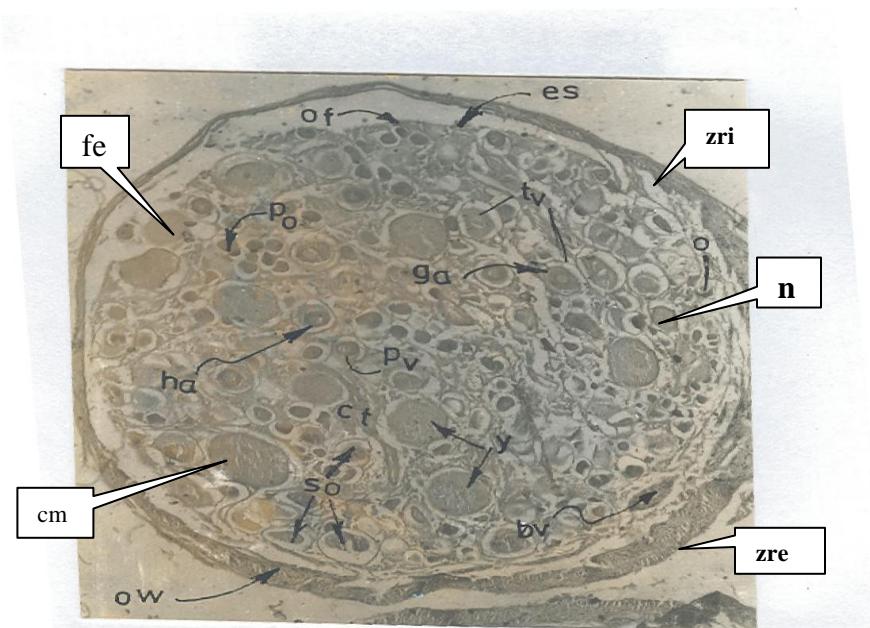


Figure 3. The vascularized and ripe stage oocytes showing different developmental characteristics. s<sup>o</sup>, secondary oocyte; po, primary oocyte; pv, primary vitellogenic oocyte; es, empty space; ow, thick ovarian wall; tv, tertiary vitellogenic oocyte; ga, gap between ovigerous fold; o, oogonium; of, ovigerous fold; bv, blood vessel; ha, haline oocyte; y, yolk; ct, connective tissue; cm, chromatin; fe, follicular epithelium layer; zri, zona radiata interna; zre, zonal radiata externa; n, nucleus.

### 3.5. Reproductive cycle and maturity stages

In the present study seven stages of maturity were developed and validated (Figure 4). These stages were grouped into three phases as presented in Figure 4. The phases were (a) Pre-spawning phase which included stages I-III ovaries; (b) Spawning phase, the stages IV

and V; and (c) post-spawning phase which were stages VI and VII. The reproductive cycle of *P. papilio* in Lagos lagoon started from stage I and ended at stage VII then back to stage II, or from the pre-spawning through spawning to post spawning, back to pre-spawning phase in cyclic manner.

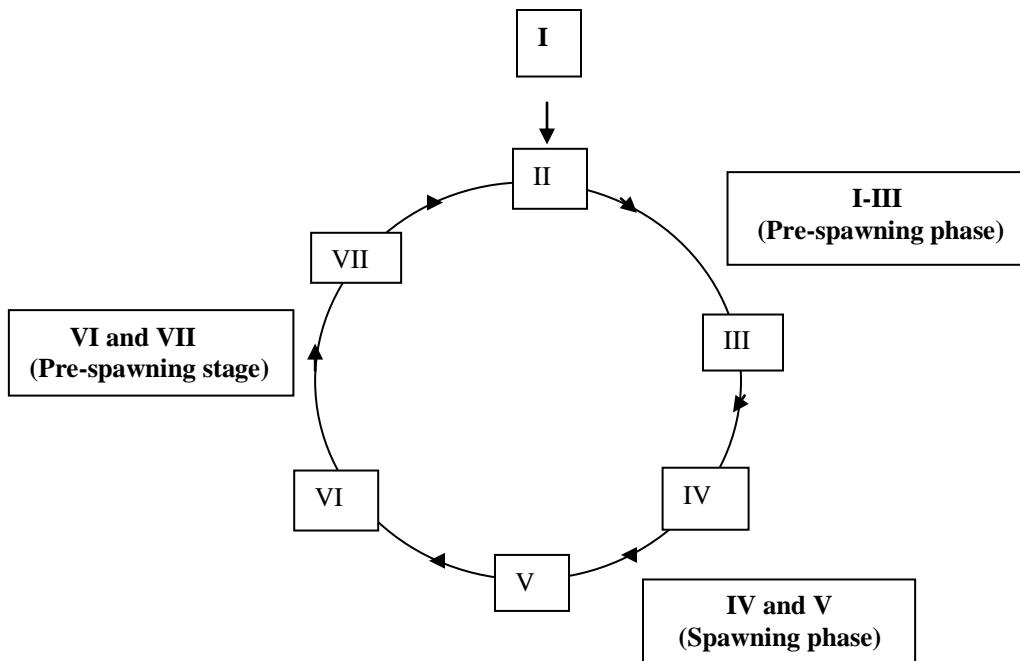


Figure 4. Reproductive cycle and maturity stage in *P. papilio* from Lagos lagoon, Nigeria.

### 3.6. Distributions of maturity stages and phases in *P. papilio*.

Of the seven (7) maturity stages and three (3) maturation phases (Figure 5) encountered in the study, The least dominant group was stage I and stage II fish were the most abundant constituting 1.15 and 47.99% of the population respectively. The pre-spawning, spawning and post spawning phases were 64.46, 29.36 and 6.19% respectively. The pre-spawners were more in number than the spawning or post-spawning fish.

### 3.7. Gonadosomatic index of *P. papilio*

Monthly changes in GSI of the fish were presented in Figure 6. GSI were high in August and October 2004. The lowest GSI value of  $1.03 \pm 0.09\%$  was recorded in May and was at the peak ( $8.4 \pm 1.67\%$ ) in February, 2005. In 2005, GSI began increasing from January ( $6.36 \pm 1.23\%$ ), and these values represented the changes similar to those of 2006, although the GSI value in February 2005 that was high was significantly lower in 2004 and 2006 than those in 2005 (t-test,  $P < 0.05$ ).

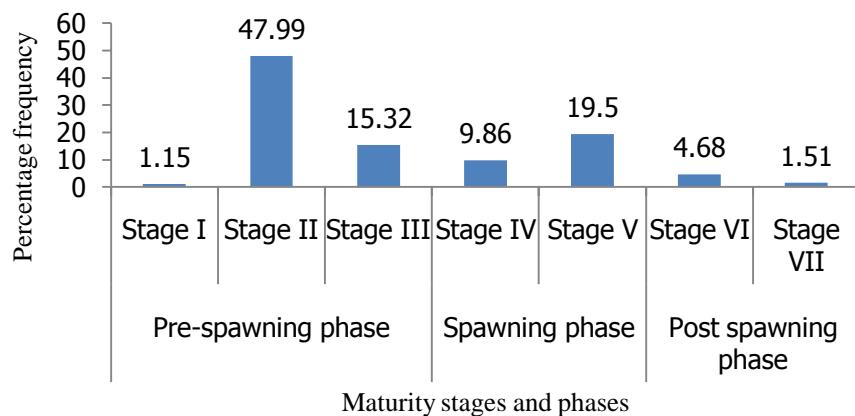


Figure 5. Histograms of percentage frequency distributions of maturity stages and phases in females *P. papilio* from Lagos lagoon, Nigeria.

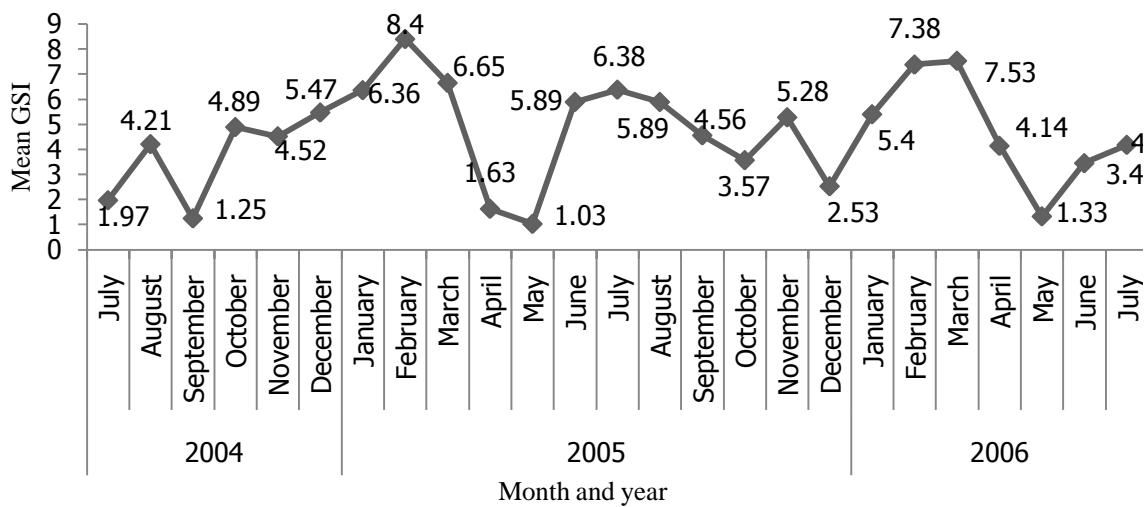


Figure 6. Monthly mean GSI in females *P. papilio* from Lagos lagoon, Nigeria.

#### 4. Discussion

In this study, seven stages of gonadal development were observed in mudskipper, *Periophthalmus papilio* (Table 1). The stages were immature, immature and developing, ripening, ripe, ripe running, spent and recovering-spent. Immature fish were those that were unable to be differentiated both micro and macroscopically as males or females. Most of the specimens were at pre-spawning phase. Fewer fish populations at the spawning and post spawning phases was an indication that the fish had

not migrated away from their spawning nests in their burrows.

The macroscopic characters and gonad differentiations occurred as maturation progress from a stage to next. Vascularisation and identification also increased with progression in size and maturity. Immature, immature and developing and ripening stage were categorized as pre spawning period, i.e a period when the fish were virgin, or maturing or were in their early or mid or late maturation phase. A scale

generated from the present study was a modification of the ICES, BIT, and IBTS scales that were used in the current study (Table 2). The general pattern of histological development of the ovaries of the present study conforms to that of the most teleosts (El-Gharabawy, 19996; Assem, 2000 and 2003). A 4-stage maturity scale was generated by IBTS, 5 by BITS, 7 by ICES and 8 by Bucholts et al 2008 for Herring and Cod. These scales were reportedly applied in histological study of many teleosts. The maturity stages are hardly discernible by the naked eye and consequently the most susceptible to misclassification.

The six (6) developmental stages (Figure 3): oogonium, primary oocyte, primary, secondary, tertiary vitellogenic and hyaline oocytes were represented the various stages of the oocyte growth and development in *P. papilio*. This also confirmed progressive process in stages of formation, growth or development of eggs (oogenesis). These developmental stages were well documented by Gardner and Snustad (1984). Oocyte is the mother cell, the cell that undergoes two meiotic divisions to form the egg cell. The primary oocyte occurs before the completion of the first meiotic division; second oocyte, after the completion of the first meiotic division. Oogonium is a germ cell of the female before meiosis begins. Oogenesis in fish according to Jackson and Sullivan (1995) is accompanied by conspicuous cellular, biochemical, molecular and endocrinological changes.

The present study confirmed that the maturation period in *P. papilio* was characterized by appearance of isolated follicular epithelial cell around the oocyte and formation of yolk nuclei. The yolk nuclei appear first as a small spherical corpuscle in close adherence to one side of the nucleus and then migrate to the periphery of the cytoplasm, where it finally disintegrates and disappears. This was in agreement with reports of Mohamed (2010) on *M. merluccius*. Herrera et al (1988) pointed out that the follicular epithelial cells are considered as a good proof for synthesis of sexual steroids in fish.

The vacuolization period may be characterized by the presence of marginal vacuoles and by the fact that the oocyte wall consisted of *zona radiata* coated with follicular epithelial layer (Mohamed, 2010). Grant (1990) characterized the vacuolization stage by cortical alveoli formation.

The yolk deposition as presented in Figure 3 was a period characterized by the presence of yolk granules in the periphery of the oocyte cytoplasm. The yolk deposition in the oocytes in *P. papilio* showed the same picture described by many authors for some fishes (El-Gharabawy, 1996; Assem, 2000

and 2003) most of cytoplasm is filled with yolk granules of various sizes.

Examination of the ovaries of this species in this study showed presence of oocytes at different stages of development. This is an indication that the fish has prolonged and fractional spawning season. Therefore, the fish may spawn more than once along the spawning period. This was supported by Salem et al (1994) for *Mugil seheili*, El-Greisy (2000) for *Diplodus sargu*, Honji et al (2006) for *Merluccius hubbi*; Garcia Diaz et al (2006) for *Serranus atricauda*; and Mohamed (2010) for *Merluccius merluccius*.

Teleosts attain sexual maturity at various ages depending on the species, latitude, water temperature, salinity. The age, at which fish living in a water body under natural environmental conditions (in regard to age and season) attain maturity depends on the latitude, the more south a water body in the northern hemisphere is found, the earlier the fish mature. The environmental factors such as temperature, photoperiod, nutrient supply, dissolved oxygen, diseases or parasites) are well known to influence reproductive maturity and oogenesis in fish (Cambray, 1994; Joy et al 1999). But the mechanism of action of various environmental factors as well as the sites of their action remains to be determined at the cellular and molecular levels.

The fish burrowed and spawned in the mud flats, this was responsible for fewer populations of the spawners and post spawning fish. Fish close to spawning phase enter the spawning nests and stayed there for some while even at spent stage. This may be reason for large number of pre-spawners than either spawning or post-spawning fish as reported in the present study. Nest spawning behavior was reported in *B. pectinirostris*, *P. cantonensis* and *P. modestus* by Uchida (1932); and Dotsu and Matoba (1977) in Ariake sound and Washio et al (1991) in Midori River, Kumamoto prefecture in Japan. The maturation following their migration to the spawning nest could also responsible for their inability to be collected with traps.

GSI values were higher in 2005 than in 2004 or 2006 (Figure 5), the difference according to Washio et al (1991) reports on mudskipper species, *B. pectinirostris* is closely related to the annual changes in reproduction. The GSI had been used to describe the development of gonads in Pike, *Esox lucius* by Danilenko (1983). However, determination of reproductive maturity using only the GSI is not enough because the structures within the ovary, such as oocytes at different stages, interstitial tissues with accumulation of yolk materials, can not be interpreted by weight (Srijunngam and Wattanasirmkit, 2001). GSI increases progressively

with increases in the percentages of ripe individuals towards the spawning seasons (Mohamed, 2010). The most common practice for determination of a species spawning season is the establishment of its GSI and the histological examination of the gonads (El-Greisy, 2000; Assem, 2000 and 2003; Honji *et al.*, 2006). High values of GSI for the months of October 2004 ( $4.89 \pm 1.06\%$ ); February 2005 ( $8.4 \pm 1.67\%$ ); and March 2005 ( $7.53 \pm 2.56\%$ ) demonstrated that the species was a multiple spawner and spawned several times within a spawning period. Less than  $8.40 \pm 1.67\%$  of the body mass was converted to gonad development in the fish. GSI varied with species, sex, seasons and availability of food and these were in conformity with reports from other teleosts (Lawson, and Aguda 2010; Lawson and Jimoh, 2010; Lawson *et al.*, 2010; Lawson, 2011) in some Nigerian waters.

Therefore, the study provides information on the maturation process and histological characteristics in a species of mudskipper, *P. papilio*, an economically valued fish from Lagos lagoon, Nigeria. There is an on going research work of the ultrastructural characteristics of the gonads in this species using a transmission electron microscope. The reports of the study will be reported in the next paper.

### Acknowledgements

Author acknowledges Department of Zoology, Fisheries and Marine Biology, University of Lagos, and Department of Fisheries, Lagos State University, Lagos, Nigeria for the use of their laboratories for this study.

### Correspondence to:

Dr. LAWSON, Emmanuel O.  
Department of Fisheries  
Lagos State University, Lagos  
P.O. Box 001, LASU Post office Box, Lagos, Nigeria

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12/10/2010

## Residual Available Copper and Boron in Soil as Affected by Zinc sulfate and Boric acid in a Zinc and Boron Deficient Soil

Farshid Aref

Department of Soil Science, Firouzabad Branch, Islamic Azad University, Iran

Tel: +989173383896, [farshidaref@yahoo.com](mailto:farshidaref@yahoo.com)

**Abstract:** Micronutrients such as copper (Cu) and boron (B) are needed in small amounts, and there are also likely to be residual effects for some years after their application. A field experiment with maize plant grown on Zn and B deficient soil was conducted to evaluate the effect of Zn and B interaction on the residual available Cu and B content in the soil during 2009 at Fars Province, Iran. Treatments including five levels of Zn (0, 8, 16 and 24 kg ha<sup>-1</sup> and Zn foliar spray) and four levels of B (0, 3, and 6 kg ha<sup>-1</sup> and B foliar spray) in a completely randomized block design were set up. The findings showed that the in all treatments, the residual available Cu and B in the soil increased compared to its initial levels (before culture). The main effect of Zn and B on the residual Cu was insignificant relative to the no Zn and B level. No treatments, showed a significant difference on the residual Cu in the soil as compared with the control and also the effect of Zn-B interaction on the residual Cu was insignificant. In most treatments, the residual B in the soil decreased compared to its initial level levels (before culture). The Zn-B interaction was significant on the residual available B content in the soil. The presence of Zn prevented from increase of the available B remaining in the soil by B use relative to the soil B content before culture. Application of a high amount of Zn in the soil decreased residual available B in the soil relative to the no Zn level.

[Farshid Aref. Residual Available Copper and Boron in Soil as Affected by Zinc sulfate and Boric acid in a Zinc and Boron Deficient Soil. Journal of American Science 2010;6(11):977-984]. (ISSN: 1545-1003).

Keywords: Interaction, Zinc, Boron, Copper, Residual available nutrients

### Introduction

Zinc (Zn) deficiency is a very important nutrient problem in the world's soils. Total Zn concentration is in sufficient level in many agricultural areas, but available Zn concentration is in deficient level because of different soil and climatic conditions. Soil pH, lime content, organic matter amount, clay type and amount and the amount of applied phosphorus fertilizer affect the available Zn concentration in soil (Adiloglu, 2006). Zinc is an essential nutrient for all plant crops. Chemically, Zn has some similarities with Fe and Mn, and in plant uptake there can be competition between these elements (Neue et al., 1998). Furthermore, high levels of phosphate in soils can strongly reduce Zn availability (Marschner, 1995). As regards agriculture, according to Cakmak (2002) Zn deficiency is the most widespread soil micronutrient deficiency in the world. Availability of Zn for plants is particularly low in calcareous and alkaline soils, while absolute Zn contents tend to be low in highly weathered acid tropical soils. Almost half of the agricultural soils from India, one third of the agricultural soils in China, and 50 per cent of cultivated land in Turkey are considered Zn-deficient for plants (Frossard et al., 2000; Gupta, 2005). Other more location specific studies report on low soil Zn contents in, for example, Iran (Aref, 2010). In spite of

the fact that the total amount of Zn in the soil is relatively high, but a small fraction of it is available to the plant. Numerous factors affect Zn availability, including the soil calcium carbonate content, which reduces the Zn availability in the soil (Mandal et al., 1992). Among the micronutrients, Zn deficiency is perhaps most extensive in the world. Zinc deficiency is most common in low- and high pH soils, low- and high organic matter, sandy, sodic, calcareous soils and waterlogged without ventilation (Takkar and Walker, 1993). Corn is among the plants most sensitive to Zn deficiency (Tandon, 1995).

Boron regulates transport of sugars through membranes, cell division, cell development, and auxin metabolism. Without adequate levels of B, plants may continue to grow and add new leaves but fail to produce fruits or seeds. A continuous supply of boron is important for adequate plant growth and optimum yields (Mahler, 2010). The total boron content of most agricultural soils ranges from 1 to 467 mg kg<sup>-1</sup>, with an average content of 9 to 85 mg kg<sup>-1</sup>. Available boron, measured by various extraction methods, in agricultural soils varies from 0.5 to 5 mg kg<sup>-1</sup>. (Gupta, 2007). It appeared that the percentage of soils deficient in B varied from 0 to 69 percent, thus suggesting that multiple micronutrient deficiencies at more localized level might be much more common

than based on only Zn, Fe, Cu and Mn (Nube, 2006). Boron deficiency is common in sandy and highly calcareous rich soils since there is an interaction between the Ca ion and the available B and high Ca levels at high pH reduces B uptake (Marschner, 1995). Soil texture, soil organic matter content, and soil moisture (annual precipitation, irrigation) are the three most important factors affecting boron availability in soils. Coarse textured soils (sands, loamy sands, sandy loams) that are low in organic matter are often low in plant-available boron. Boron deficiencies are especially pronounced in high rainfall areas (greater than 25 inches) where boron may have been leached from the soil profile. Over-irrigation may cause the same results. The availability of boron in the soil is also influenced by pH. Maximum boron availability occurs between soil pH 5 and 7 (Mahler, 2010). Copper is an essential element for plant growth. However, its presence in the soil in quantities lower or greater than the optimal amount could adversely affect plant growth (Tucker et al., 1995).

Soil fertility is an important factor, which determines the growth of plant. Soil fertility is determined by the presence or absence of nutrients i.e. macro and micronutrients. The availability of micronutrients is particularly sensitive to changes in soil environment. The factors that affect the contents of such micronutrients are organic matter, soil pH, lime content, sand, silt, and clay contents revealed from different research experiments. There is also correlation among the micronutrients contents and above-mentioned properties (Nazif et al. 2006). Iron, aluminum, and manganese oxides; organic matter; and phosphates, carbonates, and sulfides are important sinks for trace elements in soil–residual systems. The pH of the soil–residual system is often the most important chemical property governing trace element sorption, precipitation, solubility, and availability (Basta, 2005). In agriculture, micronutrients are an issue of increasing interest and concern. Many research activities are being undertaken which address the relationships between micronutrient provision to plants and associated crop growth, and trace elements such as Zn, Mn and Cu are increasingly recognized as essential when aiming for better yields (Mann et al., 2002; Bhadoria et al., 2003; Rashid and Ryan, 2004; Welch and Graham, 2004; Gupta, 2005; He et al., 2005).

The interaction among nutrient elements is very important for plant nutrition. Boron x Zn interaction among these interactions has been curcial in the Zn

deficient soils, in recent years (Alkan et al., 1998). Copper, Zn, Mn and P are Fe antagonists, and high levels of these elements in soils (or in fertilizer) can reduce Fe uptake by plants. Thus, information on extractable Fe, for example using DTPA4, is generally of much more relevance than information on the absolute levels of Fe contents in soils (Nube, 2006). The negative effect of high phosphorus suggest an induced deficiency of another element, possibly Zn, Cu, Fe or Mn (or a combination of these micronutrients), as P is known to have an antagonistic effect on these micronutrients. (Nube, 2006).

In a study in the China the residual effect of 1.1 kg B/ha remained fully effective in correcting B deficiency in oilseed rape for 2 years in the Inceptisols, whereas the residual effect of 1.65 kg B/ha continued to correct B deficiency for at least 3 years in both the Inceptisols and the Ultisol (Yang et al., 2000). Aref (2007) by studying the effect of Zn and B on the residual nutrients in the soil observed that P, Fe, Mn and Zn increased relative to its initial levels. Yang et al., (2000) reported that foliar application of B fertilizer generally corrected B deficiency for oilseed rape but showed limited residual effect in the following years after application.

In agriculture, very little research considers the entire range of micronutrients that are essential for plants (Nube, 2006). The critical level of Zn in the soil in  $\text{mg kg}^{-1}$  by DTPA extraction, has been reported by Darajeh et al. (1991) for corn to be 0.8, by Agrawala (1992), 0.8, Sharma and Lai (1993), 0.6, Terhan and Gerval (1995), 0.75. Lindsay and Norwell (1978), using DTPA extraction introduced critical limits of the soil Zn content as low if less than  $0.5 \text{ mg kg}^{-1}$ , medium if between  $0.5$  to  $1 \text{ mg kg}^{-1}$  and sufficient if more than  $1 \text{ mg kg}^{-1}$ . Nijjar (1990) has reported the critical B level by the hot water method in calcareous soils as  $0.5 \text{ mg kg}^{-1}$ . The critical level of Zn in the soil by DTPA extraction, has been reported by Agrawala (1992), 0.78, and Sims and Johnson (1991), 0.1-2.5  $\text{mg kg}^{-1}$ .

By measuring the residual nutrients in the soil after harvesting the crop, we can use many desired relations for better management in the culture. If the amount of the residual available element in the soil is at a high level, this represents less uptake by the plant or less fixation by soil particles and if it is at a low level, this represents more uptake by the plant or more fixation by soil particles. Of course, in addition to uptake and fixation, other factors such as uptake of

elements from unavailable form to available form or vice versa as well contribute to the amount of residual available element in the soil. Therefore, the objective of the study was examination of the effect of Zn and B on the residual available Cu and B in the soil after harvesting corn so that we are able to plan for Cu and B use in the next cultures.

### Materials and Methods

The field study was conducted at the research farm of Firouzabad University in Fars province of Iran, on the corn (*Zea mays L.*), cultivar "Single Cross 401" during 2009 cropping season. Composite surface soil samples (0-30 cm) were taken from the site before the experiment was initiated. This soil had a loam texture, pH of 8.4, 0.78 % organic matter, 210 mg kg<sup>-1</sup> exchangeable potassium (K), 9.9 mg kg<sup>-1</sup> available P, DTPA extractable Fe, Mn, Zn and Cu concentration were 1.4, 6, 0.38 and 1 mg kg<sup>-1</sup> and available B with hot water extractable was 0.9 mg kg<sup>-1</sup>. This experiment consisted of 20 treatments and 3 replications in the form of completely randomized block design and factorial that combinations of five levels Zn (0, 8, 16 and 24 kg ha<sup>-1</sup> Zn and Zn foliar spray) and four levels of B (0, 3, and 6 kg ha<sup>-1</sup> and B, and B foliar spray). Nitrogen: P: K used at 350, 200 and 200 kg ha<sup>-1</sup> according to the recommendation, from sources of urea, triple super phosphate and potassium sulfate, respectively, were added to all treatments (plots). Moreover, 50% of the urea was used when planting and the remainder two times: At vegetative growth (35 days after planting) and when the corn ears were formed. Potassium and P used before planting. Zinc and B, from zinc sulfate and boric acid sources, respectively, were used by two methods, adding to the soil and spraying. Addition to the soil was made at the time of plantation and the sprayings were made at 5 per thousand (0.5%) Zn sulfate and 3 per thousand (0.3%) B two times: one at vegetative growth stage and the other after corn ears formation. The Zn and B were both applied to the leaves with uniform coverage at a volume solution of 2500 L ha using a knapsack sprayer. Each experimental plot was 8m length and 3m width, had 5 beds and 4 rows, equally spaced, and seeds 20 cm apart on the rows. At the end of the growth stage (4.5 months after planting) the grain yield, dry matter and the residual available P and Zn in the soil after corn harvest were measured.

The soil samples were air-dried and ground to pass through a 2-mm sieve before analysis. Selected soil chemical and physical characteristics for the two

sites are presented in Table 1. Analysis of the soil was carried out using common lab procedures (Soil and Plant Analysis Council, 2004). Soil particle size distribution was determined by the hydrometer method (Gee and Bauder, 1986), organic matter (OM) content by the Walkley-Black method (Walkley, 1947), and pH was determined at a 1:1 soil/water ratio. Soil available K was determined by 1 M NH<sub>4</sub>OAc extraction and K assessment in the extract by flame photometer (Thomas, 1982). Soil P available was measured by Olsen method. Available Fe, Zn, Mn and Cu in the soil were first extracted by DTPA and then were read by atomic absorption (Shimatzu Model AA-670). The soil's available B was extracted by hot water and then was measured by spectrophotometer by curcumin method, considering the intensity of the color produced. Each variable was subjected to ANOVA using the Statistical Analysis System (SAS version 8.2, SAS Institute, 2001) for each soil. Treatment (fraction) means were separated by Duncun's multiple range test ( $P < 0.05$  level). Multiple regression analyses (stepwise procedure) (SAS Institute, 2001) was conducted to evaluate the relationships between residual available Cu and B in the soil and other factors.

### Result and Discussion

#### Soil analysis result before culture

Physicochemical characteristics of soils taken before the experiment was initiated in the spring of 2009 are presented in Table 1. While table 1 indicates the soil had high clay content. The soil P and K available in the soil is lower than the critical level suggested in scientific sources (Karimian and Yasrebi, 1995). Karimian and Ghanbari (1990) have reported the critical P level by the Olsen method in calcareous soils as 18 mg kg<sup>-1</sup>. The soil Zn and B content was lower than the critical level. High soil pH and CaCO<sub>3</sub> content induce B deficiency in the surveyed area. Similar results were found by Borax (1996) and Rashid et al. (1997). In soil, the B concentration of  $<0.65$  mg kh<sup>-1</sup> and  $>3.5$  mg kh<sup>-1</sup> are deficient and toxic levels for cotton crop, respectively (Anonymous, 1985). For many crops, a DTPA-extractable Zn level of 0.5-0.8 mg kg<sup>-1</sup> has been regarded as a soil critical level below which crop production would be limited by Zn deficiency (Martins and Lindsay, 1990). The soil Mn, Cu and Fe content was above the critical level. Sims and Johnson (1991) have reported the critical levels of Fe, Zn, Mn and Cu by the DTPA extraction method and

B by the hot water in the soil method to be 2.5-5, 0.2-2, 1-5, 0.1-2.5 and 0.1-2 mg kg<sup>-1</sup>, respectively.

Table 1. Soil mechanical and chemical analysis

Properties	Values
Depth of soil(cm)	0 -30
Soil texture	Loam
pH	8.4
EC (ds m <sup>-1</sup> )	2
Organic matter (%)	0.78
P	9.9
K	210
Fe	1.5
Mn	6
Zn	0.38
Cu	1
B	0.9

#### Residual available copper in the soil

The soil Cu content before planting was 0.5 mg kg<sup>-1</sup> and increased after harvesting in all treatments (Table 2). Considering that Cu fertilizer was not applied to the soil, the increase in soil Cu content after harvesting was due to availability of a part of the total Cu in the form of available Cu. Root secretions and reactions was carried out in the soil by activities such as irrigation and climate changes during the growing season leads into availability of a part of the total Cu in the form of available Cu. Also, by adding zinc sulfate fertilizer can increase available Cu content in the soil by replacement of Zn instead of Cu. Therefore, in addition to the presence of total Cu in the soil can meet the plant needs, also, some amount of the total soil Cu content be available to the plant after culture and operations on the soil. Of course, with the elapse of time the total available Cu content in the soil decrease to the unavailable form.

The use of different Zn levels and Zn-B interaction on the residual available Cu in the soil was insignificant at 5% level. No treatments showed significant difference from the control. Yang et al., (2000) reported that the decline in residual values of B from a single fertilizer addition was closely related to the soil and leaf B concentration. There was a relation between the leaf Cu content and the residual Cu content, so that Zn and B application had no significant effect on the leaf Cu content; that is the amount of Cu removal from the soil by plant did not change by Zn and B application and consequently residual Cu in the soil did not change as affected by Zn and B application. Considering that Zn and B application at all levels had no significant effect on

the residual Cu content in the soil relative to the Zn and B levels and also, almost all treatments showed no significant difference from the control, therefore increasing the residual Cu content in the soil depend on the many factors such as soil tillage, irrigation, root secretions and NPK fertilizers.

Several soil properties such as pH, redox potential (Eh), cation exchange capacity, organic matter, texture, oxide content, and clay mineralogy influence the relative distribution of Cu in different chemical forms (McLaren et al., 1983; Sims, 1986). Soil properties, metal characteristics, and environmental factors influence Cu and Zn concentrations and loads in surface soil (Zhang et al., 2003; He et al., 2004).

Table 2. The effect of Zn and B on residual available Cu in the soil after corn harvest (mg kg<sup>-1</sup>)\*

B (kg ha <sup>-1</sup> )	Zn (kg ha <sup>-1</sup> )					Mean
	0	8	16	24	Foliar Spray	
0	0.88 ab	0.63 b	0.69 b	0.85 ab	0.93 ab	0.80
3	0.69 b	0.65 b	0.66 b	0.88 ab	0.78 ab	0.73
6	0.71 b	0.85 ab	0.96 ab	0.61 b	0.72 b	0.77
Foliar Spray	0.97 ab	0.73 b	0.87 ab	0.80 ab	1.11 a	0.90
Mean	0.82 a	0.72 a	0.80 a	0.79 a	0.88 a	

\*Means with same letters lack a significant difference at 5% level by Duncan's test

#### Residual available boron in the soil

The B amount before harvesting was 0.81 mg kg<sup>-1</sup> and decreased after harvesting in all treatments except the three treatments of 3 kg ha<sup>-1</sup> B (with a residual B content of 1.3 mg kg<sup>-1</sup>), 6 kg ha<sup>-1</sup> B (with a residual B content of 0.83 mg kg<sup>-1</sup>), and joint use of 6 kg ha<sup>-1</sup> B and 8 kg ha<sup>-1</sup> Zn (with a residual B content of 0.83 mg kg<sup>-1</sup>) relative to the its initial level (Table 3). In fact, at no Zn level, the use of B increased residual B in the soil as compared with the its level before culture; but at presence of Zn, B application had no significant effect on the residual B in the soil relative to the its initial level. Reduction of residual B in the soil relative to the initial B has various causes: or B removal by plant has been more than the amount of B in the soil (initial soil B + B fertilizer), or large amount of boric acid was added to the soil, to become unavailable, or small amount of total soil B has been available. Due to high soil pH (calcareous soil) the

amount of boron added to the soil as B fertilizer comes unavailable.

Gupta (1993) reported that after the crop was harvested, lower quantities of hot-water-soluble boron were found in the soil. When boron is released from soil minerals, mineralized from organic matter, or added to soils by means of irrigation or fertilization, part of the boron remains in solution, and part is adsorbed (fixed) by soil particles. An equilibrium exists between the solution and adsorbed boron (Gupta, 2007). Usually more boron is adsorbed by soils than is present in solution at any one time, and fixation seems to increase with time (Jame et al., 1982). Soil factors affecting availability of B to plants are: pH, texture, moisture, temperature, organic matter and clay mineralogy (Goldberg, 1997). Soil reaction or soil pH is an important factor affecting availability of boron in soils. The availability of boron to plants decreases sharply at higher pH levels, but the relationship between soil pH and plant boron at soil pH values below 6.5 does not show a definite trend (Barker and Pilbeam, 2007). Boron retention in soil depends upon many factors such as the boron concentration of the soil, soil pH, texture, organic matter, cation exchange capacity, exchangeable ion composition, and the type of clay and mineral coatings on clays (Gupta, 2007).

Table 2. The effect of Zn and B on residual available B in the soil after corn harvest ( $\text{mg kg}^{-1}$ )\*

B ( $\text{kg ha}^{-1}$ )	Zn ( $\text{kg ha}^{-1}$ )					Mean
	0	8	16	24	Foliar Spray	
0	0.42	0.33	0.45	0.30	0.33	0.37
	b	b	b	b	b	b
3	1.30	0.63	0.78	0.35	0.39	0.69
	a	b	b	b	b	a
6	0.83	0.83	0.71	0.43	0.45	0.65
	b	b	b	b	b	a
Foliar	0.36	0.40	0.38	0.37	0.37	0.38
Spray	b	b	b	b	b	b
Mean	0.73	0.55	0.58	0.36	0.38	
	a	ab	ab	b	b	

\*Means with same letters lack a significant difference at 5% level by Duncan's test

The effect of different Zn levels on the residual B in the soil was significant at 5% level. The highest mean residual B in the soil,  $0.73 \text{ mg kg}^{-1}$ , was seen at no Zn level. The use of 16 and  $24 \text{ kg ha}^{-1}$  Zn, showed no significant difference from the no Zn level, but application of  $24 \text{ kg ha}^{-1}$  Zn and Zn solution spray decreased residual B in the soil from 0.73 at no Zn level to 0.36 and  $0.38 \text{ mg kg}^{-1}$ , respectively (50.7 and

48 percent decrease as compared with the no Zn level). There was no significant difference between the Zn spraying and applying Zn to the soil. No Zn content in the soil helped increasing residual B in the soil relative to levels of Zn applied to the soil.

The main effect of B on the residual B in the soil was significant at 5% level. At no B and spraying B levels where B fertilizer was not applied to the soil, the soil B content after harvesting ( $0.37$  and  $0.38 \text{ mg kg}^{-1}$ , respectively) was less than that of other levels. The use of 3 and  $6 \text{ kg ha}^{-1}$  B increased residual B in the soil from  $0.37$  at no B level to  $0.69$  and  $0.65 \text{ mg kg}^{-1}$ , respectively (86.5 and 75.7 percent increase relative to the no B level), but no significant difference was seen between these two B levels. The minimum residual B in the soil at  $0.37 \text{ mg kg}^{-1}$ , was seen at no B level.

At no Zn level, only application of  $3 \text{ kg ha}^{-1}$  B increased residual B in the soil from  $0.42$  to  $1.3 \text{ mg kg}^{-1}$  (209% increased relative to the no B use at this Zn level), but other B levels had no significant effect. At other Zn levels, B use had no significant effect on the residual B in the soil as compared with no B use at these Zn levels.

At  $3 \text{ kg ha}^{-1}$  B level, Zn application at all levels (to the soil and spraying), significantly decreased residual B in the soil, but at other B levels, Zn application had no significant effect on the soil B content relative to the no Zn use at these B levels. At  $3 \text{ kg ha}^{-1}$  level, the use of 8, 16 and  $24 \text{ kg ha}^{-1}$  Zn decreased residual B in the soil from  $1.3$  to  $0.63$ ,  $0.78$  and  $0.35 \text{ mg kg}^{-1}$ , respectively (51.5, 40 and 73 percent decrease relative to the no Zn use at this B level). Also Zn spraying at  $3 \text{ kg ha}^{-1}$  B level, decreased residual B in the soil from  $1.3$  to  $0.39 \text{ mg kg}^{-1}$  (70% decrease relative to no Zn use), but showed no significant difference when Zn was applied to the soil.

No treatments, except the treatment with the highest residual B in the soil (application of  $3 \text{ kg ha}^{-1}$  B) had no significant difference on the residual B in the soil from the control. Application of  $3 \text{ kg ha}^{-1}$  B, with a residual B in the soil of  $1.3 \text{ mg kg}^{-1}$ , showed 209 percent increase relative to the control, with a residual B in the soil of  $0.42 \text{ mg kg}^{-1}$ . In the control which did not use Zn and B, residual B in the soil decreased 48 percent relative to the initial soil B amount ( $0.81 \text{ mg kg}^{-1}$  B).

#### The correlation between the residual available Cu and B in the soil with other variables

The correlation coefficients ( $r$ ) between different variables by the Pearson method and the relevant equations were obtained by the step by step method using the SPSS software. One can use each of the following equations depending on what are the variables measured and  $r$  and  $r^2$ , but the last equation derived, is the most complete equation containing dependent and independent variables and we must measure more variables to derive that equation. The symbols \* and \*\* in equations and correlation coefficients ( $r$  or  $r^2$ ), are significance at 5% ( $\alpha = 0.05$ ) and 1% ( $\alpha = 0.01$ ) levels.

#### **The residual available Cu content in the soil**

There was a positive correlation between residual Cu in the soil and residual P ( $r = 0.43$ ) and Fe ( $r = 0.84^{**}$ ) in the soil, leaf Zn content ( $r = 0.33$ ), grain Zn content ( $r = 0.37$ ), the percentage of grain in the ear ( $r = 0.69^{**}$ ), and grain protein content ( $R = 0.37$ ), and a negative correlation with leaf Mn content ( $r = -0.36$ ), grain Cu content ( $r = -0.58^{**}$ ) and Cu uptake by the grain ( $r = -0.47^*$ ). The relevant equations were:

$$\begin{aligned} 1) \text{CuS} &= 0.174 + 0.157 \text{FeS} \quad r = 0.84^{**} \\ 2) \text{CuS} &= 0.253 + 0.155 \text{FeS} - 0.14 \text{BS} \\ &\quad r^2 = 0.78^{**} \end{aligned}$$

CuS, FeS and BS are residual Cu, Fe and B in the soil ( $\text{mg kg}^{-1}$ ), respectively.

#### **The residual available B content in the soil**

The residual B in the soil showed a positive correlation with the residual Mn in the soil ( $r = 0.37$ ) and leaf B content ( $r = 0.36$ ) and a negative correlation with leaf N content ( $r = -0.54^*$ ) and P content ( $r = -0.35$ ). The equation was:

$$\text{BS} = 3.232 - 1.136 \text{NL} \quad r = 0.54^{**}$$

BS and NL are residual B in the soil ( $\text{mg kg}^{-1}$ ) and N concentration in the leaf (%), respectively.

#### **Conclusion**

The residual Cu in the soil increased in all treatments relative to its initial level. The effect of Zn and B on the residual Cu in the soil was insignificant relative to the no Zn and B level. Also the Zn and B interaction on the residual Cu in the soil and the effect of all treatments relative to the control was insignificant. The residual B in the soil in all treatments decreased relative to the soil B content before planting. At no Zn level, B application to the soil increased residual B in the soil as compared with its initial level. Therefore, the presence of Zn in the soil prevented from increase of the residual B in the soil, by B application. The main effect of Zn and B

on the residual B in the soil was significant relative to the no Zn and no B levels. The highest and the lowest residual B in the soil were seen at no Zn and B levels, respectively. Application of Zn at a high amount decreased residual B in the soil relative to the no Zn level. Boron application to the soil at all levels, increased residual B in the soil relative to the no Zn level. many factors such as soil tillage, irrigation, root secretions and NPK, Zn and B fertilizers affected on the residual available Cu and B content in the soil relative to the its initial.

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10/16/2010

# Preparation of spherical silica nanoparticles: Stober silica

**Ismail A.M. Ibrahim\*, A.A.F. Zikry, Mohamed A. Sharaf**  
**Chemistry Department, Faculty of science, Helwan University, 11795 Egypt**  
 \* [ismailscience@gmail.com](mailto:ismailscience@gmail.com)

**Abstract:** The diameter of silica nanoparticles is mainly affected by the relative contribution from nucleation and growth. Once the total number of nuclei is fixed, the resultant particle size is then determined via the growth process by the total quantity of TEOS. In this work, we will demonstrate the effect of TEOS and NH<sub>3</sub> concentrations on particle size of silica nanoparticles. Experimental results indicate that the size of silica colloids decreases with increasing with TEOS and ammonia concentrations where both the rate of hydrolysis and condensation become faster and influence the solubility of intermediate [Si (OC<sub>2</sub>H<sub>5</sub>)<sub>4-x</sub>(OH)<sub>x</sub>] and hence the supersaturation for the nucleation process. With higher catalyst concentration, the number of nuclei is increased and therefore smaller silica colloids are obtained. Also surface modification of the silica nanoparticles by hexamethyldisilazane was studied to prevent the particles aggregation and to give good dispersion of silica nanoparticles in hydrophobic mediums.  
 [Ismail A.M. Ibrahim, Amina Zikry, Mohamed A. Sharaf, Chemistry Department, Faculty of science, Helwan University, Egypt. Journal of American Science 2010;6(11):985:989]. (ISSN: 1545-1003).

**Keywords:** Stober silica; nucleation; hydrolysis; condensation; nanoparticles; surface modification

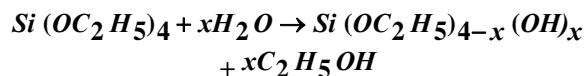
## 1. Introduction

Silicon is present in environment in different forms. Although it is not found in nascent form but it is always present in combination with oxygen (as in silica) or hydroxides (As in silicic acid). 78% of earth's crust consists of silicon and oxygen compounds, both amorphous and crystalline compounds for example quartz, flint, opal, silicates etc. Silicon is also present in dissolved form in the oceans as silicic acid. Also silica is found in living organisms like sponges, grasses, algae (for example, diatoms) (C.G. S. Phillips and R. J. P. Williams, 1965; T. L. Simpson and B. E. Volcani, 1981; R. K. Iler, 1979; S. V. Patwardhan, 2003).

The Stober process used for the preparation of monodispersed silica colloids 'white carbon black' by means of hydrolysis of alkyl silicates and subsequent condensation of silicic acid in alcoholic solutions using ammonia as catalyst was first published in 1968 (W. Stober et al., 1968). Ever since, there have been many research groups who applied those monodispersed silica colloids as model material in various applications. Sacks and Tseng utilized those colloids to pack ordered structure membrane and investigated its sintering behavior (M.D. Sacks and T.Y. Tseng, 1984). Unger et al. on the other hand applied these submicron silica colloids as packing material for capillary chromatography (S. Ludtke et al. 1997; K. K. Unger et al., 2000). In addition, there are a lot of recent investigations on using those monodispersed silica colloids to fabricate photonic crystals of 3D periodic structure (V. N. Stratov et al. 1996; H. Miguez et al., 1997). For all

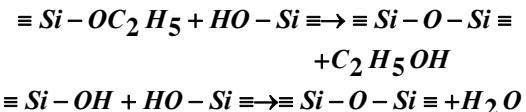
these different applications, it would always be desirable to use silica particles with a specified particle size and extremely narrow distribution.

Like any other synthesis of colloids, the diameter of silica particles from the Stober process are mainly controlled by the relative contribution from nucleation and growth. The hydrolysis and condensation reactions provide precursor species and the necessary supersaturation for the formation of particles will be briefly described as follows. During the hydrolysis reaction, the ethoxy group of TEOS reacts with the water molecule to form intermediate [Si (OC<sub>2</sub>H<sub>5</sub>)<sub>4-x</sub> (OH)<sub>x</sub>] with hydroxyl group substituting ethoxy groups. Moreover, ammonia works as a basic catalyst to this reaction; the hydrolysis reaction is probably initiated by the attacks of hydroxyl anions on TEOS molecules (C. J. Brinker and G. W. Scherer, 1990). The chemical reaction is expressed as follows:

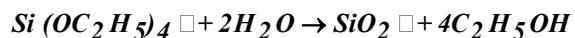


Following the hydrolysis reaction, the condensation reaction occurs immediately. The hydroxyl group of intermediate [Si (OC<sub>2</sub>H<sub>5</sub>)<sub>4-x</sub> (OH)<sub>x</sub>] reacts with either the ethoxy group of other TEOS (alcohol condensation) or the hydroxyl group of another hydrolysis intermediate (water condensation) to form Si-O-Si bridges. Furthermore, it was also claimed (K. S. Kim et al., 2002) that the rate of water condensation is thousands times faster

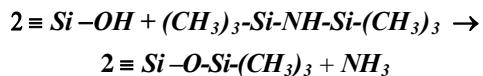
than the alcohol condensation. Both condensation reactions can be expressed as follows:



The overall reaction can then be depicted as follows:



The reactions of organosilicon compounds in acid-base reactions at oxides with surface OH groups are of technological importance in diverse fields (Leyden D. E., 1985). Because of its high reactivity with silica surface silanols, a system which has been widely used is silica reacted with hexamethyldisilazane (HMDS) to form trimethylsilylgroups and the particles become hydrophobic (Vasant E. F. et al., 1995):



Even though this system is widely employed for the synthesis of industrially useful materials, the structural element in HMDS most influential in determining its reactivity with silica surface silanols is the basic nitrogen (Dabrowski A. and Tertykh V. A., 1996).

The effect of various parameters on particle size had been reported in a previous paper (K.S. Chou and C.C. Chen, 2003). Here we discuss the effect of TEOS and catalyst concentrations on the particle size and also the effect of surface modification on the dispersion of the particles was discussed.

## 2. Material and Methods

### 2.1 Materials

The materials required for the synthesis of silica nanoparticles are described as follows. Tetraethyl orthosilicate (TEOS 98%) was obtained from the Fischer Company, Ammonium hydroxide solution 31.5% (NH<sub>3</sub>) was used as received from the Aldrich Company, Absolute ethanol 99 % from ADWIC Company. The materials required for Preparation of organically modified silica are described as follows. Silane coupling agent (hexamethyldisilazane) was obtained from fluka and used without further purification. Toluene was obtained from ADWIC Company.

### 2.2 preparations of Silica nanoparticles

Silica nanoparticles were prepared by hydrolysis and condensation of TEOS in ethanol, and in presence of ammonia as catalyst (W. Stober et al., 1968). First, solution containing appropriate quantities of absolute ethanol, ammonia and deionized water were stirred for 5 minutes to ensure complete mixing. Then a proper amount of TEOS in absolute ethanol was added to the above solution and the reaction proceeded at ambient temperature for 24 hours according to reactants concentrations. Thereafter the colloidal solution was separated by high-speed centrifuge, and the silica particles were washed by absolute ethanol for three times to remove undesirable particles, Followed by drying in oven at 100 °C for 2 hrs to prevent continuous reaction.

### 2.3 Preparation of organically modified silica

After a thermal treatment at 150°C for 2 h to remove physisorbed water, a given amount of silica is put in a glass flask equipped with an amount of toluene then the liquid reagent (10% hexamethyldisilazane in toluene) is added and the mixture is stirred and refluxed at 110°C for 3h. After treatment, the solid is filtered and repeatedly washed with toluene and acetone in order to remove any unreacted silane coupling agent, and finally dried at 150°C for 2h.

### 2.4 Transmission electron microscope analysis

TEM analyses of silica particles were carried out using JEOL JEM 2010 on silica particles to investigate the diameter of silica particles. To prepare samples for TEM analysis, silica powder is dispersed in absolute ethanol and a drop of silica colloid solution was placed on a copper grid coated with carbon. The solvent was evaporated at room temperature, leaving the silica particles on the grid.

## 3. Results and Discussions

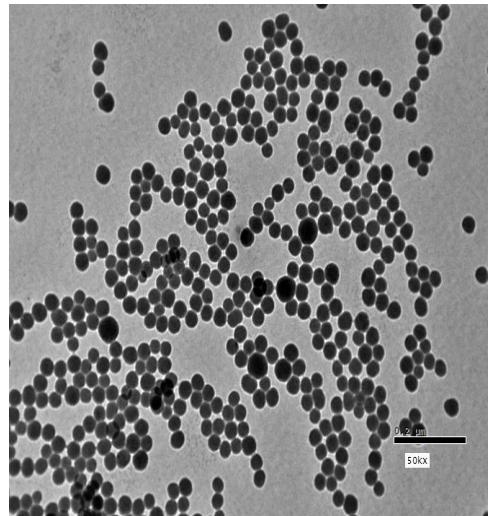
### 3.1. Mechanism of particles formation

Based on the particle formation mechanism in the sol - precipitation process (G.H. Bogush and C. F. Zukoski, 1991), a huge number of primary particles is first nucleated in the initial high supersaturated solution "induction period". Then, the primary particles are rapidly aggregated to form stable particles, which grow with the further aggregation of primary particles. After this particle induction period, any further primary particles generated under supersaturation are consumed for the growth of stable particles. As such, the resulting particles in the product suspension were highly monodispersed in size and spherical in shape,

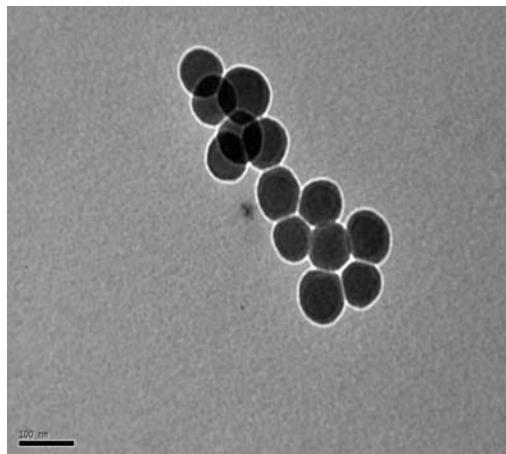
However, if the generation of primary particles by supersaturation exceeds the consumption of primary particles for the growth of stable particles during sol - precipitation, new stable particles are spontaneously formed by the self -aggregation of the extra primary particles, resulting in a multi -modal distribution of particle sizes in the product suspension (S.L. Chen et al., 1997). Yet, in the present study, the monodispersity of the particle size was maintained even over a wide range of TEOS, H<sub>2</sub>O, and NH<sub>3</sub> concentrations, implying that the population of stable particles formed during the induction period was sufficient to consume the primary particles nucleated after the induction period. Furthermore, the initial supersaturation level (supersaturation level during induction period) was critical in determining the particle size of the product suspension in the sol - precipitation, as the higher particle formation with a higher initial supersaturation resulted in a smaller particle size in the product suspension.

### **3.2. Effect of TEOS concentration on size of particles**

In Figures 1and 2 they show that the silica particles are spherical and there is no wide distribution in the particles sizes. The particle size increases with TEOS concentration, ranging from 0.2 to 0.4M while ammonia and water concentrations are fixed at 0.2M and 1.0 M respectively. When TEOS concentration is increased; both the rate of hydrolysis and condensation become faster (G.H. Bogush and C. F. Zukoski, 1991). As a result, the intermediate [Si (OC<sub>2</sub>H<sub>5</sub>)<sub>4-X</sub> (OH)<sub>X</sub>] will be increased rapidly due to the high hydrolysis reaction; however, as it reaches the supersaturation region, the consumption rate of intermediate through condensation reaction is also relatively fast (S.L. Chen et al., 1997), which probably shortens the nucleation period. Thus, the total number of nuclei formed will be less in numbers, and the final particle size of synthetic silica colloids will be relatively larger as exhibited in Figures 1 and 2 under the constraint of the same total ammonia and water concentrations in these cases.



**Figure 1** TEM of Silica nanoparticles ~50nm  
(experimental conditions: TEOS: 0.2M; NH<sub>3</sub>: 0.2M; H<sub>2</sub>O: 1M)

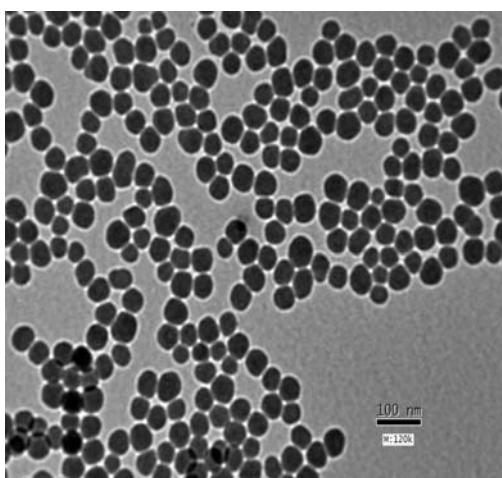


**Figure 2** TEM of Silica nanoparticles ~65nm  
(experimental conditions: TEOS: 0.4M; NH<sub>3</sub>: 0.2M; H<sub>2</sub>O: 1M)

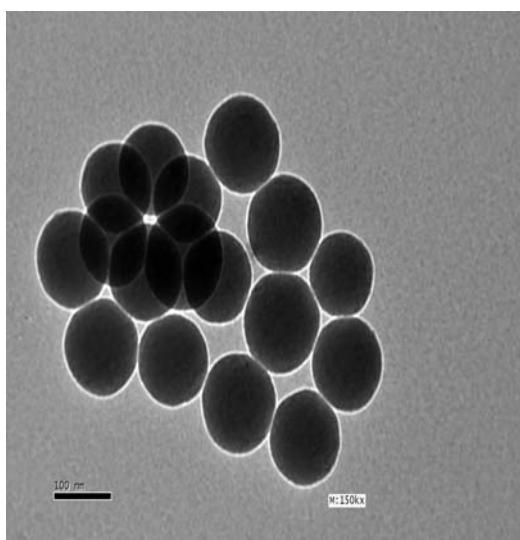
### **3.3. Effect of catalyst concentration on size of particles**

In Figures 3 and 4 they show also that the silica particles are spherical and there is a distribution in the particles sizes at low ammonia concentration. The particle size increases with ammonia concentration, ranging from 0.11 to 0.3 M while TEOS and water concentrations are fixed at 0.28M and 1.0 M respectively. When ammonia is increased, also both the rate of hydrolysis and condensation become faster. And the intermediate [Si (OC<sub>2</sub>H<sub>5</sub>)<sub>4-X</sub> (OH)<sub>X</sub>] will be increased rapidly due to the high hydrolysis reaction; however, as it reaches the supersaturation region, the consumption rate of

intermediate through condensation reaction is also relatively fast, which probably shortens the nucleation period. Thus, the total number of nuclei formed will be less in numbers, and the final particle size of synthetic silica colloids will be relatively larger as exhibited in Figures 3 and 4 under the constraint of the same total TEOS and water concentrations in these cases.



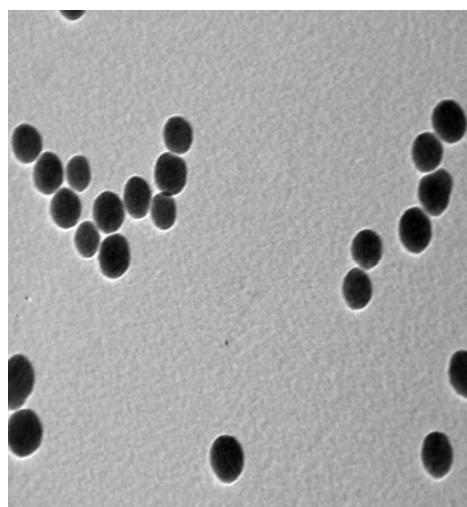
**Figure 3** TEM of Silica nanoparticles ~55nm  
(experimental conditions: NH<sub>3</sub>: 0.11M; TEOS: 0.28M; H<sub>2</sub>O: 1M)



**Figure 4** TEM of Silica nanoparticles ~130nm  
(experimental conditions: NH<sub>3</sub>: 0.3M; TEOS: 0.28M; H<sub>2</sub>O: 1M)

### 3.3. Effect of surface modification on dispersion of silica particles

Figure 5 shows the TEM image of silica nanoparticles modified with hexamethyldisilazane. The figure shows the good dispersion of the particles. This is attributed to that, the particles posses some organophilic characters after treatment with hexamethyldisilazane where the surface OH groups will convert to [-CH<sub>3</sub>)<sub>3</sub>] groups that results in the segregation of the particles.



**Figure 5** TEM of Modified Silica (experimental conditions: TEOS: 0.18M; NH<sub>3</sub>: 0.2M; H<sub>2</sub>O: 1M, 10% HMDS in toluene)

### 4. Conclusion

Herein, we have studied the effects of TEOS and catalyst (NH<sub>3</sub>) concentrations on the resulting particle size of synthetic silica colloids. Our results are discussed in terms of relative contribution from nucleation and growth processes. Any parameters, which increase the rate of hydrolysis, tend to produce fewer nuclei during the nucleation process and therefore a larger particle size in the end. The surface modification by silane coupling agent gives hydrophobic character to the silica nanoparticles.

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10/18/2010

## ***Early postpartum dietary practices among a group of Saudi women***

**Samar k. Hafez<sup>1&2</sup> and Sahar M. Yakout<sup>1&3</sup>**

<sup>1</sup>Maternity and Gynecologic Nursing Dep, Alexandria University. Alexandria, Egypt.

<sup>2</sup>Nursing Dep. Taif University, Saudi Arabia

<sup>3</sup>Nursing Dep. King Saud University, Saudi Arabia

[\\*sakamal2000@yahoo.com](mailto:sakamal2000@yahoo.com)

**Abstract:** This work aimed to study the early postpartum dietary practices among a group of Saudi women. A retrospective study was carried out on a convenient sample of 300 women during their post-partum period who attended seven primary health centers in Riyadh and Taif, KSA. The subjects were interviewed individually throughout a period of four months from September 2009 to January 2010. An interview questionnaire and a dietary scale of King and Jakobson were used for data collection. The results showed that 73.3% of the study subjects had incomplete knowledge about post-partum nutrition and an equal proportion of them (28.3%) had either excellent or borderline dietary practices during their early post-partum period and about one-fifth of them (19.3%) had dangerous dietary practices. The study concluded that Saudi women's post-partum dietary practices were significantly associated with their general characteristics such as age, education, employment and number of family member as well as with their obstetrical characteristics including their gravidity and parity.

[Samar k. Hafez and Sahar M. Yakout. Early postpartum dietary practices among a group of Saudi women. Journal of American Science 2010;6(11):990-998]. (ISSN: 1545-1003).

**Keywords:** postpartum; dietary; Saudi; women

### **1. Introduction:**

The postpartum period, or puerperium, starts about an hour after the delivery of the placenta and includes the following six week (Nian et al., 2006). By six weeks after delivery, most of the changes of pregnancy, labor, and delivery have resolved and the body has reverted to the non-pregnant state (Cunningham et al., 1997).

A good postpartum care and well balanced diet during puerperal period can influence her health for rest of her life (Pillitteri, 2007). After vaginal birth, there are no dietary restrictions for woman without underlying medical conditions or pregnancy-induced complications. Woman should be encouraged to drink 3.000ml of water and other liquids every 24 hours to restore the fluid balance altered by fluid loss during labor and birth process. Healthy food choices are encouraged with respect for ethnic and cultural preferences, while after cesarean birth, woman usually receives clear liquids until bowel sounds are present and then advance to solid foods. For each 20 calories of breast milk produced, the woman must consume an additional 30 calories. This results in a dietary increase of 500 to 1.000 calories each day for woman who is maintaining body weight (Kathleen and Patricia, 2001).

Unfortunately, many women consume less than the recommended amounts of calcium, magnesium, zinc, vitamins B<sub>6</sub>, and folate (Institute of Medicine, 1992). New mothers are likely to stop

taking prenatal vitamins which result in nutritional deficiency during their postpartum period and necessitate a restitution of prenatal nutritional supplementation. Prenatal supplements generally do not include a significant amount of calcium, in addition during lactation; 250 – 350 mg of calcium is transferred daily from the mother to the neonate through breast feeding. Woman should be apprised of the need for additional supplementation to meet requirement for this key mineral (Olson et al., 2003). Postpartum nutritional counseling can be tailored to the individual woman based on risk factors for poor nutrition such as extremes of maternal age, excessive weight gain during pregnancy, deviation from ideal body weight, multiple gestation, and history of eating disorders, close interconceptional period and highly restrictive diet due to traditional and religious practices(Olson et al., 2003).

The postpartum dietary and lifestyle habits vary greatly among different countries and cultures (Yeoun, 2003). In western countries, instead of restrictions, women are encouraged to eat a well-balanced diet from all food categories and start physical exercises during this period (Artal et al., 2003 &WHO, 1998). In Asia, postpartum maternal food restrictions (food avoidances) are common practices, which may have important health consequences in reducing the nutritional content of breast milk, inadequate breastfeeding and weaning

practices contribute to high rates of malnutrition and infant and child mortality (Barennes et al.,2007).

In gulf region, the nutritional assessment studies revealed that there are many nutritional problems. In Saudi Arabia, in spite of the vast economic advancement and availability of all types of food in the market, the general observation among the Kingdome communities surveyed was the low intake of various nutrients. On the other hand, several researchers in the Kingdome of Saudi Arabia recommended that the nationwide studies should be conducted to assess the magnitude of nutritional problems, the causative and related socio-cultural factors (Salma et al., 1990). So, this study aimed to identify the early post-partum dietary practices among a group of Saudi women.

## **2. Subjects & Methods:**

### Research design:

A retrospective study was carried out on women who interviewed individually in their post-partum period and asked to report their post-partum dietary practices within 24 hours.

### Setting:

The study was conducted at seven primary health care centers representing the different sectors of urban areas in Riyadh and Taif cities, KSA, namely, West-Naseem, East Naseem and El Rabwa health centers at Riyadh and Oudh, West El-Madina, El- Hawia and Ganoub El-Shohada El-Ganoubia health centers at Taif.

### Subjects:

A convenient sample of three hundred women attending the previously mentioned setting during their post-partum period was included in the study throughout a period of four months from September 2009 to January 2010.

### Tools of data collection:

#### Tool I:

An interview questionnaire sheet was designed for data collection and included the following parts:

Part I: Comprised the socio-demographic and obstetric characteristics of the study subjects such as age, level of education, occupation, type of the family, family income, food budget. Also, obstetrical characteristics of the study subjects such as their gravidity, parity and history of abortion were included in that part.

Part II: It included questions related to women's dietary knowledge and practices during the post-partum period with 24 hours diet recall sheet.

Tool II: A dietary scale of King and Jakobson (Zackler&Brandstadi, 1975) was translated to Arabic, modified and used to assess the dietary practices of the study subjects and to analyze the diet consumed throughout 24 hours by the puerperal women. The scale contained data related to four food groups as follows:

Group one, milk or choice from the milk food list; the amount required daily is four servings (the score was four points per serving, with a total score of 16 points).

Group two, meat or choice from the meat food list; amount required daily is three servings. (the score was 2.7 points per serving, with a total score of 8 points).

Group three, vegetables and fruits" dark green or yellow" the amount required daily is one serving scored with 3 points. Cooked or raw vegetables, the amount required daily are 2 serving (The score was 2 points for each serving with a total score of 4 points). Citrus fruits, the amount required daily are one serving scored with three points. Others fruits" fresh or canned", the amount required daily is one serving with one point score.

Group four, bread and cereals; the amount required daily is four serving (one point per serving with a total of 4 points. A total score of 60 means excellent dietary practices, 54-59 means good dietary practices, 48-53 means borderline and below 48 means danger dietary practices.

### Methods:

- 1- Official permission to carry out this study was obtained from the previously mentioned settings.
- 2- The dietary scale was translated into Arabic language by the researchers and verified by bilingual assessors specialized in the field.
- 3- A pilot study was carried out on 30 women selected from primary health care centers and was not included in the study subjects, to ascertain the relevance of the questions and to detect any further problems peculiar to the sequence and clarity of the tool. Based on the results of the pilot study, the questionnaire sheet was reconstructed and made ready for use.
- 4- Each woman was interviewed individually to collect the necessary data and asked to recall her food intake in the last 24 hours.
- 5- Women's knowledge about post-partum nutrition was scored. The correct answers were predetermined according to literature. A score of one was given to each correct answer and score of zero to the wrong answers. The score ranged from zero to two and the total score was classified according to the following: a score of 50 or above denotes good knowledge, while a

- score of 30 to less than 50 considered fair and less than 30 was scored as poor knowledge.
- 6- The dietary practices were determined by comparing the food eaten during their last 24 hours with the four food groups and the total score of the dietary practices was compared with that of King and Jacobson, 1975 scale.
  - 7- The collected data were categorized, tabulated and made ready for analysis.

#### Statistical analysis:

The collected data were coded and analyzed using SPSS version 10 for windows. Women's early post-partum dietary practices were examined for association with a variety of demographic and obstetrical characteristics of the study subjects using Chi-squared test with significance when  $p \leq 0.05$ .

### 3. Results

The general characteristics of the study subjects showed that 30% of them aged from 30 to less than 35 years old, about one-half of them (48.3%) were illiterate or just read and write, most of them (90.3%) were housewives and about three-quarters of them (75.3%) lived in extended family and more than one-half of them (55%) their family members ranged from 4 to 10 persons. Less than one-third (31.7%) of the study subjects had history of three gravida with slightly more than one-third (34%) of them had 3 deliveries and 91.7 % of them had no history of abortion (table I).

On assessing food budget and responsibility about food, it was observed that more than two-thirds (68.3%) of the study subjects had enough food budget while, less than one-third (31.7%) of them didn't have enough budget. Responsibility regarding food budget fall on father in law among 71.7 % of the study subjects while, mother in law was responsible about food choice among 45% of the study and about three-fifth (59.3%) of them were responsible about food preparation (table II).

Concerning knowledge of Saudi women about post-partum nutrition, the results of the present study revealed that more than one-half (54%) and less than one-third of them (30.7%) either had correct but incomplete knowledge or wrong answer regarding the definition of well-balanced diet respectively. Correct but incomplete answer was found among 73.3% of the study subjects regarding the importance of well-balanced diet and among more than four-fifth (83.7%) of them regarding the different food groups (table III).

It was noted that an equal proportion of the study subjects (28.3%) had either excellent or borderline dietary practices during their early post-partum period and about one-fifth of them (19.3%) had dangerous dietary practices (table IV).

The distribution of the study subjects according to food groups consumed within 24 hours showed that milk or milk group was not taken by more than two-thirds (68%). 84.7% and 76.7% had incomplete intake of meat group and vegetable group respectively (table V).

As regards food which are increased during early post-partum period, it was found that one-half (50%) of the study subjects increase chicken, slightly more than one-third of them (34%) increase honey with dates, more than one-quarter (26.7%) increases intake of vegetables and fruits, while an equal proportion of them (24%) increase intake of fish and meat. The most given reason for increased food was to replace blood loss (48%). Carbohydrates were among food to be decreased during that period by 30 % of the study subjects and more than one-half of them (53%) relate this to traditional reasons. About three-fifth of the study subjects (59%) didn't eliminate special types of food during their early puerperal period (table VI).

It was observed that only 15 % of the study subjects were more likely to drink 8 to 10 cups of fluid daily. Arabic coffee was mentioned by most of them (99%) among the different types of fluid to be taken during their early post-partum period followed by herbal tea (50%) and black dotes (46%), (table VII).

A significant association was observed between Saudi women's dietary practices during their early post-partum period with their general characteristics such as age, education, employment and number of family member as well as with their obstetrical characteristics including their gravidity and parity ( $p \leq 0.05$ ), (table VIII).

### 4. Discussion:

The postpartum period is a very special phase in the life of a woman. Her body needs to heal and recover from pregnancy and childbirth, a good postpartum care and well balanced diet during the puerperal period is very important for her health. Several studies indicated that the incidences of postpartum health problems are high and these problems maybe have relation to traditional and unscientific dietary and behavior practices in the postpartum period (Nian et al., 2009).

**Table I: Distribution of the study subjects according to their general characteristics.**

General characteristics	No. (300)	%
<b>I. Age</b>		
< 25	66	22.0
25 -	75	25.0
30 -	90	30.0
35 or more	69	23.0
<b>II. Education</b>		
Illiterate & Read and write	145	48.3
Primary school	90	30.0
Secondary school	45	15.0
University	20	6.7
<b>III. Occupation</b>		
House wife	271	90.3
Worker	29	9.7
<b>IV. Type of the family</b>		
Extended	226	75.3
Nuclear	74	24.7
<b>V. No. of the family member</b>		
4 – 10	165	55.0
11 - 15	82	27.3
> 15	53	17.7
<b>VI. Obstetrical characteristics</b>		
<b>Gravidity</b>		
1	60	20.0
2	80	26.7
3	95	31.7
4 +	65	21.7
<b>Parity</b>		
1	68	22.7
2	89	29.7
3	102	34.0
4 +	41	13.7
<b>Abortion</b>		
0	275	91.7
1	12	4.0
2	8	2.7
3	4	1.3
4+	1	0.3

**Table II: Distribution of the study subjects according to their food budget and food responsibility.**

Food budget and food responsibility	No(300)	%
<b>I. Food budget</b>		
Enough	205	68.3
Not enough	95	31.7
<b>II. Responsibility regarding food budget</b>		
Husband	16	5.3
Husband and wife	69	23.0
Father in law	215	71.7
<b>III. Responsibility regarding food choice</b>		
Husband	75	25.0
Wife	90	30.0
Mother in law	135	45.0
<b>IV. Responsibility regarding food preparation</b>		
Wife	122	40.7
Mother in law	178	59.3

**Table III: Distribution of the study subjects according to their knowledge about post-partum nutrition**

<b>Knowledge about nutrition during puerperium</b>	<b>No (300)</b>	<b>%</b>
<b>I. Definition of well-balanced diet</b>		
Correct and complete answer	46	15.3
Correct but incomplete	162	54.0
Wrong answer or did not know	92	30.7
<b>II. Importance of well-balanced diet during puerperium</b>		
Correct and complete answer	52	17.3
Correct but incomplete	220	73.3
Wrong answer or did not know	28	9.3
<b>III. Different food groups</b>		
Correct and complete answer	41	13.7
Correct but incomplete	251	83.7
Wrong answer or didn't know	8	2.7

**Table (IV): Distribution of the study subjects according to the score of their dietary practices during their early post-partum period**

<b>Score of dietary practices during puerperium</b>	<b>N= 300</b>	<b>%</b>
Excellent (60)	85	28.3
Good ( 54-59)	72	24.0
Borderline (48-53)	85	28.3
Dangerous( less than 48)	58	19.3

**Table V: Distribution of the study subjects according to food groups consumed within 24 hours**

<b>Different food groups</b>	<b>N (300)</b>					
	<b>Not taken</b>		<b>Incomplete intake of number of serving</b>		<b>Complete intake of number of serving</b>	
	<b>No</b>	<b>%</b>	<b>No</b>	<b>%</b>	<b>No</b>	<b>%</b>
Milk or choice from the milk food list	204	68.0	96	32.0	0	0.0
Meat or choice from the meat food list	6	2.0	254	84.7	40	13.3
Vegetable and fruit	0	0.0	230	76.7	70	23.3
Bread and cereals	0	0.0	152	50.7	148	49.3

**Table VI: Distribution of the study subjects according to types of food which are increased, decreased or eliminated during their early post-partum period with its given reasons.**

Type of food & given reasons	No (300)	%
<b>I. Increased food</b>		
Chicken	150	50.0
Fish	72	24.0
Meat ( Mazbay, Mandy)	72	24.0
Milk	54	18.0
Egg	48	16.0
Vegetables & Fruits	80	26.7
Garlic with Frengack	66	22.0
Honey with Dates	102	34.0
Cereals ( Hessawy rice )	18	6.0
<b>Given reasons</b>		
Replace blood loss	144	48.0
Increase immunity	102	34.0
Promote breast feeding	96	32.0
Tradition.	42	14.0
Help involution	36	12.0
<b>II. Decreased food</b>		
Salty and spicy food	48	16.0
Carbohydrates	90	30
Fried food	12	4.0
Canned food	12	4.0
<b>Given reasons</b>		
Tradition	159	53.0
To avoid wound infection	96	32
To avoid gastro intestinal trouble.	62	20.7
To avoid weight gain	18	6.0
<b>III. Eliminated food</b>		
No thing	177	59.0
Onion and garlic	51	17
Salty and spicy food	51	17
Gas forming food	33	11.0
Fish	9	3.0
<b>Given reasons</b>		
To avoid gas formation	80	26.7
Tradition	63	21.0
To avoid wound infection	30	10.0

N.B. More than one response

**Table VII: Distribution of the study subjects according to their daily fluid intake during their early post-partum period**

Amount and types of daily fluid intake		No (300)	%
<b>I. Total Daily intake of fluid</b>			
1-4 cups		142	47.3
5-7 cups		65	21.7
8-10 cups		45	15.0
> 10 cups		48	16.0
<b>II. Types of fluid intake during early puerperium</b>			
Water		21	7.0
Milk		39	13.0
Fresh juice		24	8.0
Soups		105	35.0
Arabic Coffee		297	99.0
Herbal tea		150	50.0
Black dotes		138	46.0
Fenugreek		9	3.0
Cinnamon		36	12.0
Maramia		39	13.0
Zaater		36	12.0
Somaa		9	3.0
Sesame drink		12	4.0
Mardood		102	34.0
Anzaroot		60	20.0

**Table (VIII): Association between score of early post-partum dietary practices of the study subjects and their general characteristics**

General characteristics	Excellent		Good		Border line		Dangerous		Total	p
	No.	%	No.	%	No.	%	No.	%		
<b>I. Age</b>										0.001*
< 25	36	42.4	22	30.6	5	5.9	3	5.2	66	
25 -	32	37.6	24	33.3	10	11.8	9	15.5	75	
30 -	10	11.8	15	20.8	30	35.3	35	60.3	90	
35 or more	7	8.2	11	15.3	40	47.1	11	19.0	69	
<b>II. Education</b>										0.0001*
Illiterate & Read and write	8	9.4	27	37.5	63	74.1	47	81.0	145	
Primary school	38	44.7	33	45.8	13	15.3	6	10.3	90	
Secondary school	30	35.3	5	6.9	5	5.9	5	8.6	45	
University	9	10.6	7	9.7	4	4.7	0	0.0	20	
<b>III. Occupation</b>										0.0031*
House wife	59	69.4	70	97.2	84	98.8	58	100.0	271	
Worker	26	30.6	2	2.8	1	1.2	0	0.0	29	
<b>IV. Type of the family</b>										0.103
Extended	72	84.7	60	83.3	52	61.2	42	72.4	226	
Nuclear	13	15.3	12	16.7	33	38.8	16	27.6	74	
<b>V. No. of the family member</b>										0.0021*
4 - 10	60	70.6	42	58.3	32	37.6	31	53.4	165	
11 - 15	18	21.2	20	27.8	33	38.8	11	19.0	82	
> 15	7	8.2	10	13.9	20	23.5	16	27.6	53	
<b>Obstetrical characteristics</b>										0.014*
<b>VI. Gravidity</b>										
1	30	35.3	16	22.2	10	11.8	4	6.9	60	
2	19	22.4	22	30.6	15	17.6	24	41.4	80	
3	22	25.9	30	41.7	30	35.3	13	22.4	95	
4 +	14	16.5	4	5.6	30	35.3	17	29.3	65	
<b>VII. Parity</b>										0.041*
1	25	29.4	26	36.1	9	10.6	8	13.8	68	
2	30	35.3	22	30.6	20	23.5	17	29.3	89	
3	24	28.2	21	29.2	45	52.9	12	20.7	102	
4 +	6	7.1	3	4.2	11	12.9	21	36.2	41	
<b>Total</b>	<b>85</b>		<b>72</b>		<b>85</b>		<b>58</b>		<b>300</b>	

The results of the study denoted that considerable percent (around three-quarters) of the study subjects had correct but incomplete knowledge about post-partum nutrition, while the score of the dietary practice of about one-half of them tends to be at the borderline or dangerous. These results are congruent with (Nian et al., 2009) who found that increased nutrition and health care knowledge did not lead to parallel dietary and health behavior changes, this apparent incongruence may be related to the fact that in Saudi Arabia, the tradition to support a newly delivered woman and her baby for the first month after childbirth at home is still common, where more than three-quarters of the women in the study lived in extended family and they may had an elder female of the family such as her mother or mother-in-law as the support person. The elder female who takes care of the women may have hindrance the change due to traditional believes (Nian et al., 2009).

Incomplete intake of the daily requirement of the different food groups was observed among Saudi women in this study where, milk and milk products were not taken at all by more than two-thirds of the study subjects during their early post-partum period. Such harmful practices should be negated as mothers during the post-partum period are in need for more calcium as essential component in milk production (Abraham et al., 2001 and Olds et al., 2004).

Meat group was inadequately taken by more than four-fifths of Saudi women in the study, one-half of them increase chicken intake during the post-partum period and only 24% of them increase intake of red meat and fish prepared in a unique Saudi Arabian way such as Mazby and Mandy. These results are supported by Juliana et al., (2008) who reported that, according to women in his study, some meats are problem causing for health, especially pork, fish and beef. It is not advisable to consume these animals' meat if they are neutered, nor their guts. One of the permitted meats is poultry, mainly used to prepare chicken soup and considered to be substantial light food.

The daily intake of vegetables and fruits was inadequate among more than three-quarters of the study subjects. Congruent with this, a study performed in Egypt by Wafa et al., (2004) who found that the majority of the Egyptian women in her study subjects had harmful dietary practices where, 66.3% & 82 % did not take their daily requirement of meat and vegetables group respectively. These results may attributed to the fact that mother in law was responsible about food preparation among 59.3% of the study subjects. Puerperal diet arranged by mother or mother-in-law was the negative influencing factor of vegetable intake (Nian et al., 2009).

Carbohydrates, salty and spicy food were among the food restricted or avoided by Saudi women in the study either for traditional reasons or to avoid infection and weight gain. In agreement with this Yeoun (2003), who stressed that culturally specific dietary prescriptions are common in the postpartum period among non-Western countries, the choice of certain cultural foods by non-Western women should be respected, if there are no dietary restrictions for health reasons.

A minority of Saudi women in the study had adequate daily fluid intake during their puerperium. Arabic coffee, herbal tea and nigella sativa (black seeds) were the mostly fluid taken during that period. This is a pleasant way for many women to increase fluid intake but the nurse should alert the women that excessive intake of coffee interferes with the absorption of supplemental iron and may have important health consequences in reducing the nutritional content of breast milk (Barennes et al., 2007).

A significant association was observed between the Saudi women's dietary practice and their general characteristics such as their age, education, occupation, number of family members as well as their gravidity and parity. These results are supported by Nian et al., (2009) who observed that although the traditional postpartum beliefs and practices abound, the level of adherence differs according to the socio-economic structure of the women and their families.

## 5. Conclusion:

It can be concluded that considerable percent of the Saudi women had incomplete knowledge about post-partum nutrition with an observable borderline and dangerous post-partum dietary practices that significantly related to their socio-demographic as well as their obstetric characteristics.

## Recommendations:

Antenatal clinic should develop education programs on postpartum nutrition and health care for pregnant women and their family members. Some of the antenatal visits should be extended to early postnatal visits to follow up and guide the women on contemporary postpartum practices, which will enable women to practice them. Moreover, further prospective studies are needed to explore the relationship between post-partum dietary practices and women's health outcomes.

## Corresponding author

Samar k. Hafez

<sup>1</sup>Maternity and Gynecologic Nursing Dep., Alexandria University Alexandria, Egypt.

<sup>2</sup>Nursing Dep. Taif University, Saudi Arabian  
[\\*sakamal2000@yahoo.com](mailto:sakamal2000@yahoo.com)

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10/1/2010

# Data Mining Methodology in Perspective of Manufacturing Databases

Muhammad Shahbaz<sup>1\*</sup>, Syed Athar Masood<sup>2</sup>, Muhammad Shaheen<sup>3</sup>, Ayaz Khan<sup>4</sup>

<sup>1,3</sup> Department of Computer Science & Engineering, UET Lahore, Pakistan

<sup>2</sup> Department of Engineering Management, NUST College of E&ME, Rawalpindi, Pakistan

<sup>4</sup> Forensic Expert/ Project Coordinator, National Response Center for Cyber Crimes (NR3C), FIA, Islamabad

<sup>1</sup> [m.shahbaz@uet.edu.pk](mailto:m.shahbaz@uet.edu.pk), <sup>2</sup> [atharmasood2000@hotmail.com](mailto:atharmasood2000@hotmail.com), <sup>3</sup> [m.shaheen@uet.edu.pk](mailto:m.shaheen@uet.edu.pk), <sup>4</sup> [chaudhary.ayaz@gmail.com](mailto:chaudhary.ayaz@gmail.com)

**Abstract:** In recent years data mining has become a very popular technique for extracting information from the database in different areas due to its flexibility of working on any kind of databases and also due to the surprising results. This paper is an attempt to introduce application of data mining techniques in the manufacturing industry to which least importance has been given. A taste of implementable areas in manufacturing enterprises is discussed with a proposed architecture, which can be applied to an individual enterprise as well as to an extended enterprise to get benefit of data mining technique and to share the discovered knowledge among enterprises. The paper proposes conceptual methods for better use of different data mining techniques in product manufacturing life cycle. These techniques include statistical techniques, neural networks, decision trees and genetic algorithms. An integrated and unified data mining platform is anticipated then to improve overall manufacturing process.

[Muhammad Shahbaz, Syed Athar Masood, Muhammad Shaheen, Ayaz Khan. Data Mining Methodology in Perspective of Manufacturing Databases. Journal of American Science 2010;6(11):999-1012]. (ISSN: 1545-1003).

**Keywords:** Data Mining, Manufacturing, Industrial application, Data Mining methodologies, Data Warehousing

## 1. Introduction

The progress in data acquisition and successful development of storage technology at cheaper rates, along with limited human capabilities in analyzing and understanding big databases have tempted scientists and researchers to move forward towards the specific field of knowledge discovery in databases (KDD). This recently emerged discipline, lies at the intersection of data management, artificial intelligence, machine learning and statistics. Data Mining is the search for valuable information in large volumes of data. It is a cooperative effort between humans and computers. Humans design databases, describe problems and set goals; computers sift through data, looking for relationships and patterns that match these goals. The central step within the overall KDD process is data mining, the application of computational techniques in the task of finding patterns and models in data. The major areas enjoying the benefits of KDD include banking, finance, business and medical sciences. Many companies including manufacturing enterprises all over the world, are now giving attention to the utilization of KDD technology for the improvement in their current status.

KDD has not commonly been used in manufacturing enterprises. The reasons for this are not certain, but it may be because of the long time scales and expenses involved in introducing new techniques in this area and also because of lack of awareness of

the benefits offered by this new data mining technology. An alternative possible reason might be the complexity and diversity of different manufacturing processes as these make it very difficult to devise a generic data mining process that can be used for all kinds of manufacturing processes and can handle all types of manufacturing problems. Data is stored in most manufacturing enterprises for quality control and traceability reasons or sometimes for simple statistical analysis to provide information on where the enterprise is currently standing against its past performance or its competitors. These databases may also be consulted if any problem occurs in the manufacturing process but the operational knowledge that exists within these databases is generally not exploited beyond these types of activities.

Competitive improvement can be achieved in many ways, for example by improving the quality of products or by reducing the material waste, production or overhead costs, or by decreasing the time to launch a new or improved product. Data Mining can support these improvements, through the extraction of knowledge from either existing data warehouses, or from current production data. Applying this knowledge can help to improve the quality of products by better controlling the manufacturing processes and methodologies, and by keeping product and production parameters in range.

Computer integrated manufacturing systems as well as more simply controlled enterprises, gener-

ate huge amounts of data daily but even when companies appreciate the importance and value of this information, people seldom try to explore their databases thoroughly, because of other 'urgent' commitments towards their engineering and technical duties. The usual approaches for addressing and controlling problems that arise in manufacturing areas is through the application of engineering knowledge and experience. Yet these problems may also be tackled and solved by analysing the available operational information directly. This involves sifting through past data that exists in the enterprise's databases to see if any trends exist, or are emerging that may be causing specific defects or faults. Therefore, data mining techniques could be applied to improve exploitation of valuable information and knowledge sources, to better control the system and also to check the strategic gains and losses during the manufacturing.

Competitiveness increasingly depends on the quality of decision making and improving the quality of decision making from past information and decisions [1]. Improved knowledge of manufacturing capabilities and products enables engineers to better target their future production strategies. Searching through data may identify unknown or hidden patterns and relationships in existing databases. This knowledge may then be utilised to improve the production process and hence the quality of product as well as the whole manufacturing system.

Basically data mining is concerned with the analysis of data and the use of software techniques for finding patterns and regularities in sets of data. It is the computer, which is responsible for finding patterns by identifying the underlying rules and features in the data. The idea is that it is possible to strike gold in unexpected places as the data mining software extracts patterns not previously discernable or so obvious that no one has noticed them before.

Data mining analysis tends to work from the data up and the best techniques are those developed with an orientation towards large volumes of data, making use of as much of the collected data sets as possible to arrive at reliable conclusions and decisions. The analysis process starts with a set of data and uses a methodology to develop an optimal representation of the structure of the data, during which time knowledge is acquired. Once knowledge has been acquired the process can be extended to larger sets of data working on the assumption that the larger data set has a structure similar to the sample data. Again this is analogous to a mining operation where large amounts of low-grade materials are sifted through in order to find something of value.

In the section two we will describe, at a high level, areas in manufacturing enterprises where benefit can be gained from data mining technology. We

shall also provide some details of design constraints and discuss how product life cycle data can be used to explore and discover knowledge by using data mining techniques. Section three covers data warehousing, as this plays an important part in implementing data mining and saves time spent on the initial steps required for data pre-processing. The fourth section is about the real technology of data mining, a brief background and description of some of its popular tools are given, in the perspective of manufacturing enterprises. In the last section an integrated data mining model is proposed, keeping in mind the requirements of manufacturing enterprises and how a generic data mining system can fit within a manufacturing system.

## **2. Manufacturing System Design and Performance Improvement:**

The importance of data oriented knowledge discovery techniques cannot be denied in any industry. Manufacturing enterprises in particular normally generate quantities of data at every step of the manufacturing processes from design through to the disposal of the product, and generally, most of this data is not fully exploited. Currently there is very little research being carried out in the manufacturing sector into the application of data-oriented knowledge discovery techniques.

Efforts have been made in the recent past to utilize the databases from manufacturing enterprises for design and quality control processes for example the factory data model [2][3] and data warehouses. The factory data model proposed in [2] promotes better exploitation of the information residing in the databases. It is however very difficult to design a data supported manufacturing enterprise which can gain the benefits of their historical as well as their current databases.

In manufacturing systems involving small sized products in very large volumes, for example in the semiconductor industry, quality checking has always been a problem and detecting any process abnormality as early as possible becomes more crucial than ever. Similarly in the production of very large sized products with small volumes it is desirable to maintain the non-conformities level to a minimum. The aim of current manufacturing systems is to decrease the time from occurrence of a fault to its detection. The shorter the identification time the more controlled the manufacturing system is. It is therefore important to learn from both past problems and successes, and to use this existing knowledge to improve product designs.

### ***Design and Re-usability of knowledge:***

Design can be defined as “effective allocation of resources”[4]. Alternatively, Pahl and Beitz [5] describe it as “a process of synthesis and integration”. The most comprehensive definition is given in [6] which states, “Systematic, intelligent generation and evaluation of specifications for artefacts whose form and function achieve stated objectives and satisfy specified constraints.” In their opinion, an engineering design does not directly result in a physical product compared with other design domains but rather, it provides a set of specifications to construct or fabricate the products.

Conceptual design, layout design, drafting and design analysis all create data, (which in turn may be used as the basic raw material for a data mining process). Product and process faults may occur at any stage of the product’s life cycle, hence, it is possible that certain faults can be traced back, using the data from the design and the design process. Earlier computer aided designs or old design drafts can be reused and be very helpful in the redesign of the product providing they can be analysed in a sensible way. A potential difficulty exists when attempting to re-use previous design knowledge that exists in the form of archival design documents, testing and analysis reports [7]. The difficulties lie in the forms in which the data has been archived, as some paper-based or hard forms are difficult and slow (and therefore expensive) to search and reuse. Therefore when designers or managers want to consult (and learn) from these designs or previous results the costs may be substantial in terms of time and overheads. Designers spend 20-30% of their time looking for information and same amount of time in handling information. Therefore any design system should incorporate all the designers’ own files, to make information easy to find and easy to use [7].

Data searching and retrieval become less of a problem when information is computer based. However substantially more efforts than are commonly used at present are needed to reuse such knowledge effectively. Data Mining could provide a solution to these problems by finding relationships between design problems and production problems or other aspects relating to the product life cycle. Manufacturing system design requires considerable amounts of information to be collected, and processed in order to improve the design and performance of the processes. Several information modelling techniques, and processing methodologies are already in use as described in detail within [2][3].

The popularity of computer aided design (CAD) and computer integrated manufacturing (CIM) have increased the amount of available digital data. This is easy to store and recover or search,

helping the modern manufacturing enterprise to make better use of computing power in the analysis of its valuable data. Many researchers believe that computational approaches to design should enhance, not replace, human practice [8] [9]. With the help of knowledge discovery in databases, the causes and contributory factors to faults may be more clearly identified, enabling human designers to focus and concentrate their efforts on important problem areas and thus the time to design or redesign a product can be reduced.

It is generally important to consider how a product will be produced in parallel with the design of the product. Improvements to the manufacturing system can also result in improvements to the product. The manufacturing system, modelling and design, will therefore now be considered in the context of data mining.

#### ***Advanced Manufacturing Systems:***

A primary challenge, which must be faced by manufacturing enterprises in their response to changes in world markets, is the speed at which changes need to be made. Information modelling is one approach to support changes to enterprise design and more information on enterprise modelling can be traced back from [10] [11] [12]. Successful manufacturing system designs are often created iteratively, initially from small amounts of information, which progressively gain detail as the design for the enterprise progresses. Modelling the whole enterprise is a long and complex task, and a model may not be completed in time to meet whatever challenge triggered the need for change i.e. the model may be overtaken by events [2].

Advanced manufacturing enterprises are more flexible and proactive than ever before but gaining absolute flexibility is impossible. Bartezzaghi and Turco [13] remark on the fact that the various types of flexibility may trade-off with each other (for example: a manufacturing system which is flexible in relation to volume of product may not be so in relation to the mix or the introduction of a new product). Achieving absolute flexibility requires substantial quantities of information about the current processes and related activities, which directly or indirectly, link the whole manufacturing system. Information about the product life cycle also plays an important role in designing the flexible manufacturing process. The details of the product life cycle and employment of data mining on it are discussed at the end of this section.

Flexible manufacturing can be achieved by having tools, machining programmes and parts all quickly changeable and being able to respond rapidly by minimizing the part lead times. Machine

utilization should be maximised together with an almost instantaneous response both to customers and to any problems that may occur. The philosophy behind this arrangement simultaneously aims for minimisation of lead times for the parts to be processed along with the maximisation of the utilisation of the machines doing the processing [14]. But how can this be achieved successfully?

Since it is difficult to measure manufacturing flexibility, it is often hard to financially justify investments aimed at increasing the flexibility of a manufacturing system [15]. Manufacturing enterprises are struggling to make all the steps in their operation as flexible as possible, but often without proper knowledge and information sharing between the various stages of the manufacturing processes. The hurdles in making the flexible manufacturing systems can be analysed using data that has been recorded during individual processes within the whole system. Keeping in mind the problems data mining will give the solutions to the problems that are actually indirectly involved in the brittleness of the system. Once the information about the system's brittleness is discovered the system can be made more elastic by removing the obstructions in achieving the goal.

The first step towards applying data mining techniques for achieving the maximum flexibility in the manufacturing system is to make a system to archive the data recorded during different operational stages of the manufacturing organizations. Most advanced manufacturing enterprises record such data but it is often in the form of simple database files that are not well organized. A well designed database or model is required, otherwise substantial pre-processing of the data may be necessary before it can be mined and this will consume considerable resources. The first step towards achieving this goal is to develop a data warehouse, and this will be discussed further in section three.

If a data warehouse is utilized properly it can not only help in developing the new strategies but it can also be used for repairing the strategies of an enterprise in crisis. For ongoing fault detection, it will sometimes be necessary to have an on-line data mining system, which does not have to rely on the data warehouse. The on-line systems will work on fresh data direct from the manufacturing system to find out any developing trends towards bad quality or to help better scheduling of resources. In an advanced manufacturing system there are advantages to automating the data mining system to help directly in controlling the process.

### **Manufacturing Strategies and Data Models:**

An enterprise needs to redesign if its aims are not being achieved or if its aims or strategies change. Good strategy and a well structured enterprise result in profit whereas bad strategy or a business that does not meet the competitors' challenges, damages the company in the marketplace. Therefore management decisions should be based on accurate and reliable information that is structured within a data warehouse and a factory data model [2]. A factory model focuses on operation and infrastructure in contrast to a data warehouse provides information about the behaviour of the existing enterprise. Both these sources of information are vital for the design and redesign of an enterprise and for performance evaluation.

However, the existence of this useful information is only part of the solution. How it can be utilized effectively to produce the required results is equally important. Simple statistics normally work well to give a very good picture of the current overall manufacturing process but there may be much more hidden knowledge waiting to be discovered. Machine learning and artificial intelligence tools can be used to gain insight into the data and to discover hidden patterns and trends.

The above analysis regarding the ways of extracting information may not appear to provide adequate solutions as the results of the mining process cannot be predicted. Hence, we cannot be sure that the value of the knowledge that may be discovered in the data will be greater than the time and resources that need to be spent in the mining process. However implementations of the same technology in other areas of human sciences like banking, finance, marketing, insurance, telecommunication, health care etc. have given very good results [16] [17] and people are now benefiting from the knowledge they have gained in their respective fields – so why should manufacturing enterprise not also benefit?

### **Product Life Cycle and Data Mining:**

The product's life cycle is based on the design of the product and is important for a data collection point of view and for the analysis for the mining perspective. Information collected during a product's life cycle provides feedback on a product's performance that can be used to assess the quality of the design [18]. Nahmias [19] divided the product life cycle into the following four phases: start-up, rapid growth, maturation and decline. Hazelrigg [4] made a further in-depth division and split the whole process into seven stages: engineering and design; test and evaluation; manufacturing; distribution and sales; operations, maintenance & repair and disposal. All these stages produce data, which should be more or

less easily available for analysis to gain insight into the whole life cycle of the product. Knowledge gained through this analysis can be used for redesign or for the introduction of new products.

Once the production process is completed there are many reasons why it is difficult to collect information relating to the costs. This information is very important regarding the design or redesign of the products. Prasad [20] concluded from different studies that indirect costs could be as much as 4-5 times the amount of direct labour and material costs. In this situation if all the relevant data from the product life cycle is available then it is far easier to analyse the data and to target the areas for improvement in the design and the manufacturing process. This is the point where data mining comes into play. Figure1 shows a very high level architecture of collecting the data during the product life cycle and storing it in a data warehouse, which is actually coupled with a data-mining engine. The engine would use either one or a combination of the many available data mining techniques depending on the type of the data and the type of problem to be solved. A brief introduction and relevant details of a few of these techniques and their applicability can be

found in the fourth section of this paper. A design improvement that takes into consideration all the aspects of the product life cycle gives a much more controlled and efficient design compared with a design based on market demand and production capabilities. The advantages of mining data relating to the product life cycle are that it can improve the design or redesign of the product and also that it provides more information that is helpful for design of the production process, marketing strategy, environment effects, quality and reliability of the product. This information collected during a product's life cycle provides feedback on product's performance that can be used to assess the quality of the design [18].

Data for mining can be structured so that the overall data from all the major stages of the life cycle can be examined together. In addition, analysis should also be carried out on sections of the data relating to individual stages in the life cycle. This is necessary to detect any hidden relations and effects on subsequent life cycle stages so that corrective actions can be taken to improve the design of the product or the manufacturing process.

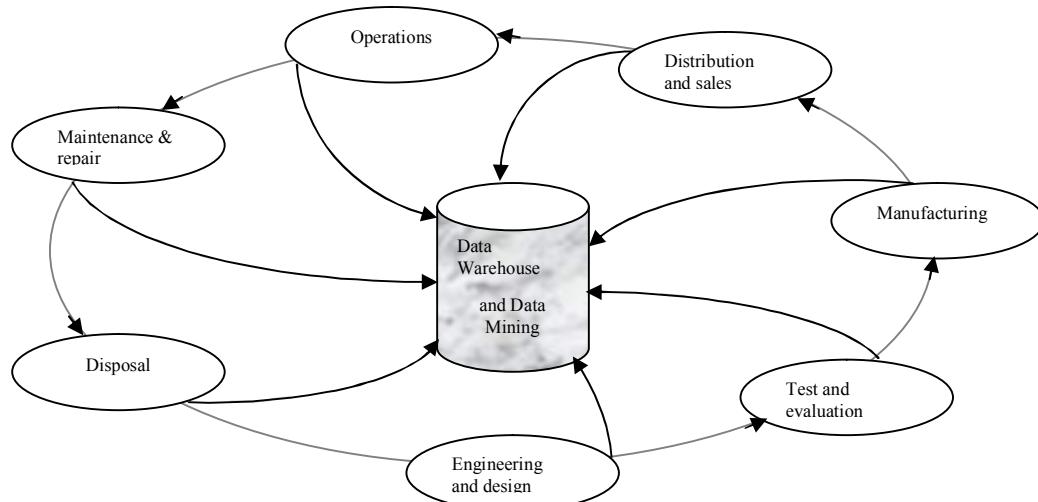


Figure1: Product Life Cycle's Data Collection Architecture.

### 3. Data Warehousing

Data warehousing has developed over the last 20 to 30 years, as they started emerging between 1984-88 [21]. The trends of today's enterprises towards data warehousing can be judged by a survey conducted by the META Group (Stanford, USA) that shows almost 95% of the corporations have plans to build data warehouses [22].

A database is a generalised and integrated collection of stored and operational data together

with their descriptions, and is managed in such a way that it can fulfil the differing needs of its different users [14]. A data warehouse can be defined as a single, complete, and consistent store of data obtained from a variety of sources and made available to end users in a way they can understand and use in a business context [21]. Mattison[23] explained data warehouse as a collection and organization of data to serve as a neutral data storage area that is non volatile, can readily be used for data

mining and/or other applications, and meets the specific business requirements. The most interesting and precise definition in terms of knowledge acquisition is concerned is given by [24] as, "an analytical database that is used as the foundation of a decision support system". A data warehouse is an organized and indexed set of stable historic data that is separated from an organization's data entry system [25]. The data warehouse concept provides integrated information, which can be used in an informational or analytical manner to provide management with reports to support or facilitate their decision-making. Data Warehouse is a subject oriented, integrated, non-volatile and time variant collection of data to support management's decision making processes [26]. It is a tactic for consolidating and sharing information [27]. The reports from the data warehouse enable management to examine aspects of business performance [2] and provides input for strategic decision-making [28].

The data warehouse must be structured to respond to queries related to different aspects of the process or business [29]. Users normally access operational databases using transactions that are also called On-Line Transactional Processes (OLTP), and often use executive information system (EIS). The disadvantage of OLTP is that the results of the queries of two users may be different if their queries are made at different times because the data is changing continuously and the second user may get updated results. However, with the data warehouse all the users will get the same results for similar queries, providing the queries are made during the intervals between updating the warehouse.

The existence of any data warehouse is very important in a manufacturing enterprise when data mining is to be used as a knowledge retrieval tool. A data warehouse saves substantial time that would be wasted in collecting data from multiple sources during the initial stages of data mining. This is particularly true in multi-site operations where manufacturing processes do not all reside at one site and the product has to move from one site to another during its manufacture (as shown in the Figure 2). In such a complicated manufacturing environment the data should be collected from each individual site and then after cleaning the integrated information should be stored in a data warehouse. If data has to be collected from the individual sites before it is used for mining, considerable effort will have to be put into pre-processing. But if the process requires the mining to be done online then a network needs to be established which shares the information extracted or any governing rules discovered on one stage or site, with the other stages or sites, using data mining tools, as explained in section 4. An example of such an

integrated online data mining system is explained in detail in the last section of this paper. For static (offline) data mining where historical data is analysed to determine any relationships between the different processes or machines to improve quality of the product, or to decrease the production time or to introduce a more flexible manufacturing system then a common data warehouse will be the best choice. Data from all the manufacturing sites and processes should be archived at a common storage location after cleaning and integrating to avoid any duplications and errors. If the requirements of data-mining implementations are considered when information is stored in the data warehouse, pre-processing effort will be reduced and in consequence, the time of the whole knowledge discovery process will also be reduced.

#### 4. Knowledge Discovery in Database

The term knowledge discovery in databases (KDD) was formalized in 1989 in reference to the general, 'high level' concept of seeking knowledge from data. This term (data mining) has been used by statisticians, data analysts and the management information systems (MIS) community. KDD has been mostly used by artificial intelligence and machine learning researchers.

Data mining is just one of several terms, used by the people in the data mining field, including knowledge extraction, data archaeology, information harvesting, and even data dredging, that actually describe the concept of knowledge discovery in databases. Data Mining includes all methods and techniques, which allow practitioners to analyse very large data sets to extract and discover previously unknown structures and relations out of huge quantities of details. Information is filtered, prepared and classified so that it will be a valuable aid for decisions and strategies [30]. The most authentic definition of knowledge discovery in database or data mining is "*non-trivial process of identifying valid, novel, potentially useful, and ultimately understandable patterns in data*" [31]. With the explosive growth of data in databases the desire to extract useful information is also increasing. The manufacturing enterprises' databases contain treasures of information that tempt analysts to detect trends or patterns in them and react flexibly to them. However useful information may be hidden within the mountains of other data, and cannot be discovered using conventional database management systems. Data mining is becoming an increasingly important research area [32] [33] [34], since knowledge, e.g. extracted knowledge trends and patterns, can be used to help and improve business decision making.

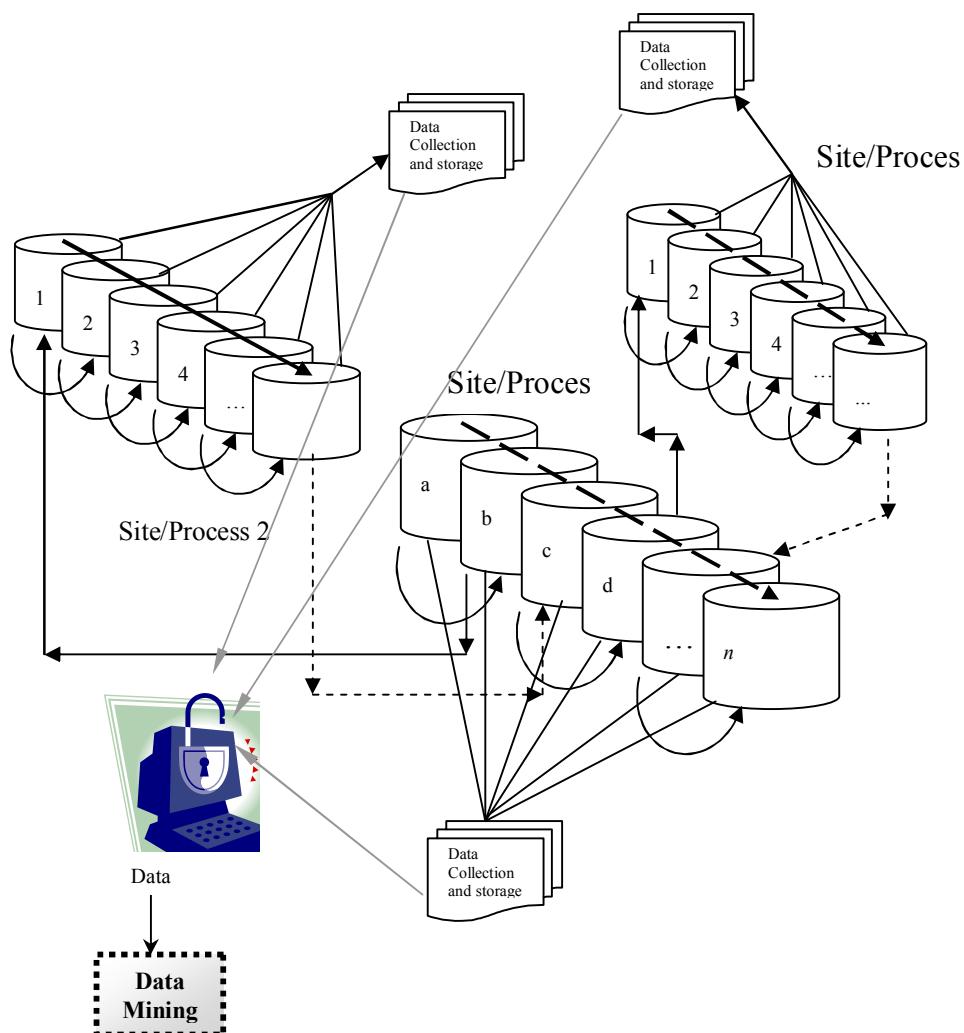


Fig2: Data warehouse supported data mining in a manufacturing process

**Legend:**

- Data Collecting stage of a Manufacturing Process
- A manufacturing process flow where product can be moved to another site and then return
- A manufacturing process flow where product continuously flows till the end of the process
- Movement of the in process product from one stage to the other
- Data Collection and recording at the local storage
- Product is being transferred from one site to
- Finalized product from one site bring back to the original production line for further operations

### **Background:**

The seed of statistics was planted in the 18<sup>th</sup> century with the probability theories of Fermat and Pascal developed through analysing games of chance [35]. Statistical learning theory developed by Vapnik and Chervonenkis in the 60's brought a new set of approaches to comparing competing models that overcame some of the difficulties of traditional statistical modelling [36].

Data Mining can trace some of its roots back to the discipline of artificial intelligence and cognitive psychology born in the 50's [37]. Hosking [38] points out that in statistics we often work with fixed conceptual and hypotheses spaces where we select a set of parameters and a model to test our hypotheses. In data mining we also have a fixed conceptual space but the hypotheses space is left to the learning algorithm, which attempts to create a model with a minimum of prediction error. Statistics involve very small databases whereas in data mining we deal with very large databases.

Most data mining programmes are based upon the machine learning algorithms similar to our own mind's discovery process of trial and error and derives its mystique from the ability of the computer to calculate more quickly than most humans. This approach requires vast computer processing power and was not practical until the early 1980s, when computers began to offer useful power at reasonable prices. Another development occurred with the introduction of machine learning techniques in the data-mining field in 80s and 90s with the commercialisation of the computers. Machine learning is a combination of the statistical techniques and artificial intelligence.

Statistics use familiar formulas for analysis but in data mining there is not a particular formula for the analysis. It works just like a black box and the degree of success that will be achieved is unpredictable. Data Mining finds its own way to the solution for a problem. New formulas are developed by the algorithms, to identify what actually governs the problem scenarios.

### **Data Mining:**

Data Mining normally works together with a data warehousing as this is necessary to organize historical information gathered from large-scale client/server bases applications as discussed in the previous section. Due to the explosive growth of data in the companies in the recent years and the non-availability of any proper technology to exploit this data in the past made data mining a very important research topic [31] [39] [40].

In order to understand how KDD can be implemented in manufacturing enterprises, it is

important to understand the whole process, especially the discovery stage and its tools. For simplicity the whole knowledge discovery process is divided into six different stages: data cleaning, integration, selection, transformation, mining and evaluation/visualization [41] [42]. Fayyad [31] proposed five steps including, retrieval of data, selecting of data, sampling and cleaning, applying the appropriate transformations and fitting models to the proposed data. IBM [43] defined the four major operations for data mining as predictive modelling, database segmentation, link analysis and deviation detection. The above divisions show the actual discovery or mining process comes at the end and takes a very small fraction of the total time involved in the discovery process. Gonzalez and Kamrani [44] conclude that as much as 80% of KDD is about preparing data, and only the remaining 20% is about mining. Pre-processing is therefore very important and the details of how to implement the pre-processing steps can be found in lots of available data mining books in the market.

Some standard methodologies like SEMMA (Sample, Explore, Modify, Model, Assess) [45] and CRISP-DM (Cross Industry Standard Process for Data Mining) [46] have also been developed for data mining process and to simplify its implementation in the industry.

Data mining in its traditional forms has been used to find patterns in the historical databases like banking, insurance, fraud detection, telecommunication data etc keep their old data for future strategy and planning. In the manufacturing process both the old and current trends of the process; policies & strategies and quality are important. The traditional way of data mining can help in finding the faulty processes and bad strategies in the manufacturing process and suggest remedies for them but finding the online or current trends is very important in any kind of manufacturing processes to control the whole process for better scheduling and quality. Data mining can also be used to solve this problem by embedding it in the process to find out the run time errors of the process if they occur [47]. In this kind of mining process, all the pre-processing steps are eliminated by automating the whole process for collecting the data, analyzing it and making corrective actions.

### **Data Mining Techniques for Manufacturing Enterprises:**

The analysis or mining of the manufacturing enterprise data can be done using all the popular data mining techniques. Some of the effective techniques for data mining like association rule, rule induction etc. are mostly used for retail market or basket

analysis [48] [49] but are helpful in any kind of manufacturing databases too. The data mining techniques can be divided into three main categories, statistical techniques which uses simple to complex statistics to analyses the databases, the second category is artificial intelligence tools which become popular with the increase in the computing power over the last two decades and the third is machine learning tools which are actually a combination of statistics and artificial intelligence tools.

A very interesting survey has been done by [50] analyzing the efficiency and productivity of different data mining techniques with different kinds of problems. For example the survey shows that for data that has many attributes, like manufacturing data, and that is numeric in nature, the most suitable algorithms are decision trees, nearest neighbors and neural networks. But it really depends on what kind of problems are being examined in the manufacturing enterprises. The above techniques can be used to search for any kind of trends in the past data but in any specific problem the choice of the technique really also depends on other factors of the problem and algorithms.

A few of the most common data mining tools are listed here in the context of manufacturing databases. Only a brief introduction is given, as details can be traced back the references provided.

#### **Statistics:**

Statistics can be counted as a data mining tool since statistics is actually the origin of data mining. There are lots of statistical techniques including regression, discriminant analysis, classification, clustering and time series which are very popular in the data mining community and are extensively used for the large database analysis. With the passage of time these techniques are now mixed with the artificial intelligence tools to give even better, more reliable and faster results than the current simple statistical methods.

Statistics has always been a very popular tool in the manufacturing enterprises for process and quality controls. In any kind of data mining process the preprocessing stage commonly uses different statistical techniques and the initial analysis of the data is also done using statistics and Structured Query Language (SQL). [42] [51] and [52] have stated that for the most part, about 80% of the interesting information can be abstracted from a database using SQL commands. However, as [30] stress, extraction of the remaining 20% of hidden information requires advanced techniques like expert systems, fuzzy expert systems, case-based learning, decision trees, neural networks, genetic algorithms etc. Hence statistics are a good starting point for the

analysis of the data, to try to identify some trends for further detailed analysis.

#### **Decision Trees:**

Decision trees are normally used for classification purposes. These are tree shape structures resulted by the decision taken at each node. The database is divided into different fields that enable the analysts to look at the behavior of the database at different stages or to distinguish among different patterns present in the data. Different decision tree methods used as a data mining technique are Classification and regression Trees (CART) [53] and Chi Square Automatic Interaction Detection (CHAID) [54]. The first efficient decision tree model called ID3 [55] was based on the concept of entropy means the choice of the next feature used for branching should increase knowledge [56]. Decision trees are simple enough to understand and explain, and are easy to build, have relatively short training time and need very low memory [7].

#### **Neural Networks:**

Neural networks, is a very popular AI technique that mimics the working of neurons of human brain. Neural networks are not new as they trace back their history about 50 years ago when McCulloch and Pitts started working on them. [57], [58]. Neural networks are complex to interpret but very good in terms of accuracy. Carol [7] tabulated different data mining techniques such as neural networks, rule induction, decision trees, nearest neighbour etc and tabulated their important characteristics. It is a good idea to keep in mind the different characteristics of the data before choosing a specific data mining technique.

Artificial neural networks are simple computer programmes, which can automatically find non-linear relationships in data without any predefined model. According to [59], neural network-based database approach consists of three major-phases:

- 1 - Network construction and training: in this phase a layered neural network based on the number of attributes, number of classes and chosen input coding method are trained and constructed.
- 2 - Network pruning: in this phase, redundant links and units are removed without increasing the classification error rate of the network.
- 3 - Rule extraction: rules are extracted in this phase.

#### **Genetic Algorithms:**

This is one of the most recent methodologies used as a data-mining tool. Their basis is on the evolutionary computing which become very popular within the machine learning methodologies [60]. The basic concept of Genetic Algorithms comes from

Darwin's theory of evolution. A genetic algorithm is reminiscent of sexual reproduction in which the genes of two parents combine to form those of their children and only the fittest will survive. The next generation improves and is better than the previous generation only if the strongest members of the population mate together to produce the next generation. The same principle can be applied to problem solving if the population consists of possible solutions to the problem. Each of these generated solutions have some characteristics that enable them to be categorized as a more or less fit as member of the next generation of offspring. The best members of a generation are given more chance for mating and producing the subsequent generation. In this way, each successive generation consists of better solutions, until an optimal solution is generated.

Genetic Algorithms can be very helpful in finding solutions that are very difficult to optimise. Another advantage of using genetic algorithms is that they can propose many possible solutions of a problem. The main advantage of using genetic algorithms (GAs) is, they can be synthesized without making use of the detailed, explicit knowledge of the underlying process. This means they will find a pattern if any exists even if the problem is new and no previous solutions are known. However, limited or noisy training data may result in inconsistent, meaningless output. This has been known to be a severe problem of genetic algorithms [61]. Another problem for genetic algorithms is they require lot of computing power to achieve a significant solution. In data mining problems specially to find out the relationships between the different entities genetic algorithms prove to be very effective. [62] shows a successful implementation of a search technique in big databases using genetic algorithms in solving a data mining problem.

The generic genetic algorithm consists of the following steps [56]

- 1- Each offspring (generation) is evaluated for fitness
- 2- The population is increased through mating and mutation of fit members to generate the new set of rules (generation)
- 3- Weak members from the generations are eliminated (reducing the size of the population)
- 4- A terminating condition is checked and if the optimal solution is not achieved then mating and mutation are done to produce the new generation.

There are many other popular data mining techniques including fuzzy logic, rule induction, association rule, k-nearest neighbor, intelligent agents etc, and this list continues to increase.

## 5. INTEGRATED DATA MINING

As explained in the first section of this paper a manufacturing enterprise can benefit from data mining in solving problems, but there is an important need for an integrated data mining system for diagnosing and solving online manufacturing problems. Here we introduce a relatively complex integrated data-mining architecture for a manufacturing enterprise. This architecture can be used to suggest solutions to particular problems, to learn from this, and subsequently to also help solve problems at other processes/sites with similar kind of parameters.

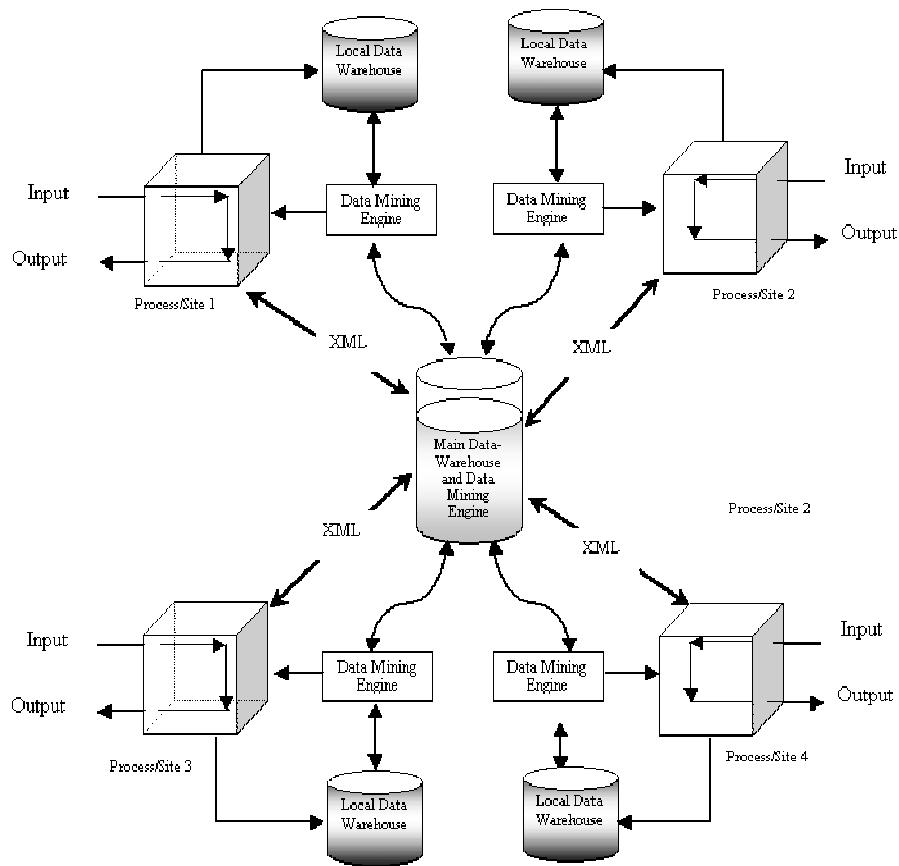
The KDD process is generally driven by individual user skills and experience and is not efficient to use in manufacturing applications where fast intervention may be required if things are going out of control. Since the production process in a factory is usually automated and manufacturing data is obtained continuously from the process, the data must be monitored without interruption so that any anomaly in the process may be detected and eradicated immediately, therefore, the KDD process must run concurrently with the production process [47]. Enterprises aim for increasing levels of accuracy and improvement as manufacturing processes become more advanced and sophisticated and data is continuously recorded.

Manufacturing databases are dynamic with very regular updates made to the records. In the proposed integrated data mining model, data mining techniques can be applied at the micro level within sections of the manufacturing processes, whilst a Main Data Mining Engine may coordinate, share and exchange knowledge between the individual data mining engines. The main Data Mining Engine when connected to individual data mining engines establishes a data-mining network (see Fig. 3), which helps to mine the whole process. The whole manufacturing process may be divided into small steps and the data relating to each step and its adjacent steps are mined independently. The same principle can be applied to the extended manufacturing enterprises where the sites are not in one premises but are located at different places, cities, countries or even continents. The whole process is therefore supported and explored by the remainder of the network. Its activities and results are consistently communicated through the Main Data Mining Engine. Thus individual areas of data mining activity are kept to a manageable size whilst still supporting related areas of activity.

In an integrated manufacturing environment where the product is developed at separate locations or in discrete steps data mining can best be used to control any individual process or step through identification of hidden information in its associated data. The data-mining algorithm say rule induction can discover the relationships within the individual process, or dependent processes. It cannot however be utilized for the other discrete steps unless a common data warehouse collects the data. The concept of the proposed integrated data mining model works on the basis that rules; principles and concepts applicable to one manufacturing stage/site may also be utilized (tested and applied) for other similar stages, (by the exchange of activity and rules information via the Main Data Mining Engine). This integrated data mining model will work where a product goes into different stages and data for each and every step is collected and stored in a pre-designed data warehouse or in the pattern warehouse as knowledge is much more compact than data [63]. Data mining activities and the main data warehouse will work in parallel during the whole activity with the production process data and company data warehouses.

Each stage will have its own local data mining engine and data warehouse where the data will be stored after cleaning. The data will also be transferred to the main data warehouse, which has a direct link with a pattern warehouse for the analysis or mining of the whole system's data.

If the same methodology is applied to an integrated system where production is being done at different sites then the central data warehouse will be built on a standard format. The data from the individual sites will be transferred to the main data warehouse using XML format where the data will be mined for the whole process and rules/knowledge extracted will be returned back in the same format.



**Figure 3. An Integrated Data Mining System Model**

The integrated data mining will be productive in the sense that if different rules are identified for two individual, but similar small manufacturing/production steps, the rules can be shared, and each data-mining engine can use its knowledge to refine the "best" one for its particular application. In this way, knowledge can be fed into the main data warehouse, so results can be reused in the future, as a pattern warehouse will be developed. Future applications can then make use of the stored patterns and rules instead of always having to return to the original manufacturing process databases.

The figure shows four manufacturing processes or sites. The input and output of the manufacturing process is shown and the data from the manufacturing process is collected at the local data warehouse and is also transferred to the main central data warehouse in a neutral format say XML. The outcome of the data mining process (if any) is implemented to the manufacturing process and same information is also reported to the central system which analyzes the kind of problem tackled by the local system and stores an index and the parameters for the problem occurred. If any of the other

processes indicate similar kinds of problem then the central or Main Data Mining Engine first tries the same solution to that problem to see if it works. This methodology will help in future to minimize the time spent on understanding the problems and finding the solution. The data-mining engine can also suggest alternative solutions, but using an old solution and refining it to suit the present requirements should help to tackle the problem in more efficient, cost effective ways.

The integrated model should also be aided with the online visualization model so that the worker who is working on the machines/products can get a clear idea about the process and products. Visualisation should allow a user to discuss and explain the logic behind the model with colleagues, internal/external customers, and other users [64]. For example if there is a recurring problem with a particular step or process on a product then visual checks for previous trends/rules or current results at other sites with the same or similar steps will help in getting a workable idea to fix the problem. Therefore visualisation of the whole mining process will help and enable the output of the data mining system to be understood qualitatively.

### **Conclusion:**

It has been showed that along with other areas, manufacturing enterprises can be benefited with the data mining techniques. There are lots of areas within manufacturing enterprises, few of them are explained in this paper, where data mining can find its ways of implementation and can give results comparable to any other corrective measures based on the engineering methodologies. The data mining architecture proposed in this paper can be refined from a small factory to an extended enterprise. Such kind of data mining approach will be an essential part during the designing of an advanced manufacturing process in future which can learn from its own mistakes and will do the corrective actions not only for its own processes but will help the other processes with its experiences.

<sup>1</sup> Corresponding Author

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10/13/2010

# Available Zn Distribution, Response and Uptake of Rice (*Oriza sativa*) to Applied Zn Along a Toposequence of Lake Gerio Fadama Soils at Yola, North-eastern Nigeria.

H. E. Shehu and G. Y. Jamala

Department of Crop Science, Adamawa State University, Mubi, P. M. B 25 Mubi, 650001, Adamawa State, Nigeria.  
[harushe2003@gmail.com](mailto:harushe2003@gmail.com)

**Abstract:** A screen house pot experiment was conducted at FAO/TCP farm of the Adamawa State University, Mubi north-eastern Nigeria, to study the response of rice to Zn fertilizer application and the distribution of Zn along toposequence of the Lake Gerio Fadama soils of North-eastern Nigeria which was used for the study. The experiment consisted of four Zn rates of 0, 5, 7.5 and 10 kg Zn ha<sup>-1</sup> as ZnSO<sub>4</sub>.7H<sub>2</sub>O. The effect of treatment on Zn concentration and dry matter yields response were determined. The 0.1N HCl and DTPA extractable Zn ranged from 8.5 to 9.5 mg kg<sup>-1</sup> soil, 2.2 to 2.7 mg kg<sup>-1</sup> soil with mean values of 9.08 and 2.35 mg kg<sup>-1</sup> soil respectively. Available Zn soil status is therefore assessed as medium. Dry matter yields and Zn uptake were optimum at 5 kg ha<sup>-1</sup> with corresponding values of 2.04 and 11.86 mg kg<sup>-1</sup> respectively.

[H. E. Shehu and G. Y. Jamala. Available Zn Distribution, Response and Uptake of Rice (*Oriza sativa*) to Applied Zn Along a Toposequence of Lake Gerio Fadama Soils at Yola, North-eastern Nigeria. Journal of American Science 2010;6(11):1013-1016]. (ISSN: 1545-1003). (<http://www.americanscience.org>)

**Key words:** Zinc uptake; Toposequence; Fadama soils; Rice

## Introduction

The need to increase food production becomes a major concern considering the rate at which population is increasing. This resulted in intensive use of land with the consequence of depleting the macro and micro nutrients faster than they can be restored. Attention has been given to the supply of macronutrients only forgetting the micronutrients.

In Nigeria and even in many developing countries, study on soil micronutrients was neglected in both soil science and crop production. Most work on soil in Nigeria has been on soils developed on crystalline basement complex, sedimentary and metamorphic rocks (Kparmwang *et al* 1995) and most savanna soils are reported to have low clay contents, organic matter and cation exchange capacities (Lombin, 1983). Zn deficiency was first diagnosed in rice on the calcareous soils of northern India (Nene, 1966). It was subsequently found to be a wide spread phenomenon in lowland rice areas of Asia, and next to N and P deficiencies.

Zn deficiency is now considered the most widespread disorder in lowland rice (Neue and Lantin, 1994). Quijano-Guerta *et al*, 2002) reported that Zn is the major cause of low rice yields in the major rice producing areas as Zn deficiency is one of the prevalent chemical stresses that is linked to coastal hydrology. With the initiation of several irrigation schemes in north-eastern Nigeria particularly the Lake Gerio Irrigation project, Gerio basin has become agriculturally important. The area

covers about 850 ha and only 320 ha are currently under cultivation and rice is the main irrigated crop.

Information on the micronutrient fertility status of these soils particularly Zn is not documented in the literature though deficiency has been noted in other crops.

Therefore, this investigation was carried out to study and determine the available Zn distribution along toposequence and responses of rice to applied Zn under pot culture conditions.

## Materials and Methods

A pot experiment was conducted in screen house in the FAO/TCP farm of the Adamawa State University, Mubi (10° 16' N and 13° 17' E) to determine the distribution of available Zn along the toposequence of lake Gerio fadama soils in Yola, north-eastern Nigeria and to asses the response and uptake of rice (*Oriza sativa*) to applied Zn from 11<sup>th</sup> August to 27<sup>th</sup> September 2008. Soil samples were collected from points using Geo-reference system satellite receiver (GPS, 2002 – GARMIN 12). The sampling points were divided into upper, middle and down slopes. The upper slope ranged between 156.4 to 159.3 m above sea level. The middle slope ranged from 153.4 to 159.0 m above sea level while the down slope ranged from 155.7 to 156.6 m above sea level. These primarily represent the soils of Lake Gerio Fadama irrigation projects of the lower Benue trough of north-eastern Nigeria. The soil samples were sieved using 2 mm sieve and analyzed for particle size, pH, electrical conductivity, organic

carbon, available P, total N, K, Ca, Na and available Zn.

Available Zn was extracted by 0.1N HCl and DPTA. Soil solution ratio was 1:10 and 1:2 for HCl and DPTA respectively. This was shaked for 2 hours and the filtrate analyzed for Zn on atomic absorption spectrophotometer (Buck Scientific model – 210 VG). To determine the response of rice to applied Zn, composite surface soil samples, 0-15 cm were used. The treatments include 0, 5, 7.5 and 10 kg Zn ha<sup>-1</sup> replicated three times in a complete randomized design. Rice seeds (IITA 2-1-2 Cv.) were sown in pots and filled with 4 kg soil and was grown for seven weeks. Deionized water was used for irrigation. Shoots were harvested and oven dried at 70 °C and dry matter yields were determined. Data was subjected to statistical analysis using SAS software (1999).

## Results and Discussion

Particle size and Zn distribution are presented in Table 1. The texture of the soil ranged from silt clay to silt clay loam. The Zn content ranged from 8.5 to 9.5 mg kg<sup>-1</sup> with mean value of 9.1 mg kg<sup>-1</sup>. Critical limits for Zn suggested by Nelson *et al* (1959), Trerweiler and Landsay, (1969) and latter confirmed by Shukla and Kwari (1990) were 0 to 4.5, 3.5 to 4.0,

4.5 and 4.5 mg kg<sup>-1</sup> HCl extractable Zn respectively. Considering these reports, all HCl extractable Zn of Lake Gerio irrigation project were higher than the 4.5 mg kg<sup>-1</sup> critical levels for Zn and thus could be classified as medium. Medium Level has also been reported in some lowland areas of Nigeria such as Sokoto, Zamfara, Kebbi, Bayelsa, Rivers and part of Niger state (FAO, 2005). There were no marked differences in the distribution of available Zn along toposequence which agrees with the findings of Voncir *et al*, (2008).

The DPTA critical limits reported by Lindsay and Norvel, (1978) and Landon, (1984) to be 0.6 to 0.8 and 0.5 to 1.0 mg kg<sup>-1</sup> soil DPTA extractable Zn respectively were lower than the values obtained at Lake Gerio fadama soils with mean value of 2.4 mg kg<sup>-1</sup>. This also could be classified as moderate confirming the HCl extractable Zn status.

Some chemical properties of the experimental soil are presented in Table 2. Soil pH ranged from 6.4 to 6.8. Organic matter content was also low (ranged from 1.7 to 2.1%). The distribution of available P ranged from 3.2 to 10.9 mg kg<sup>-1</sup> while N lower and upper values were 0.03 and 0.08% respectively. The ECEC ranged from 20.1 to 22.5 meq 100 g<sup>-1</sup> as EC ranged from 0.22 to 0.46 ms cm<sup>-1</sup>.

**Table 1: Particle size and Zn distribution of the experimental soil.**

Toposequence	Particle size (%)			Textural class	DPTA (mg kg <sup>-1</sup> )	0.1N HCl (mg kg <sup>-1</sup> )	Zn status
	Sand	Silt	Clay				
Upper slope	72.0	11.0	17.0	SL	2.4	9.5	medium
Middle slope	69.0	12.0	19.0	SCL	2.7	9.2	medium
Down slope	66.0	10.0	24.0	SCL	2.2	8.5	medium
Composite	68.0	12.0	20.0	SCL	2.4	9.1	medium
Mean	69.0	11.0	20.0	SCL	2.35	9.08	medium

SL= Silt Loam; SCL=Silty Clay Loam

**Table 2: Distribution of DPTA/HCl extractable Zn and other soil properties along toposequence of Lake Gerio irrigation Project, Yola, North- eastern Nigeria.**

Toposequence	pH 1:2.5 (H <sub>2</sub> O)	Organic C (%)	%N	Bray P (mg kg <sup>-1</sup> )	ECEC (meq kg <sup>-1</sup> )	EC (ms cm <sup>-1</sup> )
Upper slope	6.6	1.7	0.08	10.9	20.1	0.26
Middle slope	6.4	2.1	0.03	1.5	20.1	0.22
Down slope	6.8	2.1	0.03	3.2	22.5	0.46
Composite	6.6	2.0	0.05	5.0	20.2	0.31
Mean	6.6	2.0	0.05	5.15	20.7	0.313

**Table 3: Zn uptake and dry matter responses of rice plant to applied Zn.**

Zn (kg ha <sup>-1</sup> )	Dry matter (g pot <sup>-1</sup> )		Zn uptake (mg pot <sup>-1</sup> )	
	Mean	SE	Mean	SE
0	0.83 <sup>b</sup>	±0.21	5.79 <sup>b</sup>	±1.45
5	2.04 <sup>a</sup>	±0.29	11.86 <sup>ab</sup>	±2.90
7.5	1.77 <sup>a</sup>	±0.23	14.54 <sup>a</sup>	±2.79
10	2.50 <sup>a</sup>	±0.24	15.72 <sup>a</sup>	±2.07

Means with the same letter are not significantly different using Duncan's Multiple Range Test at 5% level of significance.

**Table 4: Correlation coefficient between dry matter and Zn, N, P and K uptake by rice.**

	Zn	Dry matter	Zn uptake	N uptake	P uptake
Zn	1.000				
Dry matter	0.755**	1.000			
Zn uptake	0.712**	0.700**	1.000		
N uptake	0.414	0.720**	0.709**	1.000	
P uptake	0.511	0.799**	0.493	0.786**	1.000
K uptake	0.702**	0.987**	0.678*	0.713**	0.783**

\* = Significant at 5% level of probability

\*\* = Significant at 1% level of probability

The response of dry matter to applied Zn is shown in Table 2. Though the soils showed a moderate Zn content, increase in dry matter yield was recorded. Optimum dry matter yield was obtained at 5 kg ha<sup>-1</sup> Zn application which gave a corresponding dry matter yield of 2.04 g pot<sup>-1</sup> while the highest dry matter yield was recorded at 10 kg Zn ha<sup>-1</sup> rate of 2.50 g pot<sup>-1</sup>. This is confirmed in the positive correlation (Table 4) that exists between Zn and dry matter yield ( $r = 0.755$ ). Lack of significant response between the Zn rates could be attributed to the soil Zn status and thus the demand for the crop could be met at 5 kg ha<sup>-1</sup>. Other factors that might have contributed to no marked response of dry matter to Zn application could be attributed to low organic matter content and Mg:Ca ratio as also noted by Neue and Landin, (1994).

Zn uptake response by rice plant to applied Zn (Table 3) was significant at  $P = 0.05$ . The application at 5, 7.5 and 10 kg Zn ha<sup>-1</sup> had uptake advantage of 105, 151 and 172% respectively over soils that did not receive Zn application. Optimum Zn uptake was obtained at 5 kg ha<sup>-1</sup> Zn application. This shows that

uptake of Zn in plants is a function of the amount of Zn applied. This is shown in the significant correlation between applied Zn and Zn uptake of  $r = 0.712$ . In assessing the uptake of Zn, the previous work of Dobermann and Fairhurst (2000) who also observed that ratio of P:Zn in the shoot are good indicators of Zn deficiency where values are not to exceed 20 to 60:1. The application of Zn has contributed to the positive correlation between N and P uptake ( $r = 0.786$ ) and N and K uptake ( $r = 0.713$ ).

Nutrient utilization efficiency (NUE) of Zn indicated that the rate of increase in uptake from 5 to 7.5 kg ha<sup>-1</sup> Zn rate is greater than the rate of increase from 7.5 to 10 kg<sup>-1</sup>. This shows that decline in NUE starts at 7.5 kg ha<sup>-1</sup> rate. The low utilization of this element may not be unconnected with the medium level soil status of Zn since the element is required in small quantity.

### Conclusion

The inferences drawn from the response of rice to Zn application confirms the results of soil analysis where most of the soil along the toposequence are at medium level and will require low level of Zn

application of 5 kg Zn ha<sup>-1</sup> for optimum yields in the Lake Gerio irrigation projects.

#### Acknowledgement:

We are grateful to Adamawa State University, Mubi for providing facilities for the research.

#### Corresponding author:

H. E. Shehu  
Department of Crop Science, Adamawa State University, Mubi, P. M. B 25 Mubi, 650001, Adamawa State, Nigeria.  
Email: [harushe2003@gmail.com](mailto:harushe2003@gmail.com)

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20/7/2010

# Approximate Optimal Control for a Class of Nonlinear Volterra Integral Equations

Akbar H. Borzabadi, Akram Abbasi and Omid S. Fard

Department of Applied Mathematics, Damghan University, Damghan, Iran  
[borzabadi@dubs.ac.ir](mailto:borzabadi@dubs.ac.ir)

**Abstract:** In this study an iterative approach to extract approximate solutions of optimal control problems which are governed by a class of nonlinear Volterra integral equations is presented. The structure of approach is based on the parametrization of the control and state functions. Considering some conditions on the problem, the convergence of the given approach is studied. Numerical examples illustrate the efficiency of the given approach.

[Akbar H. Borzabadi, Akram Abbasi and Omid S. Fard. Approximate Optimal Control for a Class of Nonlinear Volterra Integral Equations. Journal of American Science 2010;6(11):1017-1021]. (ISSN: 1545-1003). (<http://www.americanscience.org>).

**Keywords:** Optimal control; Volterra integral equations; iterative schemes; approximation

## 1. Introduction

The classical theory of optimal control was developed in the last years as a powerful tool to create optimal solutions for real processes in many aspects of science and technology. Complexity of applying analytical methods for obtaining fast and near optimal solutions is the reason for creating numerical approaches. An overview of numerical methods for solving optimal control problems described by ODE and integral equations can be found in (Schmidt, 2006). However these methods are not much developed for optimal control of nonlinear integral equations. Belbas (1999; 2007; 2008) has introduced and elaborated some interesting iterative schemes with their convergence for optimal control of Volterra integral equations considering some conditions on the kernel of integral equation. Also some methods based on approximating the kernel of integral equation which gives rise to a system of ordinary differential equations for approximating the Volterra integral equation can be seen in (Lukas and Teo, 1991; Wu *et al.*, 2007).

Of course it seems that the lack of general methods for solving Volterra integral equations makes serious difficulties in using of these schemes.

The idea of combination of some numerical methods for solving optimal control problems and Volterra integral equations may lead to present executable numerical approaches for obtaining near optimal solutions of optimal control problems governed by Volterra integral equations. This study intends to actualize this idea by combining the method of parameterization, (Mehne and Borzabadi, 2006; Teo *et al.*, 1999a; 1999b) and the method of power series, (Maleknejad *et al.*, 2007a; 2007b; Tahmasbi and Fard, 2008), which are successful methods for solving some classes of optimal control problems and

Volterra integral equations, respectively, for providing a numerical scheme to find approximate optimal control of systems governed by some classes of nonlinear Volterra integral equations which can be described by the following minimization problem:

$$\text{Minimize } J(x, u) = \int_0^T \zeta(t, x(t), u(t)) dt \quad (1)$$

Subject to:

$$x(t) = y(t) + \int_0^t k(s, t, x(s)) ds, \text{ a.e. on } [0, T] \quad (2)$$

where,  $x(\cdot), u(\cdot) \in C^\infty([0, T])$ ,  $\zeta \in C([0, T] \times P \times P)$  and  $k \in C([0, T] \times P \times P \times P)$ .

After this and without loss of generality we suppose  $T = 1$ . Analytical discussions about existence and uniqueness of the optimal control of systems governed by nonlinear Volterra integral equations can be found in (Angell, 1976).

## 2. The control and state parameterization

Let  $Q$  be the subset of product space  $C^\infty([0, 1]) \times C^\infty([0, 1])$  contains all pairs  $(x(\cdot), u(\cdot))$  that satisfy in the integral Eq. 2. Also let  $Q_{m,n}$  be the subset of  $Q$  consisting of all pairs  $(x_m(\cdot), u_n(\cdot))$  where  $u_n(\cdot)$  is a parameterized control function as the following polynomial:

$$u_n(t) = \sum_{i=0}^n a_i t^i \quad (3)$$

And  $x_m(\cdot)$  is extracted solution of the integral equation:

$$x(t) = y(t) + \int_0^t k(s, t, x(s), u_n(s)) ds \quad (4)$$

and it is considered as a polynomial of degree at most  $m$ :

$$x_m(t) = \sum_{j=0}^m e_j(\alpha_0, \alpha_1, \dots, \alpha_n) t^j \quad (5)$$

such that  $e_j : P^n \rightarrow P$ ,  $j = 1, 2, \dots, m$ , are continuous functions. Now we consider the minimizing of  $J$  on  $\mathcal{Q}_{m,n}$  with  $\{\alpha_k\}_{k=0}^n$  as unknowns. This is obviously an optimization problem in  $n$  dimensional space:

$$\{(\alpha_0, \alpha_1, \dots, \alpha_n) \in \square^{n+1} : \alpha_0 = u_n(0) = u_0, \sum_{k=0}^n \alpha_k = u_n(1) = u_1\}$$

and  $J(x_m, u_n)$  may be considered as a function  $J(a_0, a_1, \dots, a_n)$ .

Suppose  $(x_m^*(\cdot), u_n^*(\cdot))$  be the solution of minimizing  $J$  on  $\mathcal{Q}_{m,n}$ ,  $m = 1, 2, \dots, n = 1, 2, \dots$  then polynomial form of  $u_n^*(\cdot)$ ,  $n = 1, 2, \dots$  in (3) and considering the special form of integral equation kernel allow us applying a method based on power series for extracting polynomial solution of (4), (Tahmasbi and Fard, 2008), where applying this method give rise to obtain a sequence of state functions  $\{x_m^*(\cdot)\}_{m=1}^\infty$  as Taylor series, see Theorem 1 in (Tahmasbi and Fard, 2008) and finally to achieve a minimizing sequence  $\{(x_m^*(\cdot), u_n^*(\cdot))\}_{m,n}$ .

**Lemma 1:** If  $\alpha_{m,n} = \inf_{\mathcal{Q}_{m,n}} J$  for  $m, n = 1, 2, \dots$ , then  $\{\alpha_{m,n}\}_{m,n=1}^\infty$  is a convergent sequence.

**Proof:** By definition  $\mathcal{Q}_{m,n}$  we have:

$\mathcal{Q}_{1,1} \subset \mathcal{Q}_{1,2} \subset \mathcal{Q}_{2,2} \subset \dots \subset \mathcal{Q}_{m,n} \subset \mathcal{Q}_{m+1,n} \subset \dots \subset \mathcal{Q}$ , and therefore:

$$\alpha_{1,1} \geq \alpha_{1,2} \geq \alpha_{2,2} \geq \dots \geq \alpha_{m,n} \geq \alpha_{m+1,n} \geq \dots \geq \alpha,$$

Now it can be concluded that  $\{\alpha_{m,n}\}$  is convergent because it is a no decreasing and bounded from below sequence.

**Theorem 1:** If  $\lim_{m,n \rightarrow \infty} \alpha_{m,n} = \alpha$  then  $\alpha = \inf_{\mathcal{Q}} J$ .

**Proof:** By Lemma 1, let  $\{\alpha_{m,n}\}$  is convergent to namely  $\hat{\alpha} \geq \alpha$ . By contradiction if  $\hat{\alpha} > \alpha$ , then  $\hat{\alpha} - \alpha > 0$ . By the properties of infimum, (Rudin, 1976), there exists  $(x(\cdot), u(\cdot))$ , such that:

$$J(x(\cdot), u(\cdot)) < \alpha + \epsilon = \frac{\hat{\alpha}}{2} + \frac{\alpha}{2} \quad (6)$$

From the continuity of  $J$ , there is a  $\delta > 0$  where:

$$|J(v(\cdot), w(\cdot)) - J(x(\cdot), u(\cdot))| < \epsilon, \quad (7)$$

Whenever:

$$\|v(\cdot), w(\cdot) - (x(\cdot), u(\cdot))\|_\infty < \delta. \quad (8)$$

Here  $\|\cdot\|_\infty$  is a norm on the vector space  $C^\infty([0,1]) \times C^\infty([0,1])$  which can be defined as follows:

$$\|v(\cdot), w(\cdot)\|_\infty = \|v(\cdot)\|_\infty + \|w(\cdot)\|_\infty,$$

and one can easily check the properties of the norm for it. On the other hand side, the set of all polynomial pairs are dense in  $C^\infty([0,1]) \times C^\infty([0,1])$ , so there is a pair of polynomials  $p_m(t)$  of degree at most  $m$  and  $q_n(t)$  of degree at most  $n$  such that:

$$\|(p_m(\cdot), q_n(\cdot)) - (x(\cdot), u(\cdot))\|_\infty < \frac{\delta}{3}. \quad (9)$$

Whereas the pair  $(p_m(\cdot), q_n(\cdot))$  does not satisfy:

$(p_m(0), q_n(0)) = (x_0, u_0)$ ,  $(p_m(1), q_n(1)) = (x_1, u_1)$ , We have to define another polynomials:

$$v_m(t) = p_m(t) + (x_0 - p_m(0))(1-t) + (x_1 - p_m(1))t,$$

$$w_n(t) = q_n(t) + (u_0 - q_n(0))(1-t) + (u_1 - q_n(1))t,$$

that satisfy  $(v_m(0), w_n(0)) = (x_0, u_0)$  and

$$(v_m(1), w_n(1)) = (x_1, u_1), \text{ so } (v_m, w_n) \in \mathcal{Q}_{m,n}.$$

From (9) for  $t = 0, 1$  we have:

$$\|(p_m(0), q_n(0)) - (x_0, u_0)\|_\infty < \frac{\delta}{3},$$

$$\|(p_m(1), q_n(1)) - (x_1, u_1)\|_\infty < \frac{\delta}{3}$$

Now for  $t \in [0, 1]$  by definition  $v_m(\cdot)$  and  $w_n(\cdot)$  we have:

$$\begin{aligned} \|v_m(\cdot), w_n(\cdot) - (x(\cdot), u(\cdot))\|_\infty &\leq \|v_m(t) - x(t)\|_\infty + \|w_n(t) - u(t)\|_\infty \\ &\leq \|p_m(0), q_n(0) - (x_0, u_0)\|_\infty(1-t) + \|p_m(1), q_n(1) - (x_1, u_1)\|_\infty t \end{aligned}$$

$$< \frac{\delta}{3} + \frac{\delta}{3} + \frac{\delta}{3} = \delta.$$

Therefore:

$$\|v_m(\cdot), w_n(\cdot) - (x(\cdot), u(\cdot))\|_\infty < \delta,$$

and (7-8) imply that:

$$|J(v_m(\cdot), w_n(\cdot)) - J(x(\cdot), u(\cdot))| < \epsilon,$$

and so from (6):

$$J(v_m(\cdot), w_n(\cdot)) < \frac{\hat{\alpha}}{2} - \frac{\alpha}{2} + J(x(\cdot), u(\cdot)) < \hat{\alpha}$$

a contradiction concludes with  $(v_m(\cdot), w_n(\cdot)) \in Q_{m,n}$ , so  $\hat{\alpha} = \alpha$ .

Now we summarize the above results in a numerical algorithm for obtaining approximate optimal control of minimizing (1) subject to (2).

**Algorithm 1:** Choose  $\varepsilon_1 > 0$  and  $\varepsilon_2 > 0$  for accuracy of the solution.

Step 1: Let  $m, n, k = 1$ ,  $u_1(t) = a_0 + a_1 t$ ,  $x_1(t) = e_0 + e_1 t$  and  $\alpha_1 = J(x_1(\cdot), u_1(\cdot))$ , where  $e_0 = e_0(a_0, a_1)$  and  $e_1 = e_1(a_0, a_1)$ .

Step 2: Let  $m \rightarrow m+1$  and  $k \rightarrow k+1$  and find  $\alpha_k = \inf_{Q_{m,n}} J$ .

Step 3: If  $|\alpha_k - \alpha_{k-1}| < \varepsilon_1$  then go to Step 4, otherwise go to Step 2

Step 4: Let  $n \rightarrow n+1$  and  $k \rightarrow k+1$  and go to Step 2

Step 5: If  $|\alpha_k - \alpha_{k-1}| < \varepsilon_1$  then stop, otherwise go to Step 4

### 3. Numerical results

In this section some examples show the interesting results of the proposed iterative approach.

**Example 1:** In the first example we consider the optimal control of minimizing:

$$J = \int_0^1 \left( (x(t) - \cos(t))^2 + (u(t) - t)^2 \right) dt \quad (10)$$

subject to the following nonlinear Volterra integral equation:

$$x(t) = y(t) + \int_0^t u(s)^2 (x(s) + ts) ds \quad (11)$$

Where:

$$y(t) = \cos(1-2t) + \sin(t(2-t^2)) - \frac{1}{4}t^5$$

The exact optimal solution of problem (10-11) are  $x^*(t) = \cos(t)$  and  $u^*(t) = t$  with optimal criteria  $J^* = J(x^*(t), u^*(t)) = 0$ . We apply Algorithm 1 on this problem. Let  $m, n = 1$ , so we have:

$$\begin{aligned} u(t) &= a_0 + a_1 t, \quad x(t) = e_0 + e_1 t \\ \Rightarrow x(t) &= 1 + e_1 t, \quad (e_0 = y(0)). \end{aligned}$$

Substituting  $x(t)$  and  $u(t)$  in (11) concludes that  $e_1 = \alpha_0^2$ . To optimize  $J$  on  $(a_0, a_1)$  gives rise to  $a_0 = 0, a_1 = 1$  and so  $e_1 = 0$  and  $\alpha_{1,1} = 0.0444$ . Now let  $m = 2$  and  $n = 1$ . We have:

$$u(t) = \alpha_0 + \alpha_1 t, \quad x(t) = 1 + \alpha_0^2 t + e_2 t^2$$

and again substituting  $x(t)$  and  $u(t)$  in (11) conclude

$e_2 = \frac{1}{2}\alpha_0^4 + \alpha_0\alpha_1 - \frac{1}{2}$  and optimizing  $J$  eventuate the following results:

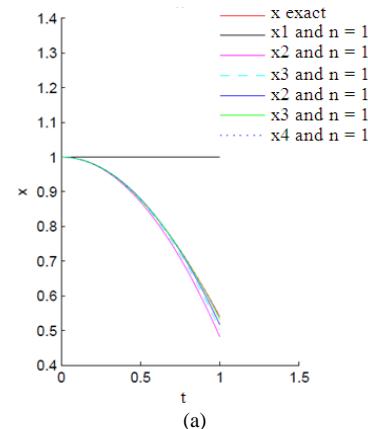
$$a_0 = -0.0187, a_1 = 0.9612,$$

$$e_1 = 3.4969 \times 10^{-4}, e_2 = -0.5180$$

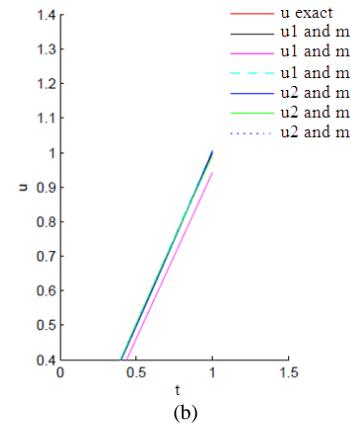
and  $\alpha_{2,1} = 0.0107$ . In Table 1 the successive applying of Algorithm 1 for some values of  $m$  and  $n$  is shown. Also the state and control functions that are obtained in process of using Algorithm 1 for problem (10-11) are shown in Fig. 1.

Table 1: The results of applying proposed algorithm in Example 1

$n$	$m$	$\alpha_{m,n}$
1	1	0.0444
1	2	0.0107
1	3	$1.5226 \times 10^{-4}$
2	2	$7.8920 \times 10^{-5}$
2	3	$2.5166 \times 10^{-5}$
2	4	$1.0922 \times 10^{-7}$



(a)



(b)

Fig. 1: The state and control functions in example 1

**Example 2:** In this example the optimal control problem of minimizing

$$\text{Minimize } J = \int_0^1 ((x(t) - \sin(t))^2 (u(t) - t)^2) dt \quad (12)$$

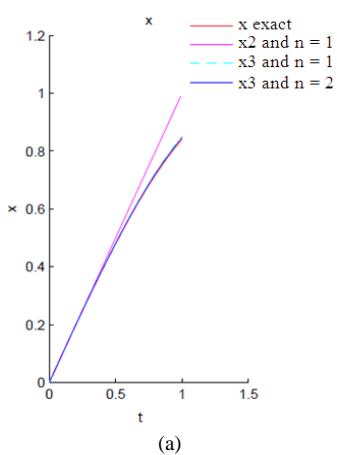
governed by the nonlinear Volterra integral equation:

$$x(t) = y(t) + \int_0^t u(s)(x(s) + t) ds \quad (13)$$

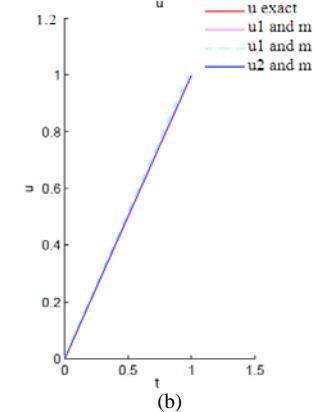
where,  $y(t) = t \cos(t) - \frac{1}{2}t^3$  is considered. The results of successive applying Algorithm 1 on this problem, as previous example, is shown in Table 2. Also the state and control functions obtained during the application of Algorithm 1 on problem (12-13) are shown in Fig. 2.

Table 2: The results of applying proposed algorithm in Example 2

$n$	$m$	$\alpha_{m,n}$
1	2	$3.5575 \times 10^{-11}$
1	3	$1.4975 \times 10^{-12}$
2	3	$3.0905 \times 10^{-14}$



(a)



(b)

Fig. 2: The state and control functions in example 2

**Example 3:** It seems to obtain suitable approximate solution for problems that have the exact solution as exponential function is difficult. In this example we consider the optimal control problem of minimizing nonlinear functional:

$$\text{Minimize } J = \int_0^1 ((x(t) - e^t)^2 (u(t) - e^t)^2) dt \quad (14)$$

on the Volterra integral equation:

$$x(t) = y(t) + \int_0^t u(s)(x(s) + t) ds \quad (15)$$

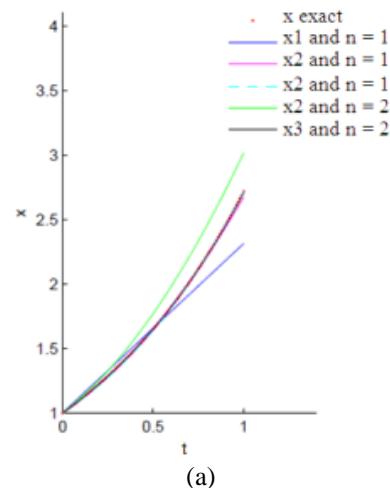
Where:

$$y(t) = e^t(1-t - \frac{1}{2}e^t) + t + \frac{1}{2}$$

The exact optimal control and state functions are  $u^*(t) = e^t$  and  $x^*(t) = e^t$  respectively and optimal criteria is  $J^* = J(x^*(t), u^*(t)) = 0$ . Table 3 shows interesting results by applying proposed approach on the problem (14-15). In Fig. 3, one can see the state and control functions that are obtained during the process of applying Algorithm 1 on problem (14-15).

Table 3: The results of applying proposed algorithm in example 3

$n$	$m$	$\alpha_{m,n}$
1	1	$2.1070 \times 10^{-4}$
1	2	$2.3385 \times 10^{-7}$
1	3	$1.9795 \times 10^{-8}$
2	2	$1.4383 \times 10^{-8}$
2	3	$4.2201 \times 10^{-10}$



(a)

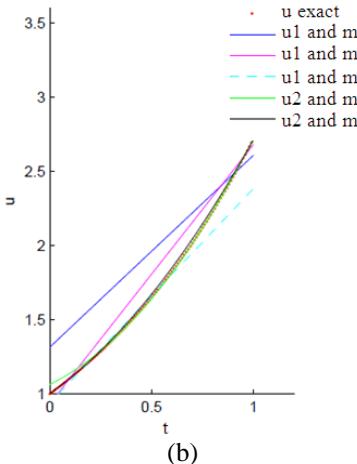


Fig. 3: The state and control functions in example 3

#### 4. Conclusion

In this study, we have proposed a numerical scheme for finding approximate solution of optimal control problems governed by a class of nonlinear Volterra integral equations. Our limitation in the application of this method depends on the type of integral equations that we face because of the power series method only for solving certain categories of integral equations may be applied. Although the presented numerical examples show the efficiency of the method for solving a wide range of problems.

#### Corresponding Author:

Dr. Akbar H. Borzabadi  
 Department of Applied Mathematics  
 Damghan University  
 Damghan, Iran  
 E-mail: [borzabadi@dubs.ac.ir](mailto:borzabadi@dubs.ac.ir)

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# Virulence Factors, Plasmid Profiling and Curing analysis of Multi-drug Resistant *Staphylococcus aureus* and Coagulase negative *Staphylococcus* spp. isolated from Patients with Acute Otitis Media.

<sup>a</sup> Akinjogunla O. J. and <sup>b</sup> Enabulele, I. O.

<sup>a</sup> Department of Microbiology, Faculty of Science, University of Uyo, P.M.B 1017, Uyo, Akwa Ibom State, Nigeria.

<sup>b</sup> Department of Microbiology, Faculty of Life Sciences, University of Benin, P.M.B.1154. Benin City, Edo State, Nigeria.

[papajyde2000@yahoo.com](mailto:papajyde2000@yahoo.com)

**ABSTRACT:** Microbiological and molecular techniques were used to determine the virulence factors, plasmid profile and antibiotic susceptibility spectrum of *Staphylococcus aureus* and CON-*Staphylococcus* spp isolated from patients with acute otitis media attending University of Uyo Teaching Hospital, General Hospital, Ikot-Ekpene and Nekede Paediatric Hospital between January, 2009 and January, 2010. 42 (30.9%) *Staphylococcus aureus* and 21 (15.4%) CON *Staphylococcus* spp were isolated from the aural swab samples collected. *Staphylococcus aureus* produced 16 (38.1%), 22 (52.4%) and 4 (9.5%) of alpha, beta and gamma haemolysis, respectively, while CON-*Staphylococcus* spp. produced more of beta haemolysis than both alpha and gamma. The results showed that 14 (33.3%) *Staphylococcus aureus* and 9 (42.9%) of CON-*Staphylococcus* spp are beta-lactamase producer. The antibiotics susceptibility testing showed that 29 (69.0%), 26 (61.9%), 27 (64.3%), 28 (66.7%) and 29 (69.0%) of *Staphylococcus aureus* were sensitive to peni-cillin, ceftriaxidime, cefoxitin, ciprofloxacin and levofloxacin, respectively. 12 (28.6%) of *Staphylococcus aureus* were resistant to streptomycin and iminipen, while about 45.2% - 50.0% were resistant to cephalothin and amoxicillin. CON-*Staphylococcus* spp also showed varied percentages of sensitivity ranging between 42.9% for streptomycin and 71.4% for moxifloxacin. The result also showed that 19.2 % of *Staphylococcus aureus* and 9.6% of CON-*Staphylococcus* spp. were resistant to more than eight antibiotics with (MAR) index ranging from 0.25 to 1.00 and 0.25 to 0.75 for *Staphylococcus aureus* and CON-*Staphylococcus* spp. respectively. The results obtained in this study are statistically significant ( $p \leq 0.05$ ). Most of the *Staphylococcus aureus* and CON-*Staphylococcus* spp were cured of their plasmids showing that they are plasmid borne. Large molecular weight plasmids ranging from 23.13kbp to 50.0kbp were harboured by both *Staphylococcus aureus* and CON-*Staphylococcus* spp obtained from acute otitis media. However, continuous surveillance to monitor antimicrobial resistance and guide the antibacterial therapy is overwhelmingly necessary.

[Akinjogunla Olajide Joseph, Enabulele Idahosa Onaiwu. Journal of American Science 2010;6(11):1022-1033]. (ISSN: 1545-1003). (<http://www.americanscience.org>)

**Key Words:** *Staphylococcus*, Plasmid, Prevalence, Infection, Otitis media, Susceptibility, Beta- lactamase

## INTRODUCTION

Otitis media is the inflammation of the middle ear due to pathogenic micro-organisms that are resident in the middle ear (Damoiseaux, 2005; Ekpo *et al.*, 2009). Otitis media which may be acute otitis media (AOM), acute suppurative otitis media (ASOM) or chronic suppurative otitis media (CSOM) occurs in the area between the ear drum and the inner

ear, including the Eustachian tube (Richard and Robert, 1996; Bluestone, 1998). Otitis media is prevalent among children because their eustachian tube is shorter, straighter, made up of more flaccid cartilage, more horizontal than adults and also they have not developed the same resistance to bacteria as found in adults (Bluestone and Klein, 2001; Ihsan *et al.*, 2010). Research showed that 83% of

children will have at least one episode of AOM by the age of three years and this accounts for a large proportion of paediatric presentations to health care professionals and is the most common cause of hearing loss in children (Bluestone and Klein, 2001). The patients with acute otitis media (AOM) and chronic otitis media (COM) present the classic "earache", pain that is severe, continuous and is often accompanied by fever (39°C or more), possibly causing febrile seizures and can lead to insomnia for patients, mild to moderate hearing loss, loss of balance, unusual irritability, unresponsiveness to quiet sounds, and draining of fluid in the ear.(Ehrlich *et al.*, 2002; Rovers *et al.*, 2006). Staphylococci are Gram positive, facultative anaerobes, spherical bacteria in cluster with diameter ranging from 0.5 to 1.5 µm (Adejuwon *et al.*, 2010; Brock and Frazier, 1996). *Staphylococcus aureus*, a worldwide pathogen with its natural reservoir in human belongs to genus of the Micrococcaceae. It is recognized as one of the major causes of severe soft tissue infections, toxic shock syndrome (TSS) and as well as scalded skin syndrome in humans (Lowy, 1998; Weems, 2001). Over time, treatment of serious *S. aureus* infections can be challenging as the widespread use of antibiotics has led some *S. aureus* becoming more resistant to antibiotics (Archer, 1998; Akinjogunla *et al.*, 2010). Recent development in the treatment of patients with otitis media include the evidence of the efficacy of antibiotics especially β-lactam antibiotics and newer topical quinolones such as ofloxacin and ciprofloxacin (Bearden and Danziger, 2001; Loy *et al.*, 2002). The most common causes of bacterial resistance to β-lactam antibiotics are the production of β-lactamases, the presence of plasmid and mutation. Incidence of β-lactamase production in *Staphylococcus aureus* has consistently been reported to be over 80% in all parts of the world (Parker and Collier, 1990). Most developed countries have reported an increase in colonization and infection in hospitalized patients by CON-*Staphylococcus* spp. while there are scanty data on infections caused by CON-*Staphylococcus* spp. in developing countries. The levels of antibiotic resistant infections in the developing world have increased steadily in the last few decades as a result of combination of microbial characteristics and the selective pressure of antimicrobial use (Blondeau and

Tillotson, 2002). Microorganisms' mechanisms of overcoming the activities of antimicrobial agents include the production of structure-altering or inactivating enzymes (e.g. beta-lactamase or amino glycoside-modifying enzymes), alteration of penicillin-binding proteins or other cell-wall target sites, altered DNA gyrase targets, permeability mutations, active efflux and ribosomal modification (Gold and Moellering, 1996; Aaterson, 2001; Levy, 2002). Multidrug-resistant bacteria in both the hospital and community environment are important concern to the clinician, as it is the major cause of failure in the treatment of infectious diseases, increased morbidity, and mortality and the evolution of new pathogens (Hacker *et al.*, 1997; Jones and Phaller, 1998).

Plasmids allow the movement of genetic material, including antimicrobial resistance genes between bacterial species and genera (Miranda *et al.*, 2004). Plasmid profiles determination is the earliest DNA-based method used as serotype-specific reference patterns for detecting certain strain with possible variation in plasmid content which is very important in epidemiological studies. This study was carried out to determine the virulence factors, plasmid profile and curing analysis of multi-drug resistant *Staphylococcus aureus* and coagulase negative *Staphylococcus* spp. isolated from patients with acute otitis media.

## MATERIALS AND METHODS

Middle-ear swabbed samples from 136 patients with acute otitis media attending University of Uyo Teaching Hospital, General Hospital, Ikot-Ekpene and Nekede Paediatric Hospital in Akwa Ibom, were collected from January, 2009 to January, 2010 under aseptic conditions and inoculated into broth cultures for 4-6hrs and later inoculated onto plates of Mannitol Salt Agar (MSA). The plates were incubated aerobically for 24 hrs at 37°C. After overnight incubation, the plates were examined for fermentation of mannitol indicated by colour change of the medium around each colony from red to yellow. The organisms on the positive plates were sub-cultured onto nutrient agar slants and further speciated by conventional laboratory techniques including Gram staining; catalase test, coagulase test, urease production, indole production, citrate

utilization and Voges-proskauer test and coagulase test and isolates that were Gram-positive cocci in cluster, indole negative, catalase positive, citrate positive and coagulase positive were considered as *Staphylococcus aureus* while the coagulase negative were considered as coagulase negative *Staphylococcus* spp (CON-*Staphylococcus* spp).

#### THE ANTIBIOTIC SUSCEPTIBILITY TESTING

The antibiotic susceptibility of the bacterial species isolated was performed on Muller-Hinton agar (MHA) (Merck) plates by disk diffusion method as described by the National Committee for Clinical Laboratory Standards with slight modification. 0.1 ml of each bacterial isolates was seeded into each of the Petri dishes containing Mueller-Hinton agar and were allowed to stand for 30 mins to enable the inoculated organisms to pre-diffuse. The commercially available discs containing the following antibiotics: Penicillin (PEN,10ug), streptomycin (STR,10ug), amoxicillin (AMY,10ug), iminipen (IMI,10 ug), ceftriaxone, (CEF,30ug), cephalothin (CEP,30ug), ceftazidime, (CAZ ,30ug), cefotaxime (CTX , 30ug), ofloxacin (OFL,5ug), ciprofloxacin (CIP, 5ug), levofloxacin (LEV, 5ug) and Moxifloxacin (MOX,5ug) (Oxoid, UK) were aseptically placed on the surfaces of the sensitivity agar plates with a sterile forceps and were incubated at 37°C over night. Zones of inhibition after incubation were observed and the diameters of inhibition zones were measured in millimeters. The interpretation of the measurement as sensitive and resistant was made according to the manufacturer's standard zone size interpretative table. The percentage resistance was calculated using the formula  $PR=a/b \times 100$ , where 'PR' was percentage resistance, 'a' was the number of resistant isolates and 'b' was the number of isolates tested with the antibiotic. The percentage sensitivity was calculated using the formula  $PS=c/d \times 100$ , where 'PS' was percentage sensitivity, 'c' was the number of sensitive isolates and 'd' was the number of isolates tested with the antibiotic.

#### DETERMINATION OF MULTIPLE ANTIBIOTIC RESISTANCE INDEX (MAR)

Multiple antibiotic resistance index (MAR) was determined using the formula  $MAR=x/y$ , where  $x$

was the number of antibiotics to which test isolate displayed resistance and  $y$  is the total number of antibiotics to which the test organism has been evaluated for sensitivity.

#### TEST FOR HEMOLYTIC ACTIVITY

The hemolytic activities of the bacterial species (*Staphylococcus aureus* and Coagulase negative *Staphylococcus* spp.) were identified by the presence of diffuse ( $\alpha$ -hemolysis) or clear ( $\beta$ -hemolysis) halos around the colonies. A colony of each of the bacterial isolates was subcultured onto freshly prepared blood agar (nutrient agar containing human blood) plates incubated at 37°C for 24 hours, after which the colonies were examined for hemolytic activity.

#### TEST FOR BETA LACTAMASE PRODUCTION

Beta-lactamase test was carried out using the Starch Paper Method (SPM) described by Odugbemi et al. (1977). Strips of starch paper about 4 – 6 cm were cut and sterilized using 70% ethanol, the strips were soaked for about 10 min in benzyl penicillin dissolved in phosphate buffer. The cut strips were then spread evenly on Petri dishes and about 18 – 24 hrs old cultures grown on Nutrient Agar were inoculated on the surface of the test starch paper and spread over an area of 2 -3 mm. The Petri dishes were incubated at 37°C for 30 min then Gram's iodine solution was used to flood the plate and drained off immediately. The starch paper turns uniformly black within 30seconds of application. Colonies with decolourized zones are positive for beta-lactamase but colonies with black background show beta-lactamase negative.

#### PLASMID CURING EXPERIMENT:

Plasmid curing was carried out to determine in order to determine the location (plasmid-borne or chromosomal) of the drug resistance marker(s). The curing (elimination) of the resistant plasmids of the (*Staphylococcus aureus* and Coagulase negative *Staphylococcus* spp.) isolated was done using sub-inhibitory concentration of 0.10 mg/ml of acridine orange as described by Sheikh et al. (2003); Yah et al (2007); Akortha and Filgona (2009) with slight modification. Isolates *Staphylococcus aureus* and

Coagulase negative *Staphylococcus* spp. isolates were grown for 24hrs at 37°C in nutrient broth containing 0.10 mg/ml acridine orange. After 24hrs, the broth was agitated to homogenize the content and loopful of the broth medium were then subcultured onto Mueller Hinton Agar (MHA) plates and antibiotic sensitivity testing was carried out as previously described. Absence of zone of inhibition on Mueller Hinton agar was indicative of plasmids-mediated resistance (plasmid cured), while presence of zone of inhibition on Mueller Hinton agar was indicative of chromosome-mediated (plasmid not cured).

### **PLASMID PROFILING AND AGAR GEL ELECTROPHORESIS**

Plasmid extraction was carried out using the method described by Ehrenfeld and Clewell 1987 with slight modification. Pure isolates were inoculated on MRS broth and incubated. The grown cells were harvested and suspended in 200µl of solution A (100mM glucose-50mM Tris hydrochloride (pH 8)-10mM EDTA) containing 10 mg of lysozyme per ml and 10µg/ml mutanolysin and incubated for 30 min at 37°C in an incubator. 400µl of freshly prepared 1% sodium dodecyl sulfate in 0.2 N NaOH was added and the samples were mixed by inverting tubes. 300µl of a 30% potassium acetate solution (pH 4.8) was added and the samples were mixed by vortexing. After incubating on ice for 5 minutes, the debris was removed by a 5-minute centrifugation in a centrifuge (model 5415R; Eppendorf). The supernatant was removed and extracted once with a phenol-chloroform mixture (1:1) and precipitated with an equal volume of isopropanol. The plasmid DNA was then dissolved in 100µl of TE buffer. Electrophoresis of the DNA was carried out on a 0.8% agarose gel in a 0.5X concentration of Tris-Borate-EDTA (TBE) buffer. Agarose gel was prepared by boiling 0.8g of agarose powder in 100mls of 0.5X TBE buffer. After boiling, the solution was allowed to cool and 10µl of ethidium bromide was added to the cooled agarose solution. This was poured into a casting tray with a comb placed across its rim to form wells. The gel was allowed to set for 30 minutes and the comb was removed. 20µl of the plasmid DNA samples were then loaded into the wells after mixing with 2µl of bromophenol blue. A DNA molecular weight marker

was also loaded into one of the wells. The gel was thereafter electrophoresed in a horizontal tank at a constant voltage of 60V for about 1 hour 30 minutes. After electrophoresis, plasmid DNA bands were viewed by fluorescence of bound ethidium bromide under a short wave ultraviolet light transilluminator and the photograph were taken using a digital camera. The DNA bands were matched with those for Lambda DNA Hind III digest molecular weight marker in the range 0.1 - 23.1kb. The approximate molecular weight of each plasmid was consequently obtained by extrapolation on graphical plots of molecular weight of marker against the distance traveled by the respective band

### **STATISTICAL ANALYSIS OF RESULTS**

Frequencies and percentages were calculated for study variables. Chi-square ( $\chi^2$ ) test was used to calculate probabilities and determine significance. A p-value of less than or equal to 0.05 was considered to be statistically significant ( $p \leq 0.05$ ), while p-value more than 0.05 was considered to be statistically not significant (NS).

### **RESULTS AND DISCUSSIONS**

The results of the morphological and biochemical characteristics of *Staphylococcus aureus* and CON-*Staphylococcus* spp. isolated from acute otitis media are shown in Table 1. The occurrence of the Bacterial spp. Isolated from 136 Patients with Acute Otitis Media are shown in Table 2. The virulence factors produced by *Staphylococcus aureus* and CON *Staphylococcus* spp. isolated are shown in Tables 3 and 4, with 16 (38.1%), 22 (52.4%) and 4 (9.5%) of *Staphylococcus aureus* producing alpha (diffuse haemolysis), beta (clear haemolysis) and gamma (absence of haemolysis), respectively, while CON-*Staphylococcus* spp. produced more of beta haemolysis than both alpha and gamma. The results showed that 14 (33.3%) *Staphylococcus aureus* are  $\beta$ -lactamase ( $\beta$ L) producer, while only 9 (42.9%) of CON-*Staphylococcus* spp. produced  $\beta$ -lactamase (Table 4).

The antibiotic susceptibility testing data are shown in Table 5. Of the forty-two *Staphylococcus aureus* screened for susceptibility to the 12 antibiotics the results showed that 29 (69.0%), 26

(61.9%), 27 (64.3%), 28 (66.7%) and 29 (69.0%) of *Staphylococcus aureus* were sensitive to penicillin, ceftriaxidime, cefoxitin, ciprofloxacin and levofloxacin, respectively. A total of 12 (28.6%) of *Staphylococcus aureus* were resistant to both streptomycin and iminipen, while about 45.2% - 50.0% were resistant to cephalothin and amoxicillin. The results of the antibiotic susceptibility profile of the twenty-one CON-*Staphylococcus* spp also showed varied percentages of sensitivity ranging between 42.9% for streptomycin and 71.4% for moxifloxacin. The resistant of CON-*Staphylococcus* spp to ciprofloxacin, levofloxacin, ceftriaxidime and moxifloxacin were low compared to the result obtained when tested with streptomycin and iminipen (Table 5). The most effective antibiotic against *Staphylococcus aureus* and CON-*Staphylococcus* spp isolated from acute otitis media was moxifloxacin as only 26.0% of the bacteria were resistant to it. The multiple antibiotic resistance (MAR) indexes of the *Staphylococcus aureus* and CON-*Staphylococcus* spp are shown in Table 6. The antibiotic resistant *Staphylococcus aureus* have MAR index of 0.25 to 1.00, while the antibiotic resistant CON-*Staphylococcus* spp have MAR index of 0.25 to 0.75. The result showed that 19.2 % of *Staphylococcus aureus* and 9.6% of CON-*Staphylococcus* spp. were resistant to more than eight antibiotics. All the resistant *Staphylococcus aureus* and CON-*Staphylococcus* spp were subjected to plasmid-curing experiments using acridine orange and the results obtained showed that most of the strains lost their plasmids as a result of the cure by acridine (Mutagenic substance) (Tables 7 and 8). The plasmids molecular weights of both *Staphylococcus aureus* and CON-*Staphylococcus* spp ranged from 23.13kb to 50.0kb (Figures 1 and 2). The isolation of *Staphylococcus aureus* and coagulase negative *Staphylococcus* spp. in the middle ear of patients suffering from patient with acute otitis media in this research is in agreement with the report by Ekpo et al., 2009. The fermentation and growth of *Staphylococcus aureus* and CON-*Staphylococcus* spp on mannitol salt agar in this study could be attributed to its ability to grow on relatively high concentrations of sodium chloride, as contained in the medium (Nester et al., 1998). Pathogenicity of *Staphylococcus aureus* in acute otitis media are attributable to

virulence factors such as coagulase and hemolysin produced by the organisms and the occurrence of this virulence factors in *Staphylococcus aureus* is in conformity with the reports of Nester et al., (1998). Geary et al, (1997) reported that coagulase negative *Staphylococcus* spp resistant to beta-lactam antibiotics produced beta-lactamase and this is in agreement with our finding as some of the coagulase negative *Staphylococcus* spp isolated are resistant to penicillin, iminipen, ceftriaxone, cephalothin, ceftriaxidime, and cefotaxime (beta-lactam antibiotics).

Resistant *S. aureus* was seen in clinical practice as early as the 1950s, by acquiring a plasmid that encodes the production of beta-lactamase enzymes causing resistance to beta-lactam antibiotics. The activities of antibiotics against *Staphylococcus aureus* and coagulase negative *Staphylococcus* spp. isolated from acute otitis media patients attending the three Hospitals showed the varied levels of multiple antibiotics resistance. There is wide variation in the use of antibiotics among the physicians of different nations from as low as 31% of cases of acute otitis media in Netherland to as high as 90% in Australia and United States (Delmar et al., 2003). The exceedingly increases and emergence of multidrug resistance pathogens in the developing countries can be attributed to indiscriminate use of antibiotics, complex socio-economic, behavioral antecedents and dissemination of drug-resistant pathogens in human medicine. (Okeke et al., 1999). Antibiotic resistance of pathogens typically causative of acute otitis media continues to increase as the emergence of multi-drug resistant strains especially *Staphylococcus aureus* and CON-*Staphylococcus* spp complicate the management of acute otitis media and increase the risk for treatment failure (Leibovitz, 2003).

Plasmid replication is inhibited by various agents especially acridine (acridine orange) that intercalates between the bases of DNA, without inhibiting the chromosomal DNA replication. In order to determine whether the observed multi drug resistance pattern in the isolates was plasmid or chromosomal mediated, the isolates were screened for the presence of conjugative plasmids using acridine orange and resultantly, some of the resistance markers were stably lost, the lost of resistance markers using acridine orange is line with

that of Yah et al (2007) and Akortha and Filgona (2009). Isolation of plasmids using agarose gel electrophoresis and observation under UV transilluminator showed the various bands for the *Staphylococcus aureus* and coagulase negative *Staphylococcus* spp. with the molecular weights of plasmids ranging from 23-50 Kbp .The molecular weights seemed to be strain specific rather than species specific. In conclusion, culture and sensitivity testing will be instrumental in the management of this infection and continuous surveillance to monitor antimicrobial resistance and guide the antibacterial therapy is overwhelmingly necessary.

**Table 1: Morphological and Biochemical Tests of *Staphylococcus aureus* and CON- *Staphylococcus* spp.**

Morphology / Biochemical Tests	<i>Staphylococcus aureus</i>	CON- <i>Staphylococcus</i> spp
Shape	Coccoid in cluster	Coccoid in cluster
Gram Staining	+ve	+ve
Mannitol	A	A
Sucrose	A	A
Maltose	A	A
Lactose	A	A
Galactose	A	A
Glucose	A	A
Catalase	+ve	+ve
Coagulase	+ve	-ve
Indole	-ve	-ve
Citrate	+ve	+ve
Methyl red	+ve	+ve
Voges-proskauer	-ve	-ve
Gelatin hydrolysis	-ve	-ve

Keys: +ve = positive; -ve = negative; A = Acid production

**Table 2: Bacterial spp. Isolated From 136 Patients with Acute Otitis Media**

Bacterial spp. isolated	Number of Occurrence	Percentage (%) of Occurrence
<i>Staphylococcus aureus</i>	42	30.9
CON <i>Staphylococcus</i> spp.	21	15.4
Total	63	46.3

p≤0.05

**Table 3: Number of Occurrence and Types of Haemolysis Produced by *Staphylococcus aureus* and CON-*Staphylococcus* spp. Isolated from Patients with Acute Otitis Media**

Bacterial spp.	Number of Occurrence	Types of Haemolysis		
		α (%)	β (%)	γ (%)
<i>Staphylococcus aureus</i>	42	16 (38.1)	22 (52.4)	4 (9.52)
CON <i>Staphylococcus</i> spp.	21	6 (28.6)	8 (38.1)	7 (33.3)
TOTAL	63	22 (34.9)	30 (47.6)	11(17.5)

p≤0.05

Keys: α: alpha; β: beta; γ: gamma; CON: Coagulase negative.

**Table 4: The Prevalence of Beta-Lactamase (βL) Producing *Staphylococcus aureus* and CON-*Staphylococcus* spp Isolated from Patients with Acute Otitis Media**

Bacterial spp	Number of Occurrence	No / (%) of βL Producers	No / (%) of βL Non Producers
<i>Staphylococcus aureus</i>	42	14 (33.3)	28 (66.7)

1028

CON <i>Staphylococcus</i> spp	21	9 (42.9)	12 (57.1)
TOTAL	63	23 (36.5)	40 (63.5)

p≤0.05

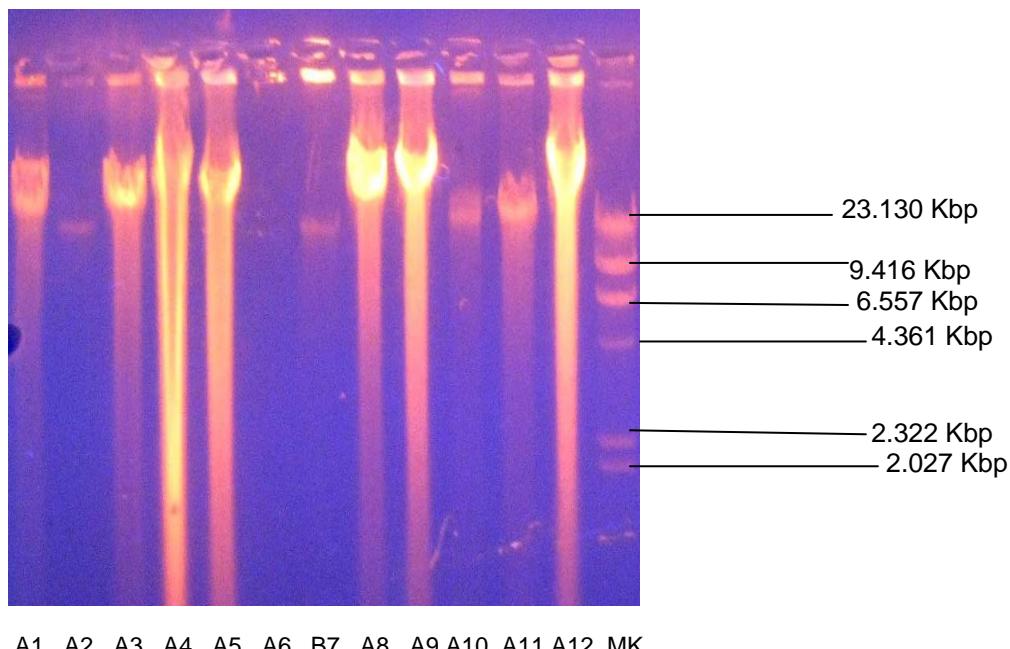
**Table 5: Antibiotic Susceptibility Spectrum of Bacterial spp. Isolated from Acute Otitis Media**

Antibiotics Used	<i>Staphylococcus aureus</i> (N=42)		CON- <i>Staphylococcus</i> spp (N=21)	
	Number /Percentage	Number /Percentage	Number /Percentage	Number /Percentage
	Sensitive	Resistant	Sensitive	Resistant
Penicillin	29 (69.0)	13 (31.0)	13 (61.9)	8 (38.1)
Streptomycin	30 (71.4)	12 (28.6)	9 (42.9)	12 (57.1)
Amoxicillin	21 (50.0)	21 (50.0)	10 (47.6)	11 (52.4)
Iminipen	30 (71.4)	12 (28.6)	9 (42.9)	12 (57.1)
Ceftriaxone	24 (57.1)	18 (42.9)	13 (61.9)	8 (38.1)
Cephalothin	23 (54.8)	19 (45.2)	11 (52.4)	10 (47.6)
Ceftriazidime	26 (61.9)	16 (38.1)	14 (66.7)	7 (33.3)
Cefoxitin	27 (64.3)	15 (35.7)	13 (61.9)	8 (38.1)
Ofloxacin	23 (54.8)	19 (45.2)	11 (52.4)	10 (47.6)
Ciprofloxacin	28 (66.7)	14 (33.3)	14 (66.7)	7 (33.3)
Levofloxacin	29 (69.0)	13 (31.0)	14 (66.7)	7 (33.3)
Moxifloxacin	31 (73.8)	11 (26.2)	15 (71.4)	6 (28.6)

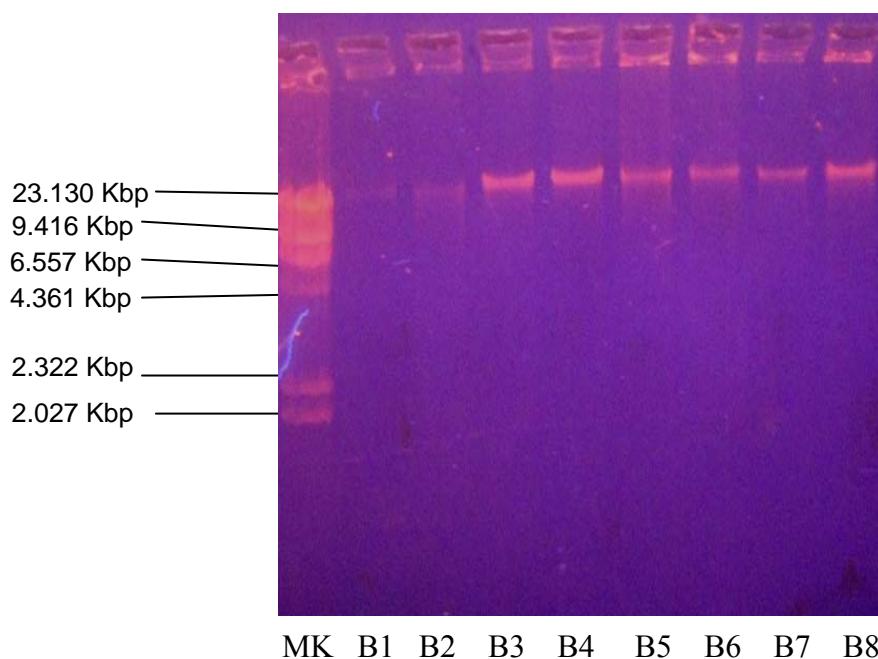
p≤0.05

**Table 6: Multiple Antibiotic Resistance Index of Bacteria Isolated from Acute Otitis Media**

Multiple Antibiotic Resistance Index (MAR)	<i>Staphylococcus aureus</i>	CON- <i>Staphylococcus</i> spp.
	Number / Percentage	Number / Percentage
0.25	6 (14.3)	2 (9.5)
0.33	7 (16.7)	3 (14.3)
0.42	4 (9.52)	2 (9.5)
0.50	6 (14.3)	2 (9.5)
0.58	3 (7.1)	3 (14.3)
0.66	2 (4.8)	1 (4.8)
0.75	2 (4.8)	1 (4.8)
0.83	2 (4.8)	0 (0.0)
0.91	1 (2.4)	0 (0.0)
1.00	1 (2.4)	0 (0.0)



**Figure 1:** Agarose electrophoresis showing plasmid profile of *Staphylococcus aureus* isolated from acute otitis media: Line A1: (>23.13 kb), A2: (>23.13 kb), A3: (>23.13 kb); A4:(>23.13 kb); A5: (>23.13 kb); A6 (No plasmid); A7: (23.13 kb) ;A8: (>23.13 kb) ; A9: (>23.13 kb); A10: (23.13 kb); A11: (23.13 kb) A12: (>23.13 kb); MK: molecular weight marker (*Hind* III digest).



**Figure 2:** Agarose electrophoresis showing plasmid profile of CON-*Staphylococcus* spp. isolated from acute otitis media. MK: molecular weight marker (*Hind* III digest).Line B1: (No plasmid), B2: (23.13 kb), B3: (23.13 kb); B4 - B8: (>23.13 kb)

**Table 7: Plasmid Curing Analysis of Resistant *Staphylococcus aureus* Isolated from Acute Otitis Media with Acridine Orange (0.1 mgml<sup>-1</sup>).**

Antibiotics Used	Number Resistant (Pre-curing)	Number /Percentage Cured	Number /Percentage Resistant (Post- curing)
Penicillin	13	8 (61.5)	5 (38.5)
Streptomycin	12	8 (66.7)	4 (33.3)
Amoxicillin	21	14 (66.7)	7 (33.3)
Iminipen	12	6 (50.0)	6 (50.0)
Ceftriaxone	18	13 (72.2)	5 (27.8)
Cephalothin	19	15 (78.9)	4 (21.1)
Ceftriazidime	16	12 (75.0)	4 (25.0)
Cefoxitin	15	9 (60.0)	6 (40.0)
Ofloxacin	19	14 (73.7)	5 (26.3)
Ciprofloxacin	14	10 (71.4)	4 (28.6)
Levofloxacin	13	9 (69.2)	4 (30.8)
Moxifloxacin	11	7 (63.6)	4 (36.4)

p≤0.05

**Table 8: Plasmid Curing Analysis of Resistant CON-*Staphylococcus* spp Isolated from Acute Otitis Media with Acridine Orange (0.1 mgml<sup>-1</sup>).**

Antibiotics Used	Number Resistant (Pre-Curing)	Number / Percentage Cured	Number /Percentage Resistant (Post- curing)
Penicillin	8	6 (75.0)	2 (25.0)
Streptomycin	12	6 (50.0)	6 (50.0)
Amoxicillin	11	7 (63.6)	4 (36.4)
Iminipen	12	9 (75.0)	3 (25.0)
Ceftriaxone	8	7 (87.5)	1 (12.5)
Cephalothin	10	7 (70.0)	3 (30.0)
Ceftriazidime	7	4 (57.1)	3 (42.9)
Cefoxitin	8	5 (62.5)	3 (37.5)
Ofloxacin	10	8 (80.0)	2 (20.0)
Ciprofloxacin	7	6 (85.7)	1 (14.3)
Levofloxacin	7	5 (71.4)	2 (28.6)
Moxifloxacin	6	4 (66.7)	2 (33.3)

p≤0.05

**ACKNOWLEDGMENTS:**

The authors remain indebted to the staff of Molecular and Biotechnology Department, Nigerian Institute of Medical Research, Yaba, for their overwhelming assistance and contributions.

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#### **Corresponding Author**

Name: Akinjogunla Olajide Joseph

Address: Department of Microbiology

Faculty of Science,  
University of Uyo,  
P.M.B 1017, Uyo, Akwa-Ibom State,  
Nigeria

E-mail: papajyde2000@yahoo.com

Phone: +2348064069404; +2348068036484

Date of Submission: 08/10/2010

## Barriers of Agricultural Development in Iran: A Case study of Fars Province

Farshid Aref

Department of Soil Science, Firouzabad Branch, Islamic Azad University, Iran  
[farshidaref@yahoo.com](mailto:farshidaref@yahoo.com), Tel: +989173383896

**Abstract:** This article attempts to illustrate the barriers of agricultural development in Fars, Iran. Agriculture is certainly a major contributor to rural development in many countries. It is one of the most important economic sectors in Iran. But, there are a significant number of barriers to effectively using agriculture industry as a tool for rural development. The findings through focus group discussion indicated that there are some organizational barriers in agricultural development in some villages in Fars. The finding can assist the local agriculture organizations for remove this problem in face of agriculture for rural development.

[Farshid Aref. Barriers of Agricultural Development in Iran: A Case study of Fars Province. Journal of American Science 2010;6(11):1034-1037]. (ISSN: 1545-1003). (<http://www.americanscience.org>).

**Keywords:** agriculture development, rural area, barriers

### Introduction

According to the U.S agriculture census, agriculture is described as a place of three acres or more on which any field forage crops were harvested or vegetables were harvested for sales of \$100 or more (Mills, 1984; Mwaijande, 2007). Agriculture can provide a way for improving the potential economic of rural communities. Agriculture is the core of the export market and it is accountable for one fourth to one-half of (GDP) gross domestic product in developing countries (Karbasioun et al., 2008).

About 25% of the Gross National Product, 33% of employment, 25% of non-oil exports and 80% of food requirements have been provided by the agricultural sector in Iran. Nevertheless, there are some evidence that agricultural development has some barriers. For instance, about 30% of the forests located in the North of Iran were destroyed during the last two decades. Furthermore, large portions of pastures and grasslands were rendered unproductive because of overuse by the cattle of the nomadic population and farmers (Darvishi, 2003; Karbasioun et al., 2008). Salinity of soil and water resources is a serious threat in many parts of the country (Siadat et al., 1997). The research of this study explores the policy constraints as institutional barriers within agriculture sectors. Roughly one-third of Iran's total surface area is suited for farmland, but because of poor soil and lack of adequate water distribution in many areas, most of it is not under cultivation. Only 12% of the total land area is under cultivation (arable land, orchards and vineyards) but less than one-third of the cultivated area is irrigated; the rest is devoted to dry farming. Some 92 percent of agro products depend on water (Wikipedia, 2010a).

### Method

In this study the general purpose is to investigate the barriers of agriculture development in Fars' villages in Iran. Fars is one of the 30 provinces and known as Cultural Capital of Iran. It is in the south of the country and its center is Shiraz. It has an area of 122,400 km. In 2006, this province had a population of 4.34 million people, of which 61.2% were registered as urban dwellers, 38.1% villagers, and 0.7% nomad tribes. Agriculture is of great importance in Fars. The major products include cereal, citrus fruits, dates, sugar beets and cotton (Wikipedia, 2010b). Iranian agriculture is thousands of years old and this reflects the length of time during which soil and water resources of the country have been utilized for crop production. This study is based on quantitative methodology to investigate the barriers of agriculture development. Hence to achieve the objectives of this study, the researcher uses quantitative method. Some villages in Abadeh Tashk in Fars Province, Iran were selected as a case study area because it provided many opportunities to develop agriculture. *Jahan Abad, Koushkak, Hassan Abbad, Khajeh Jamali, Deh murd, Tashk, Dehzir,* were villages which have been chosen for this study (see the figure 1). Focus group discussion (FGD) was performed to collection data from local farmers. FGD is probably the most widely used technique of gathering qualitative data (Aref, 2010; Grover & Vriens, 2006). According to Rafipoor (2005) FGD technique is an appropriate technique in science research in terms of Iranian culture. FGD was conducted in a group setting and was used for obtaining a better understanding of participants' attitudes towards the barriers of agriculture development. There is no consensus among qualitative researchers on the optimal number of participants in FGD. Some researchers suggest the

number of studied argued four to twelve people (Mendis-Millard & Reed 2007). But the ideal number of participants in each FGD is six to ten. Participants of FGD were classified according to their place in the villages. All respondents were male. They ranged in age from 27 to 73 years. The researcher explained to them the objectives of the study and what questions would be asked. The researchers examined, categorized participants responses from each focus group of villagers that were recorded in video tapes.

## **Result**

Information for this study was gathered from rural residents through FGD. A qualitative analysis was undertaken to determine viewed the barriers of agriculture development. According to the collected baseline data, farming is the most common occupation in the 7 villages in *Jahan Abad, Koushkak, Hassan Abbad, Deh e Murd, Khvajeh Jamali, Tashk, Dehzir, Chah-Ghaz, Ghah- Sorkh*, There were overall 65 participants with an average of 57 years old. All participants; were males. They were chosen because of their engagements in agriculture products. The questions were asked about to barriers of agriculture development. The findings showed that agriculture development in their villages is without any certain planning for rural development. Although the FGD respondent referred to variety barriers in terms of agriculture in their villages but in this study we refer to some common barriers which have been discussed in majority of FGD groups. The most barriers in terms of agricultural development were including:

1) Salinity of water: The majority of FGD participants believed there is no suitable water for irrigation in their village. They believe that their villages have many lands, but they don't have enough water to irrigation of these lands. Another problem associated with the expansion of irrigated farming is the overdraft of ground water. In support of this finding Sadat et al (1997) also state that the sustainability of our agricultural production is highly dependent on the "health" of our soil and water resources. But, the future of these resources is highly threatened by salinization and eventual desertification (Siadat et al., 1997).

2) Lack of resources: The most participants in FDG groups mentioned to this issue as main obstacles to agriculture development. Lack of credit resources also was another barrier to develop agriculture development in their area.

3) Lack of human resource development. Most of young villagers immigrated to the citied and so however, there are many educated people from the rural area of Iran but they don't have apathy to engage in agricultural activities.

4) Lack of government support: lack of government support to provide funding for poor farmer to develop their activities. Focus groups often complained about the lack of agricultural organization support to provide adequate facilities for farmers.

5) Lack of agricultural organization capacity: FGD respondents believed the lack of capacity of agriculture was behind the failure investment for their agriculture product. However in the end of any discussion they refer to the barriers of agriculture development through government policy as well as local organizations.

6) Lack of agricultural knowledge: However, in agricultural modern the role of knowledge is important. But regarding the discussion with famers, they stated that government did not support them with new knowledge. Base on my observation the farmers had traditional knowledge in agriculture. With mention to above focus group discussion about barriers of agriculture development, I summarized these barriers in two groups: community barriers and organizational barriers.

## **Conclusion**

This paper has identified the barriers agriculture development. Lack of capable organizations and community resources were an important element contributing to limited agriculture development. They refereed to government policy and lack of local organizational capacity as main barriers related agriculture development. Clearly, the described barriers may not be only specific to Fars province; some of them may also be considered as common general problems of agriculture development in other communities in Iran. Base on the findings, it can be suggest that rural empowerment can be a tool for agriculture development in Iran. The findings of this study can be useful for academics, researchers and all stakeholders involved in designing, assessing or promoting agriculture projects which are in any way associated with general development goals. An understanding of the existing barriers of agriculture provides basic information for setting a policy agenda to enhance agriculture.



Figure 1: The map of Bakhtegan (the case study)

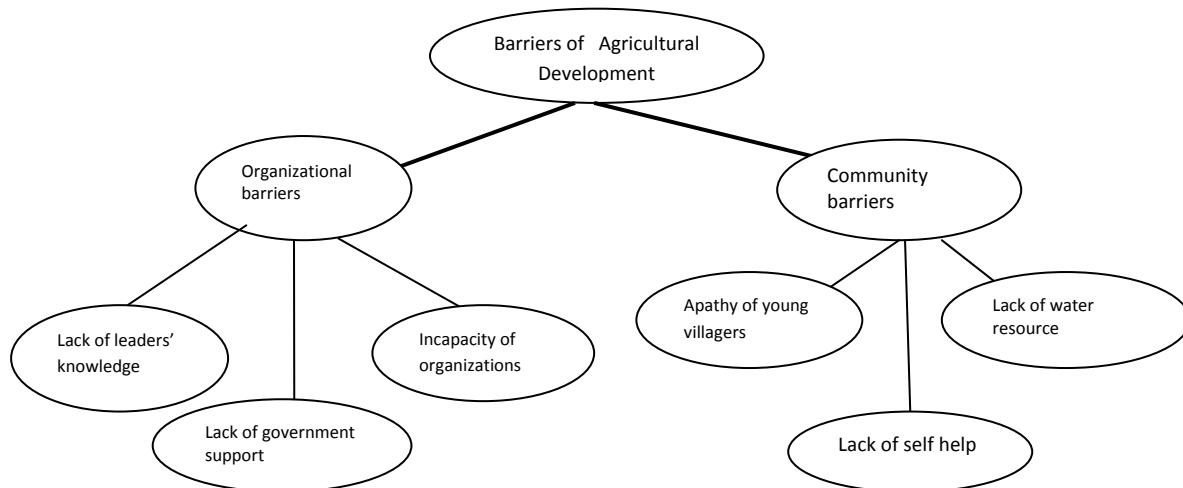


Figure 2: Summarize of barriers of agricultural development

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Date Submission: 22, October 2010

# Immunostimulatory and Protective Properties of *Lactobacillus brevis* Used as a Biocontrol Agent *in Vivo*

Agarry, O. O.

Department of Biological Sciences, Microbiology Unit, University of Abuja , P. M. B. 117, Abuja, Nigeria  
E-mail: [oluagarry@yahoo.com](mailto:oluagarry@yahoo.com)

**Abstract:** The immunostimulatory and protective properties of *Lactobacillus brevis* isolated from cassava starch were studied in vitro and in vivo. Antagonism was measured by the zone of inhibition between the bacterium streak/ring and fungus plug. Subsequent increases in inhibition were observed and complemented by a small but progressive decrease in the distance between the bacterium and the fungus. *L. brevis* significantly (>74%) inhibited the growth rate of *Fusarium moniliforme* after 168 h. Biochemical indices of albino rat plasma showed that the bacterium had liver improvement functions. Plasma aspartate aminotransferase (AST) activity of the rats dosed with *L. brevis* alone was lower (8.33 IU/L) than the control. A mild elevation of AST and alanine aminotransferase (ALT) activities was observed in rats administered with *L. brevis* and *F. moniliforme* implying that the bacterium possesses antimycotic properties capable of reducing the severity of pathogen attack on the host. However, there was a significant ( $P<0.05$ ) decrease in the plasma globulin and protein levels. There was a reduction in the count of *F. moniliforme* in rats dosed with both organisms during feeding trials. The weight gain by rats in the treatment group compared favourably with the control. Further pathological investigation confirmed a pale and friable liver while the small intestine was inflamed. The administration of *L. brevis* had an immunostimulatory effect. *Lactobacillus brevis* has not only potent in vitro antifungal activity against *F. moniliforme* but also in vivo control efficacy against *Fusarium* infection. Further evaluation of its effectiveness for disease control and applications should be done

[Agarry, O. O. Immunostimulatory and Protective Properties of *Lactobacillus brevis* Used as a Biocontrol Agent *in Vivo*. Journal of American Science 2010;6(11):1038-1045]. (ISSN: 1545-1003). (<http://www.americanscience.org>)

**Keywords:** Immunostimulatory properties, *Fusarium moniliforme*, *Lactobacillus brevis*

## INTRODUCTION

*Lactobacillus brevis* is a species of lactic acid bacteria. Ingestion has been shown to improve human immune function, and it has been patented several times (Yoshindo, 2005). While interferon is attracting international attention as a specific medication for the treatment of cancers and viral diseases, *Lactobacillus brevis* has been observed to increase the production of interferon in the body (Akihiko, 1994; 1995). In addition to strengthening the specific immunity, lactic acid bacteria also seem to reinforce the non-specific mechanisms of defense such as phagocytosis and cytokine production. Secretion by these organisms of compounds having anti-inflammatory or antimicrobial effects has also been suggested (Heyman, 2000). *Fusarium* is one of the emerging causes of opportunistic mycoses (Anaissie *et al.*, 1988; Guarro and Gene, 1995). Infections due to *Fusarium* spp. are collectively referred to as fusariosis. Outbreaks of nosocomial fusariosis have also been reported. Existence of *Fusarium* in hospital water distribution systems may result in disseminated fusariosis in

immunosuppressed patients. *Fusarium* is one of the most drug-resistant fungi. *Fusarium* infections following solid organ transplantation tend to remain local and have a better outcome compared to those that develop in patients with hematological malignancies and bone marrow transplantation patients (Deshpande and Koppikar, 1999; Tanure *et al.*, 2000 and Schell, 2000).

The colonic microflora microflora is important to health. The growth and metabolism of the many individual bacterial species inhabiting the large bowel depend primarily on the substrates available to them, most of which come from the diet. The microflora normally presents a barrier to invading organisms; but pathogens often become established when the integrity of the microbiota is impaired through stress, illness, antibiotic treatment, changes in diet, or physiological alterations in the gut. Innovative approaches have been tried as alternative to antibiotics in treating gastrointestinal diseases and these include using live biotherapeutic agents such as yeast (*Saccharomyces* spp.) and bacterial isolates (*Lactobacillus* spp.) or faecal enemals (Fuller 1992).

*Lactobacilli* have been widely used in treating diarrhoeal diseases such as pseudomembranous colitis, but the results have been mixed. Feeding freeze-dried powders of *L. acidophilus* NCDO 1748 had no effects on patients with pseudomembranous colitis (Aronsson *et al.* 1987). The presence of this group of bacteria in the gut is considered to have several potential benefits such as protection from pathogens (Casas and Dobrogosz 2000), anticholesterolaemic effect (Bertazzoni *et al.* 2001) and immunostimulation (Aattouri *et al.* 2001). However, not all lactobacilli are effective in combating enteric pathogens.

*Fusarium* is listed as one capable of causing mycetomas (Schell, 2000) and it has repeatedly been isolated from human keratitis (Deshpande and Koppikar, 1999) and corneal ulcers. Experimental animals often experience hepatotoxicity, nephrotoxicity or both; rats have also been shown to experience necrosis of stomach mucosa and myocardium due to the toxins, fumonisins. Liver cancers are induced. Fumonisins are also among the chief suspects for the agent(s) of elevated levels of esophygael cancer in certain parts of the world (Pitt, 2000).

In the current situation where the discovery of new antimicrobial agents are becoming increasingly difficult, the present study suggests that investigation of lactic aid bacteria may offer some potential applicability to chemotherapy.

## MATERIALS AND METHODS

### Microbial culture

*Lactobacillus brevis* and *Fusarium moniliforme* were isolated from cassava starch on de Mann Rogosa and Sharpe (MRS) agar and Malt Extract Agar (MEA). All growth media were supplied by Oxoid (Melbourne, Australia). The isolates were characterized using colonial, morphological and biochemical methods.

### *In vitro* antifungal activity

Modified methods of Fokkema (1973) and Adetuyi and Cartwright (1985) were used for the detection of antagonistic activity of bacterial isolate towards the growth of the fungal culture as adapted by Agarry and Osho (2005).

### *In vivo* feeding

Sixteen albino rats (Wistar strain) Aged 6-8 weeks were obtained from the Department of Biochemistry, University of Ilorin, Nigeria. The rats were fed on basal diet broiler starter (Amo-Byng Feeds and Concentrates, Oyo State, Nigeria). They were randomly assigned to 4 treatment groups designated as BUU (Basal diet only, uninfected with

fungus and untreated with bacterium (control)), BUT (Basal diet, uninfected with fungus but treated with bacterium), BIU (Basal diet, infected with fungus and untreated with bacterium), and BIT (Basal diet, infected with fungus and simultaneously treated with bacterium , and each was made up of 4 rats per group. Lyophilised *Lactobacillus* cells were reconstituted by dissolving 1 g in 10 ml of sterile water.

Adult Wistar albino rats were held under specific pathogen free conditions. Group BUU animals were kept on the basal diet alone (control). Animals in Groups BUT and BIU were fed on the basal diet and were orogastrically dosed with *L. brevis* (0.3 ml) and *F. moniliforme* (0.3ml) respectively. Group BIT were fed with basal diet, orogastrically challenged with  $10^8$  cfu/spores of *F. moniliforme* and then treated with the administration of 0.3 ml of *L. brevis* ( $10^5$  cfu/g). . The above treatments were repeated for a second day and a post-ingestion period of 14 days was observed after the administration of the cultures. Rats were then killed by cervical dislocation and blood samples collected into EDTA bottles for analysis of some plasma biochemical markers. The liver kidney, spleen, stomach and small intestine were removed for examination.

### Biochemical assay

The biomarkers assayed for: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), albumin, globulin and total proteins were conducted according to the conventional methods reported by Mokady *et al.* (1989). The haematological parameters namely packed cell volume (PCV), haemoglobin (Hb) count, white blood cell count (WBC) and differential counts were conducted using the methods of Aning *et al.* (1998).

### Monitoring the progress of infection and faecal levels of *A. fumigatus*

The body weights of animals were recorded daily up to 14 days post-pathogen challenge. The data gathered were used to calculate the following parameters: (i) weight gain = final weight – initial weight (ii) percentage weight gain = weight gain/final gain x 100.

For enumeration of viable faecal *Aspergillus fumigatus*, freshly voided faecal pellets were collected and pooled from each rat (0.3-0.4 g per rat) at 1, 2, 7 and 14 days post-dosing (Chang *et al.* 2001). Faeces were weighed and homogenized. Faecal homogenates were serially diluted in sterile water, and a0.1 ml aliquot was added in duplicate onto MEA plates. Plates were incubated in aerobic condition for 4 days at 25°C. Colonies were

characterized on the basis of morphology and pigmentation. The population levels were converted to log values before plotting out in graphs.

#### Histopathological tests

At autopsy the internal organs were inspected for morphological lesions. Samples of the liver, kidney, stomach, spleen and small intestine from each animal were fixed in 10% formalin, dehydrated in different percentages of alcohol, cleared in xylene for 2 h and impregnated in liquid for 2 h for embedding. The embedded organs were sectioned to 2 $\mu$ m using a microtome and stained with haematoxylin eosin (Silva *et al.* 1999).

#### Statistical analysis

Results are expressed as means  $\pm$  standard error of the mean. For statistical comparison, the data gathered were processed by one-way analysis of variance (ANOVA), SPSS 10.0. Means were compared by Duncan Multiple Range Test Statistical analysis was conducted with the Statistical Analysis System for personal computers (SAS Institute, Cary, NC, USA) software with the level of significance set at  $p < 0.05$ . Ethical declaration The study protocol was conducted in accordance with internationally accepted principles (European Community guidelines EEC Directive of 1986, 86/609/ee; US guidelines, NIH publication H85-23, revised in 1985) for laboratory animal use and care.

## RESULTS AND DISCUSSION

#### Antifungal activities

*Lactobacillus brevis* inhibited the growth of food spoilage and phytopathogenic fungus, *Fusarium moniliforme* (**Plate I**).

Physical contact between the bacterium and the fungus mycelium was never observed during the incubation period. An uncolonized zone between the bacterium and fungus was maintained throughout suggesting that diffusible metabolites of bacterial origin were responsible for inhibition of mycelium growth (**Plate I**). The bacterium was not directly lethal to the fungus under the conditions of the bioassay but strongly inhibitory to the mycelial growth and spore formation. The use of a separate control apart from the Fokkema "control" side of the culture in the Fokkema-type bioassay was made to judge its true presentation of normal unchallenged mycelial growth. When compared to the 'true' control plate which has no bacterial inoculum on it, there appeared to be no significant difference (Fig. 1) in the two controls. Measurements of growth rate inhibition showed variations between the two techniques, and overall levels of activity were essentially similar (**Fig. 1**) but the streak bioassay of Fokkema (1973) method gave an erratic pattern of

inhibition over the bioassay period than did the concentric ring method of Adetuyi and Cartwright (1985). The points at which inhibition was first detected and at which complete cessation of fungal growth (streak method: 96 h; ring method: 72 h) occurred in the two methods were also different with . The ring bioassay detected inhibition earlier(48h) than the streak bioassay(72h) (**Fig 1**). It also facilitated measurements of the distance between the bacterium and the fungus, and allowed accurate determination of fungal colony radius, since inhibitory effects were exerted equally around the colony. *L. brevis* inhibited fungal growth up to >74% in the ring method and >92% in the streak method (**Fig 1**). Oyetayo (2006) reported the inhibition of growth of pathogenic and food spoilage bacteria by *L. plantarum*.

#### Hepatoprotective effect

Plasma biomarkers in experimental animals after orogastric dosing with *L. brevis* reveal a significant reduction in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels as compared with those placed on basal diet and dosed with *F. moniliforme* (**Table 1**). Experimental animals often experience hepatotoxicity, nephrotoxicity or both due to the toxins, fumonisins produced by *F. moniliforme*. Fumonisins are also among the chief suspects for the agent(s) of elevated levels of esophageal cancer in certain parts of the world (Pitt, 2000). The rise in AST in rats fed basal diet and *F. moniliforme* could be attributed to possible secretion of mycotoxins. Oboh *et al.* (2000) reported aflatoxin B1 to be implied in liver damage.

AST values were reduced in rats dosed with the bacterium more than control rats (**Table 1**). Plasma AST and ALT are important enzymes used in monitoring liver damage (Johnston 1999). An increase in the level of these enzymes in the serum/plasma is an indication of hepatocellular damage (American Liver Foundation 1995).

AST and ALT are enzymes located in the liver cells and leak out and make their way into the general circulation when liver cells are injured (David and Johnston 1999). Mild or moderate elevations of AST or ALT are non-specific and may be caused by a wide range of liver diseases (American Liver Foundation 1995, 1997; David and Johnston 1999)

There was significant change(Table 1) ( $P<0.05$ ) in plasma albumin, globulin and protein of the albino rats in all groups when compared with that of the control diet. Albumin measures the main protein made by the liver and tells how well the liver is making this protein. Low albumin as reported for Group BIU animals (11.60g/dL) may be caused by acute or chronic inflammation or liver disease (David

and Johnson, 1999; Younossi and Mehta, 1998). Slight elevation of albumin in Group BUT animals indicated an increase in the protein production made by the liver. The bacterium has the ability to stimulate the immune system of the rats. Albumin is produced mainly in the liver and therefore is a test of liver function. Low albumin levels and no other liver function test abnormalities are likely to result from a nonhepatic cause (David and Johnson 1999). Total protein measures albumin and other proteins in blood, including antibodies made to help fight off infections (Liver Function Tests 2005; Younossi and Mehta 1998).

### Immunostimulatory effect

The WBC count increased in groups BIT and BUT (**Table 2**). This might result from the production of more white blood cells to engulf the antigen. T lymphocyte and other key cells of the immune system are known to activate production of antibody polymorphonuclear granulocyte to destroy an invading pathogen (Prescott *et al.*, 1999). Differential leucocyte counts in Wistar rats dosed with *L. brevis* reveal a significant increase in neutrophil and ensinophil counts and mild decrease in lymphocyte counts as compared with the control (**Table 2**). The primary role of lymphocytes is in humoral antibody formation and cellular immunity (Baker and Silver 1985). An absolute increase in lymphocytes had been found in most bacterial infection such as Staphylococcal infection (Monica 2000). The administration of *L. brevis* to Group BUT animals had an immuno-stimulatory effect. Immunoglobulins are often sought in children with recurrent infections or a combination of infections with injury (Baron *et al.* 1994, pg 158). The PCV and Hb compared favourably with the standard (Mitraka and Rawnsley 1977; Baker *et al.* 1979; Weihe e 1987). Aning *et al.* (1998) and Oboh and Akindahunsi (2004) reported similar findings on the haematological parameters of albino rats fed sorghum and brewer's grains and albino rats fed *Saccharomyces cerevisiae*-fermented cassava flour diet. The PCV and Hb of the control diet and other groups were significantly different ( $P>0.05$ ) suggesting that the treatment is not haemolytic. This result also agrees with Aletor (1993) to the extent that cassava products do not have negative haemotological effects. Agarry (2006) reported an improvement in blood composition of treated Wistar rats with an antagonistic pair of microbial isolates of cassava products origin. The improvement in blood composition that followed feeding of the animals with the bacterium indicated an immunological security for the group of animals given such treatment. (Table 2).

### Other benefits

Animals singly dosed had fluctuating weight gain/loss over the 2-week period. The weight gain in the control group compared favourably with that in group BUT(Fig 2). This implies that the treatment enhanced the growth of the animals. A reduction of the faecal level of the fungus and increase in the level of the beneficial bacterium (*L. brevis*) was also observed (Fig 3). Animals of group BUT contained a low faecal number of pathogens. The faecal levels of the bacterium increased in animals of groups BUT and BIT. Although animals were doused with isolates for only 2 days after pathogen challenge, they were protected beyond that time(Fig 3). These observations are in line with observations made by Henriksson and Conway (2001), who demonstrated that a range of new bifidobacteria may provide protection against infection by *Salmonella typhimurium* in mice resulting in both an initial reduction of *S. typhimurium* levels in feaces and a reduced weight loss of animals challenged with the pathogen.

However, the pathological studies revealed possible damage to the internal organs of the animals. The small intestine, stomach and liver of rats showed significant lesions. Distention of issue parenchyma, cellular infiltration and partial erosion of the mucus membrane apparent while no significant lesions in the spleen and kidney was observed.

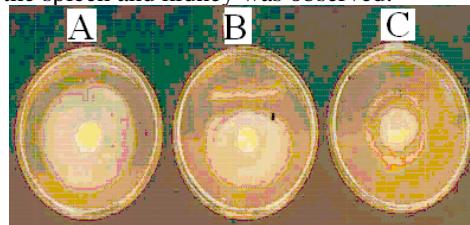


Plate 1 Antagonistic zone between *Fusarium moniliforme* and *Lactobacillus brevis*. Photograph taken 5 days after inoculation.  
A = control; B = Fokkema/conventional streak method;  
C = concentric rmg bioassay . X3

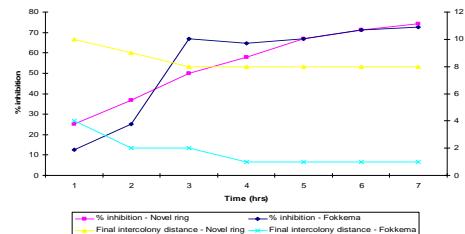


Fig. 1: Effect of antagonistic ability of *Lactobacillus brevis* on *Fusarium moniliforme* inn terms of intercolony distance and percentage inhibition of radial growth rate.

**Table 1:** Effect of the administration of *Fusarium moniliforme* and *Lactobacillus brevis* on biochemical indices of albino rat plasma

Treatment	AST (IU/L)	ALP (IU/L)	ALT (IU/L)	Albumin (g/dL)	Globulin (g/dL)	Protein (g/dL)
Basal diet alone (Control)	9.42 <sup>a</sup> ± 2.28	21.09 <sup>a</sup> ±3.89	3.38 <sup>a</sup> ±0.96	11.70 <sup>a</sup> ±0.82	29.93 <sup>a</sup> ±4.75	47.41 <sup>a</sup> ±9.04
Basal diet + fungus Alone (A)	11.11 <sup>b</sup> ±1.57	28.13 <sup>c</sup> ±5.60	5.79 <sup>c</sup> ±2.08	11.60 <sup>a</sup> ±0.16	30.25 <sup>abc</sup> ±9.49	28.42 <sup>c</sup> ±17.84
Basal diet + fungus And bacterium (B)	9.66 <sup>ab</sup> ±0.96	44.53±2.44	5.43 <sup>b</sup> ±0.46	12.36 <sup>b</sup> ±2.63	20.01 <sup>b</sup> ±6.02	33.22 <sup>ab</sup> ±9.59
Basal diet + Bacterium alone (C)	8.33 <sup>a</sup> ±3.26	40.48 <sup>d</sup> ±7.64	5.31 <sup>b</sup> ±0.97	12.37 <sup>b</sup> ±4.57	29.96 <sup>a</sup> ±5.63	38.82 <sup>ab</sup> ±7.71

AST, Aspartate aminotransferase; ALP, Alkaline phosphatase; ALT, Alanine aminotransferase.

Values are mean ± S.E. (n=4)

Means with the same superscript letter(s) along the same column are not significantly different ( $P>0.05$ )**Table 2** Effect of administration of *Fusarium moniliforme* and *Lactobacillus brevis* on the haematological parameters of albino rats.

Treatment	PCV %	Hb g/L	WBC X10 <sup>9</sup> /L	Neutrophils %	Lymphocyte %	Monocytes %	Eosinophils %
Basal diet alone (Control)	47.25±2.06 <sub>a</sub>	16.00±0.81 <sub>a</sub>	7.05±2.75 <sub>a</sub>	55.75±6.34 <sub>a</sub>	42.25±7.27 <sub>a</sub>	50±0.57 <sub>a</sub>	0.00±0.00
BUU							
Basal diet+ fungus Alone (A) BIU	49.25±1.50 <sub>a</sub>	16.75±0.50 <sub>a</sub>	6.45±4.37 <sub>a</sub>	59.00±7.07 <sub>abc</sub>	40.25±7.13 <sub>ab</sub>	1.25±0.50 <sub>a</sub>	2.00±0.00 <sub>ab</sub>
Basal diet + fungus And bacterium (B) BUT	52.25±1.50 <sub>ab</sub>	17.75±0.50 <sub>ab</sub>	7.37±2.69 <sub>a</sub>	62.00±2.58 <sub>b</sub>	37.00±1.63 <sub>b</sub>	2.75±0.95 <sub>ab</sub>	0.00±0.00 <sub>a</sub>
Basal diet + bacterium Alone (C) BIT	50.00±0.00 <sub>a</sub>	17.00±0.00 <sub>a</sub>	7.22±2.71 <sub>ab</sub>	59.00±7.62 <sub>ab</sub>	40.75±7.18 <sub>a</sub>	0.00±0.00	1.50±0.57 <sub>a</sub>

PCV, Packed Cell Volume; Hb, Haemoglobin; WBC, White Blood Cell

Values are mean of four replicates ± standard deviation

Values followed by similar alphabets along the same column are not significantly different ( $P<0.05$ )

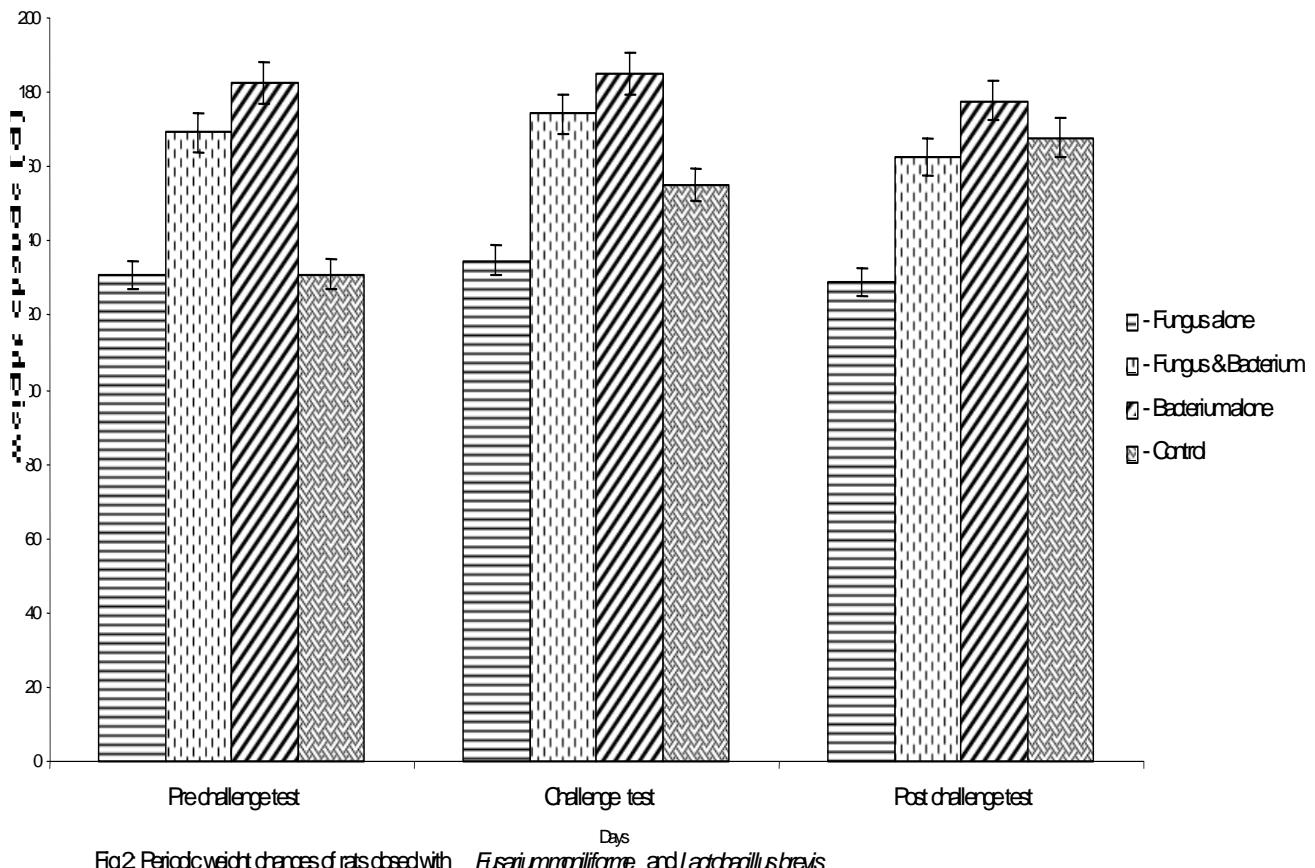


Fig 2 Periodic weight changes of rats dosed with *Fusarium moniliforme* and *Lactobacillus brevis*

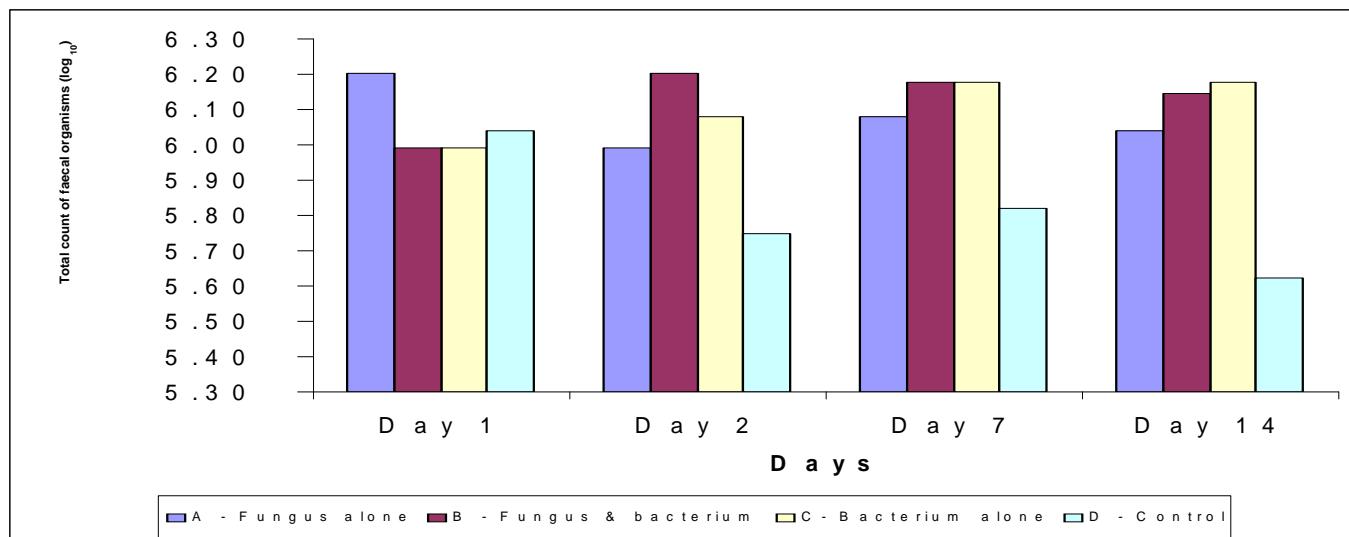


Fig. 3: Total count of faecal organisms in rats dosed with *Fusarium moniliforme* and *Lactobacillus brevis*

## CONCLUSION

*L. brevis* has a stimulatory effect on humeral immunity of albino Wistar rats with the following benefits: antimicrobial activity against important

pathogens and a food spoilage fungus: *Fusarium moniliforme*; hepatoprotective effect as a result of the ability to lower plasma aminotransferase levels and immunostimulatory effect.

**Correspondence to:**

Agarry Olubunmi Olaitan  
 Department of Biological Sciences,  
 University of Abuja, Nigeria  
 Telephone: +234-807-8160565  
 Email: oluagarry@yahoo.com

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7/27/2010

# Accelerating Vector Quantization Based Speaker Identification

Muhammad Afzal<sup>1</sup>, Shaiq A. Haq<sup>2</sup>

<sup>1</sup>Department of Computer Science and Engineering,  
University of Engineering and Technology, Lahore-54890, Pakistan

<sup>2</sup>Dean Faculty of Engineering, Wah Engineering College,  
University of Wah, Wah Cantt., Pakistan

E-mails: [shmaafzal@yahoo.com](mailto:shmaafzal@yahoo.com), [shaiq\\_haq@yahoo.com](mailto:shaiq_haq@yahoo.com)

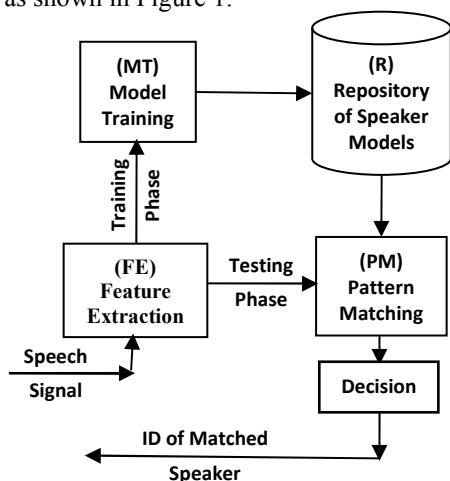
**Abstract:** Matching of feature vectors extracted from speech sample of an unknown speaker, with models of registered speakers is the most time consuming component of real-time speaker identification systems. Time controlling parameters are size and count of extracted test feature vectors as well as size, complexity and count of models of registered speakers. We studied vector quantization (VQ) for accelerating the bottlenecking component of speaker identification which is less investigated than Gaussian mixture model (GMM). Already reported acceleration techniques in VQ approach reduce test feature vector count by pre-quantization and reduce candidate registered speakers by pruning unlikely ones, thereby, introducing risk of accuracy degradation. The speedup technique used in this paper partially prunes VQ codebook mean vectors using partial distortion elimination (PDE). Acceleration factor of up to 3.29 on 630 registered speakers of TIMIT 8kHz speech data and 4 on 91 registered speakers of CSLU speech data is achieved respectively.

[Muhammad Afzal, Shaiq A. Haq. Accelerating Vector Quantization Based Speaker Identification, Journal of American Science 2010;6(11):1046-1050]. (ISSN: 1545-1003). <http://www.americanscience.org>.

**Keywords:** Speaker identification, vector quantization, partial distortion elimination, speaker pruning.

## 1. Introduction

Automated Speaker Identification (ASI) systems identify a test speaker from the database of its registered speakers (Quatieri, 2002). ASI systems have three major units namely Feature Extraction (FE), Model Training (MT) and pattern matching (PM) as shown in Figure 1.



**Figure 1. Major Components of an ASI System**

FE unit is used both by MT and PM units as front processor. The input to FE unit is a digital

speech signal which is converted by it to a sequence of  $d$ -dimensional vectors each consisting of  $d$  values of speaker specific features. Mostly Mel-frequency Cepstral Coefficients (MFCC) feature vectors of 12 to 20 elements are used (Kinnunen, 2006). MT unit of VQ based ASI systems compresses feature vector sequence  $\tilde{X} = (\tilde{x}_1, \tilde{x}_2, \tilde{x}_3, \dots, \tilde{x}_{\tilde{T}})$  of size  $\tilde{T}$  to smaller number of mean vectors by generally implementing Linde Buzo Gray (LBG) clustering algorithm (Bei and Gray, 1985). The set of  $M$  mean vectors is termed as codebook,  $C \in \mathbb{R}^{M \times d}$ . For an ASI system of  $N$  registered speakers,  $N$  codebooks are computed and stored in a repository,  $R$ , mathematically given by Expression (1).

$$R_{store} \leftarrow \left\{ \sum_{LBG}^N \tilde{X} \Rightarrow C \right\} \quad (1)$$

Where  $\mathbb{R}$  represents real number space,  $\tilde{X} \in \mathbb{R}^{\tilde{T} \times d}$ ,  $R \in \mathbb{R}^{N \times M \times d}$  and;  $d$ ,  $M$  and  $N$  are as defined above.

VQ codebook is called non-parametric model while GMM is termed as parametric model. GMM training is mostly initialized with LGB clusters

to determine its parameters using expectation maximization (EM) algorithm (Alpaydin, 2004). GMM based speaker recognition systems have been extensively studied for improving speed (Kinnunen et al., 2006). In this paper we present speeding results for VQ based systems which are as efficient as GMM (Kinnunen and Li. 2009).

Full search based PM unit of VQ system computes  $\Delta$ ,  $d$ -dimensional Euclidean distances between each vector of sequence of the test feature vectors,  $X = (x_1, x_2, x_3, \dots, x_T)$ , and each of the mean vector of each target registered speaker's codebook stored in repository,  $R$ , using Equation (2). Where  $T$  is the number of feature vectors extracted from the samples and  $X \in \mathbb{R}^{T \times d}$ . Euclidean distances are used to compute similarity measure called single vector distortion  $D_{t,s}$  between each test vector  $x_t$  and each stored target codebook of speaker  $s$ ,  $R_s$ , as given by Equation (3). Identification decision is done using Equation (4).

$$\forall \begin{cases} 1 \leq t \leq T, \\ 1 \leq s \leq N, \\ 1 \leq m \leq M \end{cases} \Delta_{t,s,m} = \sqrt{\sum_{i=1}^d (X_{t,i} - R_{s,m,i})^2} \quad (2)$$

$$\forall 1 \leq t \leq T, 1 \leq s \leq N \quad D_{t,s} = \arg \min_{1 \leq m \leq M} \Delta_{t,s,m} \quad (3)$$

$$\text{Decision Speaker id} = \arg \min_{1 \leq s \leq N} \sum_{t=1}^T D_{t,s} \quad (4)$$

Full search speaker identification as given by Equations (2)-(4) shows that  $T \times N \times M \times d$  multiplications,  $2 \times T \times N \times M \times d$  additions and  $T \times N \times M$  square root computations are required. Identification time order can be given by  $O(T \times N \times M \times d)$ . Such high time order complexity of minimum distortion slows down the identification process. Real-time speech processing systems require fast speaker identification front-end to adapt to speaker specific speech model. This emphasizes the need for research to accelerate speaker recognition task.

Brief review of existing accelerating techniques for ASI systems is given in section 2. Algorithm used to speedup ASI system that partially prunes codebooks along with its performance analysis is presented in section 3. Description of speech material used in this study,

experimental setup and its parameters are given in section 4. Results of experimental are shown and discussed in section 5 followed by conclusions in section 6.

## 2. Existing Techniques

Inserting  $\Delta_{t,s,m}$  definition for EUD from Equation (2) into Equation (3) reduces square root computations from  $T \times N \times M$  to  $T \times N$  as shown by Equation (5)

$$D_{t,s} = \sqrt{\arg \min_{1 \leq m \leq M} \sum_{i=1}^d (X_{t,i} - R_{s,m,i})^2} \quad (5)$$

Reducing  $T$  by silence detection in raw speech signal is a normal practice. Further, best speedup techniques as reported by Kinnunen et al (2006) reduce  $T$  by pre-quantization (PreQ) of test vector sequence. They have used Vantage Point Tree (VPT) indexing technique to avoid mean vectors of codebooks in searching closest of  $M$  mean vectors. They used probabilistic measure to reduce  $N$  by pruning unlikely speakers.

**Table 1. Parameters and Results of Kinnunen et al. (2006) Experiments on TIMIT Database**

Code Book Size	Speedup Technique	Error Rate %	Times (S)	Speedup Factor
32	Baseline	0.63	1.15	1 : 1
	VPT+PreQ	0.63	1.11	1.04 : 1
	VPT+ Pruning	--	--	--
64	Baseline	0.48	2.37	1 : 1
	VPT+PreQ	0.64	0.48	4.9 : 1
	VPT+ Pruning	0.48	0.43	5.5 : 1
128	Baseline	0.16	4.82	1 : 1
	VPT+PreQ	0.64	0.59	8.2 : 1
	VPT+ Pruning	0.00	1.88	2.6 : 1
256	Baseline	0.16	10.2	1 : 1
	VPT+PreQ	0.64	1.18	8.6 : 1
	VPT+ Pruning	0.00	3.28	3.1 : 1

Information specific to the test speaker is distributed all along the test vector sequence and pre-quantization of test vectors is likely to distort it as shown in Table 1 by test results by (Kinnunen, et al., 2006) for VPT+PreQ.

Table 1 shows absolute identification time and speedup ratio in (Kinnunen, et al., 2006) for

different speedup techniques exercised on a cluster of 2 Dell Optiplex G270 computers having 2.8 GHz processor and 1 GB RAM each. In Table 1 the effect of Vantage Point Tree (VPT) for speedup is multiplied with their other speedup algorithmic steps to simplify comparison with our results. We use PDE to speedup ASI system rather than VPT and speaker pruning as a whole.

### 3. Speedup Technique Used

Let ‘SI’ stand for identity number,  $\text{id}$ , of the best matching registered speaker, more specifically, the candidate speaker, and ‘Dmin’ stand for the minimum distortion of the candidate speaker. Algorithm presented next speeds up computation for Equation (5) by avoiding mathematical operations when ever possible and outputs the  $\text{id}$  of the test speaker.

Neighborhood search for closest mean vector to a feature vector, as expressed by Equation (3), is made faster by PDE algorithm proposed by Bei and Gray (1985). PDE algorithm has been largely employed in image compression for encoding and decoding images (Lee and Chen. 1994). We investigated its capability for speeding up speaker identification in partially pruning mean vectors that are unlikely to be nearest neighbor of a test feature vector,  $x_t$ , in the process of computing  $D_{t,s}$  for modeling of any speaker  $s$ . Effectively, PDE reduces parameter  $d$  in time order complexity  $O(T \times N \times M \times d)$ .

Embedded PDE in the presented algorithm avoids superfluous multiplications and twice as many additions, whenever  $D2 \geq D2m$  causes Prune Events (PE1) or (PE2) by termination of EUD computation for current value of  $m$  and initiation of distance computation for  $(m+1)$ . Line labels PE1 and PE2 used in the algorithm correspond to prune events that occur during the algorithm execution. In hypothetically best case  $(M-1)(d-1)$  multiplications are avoided if PE1 or PE2 occur at  $i=1$  for  $\forall 2 \leq m \leq M$ . In the worst case no multiplication or addition is avoided if PE1 or PE2 never occurs. In general  $D_{t,s}$  is computed with partial scan through the speaker model. It follows from best and worst cases that average case speedup

factor, given by  $\frac{2 \times M \times d}{M \times d + M + d - 1}$ , is less than 2.

The following algorithm outputs  $\text{id}$  of test speaker and requires input of test feature vectors,  $X \in \mathbb{R}^{T \times d}$ , and repository of codebooks of registered speakers,  $R \in \mathbb{R}^{N \times M \times d}$ . Square brackets are used for indices rather than subscripts.

**Algorithm:** VQ ASI with embedded PDE

```

SI ← 1
Dmin ← 0
for t ← 1 to T do D2m ← 0;
  for i ← 1 to d do
    dif ← X[t][i] - R[1][1][i]
    D2m ← dif × dif + D2m
  endfor
  for m ← 2 to M do
    D2 ← 0;
    for i ← 1 to d do
      dif ← X[t][i] - R[1][m][i]
      D2 ← dif × dif + D2
      if D2 ≥ D2m goto PE1
    endfor
  if D2 < D2m then D2m ← D2
  endif
  Dmin ← sqrt(D2m) + Dmin
endfor
for s ← 2 to N do
  Dsum ← 0
  for t ← 1 to T do
    D2m ← 0;
    for i ← 1 to d do
      dif ← X[t][i] - R[s][1][i]
      D2m ← dif × dif + D2m
    endfor
    for m ← 2 to M do
      D2 ← 0;
      for i ← 1 to d do
        dif ← X[t][i] - R[s][m][i]
        D2 ← dif × dif + D2
        if D2 ≥ D2m goto PE2
      endfor
    if D2 < D2m then D2m ← D2
    endif
    Dsum ← sqrt(D2m) + Dsum
  endfor
  if Dsum < Dmin
  then
    Dmin ← Dsum;
    SI ← s
  endif
endfor
OUTPUT(SI);

```

#### 4. Experiment

TIMIT (Garofolo et al., 1993) speech data was down sampled to 8kHz using anti-aliasing filter to match with sampling frequency of CSLU (Cole et al., 1998) data. Three TIMIT ‘si’ files were concatenated to get 8.4 seconds long test sample on the average. TIMIT data consists of read speech of microphone recordings. Hence speaker recognition results for TIMIT data are highly optimistic. For the purpose of validation we used CSLU speaker recognition corpus that consisted of telephonic speech in response to prompts. In total 40 prompts, labeled by two letters e.g., ‘aa’, ‘aq’ etc., were sent to participant speakers and their response speeches mostly repeated 4 times by the speakers were recorded over telephone. Some prompts were not sent to all the participants for unknown reasons in each of 12 sessions distributed over two year interval. Speech data of first four sessions was used in our experiments. For testing speech data files with prompts labeled as ‘aa’, ‘ab’, ‘ac’, ‘am’, ‘an’, ‘ao’ and ‘av’ were selected from sessions 2, 3 and 4. Average duration of speech per speaker was 28 seconds.

For system training all ‘sa’ and ‘sx’ TIMIT files were concatenated to get approximately 23 second long speech samples. While from CSLU corpus files corresponding to prompts labeled as ‘aq’, ‘ar’, ‘as’, ‘at’, ‘au’, ‘be’, ‘bf’, ‘bg’, ‘bh’ and ‘bi’ from sessions 1-4 were used. Total average duration of speech data per speaker was 99 seconds. Speech data selection thus made, allowed all the experiments for speaker identification to be conducted in text independent mode.

MFCC feature vector extraction was done by standard process (Deller et al., 2000). Hamming window was applied on 33% overlapping frames. Energy based silence detection was used for all tests. Raw speech frames were reduced by 9% and 8% from training and testing samples respectively for TIMIT while for CSLU data the values were 8.4% and 4.8% respectively.

A bank of 19 triangular filters was applied on magnitude real DFT spectrograms of 30 millisecond speech frames. MFCC vectors of size  $d=12$  were computed from response of triangular filterbank once and stored for use both in training and testing for TIMIT. For CSLU speech data that had undergone telephone degradation, first 3 and last 2 triangular filters were not applied. Consequently frequencies between approximately 230 Hz to 3185 Hz were processed.

VQ codebook repository was prepared using LBG algorithm for all 630 TIMIT and 91 CSLU speakers from MFCC feature vectors extracted from training data. LBG trained codebooks with  $M = 32, 64, 128, 256, 512$  were computed once and stored to use in testing. For CSLU data, codebooks with  $M=1024, 2048$  were also trained. All algorithms were coded in Microsoft C#. Programs were run on 32-bit Windows Vista(TM), installed on HP Compac DX7400 with Intel(R) Core(TM)2 Duo CPU E6550 @2.33 GHz with 2 GB RAM. Time intervals were computed by calling ‘System.DateTime.Now’ method of C#.

#### 5. Results and Discussion

Test results for speaker identification for TIMIT and CSLU corpora are shown in Table 2 and Table 3 respectively.

**Table 2: Average Speaker Identification Performance for TIMIT data**

VQ System		TIMIT DATA		
Model Size	Search Type	Error %	Time (S)	Speedup Factor
32	Baseline	15.71	1.25	1 : 1
	PDE	14.92	0.52	2.40 : 1
64	Baseline	5.40	2.45	1 : 1
	PDE	4.92	0.95	2.58 : 1
128	Baseline	1.27	4.84	1 : 1
	PDE	1.27	1.74	2.78 : 1
256	Baseline	0.32	9.61	1 : 1
	PDE	0.32	3.17	3.03 : 1
512	Baseline	0.48	19.15	1 : 1
	PDE	0.48	5.83	3.29 : 1

**Table 3: Average Speaker Identification Performance for CSLU data**

VQ System		CSLU DATA		
Model Size	Search Type	Error %	Time (S)	Speedup Factor
32	Baseline	6.59	0.31	1:1
	PDE	6.59	0.12	2.58:1
64	Baseline	2.20	0.64	1:1
	PDE	2.20	0.25	2.56:1
128	Baseline	0.00	1.13	1:1
	PDE	0.00	0.38	2.97:1
256	Baseline	0.00	2.21	1:1
	PDE	0.00	0.69	3.20:1
512	Baseline	0.00	4.35	1:1
	PDE	0.00	1.27	3.43:1
1024	Baseline	0.00	9.69	1:1
	PDE	0.00	2.73	3.55:1
2048	Baseline	0.00	19.36	1:1
	PDE	0.00	4.95	3.91:1

VQ models larger than 512 for TIMIT are not made since count of feature vectors extracted from training sample is less than 1024.

Accuracy of speaker identification increases with codebook size from 32 to 256. Systems, with codebook size 512 of TIMIT data, show over fitting degradation effects, as reported by Kinnunen et al (2006). Test results of our speedup technique with PDE show that it did not degrade accuracy when compared with corresponding full search (Baseline) systems. The technique is applicable on larger as well as smaller models. Speedup factor increases with increase in model size.

Identification accuracy for CSLU data is higher than corresponding TIMIT data that may be due to less number of speakers in CSLU data than that in TIMIT data. Speedup factor of PDE increases monotonously with codebook size for both TIMIT and CSLU data. Whereas in (Kinnunen, et al., 2006) speedup factor decreases from model size 64 to 128 and then increases for 256. In case of CSLU data there is no over fitting accuracy degradation for larger codebooks. PDE speedup factors of our systems corresponding to VPT+Pruning speedup factors shown in (Kinnunen, et al., 2006) are better in general. It is noteworthy that experimentally achieved average speedup factors of FDE for all codebook sizes are greater than theoretically possible factor 2.

## 6. Conclusions

Performance of a simple to implement technique, PDE, as compared to VPT and speaker pruning techniques given in (Kinnunen, et al., 2006), for speeding up VQ based real-time speaker identification systems, is presented in this paper. PDE is used to partially prune speaker models by obviating full scan of mean vectors of codebooks. Overall speedup factor of up to 4 is achieved. The time order,  $O(T \times N \times M \times d)$  parameter  $d$  that is ignored in (Kinnunen, et al., 2006) can be successfully manipulated to speedup ASI systems. PDE can be applied to substantially speedup VQ based speaker identification for small as well as large sized models.

## Acknowledgements:

This research was fully supported by the University of Engineering and Technology, Lahore, Pakistan. Their support is gratefully acknowledged.

TIMIT data was provided by Linguistic Data Consortium, University of Pennsylvania, USA. Their support is also gratefully acknowledged.

## Corresponding Author:

Muhammad Afzal

Department of Computer Science and Engineering,  
University of Engineering and Technology,  
Lahore-54890, Pakistan  
E-mail: [shmafzal@yahoo.com](mailto:shmafzal@yahoo.com)

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10/5/2010

# Curcumin/Irbesartan Combination Improves Insulin Sensitivity and Ameliorates Serum Pro-inflammatory Cytokines levels in Diabetes Rat model

Mohamed Khaled Mohamed. Mahfouz

Department of Biochemistry, Faculty of Vet Medicine, Benha University, [Banha, Al Qalyubiyah](#), Egypt  
[drm\\_mahfouz@hotmail.com](mailto:drm_mahfouz@hotmail.com)

**Abstract:** To evaluate the impact of chronic administration of an angiotensin II receptor antagonist (Irbesartan) and/or curcumin on fasting blood glucose level (FBG), insulin sensitivity, and proinflammatory cytokines in experimentally-induced diabetes in albino rats. Materials and Methods: The study included 80 (20 as control group) male albino rats; diabetes mellitus (DM) was induced using intraperitoneal injection of a single dose of 50 mg/kg of streptozotocin (STZ) after animals were maintained on high-fat diet for 2-weeks (30 rats) for induction of non-insulin dependent DM (NIDDM) or without dieting regimen (30 rats) for induction of IDDM. One-week later, rats received oral irbesartan (2.5 mg/kg/day), oral curcumin (200 mg/kg) or both lines for 6 weeks. Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) and rapid insulin sensitivity test (RIST) were used for clinical assessment. Two fasting venous blood samples were obtained prior to initiation and at 6-wks after treatment for estimation of FBG and ELISA estimation of fasting plasma insulin (FPI), serum interleukin (IL)-1 $\beta$  and -6 and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ). Results: Both lines of treatment induced significant reduction of FBG and FPI levels compared to pre-treatment levels with significant reduction of FBG on using curcumin compared to irbesartan, but combination therapy significantly lowered FPI levels compared to either drug alone. Post-treatment serum levels of studied cytokines in all groups were significantly lower compared to pre-treatment levels, but curcumin alone significantly reduced serum levels of IL-6 and TNF- $\alpha$  compared to irbesartan alone. Post-treatment HOMA-IR and RIST indices were significantly improved compared to pre-treatment levels. Conclusion: Chronic administration of irbesartan/curcumin combination showed anti-diabetic effect manifested as decreased FBG and FPI levels and ameliorated the increased serum levels of pro-inflammatory cytokines. The use of such combination could be recommended for clinical trials so as to document its use for control of both types diabetes.

[Mohamed Khaled Mohamed. Mahfouz. **Curcumin/Irbesartan Combination Improves Insulin Sensitivity and Ameliorates Serum Pro-inflammatory Cytokines levels in Diabetes Rat model.** Journal of American Science 2010;6(11):1051-1059]. (ISSN: 1545-1003). (<http://www.americanscience.org>).

**Keywords:** Curcumin, Irbesartan, Proinflammatory cytokines

## 1. Introduction

Insulin resistance is typically defined as decreased sensitivity or responsiveness to metabolic actions of insulin, such as insulin-mediated glucose disposal in skeletal muscle and adipose tissue and inhibition of hepatic glucose production, (Muniyappa et al., 2008). Cross-talk between inflammatory signaling pathways and insulin signaling pathways causes metabolic insulin resistance and endothelial dysfunction, (Kim et al., 2006).

Insulin resistance plays a major pathophysiological role in type 2 diabetes and is tightly associated with major public health problems, including obesity, hypertension, coronary artery disease, dyslipidemias, and a cluster of metabolic and cardiovascular abnormalities that define the metabolic syndrome, (Petersen et al., 2007). The metabolic syndrome is

considered to be a pro-inflammatory state because it is associated with elevated levels of high-sensitivity C-reactive protein, IL-6, fibrinogen, and plasminogen activator inhibitor-1, all of which promote the development of atherosclerotic cardiovascular disease, (Salmennienmi et al., 2008). Therefore, improvement of insulin sensitivity is an important therapeutic goal.

Improvement of insulin sensitivity has been suggested in many reports to be feasible by certain herbs and drugs. For instance, it was reported that curcumin improve blood glucose and insulin sensitivity in rat models of diabetes, (Weisberg et al., 2008). Curcumin, a polyphenolic compound, is the major yellow-colored pigment found in the spice, turmeric. It has been used in traditional Indian medicine for centuries, and has numerous pharmacological activities, including potent anti-inflammatory, antioxidant,

chemopreventive and chemotherapeutic actions, (Hatcher et al., 2008, Bengmark et al., 2009).

Angiotensin II (Ang II), the main effector peptide of the renin–angiotensin system (RAS), is implicated in the development of vascular, cardiac, and renal pathologies. Several lines of evidence suggest that Ang II impairs insulin sensitivity and provoke glucose intolerance, (Ogihara et al., 2002). Furthermore, angiotensin type-1 receptor (AT1R) blockers (ARBs) have recently been demonstrated to exert beneficial effects on glucose and lipid metabolism in adipocytes and adipose tissue, (Clasen et al., 2005). The RAS by blockade of the AT1R substantially lowers the risk for type 2 diabetes, (Dahlof et al., 2002). Additionally, blockade of the AT1R has been shown to improve insulin sensitivity in animal models of insulin resistance, (Henriksen et al., 2001). However, the mechanisms underlying the insulin-sensitizing and antidiabetic effects of the ARBs have not been defined. Findings from in vitro and in vivo studies have revealed that two newer ARBs, telmisartan and irbesartan, have the potential to improve insulin sensitivity and beta-cell responsiveness, (Schupp et al., 2005).

The present study was designed to evaluate the impact of chronic administration of an angiotensin II receptor antagonist (Irbesartan) and/or curcumin on blood glucose level, insulin sensitivity, and pro-inflammatory cytokines in experimentally-induced diabetes in albino rats.

## 2. Materials and Methods

**Animals:** The present study comprised 80 male albino rats with weight range of 250-300 grams. Rats were grouped and kept in separate animal cages, under the prevailing atmospheric conditions and maintained on a balanced diet (bread, barely, carrots, lettuce, milk) and fresh-water supply.

### Induction of diabetes

- A) Type 1 diabetes mellitus (IDDM group) was induced by injecting rats intraperitoneally with a single dose of streptozotocin (STZ) (Sigma) in a dose of 50 mg/kg body weight dissolved in 0.2 ml of citrate buffer (pH 4.5), (Islam and Choi, 2007) without dieting regimen.
- B) Type 2 diabetes mellitus (NIDDM group) was induced by feeding rats with high-fat diet (HFD) consisting of 22% fat, 48% carbohydrate and 20% protein. After two weeks, rats were injected intraperitoneally

with a single dose of streptozotocin (STZ) (Sigma) in a dose of 50 mg/kg body weight dissolved in 0.2 ml of citrate buffer (pH 4.5), (Islam and Choi, 2007).

**Diagnosis of diabetes:** On the third day of injection, the animals were checked for the presence of glucose in the urine using enzymatic test strips as STZ induces diabetes within 3 days by destroying the beta cells, (Karunananayake et al., 1975). Confirmation was done by measuring fasting blood glucose levels by taking a drop of blood from the rat-tail using a glucose-measuring device (Glucocard). Rats had FBG of  $\geq 200$  mg/dl were considered diabetic, (Islam and Choi, 2007).

**Drugs:** Irbesartan (Sanofi-Aventis) and curcumin (Sigma chemicals) were dissolved in 1% gum acacia so that 0.5 to 1 ml contained the desired dose. The therapeutic human dose of irbesartan was converted to rat dose according to Paget converting table, (Paget and Barnes, 1964) and about half of the therapeutic dose was used in this study.

### Grouping & Dosing:

**Group I (Control group):** 20 animals were considered as a control group for estimated parameters and were divided into 2 subgroups:

- a) Group I-A: included 10 rats received no medications and kept under the same conditions as prior to start of the study.
- b) Group I-B: included 10 rats were injected intraperitoneally with one injection of citrate buffer and received 1ml/rat of 1% gum acacia orally for 6 weeks.

**Group II:** included 30 rats had induced IDDM and were subdivided into 3 equal subgroups:

- a) Group II-A: 10 rats were administered irbesartan in a dose of 5 mg/kg/day, (Richer et al., 1999) in the drinking water for a period of 6 weeks, (O'Donnell et., 1997).
- b) Group II-B: 10 rats were administered 200 mg/kg body weight of curcumin in 1% gum acacia, orally/day for a period of 6 weeks
- c) Group II-A/B: 10 rats were administered both irbesartan in a dose of 5 mg/kg body weight in the drinking water and curcumin in a dose of 200 mg/kg body weight in 1% gum acacia; orally/day for a period of 6 weeks.

**Group III:** included 30 rats had induced NIDDM and were subdivided into 3 equal subgroups:

- a) Group III-A: 10 rats were administered irbesartan in a dose of 5 mg/kg/day in the drinking water for a period of 6 weeks.
- b) Group III-B: 10 rats were administered 200 mg/kg body weight of curcumin in 1% gum acacia, orally/day for a period of 6 weeks.
- c) Group III-A/B: 10 rats were administered both irbesartan in a dose of 5 mg/kg body weight in the drinking water and curcumin in a dose of 200 mg/kg body weight in 1% gum acacia; orally/day for a period of 6 weeks.

Biochemical Evaluation: Two fasting venous blood samples, withdrawn from the tail vein, were obtained, the 1<sup>st</sup> after induction of diabetes and prior to initiation of therapy and the 2<sup>nd</sup> at the end of the 6-wks treatment period. Blood samples were divided into 2 parts:

- A) The first was put in a tube containing sodium fluoride (2 mg sodium fluoride/ ml blood) to prevent glycolysis. Plasma was separated by centrifugation and used for estimation of glucose by glucose oxidase method, (Tinder, 1969).
- B) The second part was allowed to clot then serum was separated by centrifugation at 3000 rpm for 10 min. Serum was removed, divided into 2 parts: the first for RIA determination of serum level of insulin, (Gordon et al., 1985) and the second part was placed in pyrogen-free Eppendorf tubes and stored at -80°C until ELISA assayed (within one month) for estimation of serum levels of IL-1 $\beta$ , (Dinarello et al., 1992), IL-6, (Engvall et al., 1972) and TNF- $\alpha$ , (Beutler et al., 1985) using Quantikine ELISA kits from R & D Systems, Inc., (Minneapolis, MN).

Insulin sensitivity Evaluation: Insulin sensitivity of control and studied animals was evaluated by both tests, for comparison with IDDM using RIST and NIDDM using HOMA-IR test

- a. Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) (Matthews et al., 1985) on the basis of fasting insulin and glucose levels and according to the formula HOMA-IR= I x G/22.5, where I is fasting plasma insulin level ( $\mu$ IU/ml) and G is fasting blood glucose in mg/dl divided by 18, considering an abnormal HOMA-index >2, (Ascaso et al., 2001).

- b. Rapid insulin sensitivity test (RIST): The RIST starts with the administration of an insulin bolus (50mU/kg i.v.), over 5 min. At 1 min after initiating the insulin infusion, arterial blood glucose was measured and glucose infusion (D-Glucose/saline, 100 mg/ml, i.v.) was started at a rate of 5mg/kg/min. According to arterial glucose concentrations measured at 2 min intervals, the infusion rate of the glucose was readjusted to maintain euglycemia. When no further glucose infusion was required, usually within 35 min, the test was concluded. The amount of glucose necessary to maintain euglycemia along the test quantifies insulin sensitivity and is referred to as the RIST index (mg glucose/kg) (Lautt et al., 1998).

Statistical analysis: obtained data were presented as mean $\pm$ SD, ranges, numbers and ratios. Results were analyzed using one-way ANOVA test and Chi-square test. Statistical analysis was conducted using the SPSS (Version 10, 2002) for Windows statistical package. P value <0.05 was considered statistically significant.

### 3. Results

Estimated variables showed a non-significant ( $p>0.05$ ) difference between both subgroups of control rats, (Table 1), so all statistical analyses of study groups were compared versus control group I-A that arbitrary named control group.

Table (1): Mean values of estimated in both control subgroups

Variable	Group I-A	Group I-B
FBG (mg/dl)	77.4 $\pm$ 9.1	81 $\pm$ 9.3
FPI( $\mu$ IU/ml)	0.9 $\pm$ 0.2	0.82 $\pm$ 0.21
HOMA-IR index	0.17 $\pm$ 0.03	0.16 $\pm$ 0.05
IL-1 $\beta$ (pg/ml)	1.28 $\pm$ 0.23	1.19 $\pm$ 0.31
IL-6 (pg/ml)	12.2 $\pm$ 3.3	11.9 $\pm$ 4.2
TNF- $\alpha$ (pg/ml)	1.82 $\pm$ 0.6	1.86 $\pm$ 0.52

Fasting blood glucose levels estimated either prior to or at end of therapy, were significantly higher in all studied animals compared to control levels. Both lines of treatment either alone or in combination induced significant reduction of FBG levels in both study groups, irrespective of type of diabetes. However, administration of curcumin either alone or in combination with irbesartan induced significant

reduction of FBG, irrespective of type of diabetes, compared to irbesartan alone with non-significant difference between animals received curcumin, (Table 2).

Table (2): Mean ( $\pm$ SD) of FBG levels estimated in studied animals pre- and post-treatment compared to control levels

		Pre-ttt	Post-ttt
Control		77.4 $\pm$ 9.1	
Group II (IDDM)	Irb	164.8 $\pm$ 30.7*	145.4 $\pm$ 9.6*†
	Cur	166.9 $\pm$ 37.5*	134.4 $\pm$ 6.9*†#
	Irb/Cur	173.4 $\pm$ 28*	127.7 $\pm$ 6.5*†#
Group III (NIDDM)	Irb	176.3 $\pm$ 24.9*	144.9 $\pm$ 17.8*†
	Cur	167.7 $\pm$ 23*	126.9 $\pm$ 9.2*†#
	Irb/Cur	176.3 $\pm$ 24.9*	124.5 $\pm$ 6.4*†#

Pre: before start of therapy Post: at 6-wks of therapy

\*: significant difference versus control group

†: significant difference versus pre levels

‡: significant difference versus counterpart IDDM group

#: significant difference versus Irb subgroup

ƒ: significant difference versus Cur subgroup

Fasting plasma insulin (FPI) levels estimated either prior to or at end of therapy, were significantly higher in group III animals compared to both control and group II animals that had significantly lower FPI levels compared to control animals. As regard treatment subgroups, there was non-significant difference between group II subgroups with non-significant difference between pre and post-treatment levels. However, group III animals administered combination therapy showed significantly lower FPI levels compared to animals received either irbesartan or curcumin alone with a non-significant difference of FPI levels in animals received irbesartan compared to those received curcumin alone, (Table 3).

Table (3): Mean ( $\pm$ SD) of FPI levels estimated in studied animals pre- and post-treatment compared to control levels

		Pre-ttt	Post-ttt
Control		0.9 $\pm$ 0.2	
Group II (IDDM)	Irb	0.22 $\pm$ 0.08*	0.23 $\pm$ 0.08*
	Cur	0.25 $\pm$ 0.1*	0.26 $\pm$ 0.1*
	Irb/Cur	0.28 $\pm$ 0.1*	0.29 $\pm$ 0.1*
Group III (NIDDM)	Irb	5.1 $\pm$ 1.3*‡	3.61 $\pm$ 0.35*‡‡
	Cur	4.9 $\pm$ 1*‡	3.14 $\pm$ 0.41*‡‡
	Irb/Cur	5.3 $\pm$ 1.2*‡	2.55 $\pm$ 0.54*‡‡#

Pre: before start of therapy Post: at 6-wks of therapy

\*: significant difference versus control group

†: significant difference versus pre levels

‡: significant difference versus counterpart IDDM group

#: significant difference versus Irb subgroup

‡: significant difference versus Cur subgroup

Pre- and post-treatment estimated levels of studied pro-inflammatory cytokines were significantly higher in groups II and III compared to control level, irrespective of type of induced diabetes or line of treatment used. However, post-treatment serum levels of studied cytokines were significantly lower compared to pre-treatment levels, irrespective of line of treatment applied. Post-treatment serum levels of IL-1 $\beta$  were significantly lower in group III animals compared to group II animals, irrespective of line of treatment, (Table 4, Figure 1).

Table (4): Mean ( $\pm$ SD) of serum levels of IL-1 $\beta$  estimated in studied animals pre- and post-treatment compared to control levels

		Pre	Post
Control group		1.28 $\pm$ 0.23	
Group II (IDDM)	Irb	2.62 $\pm$ 0.42*	2.07 $\pm$ 0.56*†
	Cur	2.82 $\pm$ 0.41*	1.92 $\pm$ 0.58*†
	Irb/Cur	2.57 $\pm$ 0.36*	1.75 $\pm$ 0.4*†
Group III (NIDDM)	Irb	2.29 $\pm$ 0.3*‡	1.7 $\pm$ 0.41*‡‡
	Cur	2.14 $\pm$ 0.51*‡	1.58 $\pm$ 0.4*‡‡
	Irb/Cur	2.22 $\pm$ 0.46*‡	1.4 $\pm$ 0.32*‡‡

Pre: before start of therapy Post: at 6-wks of therapy

\*: significant difference versus control group

†: significant difference versus pre levels

‡: significant difference versus counterpart IDDM group

On contrary, there was non-significant difference between post-treatment serum levels of IL-6 and TNF- $\alpha$  between studied animals, irrespective of type of diabetes. However, administration of curcumin, either alone or in combination with irbesartan significantly reduced serum IL-6 in comparison to irbesartan alone, irrespective of type of diabetes and in IDDM animals, combination therapy significantly reduced serum IL-6 compared to curcumin alone and significantly reduced serum TNF- $\alpha$  compared to irbesartan alone, but non-significantly compared to curcumin alone, (Tables 5 & 6, Figures 2 & 3).

In group III, HOMA-IR index calculated prior to initiation of therapy was significantly higher in studied subgroups compared to control index with non-significant difference among studied subgroups. Post-treatment HOMA-IR index was significantly decreased in the three subgroups compared to pre-treatment levels, despite still being significantly higher compared to control group. Combination therapy significantly reduced HOMA-IR index compared to either irbesartan or curcumin alone with a significant difference in favor of irbesartan, (Table 7).

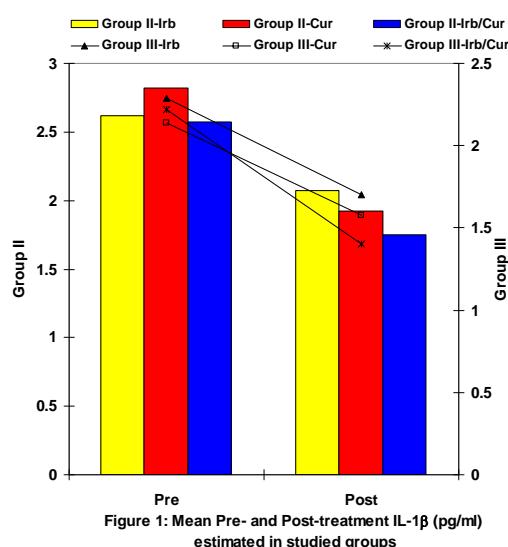


Table (5): Mean ( $\pm$ SD) of serum levels of IL-6 estimated in studied animals pre- and post-treatment compared to control levels

		Pre	Post
Control group		$12.2 \pm 3.3$	
Group II (IDDM)	Irb	$54.8 \pm 9.1^*$	$30.5 \pm 4^{*\dagger}$
	Cur	$49.6 \pm 8.8^*$	$26.4 \pm 2.1^{*\dagger\#}$
	Irb/Cur	$50.2 \pm 7.5^*$	$23.5 \pm 1.7^{*\dagger\#}$
Group III (NIDDM)	Irb	$40.7 \pm 14.1^*$	$28.5 \pm 1.9^{*\dagger}$
	Cur	$43.9 \pm 11.6^*$	$24 \pm 2.3^{*\dagger\#}$
	Irb/Cur	$43.2 \pm 9.3^*$	$21.9 \pm 2.3^{*\dagger\#}$

Pre: before start of therapy      Post: at 6-wks of therapy

\*: significant difference versus control group

†: significant difference versus pre levels

#: significant difference versus Irb subgroup

‡: significant difference versus Cur subgroup

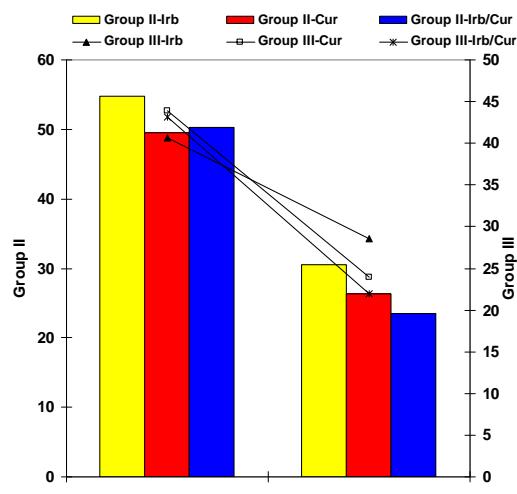


Figure 2: Mean Pre- and Post-treatment IL-6 (pg/ml) estimated in studied groups

Table (6): Mean ( $\pm$ SD) of serum levels of TNF- $\alpha$  estimated in studied animals pre- and post-treatment compared to control levels

		Pre	Post
Control group		$1.82 \pm 0.6$	
Group II (IDDM)	Irb	$6.36 \pm 1.7^*$	$4 \pm 1^{*\dagger}$
	Cur	$6.51 \pm 1.4^*$	$3.5 \pm 0.7^{*\dagger}$
	Irb/Cur	$6.54 \pm 1.7^*$	$3.1 \pm 0.5^{*\dagger\#}$
Group III (NIDDM)	Irb	$6.86 \pm 1.8^*$	$3.82 \pm 0.7^{*\dagger}$
	Cur	$6.4 \pm 1.9^*$	$3.6 \pm 0.8^{*\dagger}$
	Irb/Cur	$6.7 \pm 2^*$	$3.4 \pm 0.6^{*\dagger}$

Pre: before start of therapy      Post: at 6-wks of therapy

\*: significant difference versus control group

†: significant difference versus pre levels

#: significant difference versus Irb subgroup

In group II, post-treatment RIST index was significantly lower ( $p=0.009$ ) in animals pre-treated with irbesartan ( $17.5 \pm 3.9$ ) compared to control index ( $30.6 \pm 7.8$ ) with non-significant difference between both other subgroups compared to control index. Moreover, RIST index calculated in animals pre-treated with either curcumin alone ( $28.8 \pm 6.6$ ) or in combination with irbesartan ( $34.5 \pm 3.9$ ) was significantly higher ( $p=0.005$ , respectively) compared to those pre-treated with irbesartan only with significantly higher ( $p=0.028$ ) RIST index in animals received combination therapy compared to those pre-treated with curcumin alone.

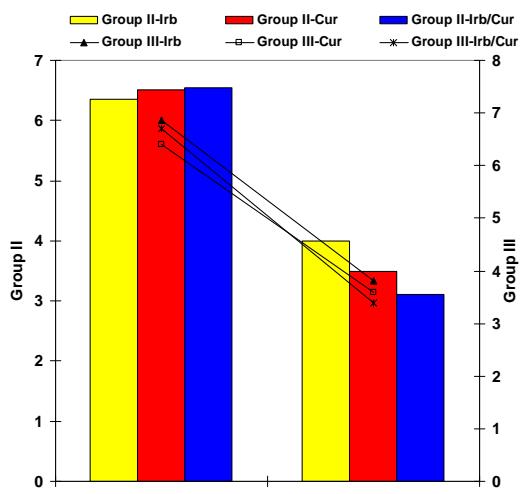


Figure 3: Mean Pre- and Post-treatment TNF- $\alpha$  (pg/ml) urestimated in studied groups

Table (7): Mean ( $\pm$ SD) of HOMA-IR index calculated in Group III compared to control levels

	Pre-ttt	Post-ttt
Control	0.17 $\pm$ 0.03	
Group III (NIDDM)	Irb	1.98 $\pm$ 0.54*
	Cur	2.11 $\pm$ 0.6*
	Irb/Cur	2.3 $\pm$ 0.08*
		0.81 $\pm$ 0.19*†#]

Pre: prior to initiation of therapy

Post: at end of 6-wks therapy

\*: significant difference versus control group

†: significant difference versus pre levels

#: significant difference versus Irb subgroup

‡: significant difference versus Cur subgroup

#### 4. Discussion

Both applied lines of treatment either alone or in combination induced significant reduction of FBG levels in both study groups, irrespective of type of diabetes. However, administration of curcumin either alone or in combination with irbesartan induced significant reduction of FBG, irrespective of type of diabetes, compared to irbesartan. These findings spotlight on the fact that both of curcumin and irbesartan induced lowering of FBG by a different mode of action towards one target, i.e. lowering FBG and both could act synergistically and that the response for either curcumin or irbesartan differed between both types of diabetes.

In NIDDM animals, FPI levels estimated at end of therapy despite being still significantly higher compared to control levels, were significantly lower compared to their pre-

treatment levels and animals administered combination therapy showed significantly lower FPI levels compared to animals received either irbesartan or curcumin alone. These finding indicated that the effect of the studied drugs on diabetic animals was conducted through increasing the sensitivity of insulin receptor to the available secreted amount of insulin and consequently increased glucose metabolism with lowering FBG without any impact on insulin secretion.

The obtained results coincided with and supported that previously reported by Pari and Murugan, (2005), who investigated the effect of tetrahydrocurcumin (THC), one of the active metabolites in curcumin, on the key hepatic metabolic enzymes involved in carbohydrate metabolism in streptozotocin-induced diabetic rats and found that in untreated diabetic control rats, the activities of the gluconeogenic enzymes were significantly increased, whereas hexokinase and G6PD activity and glycogen levels were significantly decreased, while both THC and curcumin were able to restore the altered enzyme activities to near normal levels and normalize blood glucose in diabetic rats. Also, Murugan and Pari, (2006), investigated the effect of THC on lipid profile and lipid peroxidation in type-2 diabetic rats and reported a significant reduction in blood glucose, which proved its antidiabetic effect and caused a significant reduction in lipid peroxidation and lipids in serum and tissues, suggesting its role in protection against lipid peroxidation and its antihyperlipidemic effect.

Thereafter, Murugan and Pari, (2007), and Suryanarayana et al., (2007) examined the effect of THC and curcumin on erythrocyte membrane bound enzymes and antioxidants activity in type-2 diabetic model and reported that administration of THC and curcumin induced increased levels erythrocyte antioxidants and the activities of membrane bound enzymes and concluded that these biochemical observations indicate that the THC and curcumin possess a significant beneficial effect on erythrocyte membrane bound enzymes and antioxidants defense in addition to its antidiabetic effect.

In support of the reported data, post-treatment HOMA-IR and RIST indices were significantly improved in the studied subgroups compared to pre-treatment levels, with the effect was more significantly pronounced with combination therapy. Such clinical implication of the obtained results goes in hand with various clinical studies; Huang et al., (2007) suggested

that a local pancreatic renin-angiotensin system and pravastatin, captopril and irbesartan treatment may be selectively controlling pancreatic islet blood flow, augmenting insulin secretion and thereby improving glucose tolerance and concluded that the antidiabetic actions of renin-angiotensin system inhibitors might occur, in part, through this beneficial direct islet effects. Cetinkalp et al., (2008), found the short-term treatment of irbesartan is effective to decrease microalbuminuria in normotensive type-2 diabetes patients independent of its antihypertensive effect and such decrease was associated with significantly decreased fasting and non-fasting blood glucose, and HbA1c compared to pre-treatment values.

Furthermore, post-treatment serum levels of studied cytokines were significantly lower compared to pre-treatment levels, irrespective of line of treatment applied; such ameliorative effect of curcumin and irbesartan administered separately or in combination on pro-inflammatory cytokines could be a possible mechanism for the reported effects on insulin sensitivity that proved to be improved irrespective of type of diabetes or the drug used.

These data go in hand with Ceriello et al., (2005), who reported an independent and cumulative effect of postprandial hypertriglyceridemia and hyperglycemia on endothelial function and inflammation, suggesting oxidative stress as a common mediator of such an effect and short-term treatment with irbesartan may counterbalance this phenomenon. Also, Persson et al., (2006), evaluated the impact of irbesartan treatment on biomarkers of low-grade inflammation in patients with Type 2 diabetes and microalbuminuria and found irbesartan treatment yielded significant changes in CRP with a 5.4% decrease per year versus a 10% increase per year in the placebo group, IL-6 showed a 1.8% increase per year compared with placebo's 6.5% increase per year and changes in IL-6 were associated with changes in albumin excretion and concluded that irbesartan reduces low-grade inflammation in this high-risk population. Vieitez et al., (2008), found systemic and local administration of irbesartan lowers glomerular expression of growth factors and TNF- $\alpha$  and concluded that part of the effect of lowering the expression of these growth factors and cytokines is due to a direct blockade of glomerular renin-angiotensin system.

The reported beneficial effects of curcumin alone or combination on IDDM could

be attributed to the anti-oxidant and anti-inflammatory effects of curcumin and go in hand with Tikoo et al., (2008), who reported that treatment of type-1 diabetic rats with curcumin significantly decreased blood urea nitrogen and creatinine and increased albumin; variables associated with the development of diabetic nephropathy and prevented the increased levels of HSP-27 and MAP kinase (p38) in diabetic kidney and at nuclear level curcumin prevented the decrease in dephosphorylation and increases acetylation of histone H3. Moreover, Kanitkar et al., (2008), demonstrated that curcumin in vitro protects pancreatic islets against cytokine-induced death and dysfunction by scavenging ROS and normalized cytokine-induced NF-kappaB translocation by inhibiting phosphorylation of inhibitor of kappa B alpha (IkappaBalpha) and in vivo curcumin prevents STZ-induced diabetes.

Kang and Chen, (2009) found curcumin dose-dependently eliminates insulin-induced hepatic stellate cells (HSC) activation by suppressing expression of type I collagen gene, interrupts insulin signaling in HSC by reducing the phosphorylation level of insulin receptor and suppressing its gene expression. Furthermore, curcumin attenuates insulin-induced oxidative stress in HSC by inducing gene expression of glutamate-cysteine ligase leading to de novo synthesis of glutathione. Also, Lin et al., (2009) found curcumin suppresses gene expression of lectin-like oxidized LDL receptor-1, leading to the blockade of the transport of extracellular oxidized LDL into cells through interruption of Wnt signaling and the activation of peroxisome proliferator-activated receptor-gamma

It could be concluded that chronic administration of irbesartan/curcumin combination showed anti-diabetic effect manifested as decreased FBG levels with concomitant decreased FPI and ameliorated the increased serum levels of pro-inflammatory cytokines and such effects are manifested in both types of diabetes. The use of such combination could be recommended for clinical trials so as to document its use for control of both types diabetes.

#### Acknowledgements:

Authors are grateful to Faculty of Vet Medicine, Benha University for financial support to carry out this work.

**Corresponding Author:**

Dr. Mohamed Khaled Mohamed Mahfouz  
Department of Medical Biochemistry  
Faculty of Vet Medicine, Benha University  
Mushtohor, Kaluobia, Egypt  
E-mail: [drm\\_mahfouz@hotmail.com](mailto:drm_mahfouz@hotmail.com)

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# Evaluation of some Inflammatory Cytokines in Children with Type1 Diabetes Mellitus

Azza A.A<sup>\*1</sup>, Mohga S. A<sup>2</sup>, Wafaa GH. SH<sup>2</sup>, Karam AM<sup>3</sup>, Enas R.A<sup>1</sup>, Tarek AS. H<sup>4</sup>, Salwa M E<sup>5</sup>

<sup>1</sup> Child Health Department, National Research Centre, Cairo, Egypt

<sup>2</sup> Biochemistry Department, Faculty of Science Helwan University, Helwan, Egypt

<sup>3</sup> Medical Biochemistry Department, National Research Centre, Cairo, Egypt

<sup>4</sup> Biology Department, Animal Reproduction Research Institute, Agriculture Research centre, Cairo, Egypt

<sup>5</sup> Biochemistry Department, Faculty. of Science, Ain shams University, Cairo, Egypt

<sup>\*</sup>[drazzaaa@yahoo.com](mailto:drazzaaa@yahoo.com)

**Abstract:** Objectives: Recent evidence favors primary role of cellular autoimmunity and its humoral mediators in pathogenesis and following Type I diabetes mellitus (IDDM) The present study is carried out to investigate serum concentration of TNF- $\alpha$ , IL-6 and sIL-2 R in children with IDDM. Potential role of glycemic control, body mass index and disease duration were evaluated. Design and Methods: Thirty five children with IDDM and 30 age and sex matched non diabetic healthy subjects were recruited for this study from the out patients Clinic of diabetes of National Institute of Diabetes and Endocrinology. Results: Circulating level of TNF- $\alpha$  IL-6 and sIL-2R were elevated in children with type I DM ( $39.91 \pm 17.46$  pg/ml,  $14.89 \pm 10.69$  pg/ml and  $779.0 \pm 467.06$  pg/ml respectively). Compared with nondiabetic controls ( $5.67 \pm 1.88$  pg/ml,  $6.23 \pm 2.78$  pg/ml and  $254.33 \pm 173.6$  pg/ml respectively). These differences were statistically highly significant ( $<0.0001$ ). Glycemic control, Insulin dose and disease duration were not significant predictors of cytokine concentration in children with IDDM. A significant negative correlation was obtained between TNF - $\alpha$  with age, weight, BMI and sIL-2R in diabetic patients. However there was a significant positive correlation between IL-6 with weight and BMI in those children. Conclusion: Circulating levels of inflammatory cytokines were elevated in patients with IDDM suggesting activation of the inflammatory immune response system. Their levels were not affected by glucose level , insulin dose or duration of the disease.

[Azza A.A, Mohga S. A, Wafaa GH. SH, Karam AM, Enas R.A, Tarek AS. H, Salwa M E. Evaluation of some Inflammatory Cytokines in Children with Type1 Diabetes Mellitus. Journal of American Science 2010;6(11):1060-1067]. (ISSN: 1545-1003). (<http://www.americanscience.org>).

**Keywords:** Inflammatory; Cytokines; children; Diabetes Mellitus

## 1. Introduction:

Diabetes Mellitus is a primary error of carbohydrate metabolism characterized by hyperglycemia with or without glycosuria with secondary disturbance of protein and fat metabolism, which is believed to result from autoimmune destruction of B-cells of the pancreas. (Hussain *et al.*, 1988).

Cytokines are regulatory proteins produced and secreted by lymphocytes and monocytes. Those produced by lymphocytes are called lymphokines. (Karlsson *et al.*, 2004).

According to increasing experimental and clinical evidence, proinflammatory cytokines may play important roles alone or in combination with the pathogenesis of type I diabetes mellitus.

Interlukin-2 is a lymphokine produced by T-helper cells after its stimulation by interlukin-1 (IL-1). The action of IL-2 is mediated through its binding to specific IL-2 receptors (IL-2R) that are variably present on T-cells depending on their degree of activation by antigen. The lymphocytes can shed their IL-2 R in soluble form, so many investigators have

proposed that measurements of IL-2R concentration may be useful in assessing immunological function in autoimmune disorders. (Blandino 2008 et al.).

Plasma concentration of (IL-6) was found to be elevated in diabetic patients. IL-6 through its effects on soluble intercellular adhesion molecule-1 (s ICAM-1) and tumour necrosis factor (TNF-alpha), may promote vascular adhesion adding to vascular disease risk.

Aim of the work

- To investigate serum concentrations of interleukin-6 (IL-6), soluble interleukin-2 receptor (sIL-2R) and tumour necrosis factor alpha (TNF-alpha) in children with IDDM.
- To evaluate potential role of glycemic control, body mass index (BMI) and disease duration on serum levels of these cytokines.

## 2. Subjects and Methods:

### Patient Group

This group included 35 children suffering from IDDM. They were selected from the out patients Diabetes Clinic of International Institute of

Diabetes and Endocrinology. Their ages ranged from 7-17 years, with a mean ages of  $12.03 \pm 2.2$  years.

#### Healthy subjects

30 apparently healthy children with no family history of diabetes were taken as a control group. They were free from any systemic disease. Their ages ranged from 7-16 years with a mean age of  $12.77 \pm 2.76$  years.

#### Methods

All participants were subjected to the following :

- 1- Full history taking laying stress on the age, the duration of the insulin dose.
- 2- Thorough clinical examination.
- 3- Anthropometric measurements weight, height and BMI ( $\text{kg}/\text{m}^2$ ) measurement were done
- 4- Laboratory assessment of random blood sugar glycohemoglobin and serum, sIL-2R, serum IL-6 and TNF- $\alpha$ .

#### Sampling

Five milliliters of venous blood were collected from each child 4 ml of blood in a clean dry tube without the addition of any anticoagulant. It was left to clot for 15 minutes, centrifuged and serum was separated. Assessment of random blood sugar was done immediately and the rest of the serum was kept frozen at  $-20^\circ\text{C}$  for the subsequent assay of IL-2R, IL-6 and TNF - $\alpha$  and 1 ml was added in tube with anticoagulant as EDTA to made glycotheamoglobin.

#### Analytical Methods

##### A- Random Blood sugar:

Assay was done by quantitative determination of glucose IVD using a kit provided by SPINRE ACT Inc.

##### B- Glycohemoglobin:

Assay was done by quantitative colorimetric determination of glycohemoglobin in whole blood using a kit provided by STANBIO LABORATORY INC cat. No 0350.

##### c- Tumour Necrosis factor- $\alpha$ :

This was done by sandwich enzyme immunoassay using a Kit provided by INSTRUCTION for the quantitative determination of human TNF- $\alpha$  in plasma, serum, and culture supernatant fluids. cat. No. 1121

##### D- IL-6 assay:

This was done by a solid phase sandwich enzyme linked immunosorbent assay using a kit provided by Biosource International, Inc. cat No KHC0062

##### E- sIL-2R assay:

This was done by a salid phase sandwich enzyme linked immunosorbent assay using a kit provided by Biosource International, Inc. cat. No. KHR0022

#### Statistical analysis

Data were analyzed by computer using the statistical program SPSS v12 for Windows. Group was compared by the Student's test for normally distributed data or by the Mann-Whitney test otherwise. The linear relationship between variables was assessed by Pearson's correlation coefficient (r). For all tests, P values less than 0.05 were considered statistically significant. Sensitivity, specificity and ROC curves were done for all inflammatory cytokines.

#### 3. Results

Clinical and physical characteristics of children with IDDM and healthy subjects were demonstrated in table (1). There was a significant change in weight and BMI in diabetic patients as compared with healthy group. However, Age and height of diabetic patients didn't have significant change than healthy one.

Comparison of serum glucose levels and glycosylated hemoglobin (Hb A<sub>1c</sub>) between children with diabetes mellitus and healthy subjects were shown in table (2). The results indicated that serum glucose and Hb A<sub>1c</sub> levels were highly significant in diabetic patients than healthy subjects ( $P<0.001$ ).

As can be seen from table 3 & Fig. 1, the mean values of serum inflammatory cytokins [TNF- $\alpha$  , IL-6 and sIL-R] levels were highly significant in diabetic patients than healthy one ( $P<0.001$ ). The recorded increase was 603.9, 139 and 206.3 respectively.

**Table 1: Physical characteristics of children with IDDM and control [Mean  $\pm$  SD (Range)]**

	Diabetics (n=35)	Control. (n=30)	% of change	P value
Age (years)	$12.03 \pm 2.2$ (7-17)	$12.77 \pm 2.76$ (7-16)	-5.8	n.s
Weight (kg)	$41.69 \pm 11.31$ (22-64)	$52.93 \pm 13.94$ (29-95)	-21.2	0.0006
High (cm)	$146.57 \pm 12.12$ (125-168)	$150.57 \pm 16.54$ (125-175)	-2.7	n.s
BMI ( $\text{Kg}/\text{m}^2$ )	$19.04 \pm 2.94$ (14.1-26.2)	$23.06 \pm 2.98$ (18-32.1)	-17.4	<0.0001

**Table 2: Serum glucose levels and glycosylated haemoglobin level in patients and control [Mean ± SD (Range)]**

	Diabetics (n=35)	control. (n=30)	% of change	P value
Glucose (mg/dl)	224.34 ± 118.78 (90 – 516)	86.0 ± 7.97 (72-107)	160.9	<0.0001
Hb A <sub>1c</sub> (%)	9.01 ± 1.61 (6.6-15)	6.6±0.34 (6.0-7.1)	36	<0.0001

**Table 3: Serum inflammatory cytokines levels in patients and control [Mean ± SD (Range)]**

	Diabetics (n=35)	control. (n=30)	% of change	P value
TNF - α (Pg/ml)	39.91 ± 17.46 (12 – 80)	5.67 ± 1.88 (2.5-10)	603.9	<0.0001
IL-6 (Pg/ml)	14.89± 10.69 (6-48)	6.23 ± 2.78 (3-15)	139.0	<0.0001
sIL-2R (Pg/ml)	779.0 ± 467.06 (100- 1750)	254.33±173.06 (50-700)	206.3	<0.0001

**Table 4: Inflammatory cytokines in children with newly diagnosed ( $\leq 1$  year) and long Standing ( $>1$  year) type 1 diabetes mellitus [Mean ± SD (Range)]**

	Duration≤ 1 year (n=12)	Duration>1 year (n=23)	P value
TNF - α (Pg/ml)	43.0 ± 19.12 (22 – 80)	38.3 ± 16.74 (12-80)	>0.05
IL-6 (Pg/ml)	12.17± 5.69 (6-23)	16.3± 12.43 (6- 48)	>0.05
sIL-2R (Pg/ml)	677.5 ± 467.28 (100-1700)	831.96± 468.41 (150-1750)	>0.05

On trying to elucidate the effect of duration of illness on the inflammatory markers we compared serum level of TNF- $\alpha$ , IL-6 and sIL-2R in Diabetic patients with duration of illness less than one year and those in patients with duration of illness greater than one year (table 4).

The relationships between serum inflammatory cytokines and all clinical and biochemical indices in children with children with IDDM and healthy subjects were obtained in Table 5. A significant negative correlation was obtained between TNF- $\alpha$  with age, weight, BMI and sIL-2R in diabetic patients. However, there were significant positive correlations between IL-6 with weight and BMI in both diabetic children and healthy subjects. Insulin dose and glycosylated hemoglobin were not correlated to any of the inflammatory markers.

**Table (5): Correlation coefficient between inflammatory cytokines and all clinical and biochemical characteristic in children with IDDM and healthy subjects.**

Group	Diabetic patients (n=35)			Healthy group (n=30)		
	Variables	TNF- $\alpha$ (pg/ml)	IL-6 (pg/ml)	sIL-2R (Pg/ml)	TNF- $\alpha$ (pg/ml)	IL-6 (pg/ml)
Age (year)	-0.37*	0.45**	0.17	-0.08	0.24	0.13
Weight (kg)	-0.39*	0.47**	0.01	0.05	0.52**	0.28
Hight (cm)	-0.29	0.30	0.02	-0.01	0.31	0.10
BMI (kg/m <sup>2</sup> )	-0.42*	0.50**	0.02	0.08	0.46**	0.33
Duration	-0.15	0.19	0.13	-	-	-
Glucose (mg/dl)	0.28	-0.11	-0.12	-0.04	0.20	0.22
Hb A <sub>1c</sub> (%)	-0.12	0.08	-0.02	-0.07	0.30	-0.02
Insulin dose (Iu)	-0.28	0.14	-0.05	-	-	-
TNF- $\alpha$	-	-0.09	-0.39*	-	0.04	0.21
IL-6	-0.09	-	0.31	0.09	-	0.30
sIL-2R	-0.39*	0.31	-	0.21	0.30	-

\* P <0.05    \*\* P <0.01

Roc analysis was used to determine the accuracy of each serum cytokines as a marker for diagnosis of children with IDDM in 35 patients and 30 healthy subjects. The data obtained in tables 6, 7 and fig. 1, proved that TNF- $\alpha$  test was the best test to discriminate children with diabetes mellitus from healthy subjects with an area under the curve of 1 and cut-off value of 10 Pg/ml. The sensitivity and specificity were 100 %. IL-6 showed an area under the curve of 0.86 for cut-off value of 6pg/ml, the sensitivity was 88.6% and

specificity was 66.7% (tables 6, 7 and fig. 2). The data illustrated in tables 6, 7 and fig. 3, proved that sIL-2R had an area under the curve 0.87 for cut-off value of 330 pg/ml. The sensitivity was 85.7% and specificity was 86.7%.

Multivariate analysis using logistic regression indicated that all tested inflammatory cytokines [TNF- $\alpha$ , IL-6 and sIL-2R] were found to be independently associated with diabetes mellitus ( $P<0.001$ ) fig 4.

**Table (6): Results of ROC analysis to differentiate between patients and healthy subjects.**

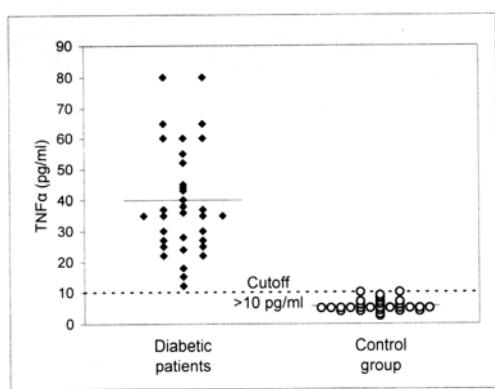
	Cut-off	AUC	$\pm$ SE	95% CI	Sensitivity	Specificity
TNF	>10pg/ml	1.00	0.00	0.94-1*	100%	100%
IL-6	>6 pg/ml	0.86	0.05	0.75-0.94*	88.6%	66.7%
SIL-2R	>330 pg/ml	0.87	0.04	0.77-0.94*	85.7%	86.7%

\* Significant ( $P<0.05$ ) AUC = area under the curve SE = standard error

95% CI=95% confidence interval for AUC.

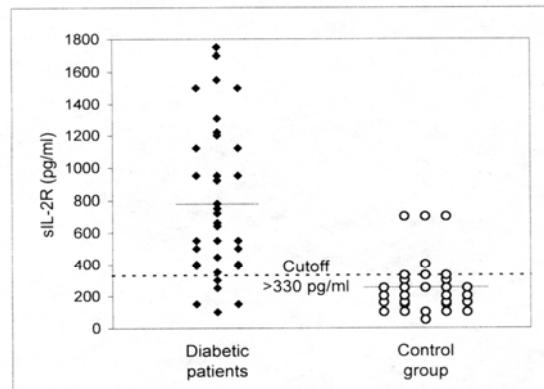
**Table (7): Chi-square analysis using determined cut-offs**

	Patients		Controls		Chi square	P value
	N	%	n	%		
TNF	>10	35	100%	0	0%	<0.001
	<=10	0	0%	30	100%	
IL-6	>6	31	88.6%	10	33.3%	<0.001
	<=6	4	11.4%	20	66.7%	
SIL-2R	>330	30	85.7%	4	13.3%	<0.0001
	<=330	5	14.3%	26	86.7%	



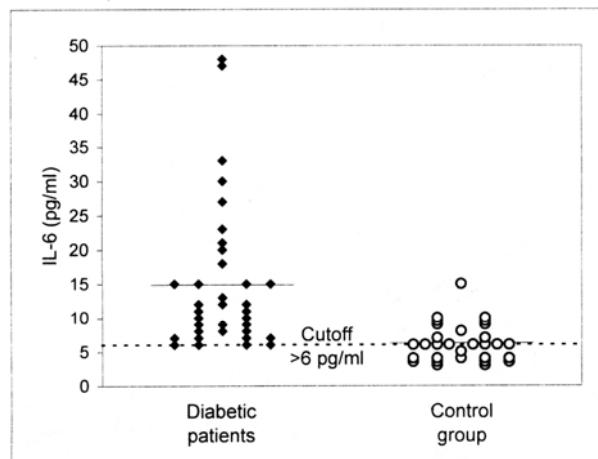
**Fig. 1: Scatter diagram of TNF- $\alpha$  (pg/ml) level in diabetic patients and healthy group.**

The horizontal line represents the cut-off value of 10 pg/ml. The sensitivity is 100% at specificity 100%.



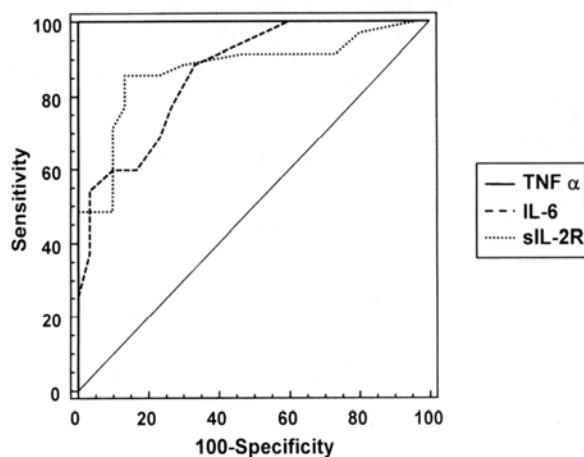
**Fig. (2): Scatter diagram of sIL-2R (pg/ml) level in diabetic patients and healthy group.**

The horizontal line represents the cut-off value of 330pg/ml. The sensitivity is 85.7% at specificity 86.7%.



**Fig. (3): Scatter diagram of IL-6(pg/ml) level in diabetic patients and healthy group.**

The horizontal line represents the cut-off value of 6pg/ml. The sensitivity is 88.6% at specificity 66.7%.



**Fig. (4): Roc curve testing the ability of TNF- $\alpha$ , IL-6 and sIL-2R.**

Differentiate between patients with type (1) diabetes mellitus and healthy group. Areas under the curve (AUC) are 1.0, 0.86 and 0.87 for TNF- $\alpha$ , IL6 and sIL-2R respectively P<0.0001 for comparison of the markers.

#### 4. Discussion:

Insulin dependent diabetes mellitus is the effect of T cell dependent autoimmune destruction of insulin production beta cells in the pancreas. Insulin is one of the islet autoantigens responsible for activation of T-lymphocyte functions, inflammatory cytokine production and development of IDDM (Tchorzewski et al., 2001).

Information about the inflammatory state of an individual can become of clinical relevance since factors that determine inflammation can be modified,(Chatz et al,2010).

The proinflammatory cytokines TNF- $\alpha$  and IL-6 play an important role in the pathogenesis of insulin- development diabetes mellitus (Alexandrak et al.,2008) while TNF- $\alpha$  is also involved in promoting insulin resistance, development or progression of IDDM (Shbaklo et al., 2003).

Data of the present study revealed that serum cytokines (TNF $\alpha$ , IL-6 and sIL-2R) levels were significantly higher in patients than healthy control. These results are in agreement with those of Wasmuth et al., 2004, Dondona et al., 2004, Glowinska & Urban, 2003 and Miranda et al., 2003 who reported that the inflammatory activity is increased in individuals with type-1 diabetes, may be

due to hyperglycemia and the formation of advanced glycation end products. Another report indicated that TNF- $\alpha$  is identified as the unifying principle linking the pathogenesis of insulin-dependent diabetes mellitus and non insulin-dependent diabetes mellitus. Elevated TNF- $\alpha$  initially increases and then inhibits, the activity of a number of key enzymes involved in energy metabolism and major histocompatibility (MHC) class I molecule expression. These enzymes include protein-tyrosine kinase (PTPase). Enzymes involved in energy metabolism, cell proliferation and stimulation of MHC class I molecule pathway (Foss et al.,2007) and concomitant destruction of pancreatic beta cells. So, TNF- $\alpha$  can be implicated as indicator of continuing autoimmune aggression against beta-cells before the development of extensive beta - cell destruction (Haller and Schatz 2008).

It is well documented that TNF- $\alpha$  can be cytotoxic, cytostatic since it inhibits insulin synthesis and secretion (rabinovitch 2002 & Suarez-Pinzon, 1998). Additionally TNF- $\alpha$  and IL-6 mediated damage to micro-and macrovascular tissues, altered insulin secretion directly or through stimulation of free fatty acids production and altered glucose homeostasis (Peraldi & Spiegelman, 1998, Schmidt et al., 1999, Corbett et al., 1997 and Unger, 1995).

About the molecular mechanisms for increased IL-6 under hyperglycemia, Igarashi et al. (1999) found that high glucose has been shown to activate p38 MAPK (Mitogen Activated Protein kinase) which regulate the production of inflammatory cytokines such as TNF- $\alpha$  and IL-6 (Yamakawa et al., 1999). On the same line, Sridevi et al. (2005) reported that under high glucose, monocytes secrete increased amounts of IL-6, via upregulation of protein kinase (PKC- $\alpha$ - and  $\beta$ ), P38 MAPK and Nuclear factor (NF- $\kappa$ B) activity leading to increased IL-6 transcription and release .

There is a discrepancy between the data obtained by our work and that of Haller and Schatz. (2008) who found non significant change in serum TNF- $\alpha$  levels of diabetic children when compared with age-matched healthy controls. Similarly,) Todd et al. (2005), indicated that serum TNF- $\alpha$  and IL-6 levels were comparable in diabetic and non diabetic groups. However, newly diagnosed (<1yr ) cases had higher TNF- $\alpha$  and IL-6 levels compared with larger standing DM<sub>1</sub>. Another study obtained by Chatzi et al. (2010) found that the duration of diabetes was associated with TNF- $\alpha$  and general scores of inflammatory markers. Unlike previous reports, our data indicated that the elevation of cytokine markers were comparable in diabetic patients with a duration of disease more than one year as well as in diabetic patients with a shorter duration of diabetes ( $\leq$ 1 yr).

This results are in agreement with that given by Blandino et al. (2008). On the other hand, another study indicated that IL-6 concentrations were statistically higher at onset diabetes than in diabetic patients with long-term disease (Wedrychowicz *et al.* 2004) and Fosset *et al.* 2007. We have tested the correlation between cytokine levels and duration of disease to confirm whether these difference observed in cytokine levels have any pathophysiological consequences. Inflammatory, diabetes preventing or diabetes promoted effects have been suggested for altered cytokine (Rabinovitch *et al.*, 2002). On the other hand there is increasing evidence that a number of acute phase proteins such as CRP and IL-6 itself are antiinflammatory and immuno suppressive being involved in the resolution of the inflammatory response (Haller and Schatz 2008).

Adipocytes can produce IL-6 and TNF- $\alpha$  and many studies in non diabetic (Onate *et al.*, 2001, & Yamada *et al.* 2001) and diabetic (Festa *et al.*, 2000, Saraheimo *et al.*, 2003) individuals, have shown an association between estimates of body fat and inflammatory activity. Our data revealed that BMI was negatively correlated with TNF- $\alpha$  levels ( $r=-0.42$ ,  $P<0.05$ ) in diabetic children and positively correlated with IL-6 levels ( $r=0.50$ ,  $P<0.01$ ) in the same group. BMI showed also positive correlation with IL-6 levels in non diabetic children. The data obtained by Karlsson *et al.* 2004 suggesting that BMI was associated with all inflammatory markers (TNF $\alpha$ , IL-6) in diabetic children that support our data. On the other hand, there is no evidence correlation between these cytokines and BMI. was reported in other reports (Ayse *et al.* 2001).

Many cytokines play an important role in the etiopathogenesis of type 1-DM, among these is IL-2 which induces its action through its binding to specific interleukin-2 receptors (IL-2Rs) that are present on the surface of T-cells (Zhenge *et al.*, 1999 & Kretowski *et al.*, 1999).

Interleukin-2 system which involves IL-2 production, IL-2 receptor expression and response to IL-2, is associated with autoimmune phenomena. Immunological abnormalities including autoimmune phenomena are believed to contribute to the pathogenesis of IDDM (Blandino *et al.*, 2008). It was found that the percentage of IL-2 receptor positive circulating T-cells was significantly increased in diabetic children than non diabetic group (Gartner *et al.*, 1995). Similar results were obtained by previous study of Chatz and his co-workers (2010) who indicated that there was no correlation between sIL-2R and any metabolic parameters in type-1 diabetic patients. This results are in accordance with our data that revealed serum sIL-2R levels were significantly higher in diabetic children than non diabetic group.

On the other hand, correlation was not detected between level of sIL-2R and any clinical or biochemical parameters in diabetic patients except TNF- $\alpha$  that showed significant negative correlation with sIL-2R ( $r=-0.39$ ,  $P<0.05$ ). Supportive to our results, is the evidence that TNF- $\alpha$  cytokine regulate production of other soluble factors (Foss *et al.*, 2007). Furthermore, the TNF- $\alpha$  gene is located on chromosome 6 in close proximity to the MHC class II region. Thus further studies must address the question as to whether there is an association between TNF- $\alpha$  and the abnormalities of cytokine production and its soluble receptors such as IL-2R as observed in the present study.

Previous data indicated that 46% of diabetic patients, 40% of their parent and 55% of their sibling had sIL-2R levels exceeding the highest normal value. Moreover, the authors found increased levels of TNF- $\alpha$  and IL-6 in the diabetic patients and their healthy relatives. They explained such spectrum of immunological abnormalities in diabetic patients and their family members by heightened immune response in these individuals in which activated T-lymphocytes express IL-2R with shedding of these receptors in the serum (Hussain *et al.*, 1998).

Contradictory results, indicating decrease in the concentration of sIL-2R in pre diabetic and diabetic patients with newly diagnosed type-1-DM. when compared with age-matched control subjects). Such inconsistency and contradiction might be due to a difference in stages of the autoimmune process (Alexandraki *et al.*, 2008).

Regarding our study that revealed level of IL-2R were not correlated with disease duration, random blood sugar or age in the diabetic patients or non diabetic group. These results are in agreement with other previous studies (Rabinovitch *et al.*, 2002, Kukrega *et al.*, 2002 & Haller and Schatz *et al.*, 2008).

In order to determ if the inflammatory cytokines (TNF- $\alpha$ , IL-6 and sIL-2R) were useful as markers in screening for early IDDM and in monitoring immunological treatment, diagnostic reliability was performed to choose the best cut-off value within the patient group (calculated from control group). Our data proved that TNF- $\alpha$  was the best to discriminate type 1 DM. and its cut-off value of 10 Pg/ml and at this value, the sensitivity was 100% and specificity was 100%. The next most useful test for predicitng type-1 DM, among our patients, was IL-6 with cut-off value of 6 pg/ml at which the sensitivity was 88.6% and specificity was 66.7%. the data obtained also, proved that sIL-2R with cut-off value of 330 pg/ml had a sensitivity of 85.7% and specificity of 86.7%.

In conclusion, circulating levels of tumor necrosis factor, interleukin-6 soluble interleukin-2 receptor are significantly increased in patients with IDDM as compared to healthy subjects and their levels are not affected by glucose level, insulin dose or disease duration. This is highly suggestive of the availability of these non invasive indices to help in further examining Type 1DM pathophysiology and monitoring pharmacological interventions to interfere with disease development and progression.

### **Corresponding author**

Azza A.A  
Child Health Department, National Research Centre,  
Cairo, Egypt  
[drazzaaa@yahoo.com](mailto:drazzaaa@yahoo.com)

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10/1/2010

# Postoperative Pain Control in Patients after Lower Third Molar Extraction

\*Hanaa El Shenawy ; \*\*Neveen Helmy Aboelsoud; \*\*\*Ahmed Abbass Zaki; ;\*\*\*\*Mohamed El Zahahry ; \*Amr Shaibeta .

\*Oral Surgery and Medicine , National Research Centre

\*\*Complementary Medicine , National Research Centre

\*\*\*\* Fixed and Removable Prosthodontics Departments –National Research Centre –

\*\*\* Oral Surgery Departments -National Institute of Laser Enhanced Sciences- Cairo-Egypt.

Corresponding author: Name: Prof. Dr. Neveen Helmy Aboelsoud.

Prof. of Complementary Medicine/ Complementary Medicine Department

National Research Centre – 33 El Bohouth Street – Dokki- Cairo- Egypt-12311

Phone: +202 0124359509; E-mail: neevenster@gmail.com

**Abstract:** The most valuable treatment objective in dental practice is to afford the patient a pain-free treatment. The **aim of this study** was to compare the use of low-power laser irradiation and the non-steroidal anti-inflammatory drug diclofenac sodium, as dental analgesic postoperative tools. **Materials and Methods:** Ninety patients undergoing non- surgical extraction of lower third molar with local anaesthesia (2% lidocaine with epinephrine 1:80.000) were enrolled in this study. Sixty received a preoperative single dose of 100 mg diclofenac sodium; thirty patients of them had postoperative low power laser irradiation in addition. They were compared to a third group with only regular postoperative recommendations (30 patients). **Results** showed that low-power laser irradiation significantly reduced postoperative pain intensity than in patients pre-medicated with diclofenac alone, or depend only on regular recommendations (controls).**In conclusion:** We suggested that the use of low-power laser irradiation enables the best postoperative analgesic effect and the most comfortable postoperative course after non surgical extraction of lower third molar than non-steroidal anti-inflammatory drugs or regular postoperative treatment.

[Hanaa El Shenawy; Neveen Helmy Aboelsoud; Ahmed Abbass Zaki; Mohamed El Zahahry; Amr Shaibeta. **Postoperative Pain Control in Patients after Lower Third Molar Extraction.** Journal of American Science 2010;6(11):1068-1072]. (ISSN: 1545-1003). (<http://www.americanscience.org>).

**Key words:** Post operative pain- laser therapy- Diclofenac sodium - VAS.

## 1. INTRODUCTION

The primary obligation and ultimate responsibility of oral health care providers is not only to restore function, but also to relieve pain. Currently available analgesic agents include aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs). The efficacy and safety of NSAIDs have been reviewed extensively, Shapiro and Cohen (1992). Potential adverse effects of NSAIDs included peptic ulcer disease, gastrointestinal (GI) bleeding, GI perforation, impaired renal function and inhibition of platelet function. So, there is a need to depend on another analgesic tool with minimal side effects, Fisher et al., (1988). The application of low energy lasers in the field of dentistry and oral surgery has been described since the 1970s. Low energy laser light was supposed to reduce pain, to accelerate wound healing and to have a positive effect on

inflammatory processes, Neckel et al.,(2001). The aim of this study was to compare the use of low-power laser irradiation and the non-steroid anti-inflammatory drug diclofenac sodium, which are claimed to be among the most successful aids in postoperative pain control.

## 2. Materials and Methods:

### 2.1.Materials:

#### Patients

Ninty healthy patients of both sexes, randomly selected among patients undergoing non surgical third molar extraction with local anesthesia (2% lidocaine with epinephrine 1:80.000) in the outpatient oral surgery clinic- National Research Centre-Cairo. Informed consent was obtained from participating patients. The study was approved by the local ethical committee. Exclusion criteria were

chronic diseases – pregnancy- known allergy to local anaesthetics – recent history of chronic pain medication.

## 2.2.Methods

### 2.2.1.Procedure

Sixty patients received a preoperative single dose of 100 mg diclofenac sodium, one hour before surgical procedure, thirty of them had postoperative low power laser irradiation in addition. They were compared to a third group with only regular postoperative recommendations (30 patients) (cold packs, soft diet, etc.) which is also given after extraction procedure to all the investigated patients. The laser group received a low-power laser using a soft laser SL-202 (PETRO LASER, Pr. Stachen, Saint-Petersburg, 198097, Russia) with an 870 nm wave length applied intra-orally from a distance of 1 cm for 10 minutes after extraction procedure. The energy output was 4 J/cm<sup>2</sup>, with constant power density of 80 mW. Laser treatment was performed once. The extraction was performed by a single surgeon to minimize individual technical differences to prevent pain bias. Postoperatively, extraction wounds were primarily closed by interrupted sutures. Although the patients were grouped randomly, the duration of surgical procedure and its complexity, based on the need for root separation, were again comparable among all the investigated patients, regardless of the used analgesic regimen (Table 1). After surgical procedure, all the patients were instructed to note pain intensity (using visual analogue scale [VAS]), and any possible side effects, for example, dizziness and nausea. Postoperative analgesic efficacy was estimated by the postoperative VAS of 100 mm length, where patients marked the maximal pain intensity they experienced during the postoperative period.

### 2.2.2. Statistics:

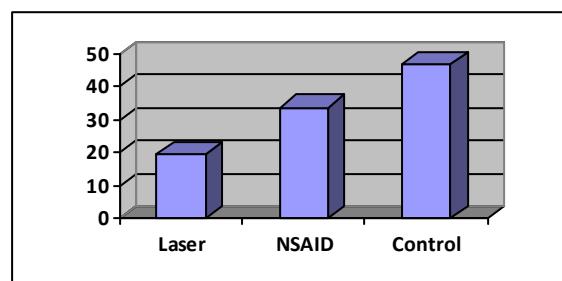
Data was analyzed using professional statistics package (SPSS for windows, Release 7.5, SPSS Inc., and Chicago, IL, USA). Descriptive data represented as mean  $\pm$  SD for numeric data. Data of the three studied groups were compared using one-way analysis of variance (one way ANOVA) test. sig. (2-tailed) p<0.05 was considered significant.

**Table (1): General and operation characteristics among the three studied groups**

Parameter	Laser N = 30	Diclofenac N = 30	Control N = 30	P
<b>Age</b>	28 $\pm$ 7.9	29 $\pm$ 6.5	27.5 $\pm$ 5.8	> 0.05
<b>Sex distribution</b>	18 (60%) 12 (40%)	17(56.7%) 13(43.3%)	19(63.3%) 11(37.7%)	> 0.05
<b>Duration of surgery</b> (mean $\pm$ SD)	27.9 $\pm$ 13.5	30.5 $\pm$ 10.6	28.9 $\pm$ 11.5	> 0.05
<b>Distribution of the duration of surgery:</b> < 30 min. 30 – 60 min.	26(86.7%) 4 (13.3%)	25(83.3%) 5 (16.7%)	26 (86.7%) 4(13.3%)	> 0.05
<b>Tooth separation:</b> Yes No	13(43.3%) 17(56.7%)	15 (50%) 15 (50%)	14 (46.7%) 16(53.3%)	> 0.05

## 3. Results

The general and operative characteristics of the studied groups were presented in table (1). There was no significant difference between the three groups regarding their mean age, sex distribution, mean duration of surgery, the distribution of the duration of surgery and the incidence of tooth separation (one way ANOVA test) (P > 0.05). The results showed that there was significant reduction of pain intensity in patients treated with low-power laser irradiation, in comparison to patients medicated with diclofenac sodium alone and to the controls (fig 1).



**Fig. (1): Mean Pain score as assessed by VAS (visual analogue scale) among the three groups**

In laser group, the mean pain intensity obtained by VAS was 19.7  $\pm$  24.8 mm, the maximal value was 65 mm and the minimal was 3 mm. While in patients preoperatively medicated with diclofenac sodium only, the average pain intensity was 33.8 mm,

the maximal value was 85 mm and the minimal was 10 mm. In control patients, the average pain intensity was 46.7 mm, the maximal value was 90 mm and the minimal was 15 mm (Table 2).

**Table (2): Comparison of post-operative analgesic effect (Assessed by \*VAS) among the studied groups**

Group	N	Minim um	Maxim um	Mean ± SD	P
Laser	30	3	65	19.7 ± 24.8	< 0.01
Diclofenac	30	10	85	33.8 ± 22.9	< 0.01
Control	30	15	90	46.7 ± 38.6	< 0.05

\*VAS: visual analogue scale

These differences were statistically significant for pain reduction in patients treated with low-power laser irradiation compared to patients medicated with diclofenac sodium alone or in controls. There were no clinically evident side effects that could be attributed to the used diclofenac sodium or low level laser therapy.

#### 4. Discussion

It has been emphasized that one of the most valuable treatment objectives in dental practice is to afford the patient a pain-free treatment Ngan et al., (1999). By the evolution of the laser applications, the dental committee aimed to achieve this goal without analgesic drugs and painful methods , Walsh (1997).

The use of laser as a non-surgical medical treatment modality for assisting the normal processes of healing has increased over the last few years. However, the efficacy of laser in reducing pain or promoting tissue repair still remains controversial, Enwemeka et al.,(2004).

Laser therapy aims to restore the normal biological function of injured or stressed cells so ‘Normalization’ is the keystone of laser therapy Tunér and Hode (2002). The stimulatory effect of laser therapy can be seen in wounded cells or in cells that are growing suboptimally whereas cells that are normal or fully functional remain unaffected and no therapeutic effect can be observed ,Smith (1991).

Laser light has the unique properties of monochromaticity (a single wavelength), collimation (travels in a single direction without divergence) and coherence (with all waves in phase) Denise and Heidi (2007). These properties are what allows laser light to penetrate the skin surface non-invasively, Matic et al.,(2003); Theralase. (2003) and Schindi (1999).

Therapeutic lasers are athermal with no appreciable heat transfer (<0.65 °C) so the photonic energy is transferred directly to the target cells and thermal damage is avoided Matic et al.,(2003); Theralase. (2003). Therapeutic lasers use monochromatic light in the 630 to 905 nm range, known as the “therapeutic window” Stadler et al.,(2004) .

The unique pain reduction abilities of LLLT (Low Level Laser Therapy) have been extensively researched and documented in numerous clinical studies and medical papers. Because the pain amelioration capabilities of LLLT are accomplished via the combination of local and systemic actions — utilizing enzymatic, chemical and physical interventions — the process is very complex. However, there is a preponderance of medical evidence that justifies a conclusion that effective pain reductions can be achieved via increase in

b-Endorphins, blocked depolarization of C-fiber afferent nerves, Ohno(1997), increased nitric oxide production, increased nerve cell action potential, axonal sprouting and nerve cell regeneration, decreased Bradykinin levels, increased release of acetylcholine or ion channel normalization, Byrnes et al.,(2002)and Rochkind et al.,(1997).

Many clinical studies and case reports investigated the use of oral soft laser applications. Positive laser effect was used for the prevention of pain, swelling or trismus after removal of third molars and periodontal surgery procedures as well as for reducing orthodontic post-adjustment pain Kreisler et al., (2004)and Roynesdal et al.,(1993).

Moreover, soft lasers were used for the treatment of craniomandibular disorders, chronic facial pain, chronic sinusitis, gingivitis, herpes simplex, dentinal tooth hypersensitivity, and sensory aberrations in the inferior alveolar nerve. The results were controversial. While some studies reported on a positive laser effect with regard to the investigated parameters others showed no or only negligible clinically relevant influence of LLLT, Youssef et al., (2008)

Amarillas-Escobar et al. (2010) found that the use of therapeutic laser in the postoperative management of patients having surgical removal of impacted third molars, decreased postoperative pain, swelling, and trismus, but without statistically significant differences. In agreement with Douglas et al.(2004) and Little et al.(1997).

Aras and Güngörümüş (2009) Stated that extraoral LLLT is more effective than intraoral LLLT for the reduction of postoperative trismus and swelling after extraction of the lower third molar.

Fernando et al. (2001) in their randomized double blind comparative study of low level laser

therapy following surgical extraction of lower third molar teeth showed that there was no evidence of a difference in pain and swelling on the third day after operation between laser and placebo sides. There was no difference between the two sides when they were assessed for healing 7 days after surgery.

Roynesdal et al. (1993) had reached similar conclusion where they found no statistically significant differences observed in comparison of the experimental side with the placebo side. They concluded that soft-laser treatment had no beneficial effect on swelling, trismus, and pain after third molar surgery.

In the current study we compared between low-power laser irradiation and a non-steroid anti-inflammatory drug diclofenac sodium in postoperative pain control after surgical removal of third molars. Postoperative analgesia is one of the most important segments of surgical extraction of third molars. Many attempts have been made to control postoperative pain, the results being satisfactory only to some extent, Seymour and Walton (1984). It seems, from the results of this study that the use low power laser irradiation is the most promising type of therapy in reducing the post-operative extraction pain.

The results of this study indicated that postoperative use of low-power laser irradiation after surgical extraction of third molars significantly reduces postoperative pain. Compared with the postoperative analgesic effect of diclofenac sodium that was beneficial but less prominent. The Influence of preoperative use of diclofenac-Na on postoperative pain after removal of impacted lower third molars was investigated before and its positive effects reported. However it had slow effect, Gregg(1992) ; Markovic and Todorovic(1995).

Also results of the current study agreed with the results of the study of Alekxa et al. (2006) who investigated the analgesic effect of low level laser therapy after lower third molar extraction and they found that LLLT was superior than non-steroidal anti-inflammatory drug diclofenac and long acting anaesthetic drug (bupivacaine). Markovic and Todorovic (2007) Suggested that low power laser irradiation after lower third molar surgery can be recommended to minimize swelling. The effect is enhanced by simultaneous local intramuscular use of dexamethasone.

The mandate for dentistry in the 21st century calls for continued efforts directed toward eliminating dental disease and enhancing the overall health and well-being of patients by translating scientific discovery into clinical practice, Kreisler et al., (2004)

This persuades us to strongly recommend the use of LLLT for molar extraction in addition to the usual conservative measures and the anti inflammatory drugs.

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10/1/2010

## Organizational, Operational and Interactional Processes of People's Participation in Community Activities in Malaysia

<sup>1</sup>Asnarulkhadi A. S & <sup>2</sup>Fariborz Aref

Dept. of Social and Development Science, Faculty of Human Ecology  
Putra University, Malaysia  
School of Management and Economics, Science and Research Branch  
Islamic Azad University, Tehran, Iran  
[fariborz.aref@gmail.com](mailto:fariborz.aref@gmail.com)

**Abstract:** This study focuses particularly on how people living as one community organize themselves to fulfill their needs and expectations through various groups, as revealed and directed by respondents in the research process. Therefore, the analysis and interpretation of the data is based on the people's expressed experiences of participating in such processes by treating those experiences as one entity, regardless of the type of groups they represented.

[Asnarulkhadi A. S & Fariborz Aref. **Organizational, Operational and Interactional Processes of People's Participation in Community Activities in Malaysia**. Journal of American Science 2010; 6(11):1073-1077]. (ISSN: 1545-1003). (<http://www.americanscience.org>).

**Keywords:** participation, community activities, planning

### Introduction

This study focuses on the people's participation process in conducting their community activities. It describes the interaction and operational processes by which community members mobilized themselves to organize and plan their activities, and subsequently participated to implement and manage them based on mutual agreement. While describing these processes, important elements in discussing people's participation, the decision making process and the way they solved their internal group problems, are also examined. It is important to note here that the decision making process not only took place at the planning stage but in some community groups this process also took place at the implementing and managing stages. Hence, the central issue in discussing people's participation will be examined in both stages; organizing and planning, and implementing and managing. The type of people's participation will be examined at these stages. This study also discusses the development of leadership and the role played by the leaders in initiating, mobilizing, enabling, facilitating, campaigning, leading, and directing the community work and community development activities. In short, this study examines the process whereby people are exercising their capabilities, individually and collectively, in participating towards achieving their common needs or expectations, and solving the shared problems faced, in their respective groups. The information gathered is mainly based on interviews and observations, supplemented by secondary recorded materials provided by them.

### Methodology

This study employs a qualitative-ethnographic approach that uses a flexibility and open-ended framework of research design. In this kind of interactive research, the basic aim of the whole process of the research activity is not only to gain information and to understand the meaning and process of participation. This study, which can be classified as the 'knowledge-development research' in Thomas's community work research typology (Thomas, 1980), emphasizes the process approach: describing and analyzing the people's participation experience, rather than examining participation through a snapshot approach as in a quantitative study. The interactive and responsive techniques of data collection of in-depth and follow-up interviews, and group discussions advocated by this approach allowed the people to be directly involved in the research process and helped to facilitate an understanding of their participation in the activities of the community (Asnarulkhadi & Aref, 2009).

### Literature Review

Participation is a dynamic process. Participation is considered as an important factor for successful and prosperity of local development (Aref et al., 2010). Hence, it is difficult to predict or even to quantify using a standard 'measurement'. Participation is rather molded by, and originates from, individuals' experiences in participating. As such, the qualitative-ethnographic approach employed in this study was able to assist in understanding the process of people's

participation in community development activities. This approach has also helped to deepen the knowledge about participation itself (Asnarulkhadi & Aref, 2009). Community participation processes can support and uphold local culture, tradition, knowledge and skill, and create pride in community heritage (Lacy et al., 2002). Ashley& Roe, (1998) describe community participation as a spectrum from passive to active involvement to full local participation, where there is active community participation and venture ownership. Community participation also is the mechanism for active community involvement in partnership working, decision making and representation in community structures (Chapman & Kirk, 2001). It should be noted that community participation often means the involvement of people or community with the government. Numerous studies about community participation have been published (Aref et al., 2010; Bozlul, 1994). However, this study emphasized the participation of the community as an involvement of local people in community activities processes.

## **Findings & Discussion**

### **Organizing and Planning**

It is obvious that the outcome of people's empowerment is objectified and manifested in the activities implemented by the various types of groups. However, prior to that the process of how the community or group members engaged themselves in organizing, planning, implementing, and sustaining the group activities resulted from their awareness to take their own initiative to achieve the shared goals within the existing structure to improve the community living situation is an important aspect of understanding people's empowerment. In order to understand this it is necessary to explore the people's participation and their interaction process in organizing and planning the activity. This can be examined in three ways: first, in the pre-group process; second, in the group process; and lastly, in the decision making process. In practice, the second and third stages took place simultaneously and will therefore be examined together.

#### ***Pre-group process***

Before deciding on any activity, it is common practice among all community groups to invite members to attend a group meeting or discussion. Most of the group leaders interviewed mentioned that they had invited their group members to plan and organize the activities. The practice of

informing members about group meetings was not only restricted to the younger leaders, who mostly led the formal or 'modern' type community groups as portrayed above, but was also used by the 'traditional' type of community groups that were led by middle aged women, such as the self-help Group. From the descriptions above it can be seen that inviting group members to organize or plan an activity in a meeting is a normal practice of the leaders. Although the date for the meeting is decided by the leaders themselves, their efforts to inform the members about the meeting shows that they realize the importance of a meeting and are able to organize themselves. At this point, it can be deduced that the leaders' participation in encouraging their members to attend the meeting, through his or her personal invitation shows that, from the point view of the leaders and the local activists, the group process in organizing and planning an activity is an important aspect of organizing the activity.

#### ***Group Process***

The actual group process normally takes place in a meeting. There are two types of group meeting; formal and informal. Formal group meetings are practiced by the Youth Club, Mosque Committee, and the Anti-Drugs Campaign Committee. From their files and by personal attendance, it was found that these groups have formalized their meetings. Prior to the meeting, each group produces an agenda and keeps updated minutes of meetings. The informal meetings are practiced by four neighborhood groups; the self-help groups, the Crockery Association/ Rice and Egg Cooperative, the Community Death Fund Association, and the Women's Study Circle group. In comparison with the other groups, these informal groups do not have proper agendas for their meetings. Decisions made during the meetings are recorded in a small book or diary, which is kept by the leaders. The exception is the self-help group, which does not possess any sort of documentation.

In practice, both types of group meetings (informal and formal) eventually end up as group discussions. Group meetings are a focal point for most community group members to sit together in discussing, planning and deciding on their group activities. Since different groups have different ways of planning their activities, and because within the same group different activities demand different ways of organizing, the experiences gained by the members in these meetings (at the planning stage) are different. In general, from the information gathered it could be deduced that

there are two functions of having a group meeting; first as a medium for sharing and communicating ideas and problems and making decisions, and second as a medium for requesting, directing and informing. These two distinctive functions of group meetings employed by the community groups eventually influence the group members' participation in the decision making process, as explained below. In other words, it is within group meetings that the process and level of peoples' participation in planning emerges simultaneously.

#### Type of participation

In general, from the experiences described by the respondents at the planning stage, there are four types of participation in community work practice:

- Self-directed participation
- Joint-participation
- Leader directed/induced participation
- Externally directed participation

This division is not a rigid classification, but is based on one fundamental dimension, that is the process within which the activity is decided: how and who determines the activity for the people and to what extent a group has 'a say' in the decision making process. The degree, type of participation and community activity is as shown in Table 1.

In self-directed participation, the group members themselves decide the type of activity, then design and plan it - they decide the time, identify content, budget, strategies and tactics, and finally organize members to carry out the activity. In this type of participation, members have direct control over their own project or activities, which indicates that they have the opportunity and capacity to control their own affairs to fulfill their needs and expectations. They have the chance to utilize and develop their abilities, skills, confidence and competency to shape the activity that they pursue to achieve their targeted goals and every member has the opportunity and power to decide for themselves.

**Table 1: Degree of Participation, Type of Participation and Activities**

Degree	Type of Participation	Activities
High ↑	Self-directed participation	<b>Community-Initiated</b> Self-help and community care activity. Islamic Family Course My Home My Heaven project . Meat Market project. Community sport. Religious talks/communal feasts/ religious classes Cooking, craft work & sewing classes (WSA). Religious School, Religious Camp .Campaigning for better school project .Motivational Training project .Bill Paying Service project Neighborhood watch project (for drugs abuser)
	Joint-participation	<b>Partnership</b> Group Replanting Scheme
	Leader-directed participation	<b>Leader-Induced</b> Greening the village project Book Corner project
	External-directed participation	<b>External-directed</b> All competition activities Social gathering; and Exhibitions organized by WI District level

Thus, the activity that offers the greatest opportunities for members to identify, choose and decide for themselves in the decision making process, possesses a high degree of people's participation. It can be said that community members are in control of their lives and possess the ability to affect the development process

through participating in self-initiated activities or projects.

Self-directed participation is not limited to describing the features of people's participation in the community-initiated groups. People's participation in the WSA (and its activities - cooking, craft work and sewing classes), and in the Meat Market project organized by the Mosque

Committee can also be considered as self-directed participation, even though the group is under the auspices of, sponsored and patronized by, the state agency, RISDA and the State Islamic Department respectively. This is because the initiation of the group (WSA) and its activities was decided by the women members themselves, aimed at improving their situation in the village, while the Meat Market project aims to offer services for the whole community during the festival session. Therefore, the whole process of participation, in identifying and deciding the activities is similar to that of self-directed participation in the community-initiated activities of the community-based groups.

When a project is joint-ventured, the activity is conducted along the lines of a partnership. In the case of rubber replanting program, RISDA had to accept the decision of the people. Through a series of meetings between both parties (RISDA and the settlers) and followed-up by group discussions among the settlers, they chose the GRS, rejecting the mini-estate scheme. At this point, the people (the settlers) had the power to decide and choose the option that would be most beneficial to them, and subsequently to influence the decision of the authorities. However, since the activity involved bureaucratic procedures and the funding came from the state as the patron, the people's control over the project was restricted within certain regulations and by-laws outlined by the state agency. The GRC established by the settlers provided a platform for the members to decide certain issues pertain to the activity and act as the settlers' representative body in choosing the contractors and monitoring their work in the plantation. This was based on advice given by RISDA personnel, although the GRC had the right to select one of the few bidders. It is the responsibility of the GRC to monitor the contractors work and keep account of the money allocated for the scheme. Monitoring the quality of work performed by the contractor so that, allowing for the budget, the work schedule was met in accordance with the replanting guidelines encouraged members to decide their tactics and strategies in supervising the contractor's work. It is within this context that settlers have the power to control the contractor to ensure they are not being cheated.

An activity that is put forward by a leader for the members to carry out is the basic characteristic of leader-directed/induced participation. In this kind of participation, although members are requested to carry out a particular project, they have the opportunity to decide how to carry it out: the

launch date, organizing manpower and so forth. Since this type of activity is inspired by the leader, and thought to be beneficial for the community at large, it has the potential to be more empowering if members make full use of opportunities to influence the outcome of the project. As far as collective decision making is concerned, leader-induced participation to some extent has undermined the critical stage, the group process in deciding and prioritizing the type of activity needed, where the meeting can be used to redefine and reemphasize the reason for initiating the activity.

On the other hand, in externally directed participation, the arena for members to exercise their power to choose an activity is taken away from them, and instead, they are obliged to follow the directive of the higher-level organization, as experienced by the *WI Kg. Barem*. Being under the patronage of the District WI and strictly devoted to carry out the activity planned by higher management means that they are being co-opted. The only decision they have is in determining who should be sent to represent the local WI group in the district competition. This type of decision-making is not empowering enough to encourage and motivate group members to develop their abilities and skill to determine for them, compared with the community initiated activity. Therefore, it was a wise step taken by the WI members to be involved in helping to plan, implement and manage some of the joint-ventured activities with other local community groups as this action probably complements the 'vacuum' that they experience in participating in the directed-activities. In short, these four different types of participation set the pattern for the process of members' involvement in planning, implementing and managing the group activities. It is within this participation process that people showed their commitment and ability to utilize their inherent skills and knowledge to achieve group goals, as well as developing and constructing the features of community development they want, and at the same time increase and develop their own competencies.

### Conclusion

The study showed organizational, operational and interactional processes of people's participation in community activities in Malaysia. The position between leaders and members is diffused between community groups. In one group a respondent is an ordinary member but in another group the same respondent may act as leader. Due to this,

one can see that the role of a respondent varies from being an initiator in one group or just an ordinary member in another. It is not unusual for some respondents to play different roles in different community groups. From the in-depth interviews and observations made in this study, it was found that all the respondents are involved in various forms of participation in conducting an activity in at least in one of the groups they were involved in. 'Form of participation' here is defined as an individual action in organizing the activity. There are three forms of people's participation, which emerged out of the induction and deduction process of analysis. First, giving ideas; second, task, work or role performed; and third, attending the group meetings. In general, all three forms of involvement are present in all group activities, but the degree of participation varies.

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October, 15, 2010

# Influence of Organic Matter and Different Rates of Sulphur and Nitrogen on Dry Matter and Mineral Composition of Wheat Plant in New Reclaimed Sandy Soil

**El-Fatah., M.S. and Khaled, S.M.**

Plant Nutrition Dept., National Research Centre. Dokki, Cairo, Egypt

**Abstract:** A pot experiment was carried out in greenhouse on reclamation sandy soil from (Abu-Rwash) region north of Egypt to evaluation effect of organic matter at rate 2% of soil weight and different rates of elemental sulphur at a rate, i.e. (100 and 200) ppm ( $S_1$  and  $S_2$ ) respectively and nitrogen, i.e. (50, 100 and 150) ppm ( $N_1$ ,  $N_2$  and  $N_3$ ) respectively at from ammonium sulphate  $(NH_4)_2SO_4$ . Dihydrogen potassium phosphate  $H_2KPO_4$  was added as at a rate 200 ppm as sources to phosphorus and potassium. All treatments were added before the culture of a week at one dose. The growth stages were divided to three stages (planting, elongation and maturity) each stage for two months about. The determination was performed each stage to soil and plant (whole plant). The results can be summarized as follows: (1) Soil pH decreasing at significantly especially at rates, i.e. 200 ppm S and 150 ppm N treatment in each of planting and elongation stages then began a gradual return to initial in maturity stage. (2) Electric conductivity (E.C) is rising at significantly especially with  $S_2 - N_3$  treatment then starting the gradual return to initial in maturity stage. (3) Thiosulphate  $S_2O_3^-$  was found in soil as a result sulphur oxidation as it affects inhibition on the nitrification process. (4) Available nitrogen ( $NH_4^+$  and  $NO_3^-$ ) continued a long experiment period. (5) Dry weight was more significantly with  $S_2-N_3$  treatment in comparison to other treatments. (6) Mineral contents were more significantly with  $S_2-N_3$  treatment along of time experiment except potassium and zinc elements as decreasing in maturity stage.

[**El-Fatah., M.S. and Khaled, S.M. Influence of Organic Matter and Different Rates of Sulphur and Nitrogen on Dry Matter and Mineral Composition of Wheat Plant in New Reclaimed Sandy Soil.** Journal of American Science 2010;6(11):1078-1084]. (ISSN: 1545-1003). (<http://www.americanscience.org>).

**Key Words:** organic matter-Sulphur-Nitrogen-wheat plant reclamation sandy soil-mineral composition-dry matter.

## 1. Introduction:

Now the world became at the strongest necessity to new reclamation soils to meeting the need excess to the food as a result to increase of population on the world level, they not in the front of unlike incursion of the desert soils and reclamation it. Egypt to suffer of the same problem, the majority of the desert soils decline in sandy and calcareous soil. These soils suffer of badly of physical properties such as weakness of biological activity, less of the soil structure and less available for nutrients. Elemental sulphur and organic matter of to soil beneficent as its improving of available nutrients, the soil structure, increasing to keep of the water and increasing of exchangeable capacity of soil, which enhanced on the rise of soil production.

A correlation was observed between sulphur oxidizing activity and organic matter content. (Barrow 1960 and Stewart et al 1966) showed that

the addition of organic materials and plant residues can greatly affect the process of sulphur mineralization. In this case, the C/S ratio of added organic material will determine whether mineralization or immobilization occurs.

The aim of this work is to evaluate the efficiency of sulphur and organic matter in reclamation of sandy soil under wheat plant cultivation.

## 2. Materials and methods

A pot experiment in greenhouse to evaluate the effect of different rates of sulphur (100 and 200) ppm and nitrogen (50, 100 and 150) ppm at form ammonium sulphate  $(NH_4)_2SO_4$  and plant organic matter at a rate 2% of soil weight on dry matter and mineral composition of wheat plant under sandy reclaimed soil from (Abou-Rwash) region, Egypt. Table (1) shows some chemical and physical

characteristics of the studied soil. Pot contents with 5 kg soil. All treatments under the study were added of mixed soil with culture at one dose. Potassium dihydrogen phosphate  $\text{KH}_2\text{PO}_4$  was added at a rate, i.e. 200 ppm as a source for potassium and phosphorus. Ten seeds of wheat were sown and thinned to 5 plants per pot. Wheat plant of class seeds (1). Each of growth stage continued for two months. Plant harvested after six months (All plant). The moisture contents of the pot were maintained at 80% of water holding capacity. One of the cutting samples was immediately frozen to nitrate determination. Dried plant exhibited to constant weight at 80°C in a ventilated oven. Nitrogen was determined using

devarda alloy by (Microkjeldahel distillation) according to (Jackson, 1985). The phosphours is determined by spectrophotometer, potassium using a flame photometer. Fe, Mn and Zn were determined by atomic absorption, (Jackson, 1985). Thisoulphate ( $\text{S}_2\text{O}_3$ ) was determined according to, (Nor and Tabatabai 1975) by the colorimetric method in lithium chloride 0.1 M extract after filtration added ml of 0.1 M KCN, after 15 min, add 2 ml of 0.033 M cucl<sub>2</sub> and 1ml of Fe ( $\text{NO}_3$ )- HNO<sub>3</sub> reagents. Make the volume to 25 ml with 0.1 M LiCl, invert the flask several times to mix the contents, measure to a wavelength of 460 m $\mu$ .

**Table (1): Some chemical and physical characteristics of the experimental soil.**

Parameter	Value	Parameter	Value
Soil pH (1 : 2.5)	7.98	Sulphate $\text{SO}_4^{=}$ ppm	72.4
E.C dsm <sup>-1</sup> (1 : 5)	0.185	Thiosulphate $\text{S}_2\text{O}_3^{=}$ ppm	3.0
$\text{NH}_4^+$ ppm	5.0	Total $\text{CaCO}_3$ %	0.40
$\text{NO}_3^-$ ppm	17.3	Organic matter (O.M) %	0.21
Total nitrogen %	0.042	Texture type	Sandy
Available P ppm	6.3		
Available K ppm	12.3		
Available Fe ppm	7.2		
Available Mn ppm	6.3		
Available Zn ppm	2.6		

### 3. Results and Discussion:

This experiment was carried out in sandy reclaimed soil from Abou-Rwosh, Egypt in purpose of the evaluation of the effect addition of elemental sulphur with different rates (100, 200) ppm, rates of nitrogen (50, 100, 150) ppm as ammonium sulphate ( $(\text{NH}_4)_2\text{SO}_4$ ) and plant organic matter at one rate 2% of the soil weight under wheat cultivation.

Data in table (2) contents shows that some parameters in sandy soil at three stages of wheat plant (planting, elongation and Maturity).

Soil pH and E.C status:

Soil pH was decreasing significantly due to the addition of sulphur and organic matter, which is continued to a long time of experiments. The relationship was positive between sulphur concentrations and pH decreasing from 7.86 to 6.11 about 1.75 units.

The data showed that the treatments with rate 200 ppm sulphur, 150 ppm nitrogen and 2% organic matter of soil weight at planting stage were useful. While, in the maturity stage the decreasing ratio started to lessen, but it remains continues to the end may be due to a weakness of buffering capacity of sandy soil as a result to a little of calcium carbonate and clay ratio.

**Table (2); Effect of organic matter and different rates of sulphur and nitrogen on some parameter reclamation soil under wheat plant cultivation.**

Growth Stages	Fertilization Treatments	pH	E.C $\text{dsm}^{-1}$	$\text{S}_2\text{O}_3^{\equiv}$	$\text{NH}_4^+$	$\text{NO}_3^-$	$\frac{\text{NH}_4}{\text{NO}_3}$
				ppm			
<b>Planting</b>	Control	7.86	0.185	3.00	5.00	17.3	0.29
	$\text{S}_1 \text{N}_1$	6.39	0.189	15.0	17.2	23.5	0.73
	$\text{S}_1 \text{N}_2$	6.40	0.195	12.0	28.7	44.8	0.64
	$\text{S}_1 \text{N}_3$	6.42	0.198	10.0	55.6	38.9	1.42
	$\text{S}_2 \text{N}_1$	6.15	0.217	14.9	20.3	22.7	0.89
	$\text{S}_2 \text{N}_2$	6.16	0.227	12.3	45.6	32.3	1.41
	$\text{S}_2 \text{N}_3$	6.11	0.245	18.0	59.5	39.4	1.51
<b>Elongation</b>	Control	7.83	0.188	1.93	2.21	3.54	0.62
	$\text{S}_1 \text{N}_1$	6.53	0.198	3.76	11.5	11.6	0.99
	$\text{S}_1 \text{N}_2$	6.59	0.212	5.59	21.3	19.6	1.08
	$\text{S}_1 \text{N}_3$	6.55	0.218	7.42	26.7	27.9	0.96
	$\text{S}_2 \text{N}_1$	6.39	0.213	8.41	15.3	36.1	0.42
	$\text{S}_2 \text{N}_2$	6.33	0.216	9.46	25.6	40.5	0.63
	$\text{S}_2 \text{N}_3$	6.42	0.210	10.4	29.5	48.4	0.51
<b>Maturity</b>	Control	7.92	0.178	1.52	3.71	2.83	1.3
	$\text{S}_1 \text{N}_1$	6.84	0.187	2.11	65.30	9.45	0.67
	$\text{S}_1 \text{N}_2$	6.92	0.196	2.85	8.90	16.6	0.54
	$\text{S}_1 \text{N}_3$	6.87	0.205	3.35	11.5	22.7	0.51
	$\text{S}_2 \text{N}_1$	6.69	0.200	3.05	14.7	18.6	0.79
	$\text{S}_2 \text{N}_2$	6.75	0.194	2.73	13.5	20.3	0.67
	$\text{S}_2 \text{N}_3$	6.88	0.192	2.74	13.4	25.7	0.52

Elemental sulphur:  $\text{S}_1 = 100 \text{ ppm}$ ,  $\text{S}_2 = 200 \text{ ppm}$ Nitrogen as ammonium sulphate:  $\text{N}_1 = 50 \text{ ppm}$ ,  $\text{N}_2 = 100 \text{ ppm}$ ,  $\text{N}_3 = 150 \text{ ppm}$ 

(Sallade and Sims 1992, El-Fayoumy and El-Gamal 1998) showed that, thiosulphate was oxidized to tetrathionate then to sulphate in the end; they have effect on soil pH decreasing, this effect increases with the increase of organic matter ratio and decreasing of calcium carbonate than sulphur alone.

On the other hand, soil salinity affected by sulphur and organic matter, whereas EC increased ( $0.06 \text{ dSm}^{-1}$ ) as a consequence of excess of the soluble salts which added with sulphur. As the result of the characteristics of sandy soil which poor in elements (salts), the rising was limited.

The treatment of  $\text{S}_2\text{-N}_3$  (200S – 150N) ppm at planting stage is more effected in comparison with the other treatments along the experiment, and then the gradual decrease started at elongation stage and continued to the end of maturity stage. The decrease in salt content may be due to leaching of soluble salts and/or absorption by growing plants until to reach on equilibrated level corresponding to the initial content.

Sulphur was oxidized to sulphide then to tetrathionate then to thiosulphate and then to sulphate by heterotrophic bacteria, actinomycetes and filamentous fungi (Guittonneau and ketlling 1932).

Through data at table (2) was noticed that oxidation of the sulphur ratio to thiosulphate was to be slow may be due to the weakness of the biological activities in sandy soil. Sulphur oxidation to thiosulphate at planting stage was more of a ratio than the other two stages as thiosulphate concentration was a high relatively, the highest value of thiosulphate was 18 ppm at 200 ppm S and 150 ppm nitrogen treatment, positive contact was found between sulphur concentration and thiosulphate concentration at planting and elongation stages clearly. Organic matter addition may be enhanced at slow of oxidation. (Kowalenko and Lowe 1975) they found that samples with high C:S and C:N ratios have low values of mineralizable sulphur and nitrogen. Awad (1990) studied the oxidation regime of the added sulphur in the sandy soil was delayed than the remainder of soil types.

#### Nitrogen availability status in soil:

Data in table (2) indicated that, nitrification process was occurred by slow rate at planting and elongation stages while the normal period to convert ammonium ( $\text{NH}_4^+$ ) into nitrate about two weeks.

Plant uptakes each of them but the ammonium can not be loosed from the soil easily as it hold on the surface of soil granules. Nitrate is exposed to losses by the leaching as a result to repeat of irrigation and denitrification process (nitrate convert to nitrogenous volatilization oxides). This is the positive effect due to sulphur and organic matter were added as a result of sulphur oxidation compound of thiosulphate ( $\text{S}_2\text{O}_3^{2-}$ ), which will inhibit the nitrification process as noticed that thiosulphate remained along growth period due to weakness of biological activities in the sandy soil. Thus organic matter decomposed into some organic acids and other compounds, which inhibit the effectiveness of the nitrification process. Available nitrogen (ammonium + nitrate) continued in soil along experiment period.

From the result, it can be indicated that sulphur, and organic matter are contributed to raise qualification of nitrogenous fertilizers. (Mostafa et al 1995) Indicated that elemental sulphur application reduces the extracted  $\text{NO}_3^-$  and increased the  $\text{NH}_4^+$ , due to the inhibition of nitrification. The effect was more pronounced in sandy soil. (Frgl et al 2005). Found that sulphur and organic matter application as ammonium fertilizers to cause retardation of nitrification (nitrate formation) was positively related to the rate of sulphur addition.

Data in table (3) represent the dry weight and mineral contents of wheat plant in reclamation of soil (sandy) as affected by organic matter and

different rates of sulphur (100 and 200) ppm and nitrogen (50, 100 and 150) ppm.

#### Dry weight (gm/pot) status in whole plant:

Dry weight of wheat plant is the reflection for all treatments during three stages of wheat plant (planting, elongation and maturity). The first stage show that effect of dry weight as found a positive relationship between rates of sulphur and dry weight as an increasing rate of the sulphur, increase the dry matter, same this contact was occurred between rates of nitrogen and dry matter as increasing rates of nitrogen, increase weight of dry matter. This connection was continued a long time of an experiment (three stages), this effect due to the beneficial effect of sulphur and organic matter are included in its effect on the chemical conditions of the soil such as reducing the pH of the soil and making some plant nutrients more available. (Green and Chaudhry 2006) explained the indirect effect of sulphur in soil by its conversion to the sulphuric acid which has a solvent action for several important nutrients. (Barrow 1960) showed that the addition of organic materials and plant residue can greatly affect the process of sulphur mineralization. In this case, the C/S ratio of added organic material will determine whether mineralization or immobilization occurs.

#### Nitrogen status in plant:

Data in table (3) concerning nitrogen uptake by wheat plant under the effect of organic matter addition at a rate 2% of soil weight and different rates of sulphur (100 and 200) ppm and nitrogen (50, 100 and 150) ppm during three stages of wheat plant (planting, Elongation and Maturity). It is found a positive relationship between the rates of sulphur and nitrogen added and nitrogen contents in plant as increasing of rates of sulphur and nitrogen follow up by increasing of nitrogen uptake.

The high values of nitrogen uptake were at sulphur rate 200 ppm and nitrogen rate of 150 ppm treatments and this contact was continued during three stages of plant.

These results reflect to the range of importance of sulphur for increasing of nitrogen content in plant as is entered in the structure of many amino acids. (Brazozowska et al. 1964) reported that, sulphur deficient plants of groundnut (*Aroctis hypogea* L.) had less protein N in all plant organs. There was an accumulation of arginine, a sparagine and a decrease in cystine, cysteine and methionine contents.

#### Phosphorus status in plant:

At planting stage found a positive contact between rates of sulphur, nitrogen and phosphorus contents in plant as a high value in this stage with

rate of sulphur 200 ppm and nitrogen 150 ppm. At elongation stage found that the values were a low relatively in comparison with planting stage but these differences not significantly may be due to phosphours entity at form unavailable at form limited. In maturity stage was positive contact among rates of sulphur, nitrogen and phosphour contents

may be due to increasing of the representation for phosphour element as a result of increasing plant growth. (Aulok and Posrichs 1997) showed that, the negative effect of sulphur and phosphorus fertilizers upon the uptake and utilization of each other was conspicuous when they were simultaneously.

**Table (3) Effect of organic matter and different rotes of sulphur and nitrogen on dry weight (gm/pot) and mineral contents of wheat plant in reclamation soil (sandy).**

Growth Stages	Fertilization Treatments	Dry weight	N	P	K	Fe	Mn	Zn
			%	ppm				
Planting	Control	4.58	0.15	0.191	1.59	112	35.8	32.3
	S <sub>1</sub> N <sub>1</sub>	6.75	0.28	0.223	2.81	145	38.2	36.4
	S <sub>1</sub> N <sub>2</sub>	7.09	0.39	0.257	2.42	133	41.3	33.5
	S <sub>1</sub> N <sub>3</sub>	9.72	0.48	0.279	2.88	165	41.8	37.2
	S <sub>2</sub> N <sub>1</sub>	8.32	0.56	0.365	2.98	206	46.5	42.5
	S <sub>2</sub> N <sub>2</sub>	10.14	0.61	0.388	3.16	230	44.0	44.7
	S <sub>2</sub> N <sub>3</sub>	12.4	0.65	0.401	3.25	253	487	45.3
Elongation	Control	10.4	0.22	0.265	2.22	85	42.3	38.4
	S <sub>1</sub> N <sub>1</sub>	12.9	0.40	0.285	3.15	126	54.2	41.3
	S <sub>1</sub> N <sub>2</sub>	11.5	0.59	0.320	3.66	164	60.7	46.6
	S <sub>1</sub> N <sub>3</sub>	15.4	0.79	0.356	3.95	224	59.3	47.9
	S <sub>2</sub> N <sub>1</sub>	12.9	0.53	0.312	4.24	278	56.3	53.3
	S <sub>2</sub> N <sub>2</sub>	18.2	0.97	0.350	4.28	316	68.4	56.4
	S <sub>2</sub> N <sub>3</sub>	22.7	1.33	0.397	4.53	314	75.2	58.7
Maturity	Control	17.4	0.27	0.350	1.78	964	55.4	23.5
	S <sub>1</sub> N <sub>1</sub>	19.0	1.85	0.375	2.94	229	78.2	26.4
	S <sub>1</sub> N <sub>2</sub>	22.2	2.01	0.388	2.35	223	77.3	31.0
	S <sub>1</sub> N <sub>3</sub>	28.5	2.17	0.362	2.85	231	89.0	33.5
	S <sub>2</sub> N <sub>1</sub>	18.8	2.05	0.426	2.76	275	84.7	37.3
	S <sub>2</sub> N <sub>2</sub>	35.7	2.46	0.445	2.18	322	80.2	41.2
	S <sub>2</sub> N <sub>3</sub>	36.2	2.85	0.462	2.35	328	8.7	44.0
L.S.D. 0.05		1.44	0.051	0.042	0.018	8.12	7.56	5.81

Elemental sulphur: S<sub>1</sub> = 100 ppm, S<sub>2</sub> = 200 ppm

Nitrogen as ammonium sulphate: N<sub>1</sub> = 50 ppm, N<sub>2</sub> = 100 ppm, N<sub>3</sub> = 150 ppm

### Potassium status in plant:

Potassium content in plant was at a positive contact with rates of sulphur and nitrogen in each of planting and elongation stages. Head values of potassium uptake with  $S_2 = 200$  ppm and  $N_3 = 150$  ppm treatment along period experiment. At maturity stage potassium uptake was lesser than previously of two stages may be due to maturity stage not require to more potassium as the plant complete of growth stages. (Modaihsh et al 1989 and Solimon et al 1992a) who found that the uptake of K significantly increased as a result of increasing sulphur rates application to the soil while potassium uptake was decreasing at late of growth stages.

### Micronutrients status in plant:

Positive contact was found between increasing rates of sulphur and nitrogen with the uptake of iron, manganese and zinc by wheat plant during of three stages of the growth except zinc uptake as decreasing at maturity stage. Head values of Fe, Mn and Zn uptake were with the rate of sulphur 200 ppm and nitrogen 150 ppm treatments. Sulphur application in all soil types is effective on the solubility and availability on nutrients as influenced by soil pH, organic matter and oxidation of soil amendments by lowering of soil pH and therefore, increased plant uptake of these nutrients. (Procopiou et al 1976) indicated that Fe levels in the leaves was increased at lower sulphur levels.

The tendency of sulphur to increase the level of Mn in plant was pronounced on both the concentration and the uptake levels of Zn and Fe were increased by most, if not all, levels of sulphur. They concluded that the positive effect of sulphur varied according to the level of sulphur application.

### Corresponding author

Abd El-Fatah., M.S.  
Khaled, S.M.  
Plant Nutrition Dept., National Research Centre.  
Dokki, Cairo, Egypt

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9/23/2010

# Presumed Chronological, Developmental and Clinical Classification of Human Dentitions

Said Mahmoud Hani

Oral Biology Department, Faculty of Dental Medicine, Al-Azher University, Cairo, Egypt

[hani.said@yahoo.com](mailto:hani.said@yahoo.com)

**Abstract:** Diphyodont, including man, have traditionally two dentitions the deciduous and permanent. The significances of the presence of two dentitions may lie behind the fact that once the teeth have been developed, they are unable to grow, by the common sense of the word. Since the individual organs and tissues grow by time and the jaws also do, therefore, other generation of dentition is needed to match the new situation, that is, the permanent dentition. However, this typing of dentition into two sets is oversimplified and nonindcative for the condition in which the teeth are variably represented. It also, does not exhibit the different and definite cases by which the teeth are expressed. Taking these drawbacks into consideration, a presumed classification has been presented indicating the developmental, clinical and chronological situations of the different sets of dentitions. The presumed classification may be valuable not only for pedagogic purposes but also for the developmental and clinical studies. [Journal of American Science 2010;6(11):1085-1090]. (ISSN: 1545-1003). (<http://www.americanscience.org>).

**Keywords:** Classification, Dentitions, Human

## Principles of classification

The human dentition may have two sets, three phases and two conditions which may be indicated as follow:

1 - The two sets are the deciduous and permanent dentitions.

2- The period of time for the existence of each set of dentition is not sharply distinct from the other, However, both dentitions partly share a common period is termed the phase of mixed dentition. In this phase, some of the deciduous teeth and some of the permanent teeth simultaneously exist.

3- Each set of dentition tends to be only present at a certain period of time before or after the phase of mixed dentition. Therefore, the time consumed from the start of deciduous teeth development to the beginning of the permanent teeth development, in which the deciduous teeth predominate, is termed the phase of pure deciduous dentition. Also, the time elapsed between the exfoliation of the last deciduous teeth to the loss of all permanent teeth, in which the permanent teeth predominate, is termed the phase of pure permanent dentition.

4- In respect to the condition by which the teeth exist, the distinction whether the teeth are present intraosseously inside the jaw crypt or appearing in the mouth is determined. The former situation is termed the *developmental condition* while the latter is termed the *clinical condition*.

5- The nomenclature of *developmental dentition* denotes the period of time starting with the commencement of enamel organ development and terminates with the complete root formation. The

observation of this variant of dentition can be partly determined either by radiographic or by postmortem investigations for the unerupted developing teeth and for the growing root of the teeth which have already erupted. However, the teeth in this variant of dentition continue their root development following the eruption event.

6. The nomenclature of *clinical dentition* denotes the period of time starting by the eruption of the crown in the mouth until the ultimate tooth loss. The disappearance of the teeth may occur either physiologically by exfoliation or due to the various environmental influences. The observation of this variant of dentition can be determined clinically, as its name implies, by the naked eyes.

7. The accounting for the time consumed either for the beginning of certain event of odontogenesis, was considered for either the deciduous or permanent dentition as a whole. For instance, it is accounted by the time elapsed from the commencement of enamel organ development of the firstly formed teeth in deciduous dentition, that is, the incisor teeth at about 7 WIU to the lastly developed enamel organ of the maxillary second deciduous molars at about 9 WIU.

The same also was done for accounting the time for the root completion, where the first completely formed roots are those of deciduous incisors at about 1.5 year of age and the last completely formed roots are those of the deciduous second molars at about 3 years of age. So that the time consumed for the developmental deciduous dentition is about 2-3.5 years of age. The same was also followed for the time

consumed with the developmental permanent dentition which may figure about 10.5-16 years of age. The same strategy was also followed for the time consumed with the developmental permanent dentition which may figure about 10.5 -16 years of age.

### **Classification of human dentitions**

The various human dentitions, presumed through the present study, can be mainly classified into chronological and typal ad eitherone has its proper characteristics as follow:

#### **I. Chronological Classification**

1. *Developmental dentition*: it is concerned with the completion of development of either set of teeth, whether they are deciduous or permanent. The developmental dentition would be accounted from the time of start of development of the firstly formed enamel organs in either set of teeth until the time of root completion. This is based on the last tooth to end its root development in that set.

a) *Developmental deciduous dentition*: it begins with the development of enamel organ of the deciduous teeth at 7-9 WIU in their crypts inside the jaw. However, by 1.5-3 years of age the roots of all the deciduous teeth have been completely developed. So, the developmental deciduous dentition may consume about 2- 3.5 years, that is, the time required for completing the development of all deciduous dentition.

b) *Developmental permanent dentition*: it begins with the development of enamel organ of the permanent molars at about 4 MIU - 4 years in their crypts inside the jaw. However, by the time from 10-25 years of age, all the permanent teeth have completely developed their roots. So that the developmental permanent dentition may consume about 10.5-16 years, that is, the time required for the complete development of all permanent dentition.

2. *Clinical dentition*: it is concerned with the period of appearance and maintenance of either set of teeth in the mouth. The clinical dentition, therefore, would be accounted from the start of teeth eruption until their loss by the shedding of deciduous teeth or the extraction of permanent teeth for whatever reason. This can be recorded *clinically* in a contradistinction to the developmental dentition which can be recorded *radiographically* or by the *postmortem investigation*.

a) *Clinical deciduous dentition*: it begins by the eruption of mandibular central deciduous incisors at

6-8 months of age, and the second maxillary deciduous molars at 30 month of age. It ends by about 11 years of age; the time of exfoliation of the last deciduous teeth, that is, the maxillary canines. So, the clinical deciduous dentition may consume about 10.5-8.5 years, that is, the time the deciduous teeth are physiologically represented or remain in the human mouth.

b) *Clinical permanent dentition*: it begins by the eruption of the first permanent molars at about 6 years of age, and ends by the loss of all permanent teeth. However, no definite time can be recorded for the end of the clinical permanent dentition.

#### **II. Typal classification**

1. *Pure dentition*: it refers to the exclusive presence of a certain set of teeth, that is, the deciduous or permanent dentition. The pure developmental dentition only exists in case of the deciduous teeth.

a) *Pure developmental deciduous dentition*: it begins by the development of the deciduous incisor – molar enamel organs at about 7-9 WIU to the commencement of development of the first permanent molar enamel organs at about 4 MIU. Through these two months, intervening these chronological events, only the deciduous enamel organs are seen developing intraosseously in the jaw. Therefore, the time consumed by this variant of dentition is about 2 months..

b) *Pure clinical deciduous dentition*: it begins by the eruption of deciduous incisors at about 6-8 months of age and ends by the eruption of the first permanent molars at about 6 years of age. So, the pure clinical deciduous dentition may consume about 5.5 years, that is, the time thorough which the pure deciduous dentition only appeared in the mouth.

c) *Pure clinical permanent dentition*: it begins at about 11 years of age when all the deciduous teeth are exfoliated by shedding of the maxillary canines, and continues until all the permanent teeth are ultimately lost.

2. *Mixed dentition*: it means the simultaneous presence of the two sets of teeth; the deciduous and permanent. Their existence, however, not only occurs clinically but can also be noted developmentally.

a) *Developmental mixed dentition*: since the developmental dentition is initially concerned with

the enamel organs development. So that the developmental mixed dentition begins at about 4 MIU. At which time, the enamel organs of the first permanent molars are starting their development while the previously developed enamel organs of all the deciduous teeth have already existed inside the jaw. The developmental mixed dentition ends by the complete root formation of the deciduous incisors – molar at 1.5-3 years of age. So that the time consumed for the developmental mixed dentition is about 2-3.5 years.

b) *Clinical mixed dentition:* it occurs as the child reaches the age of 6 years. At this time, the first permanent molars are clinically seen erupting distal to the second deciduous molars. The clinical mixed dentition continues to about the age of 11 years, where the maxillary deciduous canines exfoliate ending the phase of mixed dentition. So that this variant of dentition consumes about 5 years.

### Problems resolved

(1) The nomenclature of the *developmental dentition*, in this classification, denotes and accounts the period of time starting with the commencement of development of enamel organ and ends by the complete root formation. The determination of this variant of dentition can be partly performed either radiographically or by the postmortem analysis, where the developing teeth are initially present intraosseously, and partly by the naked eye following their eruption.

The eruption process may normally intervene between the commencement of enamel organ development and the root completion. So, through the time of developmental dentition, lying between the eruption and the root completion, the crown can be seen clinically while the relative amount of the root formed can be accounted radiographically. Theoretically, it is known that as the tooth erupts, about  $\frac{1}{3}$  to  $\frac{1}{2}$  the normal root length may be formed and may be completed after the eruption of permanent teeth by about 3 years and after the eruption of deciduous teeth by about 1 year.

(2) The forementioned rule was being well applied for accounting the *developmental dentition*, that is , starting with the development of enamel organ and ending with the complete root formation. However, a problem has been encountered, that is, reflected with the incapability of the application of this rule in respect to the developmental mixed dentition as indicated in the following categories:

a) The commencement of the enamel organs development of the deciduous dentition coincides with that of the deciduous incisors at about 7 WIU while it occurs for the enamel organs of the permanent dentition, coinciding with that of the first permanent molars, at about 4 MIU. Thereby, it is apparent, from this presentation, that there is unsharing period between these two chronological ages (Fig.1). This periods, unfortunately, can not be accounted in the domain or the expense of the mixed developmental dentition.

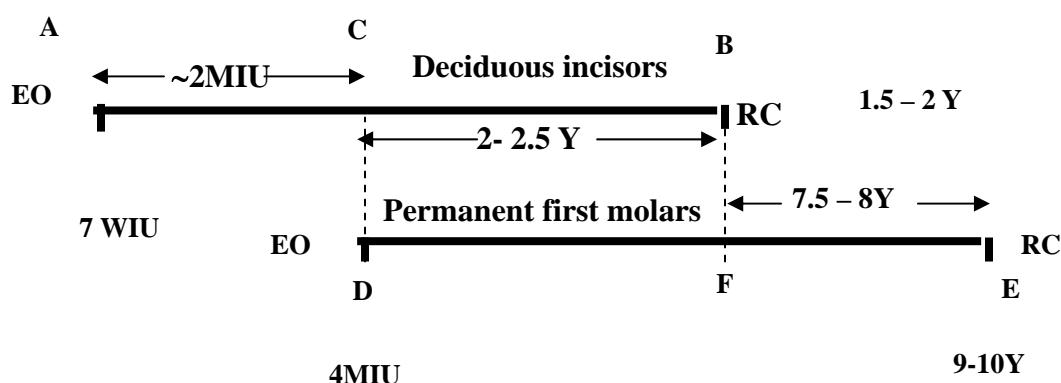
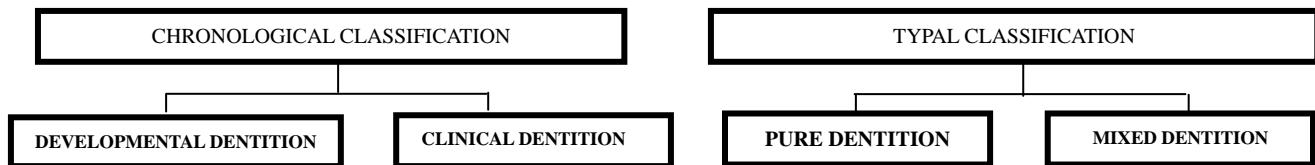


Fig. 1: diagram showing the *sharing periods of time* (2-2.5y) of deciduous dentition (C-B) with permanent dentition (D-F) during developmental mixed dentition. *Unsharing period of time* (A-C) for deciduous dentition occurs between deciduous incisor enamel organs development (A) at 7WIU to first permanent molar enamel organ development (D) at 4MIU. Other *unsharing period of time* (F-E) for permanent dentition occurs between deciduous incisors root completion (B) at 1.5-2 years of age to first permanent molars root completion (E) at 9-10 years of age. Enamel organ (EO) and root completion (RC).

## CLASSIFICATION OF HUMAN DENTITIONS



It denotes the teeth formation from the development of the enamel organ to the root completion

### **1. Developmental deciduous dentition**

- Begins by the development of incisor-molar enamel organs at about 7-9WIU
- Ends by the root completion of incisors-molars at 1.5-3Y.
- The time consumed is about 2-3.5 Y.

It denotes the appearance of teeth in the mouth which begins by the time of teeth eruption to their loss.

### **1. clinical deciduous dention**

- Begins by the eruption of incisors –molars at about 6-30M
- Ends by the shedding of maxillary canine at 11Y
- Time consumed is at about 10.5-8.5Y

It denotes the exclusive presence of one set of teeth, either the deciduous or permanent

### **1. Pure developmental deciduous dentition**

- Begins by development of incisor-molar enamel organs at about 7-9WIU
- Ends by development of the first permanent molar enamel organs at about 4MIU.
- Time consumed at about 2M

It denotes the presence of both the deciduous and the permanent teeth simultaneously

### **1. Developmental mixed dentition**

- Begins by the development of the enamel organ of the first permanent molar at about 4MIU
- Ends by the root completion of the deciduous incisors-molars at about 1.5-2Y
- Time consumed is at about 2-2.5Y.

### **2. Developmental permanent dentition**

- Begins, by the development of the first –third molar enamel organs at 4MIU-4Y.
- Ends by the root completion of the first-third molars at 10-25Y
- Time consumed is about 10.5-16Y

### **2. Clinical permanent dentition**

- Begins by the eruption of the first molars at 6Y
- End by the loss of all the teeth.
- Variable time is consumed.

### **2. Pure clinical deciduous dentition**

- Begins by the eruption of the deciduous incisors at about 6-8M
- Ends by the eruption of the first permanent molars at about 6Y.
- Time consumed is at about 5.5Y.

### **2. Clinical mixed dentition**

- Begins by the eruption of the first permanent molars at 6Y.
- Ends by the shedding of the deciduous maxillary canines at about 11Y
- Time consumed is at about 5Y.

### **3. Pure clinical permanent dentition**

- Begins as all deciduous teeth are exfoliated at about 11Y
- Ends by the ultimate loss of all permanent teeth.
- Variable time is consumed.

M: Month of age

Y: year of age

WIU: week intra-uterine

MIU: month intra-uterine

b) The complete root formation of deciduous dentition, coincident with that of deciduous incisors, ends at 1.5-2 years of age. At which time, the roots of permanent molars, partly sharing the development of deciduous incisors, are not yet completely formed, where their root development completes at 9-10 years of age. Thereby, it is apparent, from this presentation, that there is *unsharing period* between these two chronological ages (Fig.1) This unsharing period, between these two chronological ages, can not be accounted in the domain or in the expense of the mixed developmental dentition.

These dual technical discrepancies were reconciled by regarding the commencement of

developmental mixed dentition, in which the deciduous and permanent dentitions simultaneously develop and share certain period of time, at about 4 MIU meanwhile, the end of root formation for this dentition occurs at about 1.5-3 years of age. This situation will simulate an exclusive exception to the aforementioned rule for accounting of the mixed developmental dentition.

(3) In concern to the accounting of both the timing and the commencement of developmental dentition, which is designated to begin with the enamel organ development and ends by the complete root formation, two approaches have been arised:

• Firstly, *single representation concept*: it considered the start of the developmental deciduous dentition, for instance, with the time of development of the initially formed enamel organ of the deciduous incisor at about 7 WIU as a single representative for the initiation of the deciduous developmental dentition. However, this dentition is presumed to end with the root completion of the second deciduous molar at about 3 years of age. This concept may designate the chronology for the deciduous developmental dentition individually by marking the start of this dentition and its end with either the developmental events for a single tooth.

• Secondly; *dual representation concept* : it considered the beginning of developmental deciduous dentition, for instance, with the initially developed enamel organs of both the deciduous incisors group of teeth and the second molars group at about 7 and 9 WIU, respectively. With the same strategy, the consideration for the end of deciduous developmental dentition was followed. Thereby, the end of this dentition will be represented by the time of complete root formation for both the deciduous incisors and the second molars at about 1.5-2 and 3 years of age, respectively.

• The presumed classification presented, here in this study, has adopted the dual representation concept since it was the more inclusive and representative for the whole dentition in general.

(4) The accounting of time consumed by any variant of dentition is considered by recording the period of time elapsed between the two subsequent chronological events. For instance, throughout the developmental deciduous dentition, the first chronological event is the development of enamel organ for the initially formed teeth, the deciduous incisors, is at about 7 WIU. The subsequent chronological event, that is, the root completion for these incisor teeth occurs at about 1.5 years of age. Accordingly, the time consumed for the developmental dentition is about 2 years. The same strategy was also used for both the deciduous molars and the developmental permanent dentition.

(5) In case of the *clinical permanent dentition*, while it is accounted to begin with the eruption of the first permanent molar, however, there is no expectation for the end /or loss of this dentition. This is due to the absence of an actual expectation for the end of clinical permanent dentition. Crucially, any of the environmental, endocrinological or genetic factors may be highly significant in the determination of the end

of this dentition. These factors are variable between the different individuals and the various communities which certainly influence the timing of loss for the permanent teeth.

(6) Occasionally, some deciduous teeth may abnormally remain orally even after their proper time of exfoliation and so they are traditionally referred to as the *retained teeth*. In the extreme, early eruption of the permanent successors may also be encountered in association with the premature loss of deciduous teeth. All these instances are regarded abnormal departure from the ideal condition and are excluded from the present classification.

(7) Some deciduous teeth have shown a premature root resorption even prior to their complete root formation. Such situational discrepancy in the time of root resorption for deciduous teeth were coinciding with the complete crown formation for their permanent successors. This is based on the concept that the eruptive - resorptive dialogue which denotes and endorses the synchronism between the root resorption and the crown completion. That is to say that immediately after or in association with the crown completion, the root begins to form which per se is coinciding with the start of the pressure-resorption process where the crown of permanent successor contacts the root of deciduous predecessor.

(8) *The pure developmental dentition* exclusively exists in case of the deciduous dentition. In the extreme, no pure developmental permanent dentition does exist. This is due to the initiative and exclusive presence of the developing deciduous teeth while the permanent teeth initiate their development thereafter.

#### **Corresponding author:**

Said Mahmoud Hani

Oral Biology Department, Faculty of Dental Medicine, Al-Azher University, Cairo, Egypt

[hani.said@yahoo.com](mailto:hani.said@yahoo.com)

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9/25/2010

# Prognostic and Predictive Significance of Haemostatic and Angiogenic Parameters in Cancer Bladder Patients

**Madkour B. S.; Bekheet I.W.\* , El Baz A.G.\* , Ghobashy S.; El-Ganzory H. and, Essawy F.M.**

Haematology and Urology Department, Theodor Bilharz Research Institute, Imbaba, Giza, Egypt.

[iman.william@yahoo.com](mailto:iman.william@yahoo.com)

**Abstract:** Recent studies demonstrated a key role of angiogenesis, thrombosis and fibrinolysis in tumour invasion and metastasis. We aimed to clarify the potential link between angiogenic factor {vascular endothelial growth factor (VEGF)}, prothrombotic factor {von Willebrand factor (vWF)} and fibrinolytic markers {tissue plasminogen activator (t-PA), plasminogen activator inhibitor-1 (PAI-1) and D-dimer} with disease progression and metastatic dissemination in bladder cancer patients. The study enrolled forty five bladder cancer patients classified into three groups: 20 patients with locally invasive tumours, 15 patients with regional lymph nodes involvement and 10 patients with distant metastasis. In addition to 15 subjects served as a control group. Enzyme linked immunoassay method was used for measurement of VEGF, vWF, t-PA, PAI-1 and D-dimer. Enhanced angiogenesis was evident by high level of VEGF with subsequent high release of endothelial vWF. Also activation of fibrinolytic system was pronounced by elevated t-PA, PAI-1 and D-dimer. In addition, highest values of these factors were associated with relatively advanced tumour stage, as they showed a significant direct correlation with the stage of bladder cancer. Regression analysis proved that VEGF, vWF, t-PA and D-dimer are independent determinant for the stage of bladder cancer. Conclusion: These results suggest that VEGF, t-PA, PAI-1 and D-dimer are potential prognostic markers in bladder cancer patients. These findings may have future implications for the treatment of patients with metastatic disease.

**[Madkour B. S.; Bekheet I.W.\* , El Baz A.G.\* , Ghobashy S.; El-Ganzory H. and, Essawy F.M. Prognostic and Predictive Significance of Haemostatic and Angiogenic Parameters in Cancer Bladder Patients. Journal of American Science 2010;6(11):1091-1097]. (ISSN: 1545-1003). (<http://www.americanscience.org>).**

**Key words:** cancer bladder; vWF; VEGF; t-PA; PAI-1; D-dimer.

## 1. Introduction:

Some haemostasis and angiogenesis-related factors such as platelets, vWF, fibrinogen, PAI-1, D-dimer and VEGF have been highlighted as new potential response and survival predictors in cancer patients<sup>(1)</sup>.

Metastasis is a multi-step process involved in the alterations of cell-cell adhesion, angiogenesis, degradation of extracellular matrix, escape of immune surveillance and cell-matrix adhesion<sup>(2)</sup>. Degradation of extracellular matrix is important for tumour growth and invasion, which in part is regulated by the plasminogen activation system. Cell matrix adhesive interaction plays an important role in the normal organization and stabilization of the cell layer in epithelial tissue. However in tumour cells the adhesive interaction of these cells and the subendothelial matrices is essential for their invasive and metastatic capabilities, and the molecules that mediate this adhesive process may facilitate tumour cells to metastasize<sup>(3,4)</sup>.

Vascular endothelial growth factor (VEGF) is one of the most important angiogenic mediator both in physiological and pathological states<sup>(5)</sup>. Angiogenesis and coagulation system activation are

associated with tumour growth and metastasis<sup>(6)</sup>. Their aberrancies are integral parts of the pathobiology of cancer growth and dissemination<sup>(7)</sup>. We reported previously that enhanced platelet activation in cancer bladder patients associated with release of platelet derived angiogenic factor (VEGF), plays an important role in tumour growth and dissemination<sup>(8)</sup>.

Von Willebrand factor (vWF) is a glycoprotein, synthesized mainly in endothelial cells and in megakaryocytes<sup>(9)</sup>. It mediates the adherence of platelets to subendothelial matrices during vascular-endothelial damage and acts as a carrier protein for coagulation factor VIII<sup>(10)</sup>. Increased plasma vWF have been reported in patients with various types of cancer such as prostate cancer<sup>(11)</sup>, cervical and ovarian carcinoma<sup>(12)</sup>, head and neck cancer<sup>(13, 14)</sup> and colorectal cancer<sup>(1, 15, 16)</sup>. Moreover, high plasma vWF concentrations often correlates with advanced tumour staging and may have prognostic significance in these patients<sup>(11, 12, 13, 14, 15, 16)</sup>.

It has been demonstrated that the fibrinolytic system, in particular the urokinase-type plasminogen activator system (uPA), is involved in the process of

tumour cell invasion and metastasis. uPA binds to the urokinase-type plasminogen activator receptors (uPAR), which is present on tumour cells and monocytes, thus facilitating the conversion of plasminogen to plasmin. Plasmin is a protease not only able to cleave the fibrin network of a clot but also degrades the extracellular matrix, thereby allowing tumour cells and monocytes to invade the extracellular matrix and surrounding tissues<sup>(17, 18)</sup>.

Elevated tumour levels of uPA, uPAR and PAI-1 are associated with poor prognosis in various malignancies, including colorectal cancer<sup>(3, 4, 19, 20)</sup>, bladder and renal cell cancer<sup>(21)</sup>, squamous cell cancer<sup>(22)</sup> and breast cancer<sup>(23, 24, 25)</sup>.

D-dimer is a degradation product of cross-linked fibrin clots and reflects fibrin concentration. D-dimer levels are increased in patients with enhanced fibrin formation and have been reported as sensitive indicators for deep venous thrombosis and pulmonary embolism<sup>(26, 27)</sup>. In addition, preoperative plasma D-dimer level correlates with tumour stage and prognosis for patients with lung cancer<sup>(28, 29, 30, 31)</sup> and colorectal cancer<sup>(32, 33)</sup>.

Von Willebrand factor and other coagulation/fibrinolysis factors should be seriously considered as potential future prognostic and predictive indicators in bladder cancer patients. We aimed to evaluate the level of angiogenic factor (VEGF), coagulation factor (vWF), fibrinolytic parameters (t-PA, PAI-1 and D-dimer) and their possible relation to tumour progression and prognosis in cancer bladder patients. It is hoped that a better understanding of these factors will ultimately lead to the development of more targeted strategies that may have a positive effect on the process of tumour growth and dissemination.

## 2. Patients and Methods:

This study was conducted on forty five patients (32 males and 13 females having mean age of  $63.57 \pm 10.52$ ) with urinary bladder cancer admitted to Urology Department, Theodor Bilharz Research Institute, Giza, Egypt. Results were compared to those of fifteen age and sex matched healthy subjects (10 males and 5 females having a mean age of  $59.71 \pm 10.45$ ) that served as a control group.

The study protocol was approved by the international committee for the protection of human participants and confirmed by the guidelines of the 1975 Declaration of Helsinki.

All studied patients' groups were subjected to detailed history taking, thorough clinical examination, abdominal and pelvic ultrasonography, chest X-ray, Computed Tomography (CT), urine cytology and

histopathological diagnosis of urinary bladder biopsies obtained by cystoscopy.

Accordingly, patients were classified clinically into three groups:

- 20 patients (13 males and 7 females having a mean age of  $62.23 \pm 10.41$ ) with primary locally invasive urinary bladder cancer (de novo urinary bladder carcinoma) with no regional lymph nodes involvement and distant metastasis.

- 15 patients (12 males and 3 females having a mean age of  $63.26 \pm 10.34$ ) with urinary bladder cancer accompanied by regional lymph nodes involvement.

- 10 patients (7 males and 3 females having a mean age of  $65.23 \pm 8.41$ ) with urinary bladder cancer accompanied with regional lymph nodes involvement as well as distant metastasis.

According to the results of the above mentioned investigations for each case, determination of the stage using Tumour-Node-Metastasis (TNM) staging system were done.

Depending on histopathological examination of cystoscopic bladder biopsies, patients were classified into three types:

- 31 out of 45 patients (68.9%) with transitional cell carcinoma.

- 13 out of 45 patients (28.9%) with squamous cell carcinoma.

- 1 out of 45 patients (2.2%) with adenocarcinoma.

For all studied subjects, five ml blood samples were collected under complete aseptic conditions by clean venipuncture without venous stress, samples were distributed into the following tubes:

- One ml blood was collected on EDTA containing tube for complete blood picture.

- Two hundred  $\mu$ l Na citrate (3.8%) containing vacutainer was completed to 2 ml with blood in a ratio of 1:9. Centrifugation at 3000 rpm for 20 minutes was done and plasma samples were separated and preserved in small aliquots and stored at  $-20^{\circ}\text{C}$  for assay of haemostatic parameters (vWF, t-PA, PAI-1 and D-dimer). Two ml of blood was left to stand for clot formation. Serum was separated and divided into small aliquots for assay of liver and kidney function tests and serum level of VEGF.

All individuals were subjected to general investigations including an automated haemogram (using ACT differential, Beckman, France), liver and kidney function tests using autoanalyzer (Hitachi 736, Hitachi, Japan).

Haemostatic parameters were assayed for all studied groups by enzyme immunoassay, including assay of plasma level of vWF (Helena, Laboratories), tPA (Hyphen Biomed, France), PAI-1 (Stago Diagnostica, USA), Plasma D-dimer (Hyphen Biomed, France). In addition, determination of serum level of VEGF was also done by enzyme

immunoassay technique (using R&D System, VEGF, USA). All specific assays were carried out according to manufacturer's guidelines.

#### Analysis of data

SPSS for Windows version 9.0 computer program (SPSS Inc., Chicago, Illinois, USA) was used for statistical analysis. Means of different groups were compared using one-way ANOVA. Pearson correlation coefficient 'r' was used to measure the relationship between two variables. Stepwise multiple regression analysis was employed to evaluate any association between both angiogenic factor (VEGF) and haemostatic parameters (vWF, tPA, PAI-1 and D-dimer) with tumour progression and prognosis (tumour stage and distant spread) in bladder cancer patients. For all tests, a P value of less than 0.05 was considered statistically significant.

#### 3. Results:

The results of studied parameters in patients' and control groups are shown in Table (I). Table (II) shows the result of the studied parameters in control, locally invasive tumours, tumours with LN involvement and tumours with distant metastasis. Cancer Bladder patients showed increased serum angiogenic factor (VEGF) level compared to controls ( $P<0.05$ ). A significant high VEGF level was detected in patients with regional LN involvement compared to those with localized tumours ( $P<0.05$ ). Also a significant high VEGF level was noted in patients with distant metastasis compared to both locally invasive tumours ( $P<0.05$ ) and those with regional LN involvement ( $P<0.05$ ).

Our study showed a significant increase in plasma vWF in patients' groups compared to control group ( $P<0.05$ ). A significant high level was detected in locally invasive tumours compared to control group ( $P<0.05$ ). Also vWF was significantly higher in patients with regional LN involvement compared to both control group ( $P<0.05$ ) and patients with locally invasive tumours ( $P<0.05$ ). In addition, vWF was significantly higher in patients with distant metastasis compared to control ( $P<0.05$ ), locally invasive tumours ( $P<0.05$ ) and tumours with regional LN involvement ( $P<0.05$ ).

Regarding the fibrinolytic system, our data showed a statistical significant difference in patients'

group compared to control group. A significant high plasma level of both t-PA and PAI-1 was observed in locally invasive tumours compared to control group ( $P<0.05$ ). In addition, both t-PA and PAI-1 were significantly higher in tumours with regional LN involvement compared to control ( $P<0.05$ ) and locally invasive tumours ( $P<0.05$ ). Moreover, the highest levels of t-PA and PAI-1 were observed in patients with distant metastasis and their levels were significantly higher compared to control ( $P<0.05$ ), locally invasive tumours ( $P<0.05$ ) and tumours with regional LN involvement ( $P<0.05$ ).

D-dimer level was significantly raised in patients' group compared to control group ( $P<0.05$ ). A statistical significant difference was observed in locally invasive tumours compared to control group ( $P<0.05$ ). Also, a statistical significant rise was detected in tumours with regional LN involvement in comparison to both control ( $P<0.05$ ) and locally invasive tumours ( $P<0.05$ ). In addition, a statistical significant difference was noticed on comparing tumours with distant metastasis with control ( $P<0.05$ ), locally invasive tumours ( $P<0.05$ ) and tumours with LN involvement ( $P<0.05$ ).

Correlation analysis revealed a significant direct correlation between VEGF and vWF ( $r=0.734$ ,  $P<0.05$ ), suggesting the influence of angiogenic factors in increasing the production of endothelial vWF by the newly formed blood vessels. On the other hand, the angiogenic factor (VEGF), procoagulant factor (vWF) and fibrinolytic factors (t-PA and D-dimer) showed a significant direct correlation with the stage of bladder cancer ( $r=0.875$  and  $r=0.579$  respectively). These results give an evidence for the role of angiogenic and haemostatic factors in tumour growth and distant dissemination of malignant tumours (table: III).

Stepwise multiple linear regression analysis revealed that the angiogenic factor (VWGF), haemostatic factor (vWF) and fibrinolytic factors (t-PA and D-dimer) are independent determinant for the stage of bladder cancer tumour ( $f=11.982$ ,  $p=0.000$ ), ( $f=2.456$ ,  $p=0.024$ ), ( $f=4.059$ ,  $p=0.001$ ) and ( $f=12.857$ ,  $p=0.000$ ) respectively. These data give strong evidence for the importance of these parameters as prognostic markers for bladder cancer tumours.

**Table I: Results of studied parameters in control and patients' groups**

		Control Group (n=15)	Patients' Group (n=45)
VEGF (pg/ml)	Mean±S.D. Range	58.93±29.14 10-115	257.77±172.0* 52-762
vWF (%)	Mean±S.D. Range	112.46±16.80 82-130	143.51±34.08* 89-195
tPA (ng/ml)	Mean±S.D. Range	5.7±2.7 3.0-9.0	8.77±3.4.2* 4.5-19.5
PAI-1 (IU/ml)	Mean±S.D. Range	55.30±21.20 34.0-78.0	81.56±16.29* 38.0-180.0
D-dimer (ng/ml)	Mean±S.D. Range	224.40±99.7 93-342	484.44±119.26* 210-907

\*: Statistically significant from control ( $P<0.05$ )

**Table II: Results of the studied parameters in control, locally invasive tumours, tumours with LN involvement and tumours with distant metastasis.**

		Control Group (n=15)	Locally Invasive Tumour (n=20)	Tumour with LN Involvement (n=15)	Tumour with distant Metastasis (n=10)
VEGF (pg/ml)	Mean±S.D. Range	58.93±29.14 10-115	114.40±45.55 <sup>a</sup> 52-205	277.60±76.72 <sup>ab</sup> 152-390	514.80±106.60 <sup>abc</sup> 384-762
vWF (%)	Mean±S.D. Range	112.46±16.80 82-130	126.10±33.53 <sup>a</sup> 89-190	160.00±26.03 <sup>ab</sup> 100-190	174.26±14.24 <sup>abc</sup> 160-195
tPA (ng/ml)	Mean±S.D. Range	5.7±2.7 3.0-9.0	7.6±3.9 <sup>a</sup> 4.5-11.5	9.4±3.9 <sup>ab</sup> 4.3-13.5	15.9±4.3 <sup>abc</sup> 10.5-19.5
PAI-1 (IU/ml)	Mean±S.D. Range	55.30±21.20 34.0-78.0	63.5±32.9 <sup>a</sup> 38.0-100.0	98.0±26.4 <sup>ab</sup> 71.0-128.0	129.0±36.2 <sup>abc</sup> 95.0-180.0
D-dimer (ng/ml)	Mean±S.D. Range	224.40±99.7 93-342	341.1±98.8 <sup>a</sup> 210.0-443.0	631.7±112.6 <sup>ab</sup> 354-741	881.3±36.8 <sup>abc</sup> 819-907

a: Statistically significant from control group ( $P<0.05$ )

b: Statistically significant from locally invasive tumours ( $P<0.05$ )

c: Statistically significant from tumours with LN involvement ( $P<0.05$ )

**Table III: Correlation analysis between studied parameters and both stage and grade of bladder cancer tumour**

	Stage of tumour (TNM system)
VEGF	r=0.875*
vWF	r=0.427*
tPA	r=0.414*
PAI-1	r=0.113
D-dimer	r=0.579*

\*: Correlation is significant at ( $P<0.01$ )

#### 4. Discussion:

The development of metastasis is a stepwise process that starts when cancer cells separate from a primary tumour, migrate across blood vessel walls into blood stream and disperse throughout the body to generate new colonies. During the transit into the circulating system, tumour cells are exposed to fluid mechanical forces, plasma proteins and the vascular cells such as platelets. All of which may affect their survival and extravasations from the vasculature<sup>(16)</sup>.

As we previously reported<sup>(8)</sup>, our recent study demonstrated a significantly raised serum levels of VEGF in both transitional cell carcinoma and squamous cell carcinoma in comparison to control subjects. Moreover, its level showed a direct correlation with the tumour stage. VEGF has been associated with angiogenesis, lymphangiogenesis and regional lymph node metastasis and was reported to have anti-apoptotic and proliferative role<sup>(34)</sup>. Also Suzuki et al., (2005)<sup>(35)</sup>, reported that VEGF expression is an important predictive factor of pelvic lymph node metastasis in bladder cancer patients.

vWF plays a very important role in the pathogenesis of metastasis, by promoting the binding of tumour cells to platelets, and subsequently, to vascular endothelium<sup>(36, 37, 38)</sup>. The binding of vWF to several types of collagen may contribute to the attachment of platelets to the extracellular matrices of subendothelium, furthermore, a direct interaction between vWF and neoplastic cells has been demonstrated<sup>(39)</sup>. This interaction forms heterotypic cellular emboli, which are not easily recognized by the immune system and have more chance of

attaching to the endothelial surfaces than single tumour cells<sup>(36, 37)</sup>.

Elevated plasma vWF had been reported in different types of cancer. Our study demonstrated a significantly higher plasma vWF levels in bladder cancer patients compared to healthy controls, and the highest levels were observed in patients with metastatic disease. Elevations in vWF plasma levels in disseminated disease reflect the enhancement of angiogenic activity (as evident by high serum VEGF levels and its direct correlation with plasma vWF) to sustain a larger tumour cell burden and the metastatic progression<sup>(15)</sup>. In addition, the release of thrombin by tumour cells may induce vWF production in endothelial cells and enhance the adhesion of tumour cells<sup>(16)</sup>. Furthermore, the metastatic status of these patients may represent an effect of the adhesive property of vWF, which seems to play a crucial role during the course of haematological spread<sup>(16)</sup>. Accordingly, vWF may serve as a potential biological marker of disease progression in these patients.

It is well known that the fibrinolytic system is of importance in inflammation, wound healing and fibrosis development. However, it is also important in the process of tumour invasion and metastasis<sup>(3)</sup>. The present study demonstrated high plasma level of both t-PA and PAI-1 compared to control subjects and the highest levels were reported in patients with distant metastasis.

Numerous independent studies have demonstrated that patients with low levels of u-PA and PAI-1 in their primary tumour tissue have significantly better survival than patients with high levels of either factor<sup>(23, 24)</sup>. Tumour cells can express everything required for regulation of the fibrinolytic pathway on their cell surface. They possess both the urokinase-type (u-PA) and the tissue-type plasminogen activator (t-PA) and can also produce plasminogen activator inhibitor-1 (PAI-1)<sup>(40, 41)</sup>. Indeed, tumour cells are known to carry the specific PA receptors (u-PAR) (CD 87) on their membranes, which can facilitate the activation of the fibrinolytic system<sup>(42)</sup>. Extravasation and intravasation of solid malignant tumours is controlled by attachment of tumour cells to components of the basement membrane and the extracellular matrix, by local proteolysis and tumour cell migration<sup>(17)</sup>. Recent data strongly suggest that the delicate balance between plasminogen activators and their inhibitors plays a role in tumour invasion, tumour cell progression and metastasis and associated with shortened disease free and or overall survival in patients affected with malignant solid tumours. Levels of one or more of these markers have been recognized as predictors of disease-free interval and long-term survival in some patients with malignant disease<sup>(17, 22, 24, 41)</sup>. Moreover,

intravesical administration of PAI-1 significantly inhibits tumour progression in an in vivo model of bladder cancer<sup>(43)</sup>. The above mentioned data clarify the clinical impact of fibrinolytic system in tumour invasion and metastasis, and the future relevance in anti cancer therapy.

Plasma D-dimer levels have been shown to be increased in patients with various solid tumours including lung, prostate, cervical, ovarian, breast and colon cancer<sup>(33)</sup>. An elevated plasma D-dimer level indicates activation of coagulation and fibrinolysis<sup>(32)</sup>. A close interaction exists between venous thromboembolic disease and cancer. In the present study, we demonstrated a high plasma D-dimer level in cancer bladder patients compared to controls. Moreover, its level showed a significant correlation with the stage of the tumour. These data suggest that tumour progression is associated with activation of coagulation and fibrin formation, which is both implicated in cancer proliferation and metastatic dissemination<sup>(44)</sup>. Accordingly, elevated plasma D-dimer might be a sign of poor prognosis and preoperative plasma D-dimer level can be used to predict postoperative survival.

In summary, our data indicates that serum VEGF and plasma vWF, t-PA, PAI-1 and D-dimer levels are elevated in a stage-dependent manner, and higher levels correlate with metastatic diseases. Accordingly, they can serve as potential biological markers of disease progression in bladder cancer patients.

#### **Corresponding author**

Bekheet I.W

Haematology and Urology Department, Theodor Bilharz Research Institute, Imbaba, Giza, Egypt.  
[iman.william@yahoo.com](mailto:iman.william@yahoo.com)

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# Oil content and yield of *Foeniculum vulgare* Mill. cv. Soroksary seeds as affected by different plant cultivation densities

Jalal Khorshidi, Seyed Fazel Mirahmadi, Mohammad Fakhr Tabatabaei

Department of Horticulture Science, Faculty of Agricultural Science and Engineering, University of Tehran, Karaj,  
Iran  
[Mirahmadif@ut.ac.ir](mailto:Mirahmadif@ut.ac.ir)

**Abstract:** In this study, the effect of different plant cultivation densities on the oil content and yield of *Foeniculum vulgare* Mill. Cv. Soroksary seeds was studied at the Faculty of Agricultural Sciences and Engineering, Karaj, Iran (Latitude 35° 47' N and Longitude 50° 59' E) in 2008. Five plant spaces studied were 10, 15, 20, 25, and 30 cm and the distance between rows in all treatments was 40 cm using a complete randomized block design with three replicates. According to results, the effect of plant density on oil content and yield was significant ( $P<0.01$ ). The highest oil content (3.33%) and yield per hectare (116.73 liter) was obtained with the lowest plant density. [Jalal Khorshidi, Seyed Fazel Mirahmadi, Mohammad Fakhr Tabatabaei. Oil content and yield of *Foeniculum vulgare* Mill. cv. Soroksary seeds as affected by different plant cultivation densities. Journal of American Science 2010;6(11):1098-1100]. (ISSN: 1545-1003). (<http://www.americanscience.org>).

**Keywords:** *Foeniculum vulgare* Mill. cv. Soroksary; Plant density; Oil content; Oil yield

## 1. Introduction

Fennel (*Foeniculum vulgare* Mill.) is one of the most important medicinal plants, native of Mediterranean regions and belongs to the Apiaceae family (Omidbaigi, 2007). The plant has abundant applications in various industries; for instance, the essential obtained from seeds is added to perfumes, soaps, pharmaceuticals and cosmetics. Fennel oil, seeds or extracts are also used to flavor prepared foods including meats, ice cream, candy, baked goods and condiments. Recent studies have shown that essential oil of this plant can be used as an valuable antioxidant, antibacterial and antifungal agent (Lucinewton et al., 2005).

One of the major restraints in crop production is improper crop spacing in the field (Dupriez and Deleener, 1989). The effect of spacing on growth and secondary metabolites is largely due to change in the interception of radiant energy (Yao and Shaw, 1964). When crops are overcrowded, there will be competition. In the wider spacing, plants have more nutrition, water and air, but in the narrower spacing, they have restricted conditions for development (Ozar, 2003). Plant density is one of the most important factors affecting yield, yield components, oil and essential oil in medicinal plants. Masood et al (2004) investigated the effect of row spacing (40, 50, 60, and 70 cm) on morphological characters and seed yield of fennel and reported that the highest plant height, seed yield per bed, and seed yield per hectare were obtained with the lowest row spacing. Najafi and Moghadam (2002) reported that with increase in the plant density seed and biological yield increased. Arabaci and Bayram (2004) reported

that the highest effective substances yield in the Basil (*Ocimum basilicum* L.) was obtained in lower plant density. The maximum oil percentage and oil yield in Coriander (*Coriandrum sativum* L.) were obtained in density 30 plant per m<sup>2</sup> (Masood et al., 2004).

The objective of this study was to evaluate the percentage variation and oil yield of *Foeniculum vulgare* Mill. cv. soroksary in different plant population densities.

## 2. Material and Methods

A field study was conducted at the Faculty of Agricultural Sciences and Engineering, Karaj, Iran (Latitude 35° 47' N and Longitude 50° 59' E) to determine the effect of different plant cultivation densities on the oil content and yield of *Foeniculum vulgare* Mill. cv. Soroksary seeds in 2008. Results of soil analysis are shown in (Table 1). Experiment was conducted based on completely randomized block design with three replicates and five plant densities. The plot size was 2.5x1.5m. The distance between blocks and plots were 1m. Five plant spaces studied were 10, 15, 20, 25, and 30 cm. The distance between rows in all treatments was 40 cm. Each plot consisted of five rows. The bitter fennel seeds were sown on the 7th March 2008.

The following irrigation regime was followed;

1. 2-3 day's interval irrigation until germination stage,
2. 4-5 day's interval irrigation from germination to appearance of first flowers stage,

3. 7 days interval irrigation from appearance first flowers to harvest stage.

Thinning was done when plants had 4-5 leaves. All agronomic practices were kept for all the treatments. The seeds were harvested twice after ripening (20th August and 30th August) and dried in a shade for 72 hours. Afterwards, 15 grams of seeds were powdered and their oil content was extracted using a soxhlet apparatus with hexane solvent method (Laurence, 1999). After isolation, the oil was purified in a rotary vacuum evaporator apparatus (Buchi, Switzerlan).

Data collected were analyzed using Duncan Multiple Range Test (Duncan, 1955) and statistical software (SPSS).

Table 1. Results of soil analysis

Soil sample	Properties
pH (in 2:1 water)	8.1
Sand (%)	30
Clay (%)	32
Silt (%)	38
Ca (g/kg)	293
Fe (mg/kg)	12.1
Organic matter (g/kg)	0.78
N (g/kg)	0.092
P (cmol/kg)	12.83
K (cmol/kg)	305

### 3. Results

Results of present study showed that the oil content and yield of seeds was affected by change in plant density. With increasing spaces between plants, the oil content of seeds was increased but the pattern of oil yield changes was irregular. Generally with increase in spaces between plants, the oil percentage increased significantly ( $P<0.01$ ).

The maximum oil percentage (3.33%) and yield (116.73 Lit/he) were obtained in the lowest plant density. While the minimum oil percentage (1.33%) was obtained in the highest plant density and the minimum oil yield (65.27 Lit/he) was obtained in 15cm space between plants (Fig 1 and Fig 2).

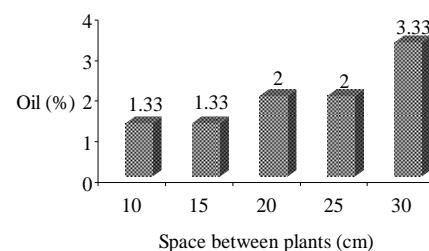


Fig 1. Relationship between plant density and oil percentage

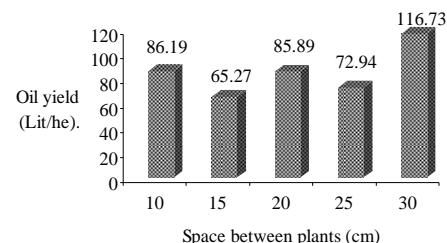


Fig 2. Relationship between plant density and oil yield

Comparison of treatments indicated that there was no significantly difference in the oil percentage between 10 and 15 cm of plant densities. Similarly, there was no significantly difference in the oil percentage between 20 and 30 cm of plant densities (Table 2).

Table 2. Oil content and yield of seeds in different plant cultivation densities

Space between Plants (cm)	Oil content (%)	Oil yield (Lit/he)
10	1.33a	86.19c
15	1.33a	65.27a
20	2b	85.89c
25	2b	72.94b
30	3.33c	116.73d

Different letters in each column indicating significant difference at  $P<0.01$

### 4. Discussions

These results are in agreement with findings Akbarinia et al (2006), and Ozer (2003). The studies in most plants have shown that plant density is an important factor affecting on yield. In the lower plant density, plants have more nutrition, water and air, and therefore have the better growth and finally produce the higher yield. But in the lower plant density, they have restricted conditions for development and thus produce the lower yield. In conclusion, to reach the

maximum oil yield of *Foeniculum vulgare* Mill. cv. Soroksary, the minimum plant density is suggested.

In this study, increases observed in the yield of seeds can be attributed to the better growth of plants and subsequently the better canopy development which led ultimately to the better use of solar irradiance and higher photosynthesis. Considering the significant effect of different plant cultivation densities, it can be argued that seed yield increases in suitable plant densities are due mainly to production of more seeds in each umbel. In other words, plant density by affecting the absorption of nutrients and exposure of the plant to the light can affect the photosynthesis rate and production of oil content.

#### **Acknowledgements:**

The authors very grateful to University of Tehran, Kaveh Mollazadeh, Khaled Ahmad Ali, Rahmat Mohammadi, Payman Salami and Salman Sharif Azari for their assistance.

#### **Corresponding Author:**

Seyed Fazel Mirahmadi

Department of Horticulture Science, Faculty of Agricultural Science and Engineering, University of Tehran, Karaj, Iran. E-mail: [Mirahmadif@ut.ac.ir](mailto:Mirahmadif@ut.ac.ir)

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## Cyanobacteria of a Tropical Lagoon, Nigeria.

Adesalu, Taofikat Abosede<sup>1</sup>, Nwankwo, Dike Ikegwu.<sup>2</sup>

<sup>1</sup>Department of Botany and Microbiology, University of Lagos, Nigeria.

<sup>2</sup>Department of Marine sciences, University of Lagos, Nigeria.

[boseadesalu@yahoo.com](mailto:boseadesalu@yahoo.com).

**Abstract:** Investigations for the first time into the blue green algae of Lekki lagoon were carried out for 24 months (June 2003- May 2005) at monthly intervals using standard plankton net of mesh size 55µm. One hundred and seventy nine species belonging to thirty genera were observed. The filamentous blue green algae *Oscillatoria* formed the most abundant genus making up twenty three species followed by *Phormidium* eighteen species. *Anabaena* and *Chroococcus* recorded thirteen species each while the genera, *Gleocapsa*, *Merismopedia* and *Microcystis* recorded ten, eight and twelve species respectively. Only one genus each of *Cyanosarcina*, *Calothrix* and *Scytonema* were encountered. Bloom forming species identified were *Microcystis aeruginosa*, *M. flos-aquae*, *M. wesenbergii* and *Anabaena flos-aquae*. In this study, thirty-nine new species were recorded for Lagos lagoon complex in which Lekki lagoon is one of it while *Cyanosarcina hueberliorum* is new record for Nigeria.

[Adesalu, Taofikat Abosede, Nwankwo, Dike Ikegwu. **Cyanobacteria of a Tropical Lagoon, Nigeria.** Journal of American Science 2010;6(11):1101-1107]. (ISSN: 1545-1003). (<http://www.americanscience.org>).

**Key words:** Cyanophytes, tropical, bloom, Lagos lagoon complex

### Introduction

The coastline of South Western Nigeria is a meandering network of lagoons and creeks of which Lagos lagoon with an area of 208sqkm is the largest (Nwankwo 1989). The geography and hydrology of various parts of Lagos lagoon complex in which Lekki lagoon is one it have been described by several workers. These include Lekki lagoon (Ikusemiju 1973); Lagos lagoon (Hill and Webb 1958) and harbour (Olaniyan 1957). Checklists of planktonic algae in some parts of Nigeria have been documented by different workers. For instance in the North, Holden and Green (1960) studied the phytoplankton of River Sokoto while Khan and Agugo (1990) studied Kongiri dam, Jos mine lakes was studied by Anadu et al. (1990).

In Southern region, studies include Opute (1990,1991,1992) who studied Warri Forcados estuary phytoplankton, New Calabar river by Nwadiaro and Ezefili (1986). Biswas (1984, 1992) had report for eastern region while western region reports include that of Imevbore (1968) on Eleiyele reservoir, Egborge and Sagey (1979) on Ibadan freshwater ecosystem . Nwankwo (1988) studied the planktonic algae of Lagos lagoon, Nwankwo (1993) reported eight cyanobacteria bloom species of coastal waters in South Western Nigeria excluding Lekki lagoon, Nwankwo (1997) reported dinoflagellates list of Lagos lagoon. Adesalu and Nwankwo (2005, 2009) reported the diatoms of Olero creek and Lekki lagoon respectively, Wujek et al. (2003) studied the chrysophytes of Lekki lagoon while Kadiri (1989, 1993, 1999, 2000) reported the rich flora of

*Micrasterias*, desmids, algae composition and euglenoids of Ikpoba reservoir respectively.

Of the entire aforementioned checklist, none specifically reported the cyanobacteria checklist in Nigeria coastal waters. The present study was undertaken to investigate the composition of cyanobacteria species of Lekki lagoon for possible biological monitoring since the lagoon is a source of fish supply for people of South Western states and beyond.

### Description of study area

Lekki lagoon (Fig.1) a large expanse of shallow freshwater extends between Lagos and Ogun states. It covers an area of about 247km<sup>2</sup>. A greater part of the lagoon is shallow (<3.0 m), while some areas are up to 6.4m deep. It lies between longitudes 4°00' E and 4°12' E and latitude 6°25' N and 6°37' N. The lagoon is fed by river Oni in the north eastern part, while rivers Osun and Saga flow into the north western part. Two peaks of rainfall are associated with this lagoon, a major peak in July and a lesser peak in September. There are two peaks of sunshine hours which approximately correspond to the equinoxes. The mainstay of communities that live around this environment is artisanal fishing.

### Materials and methods

#### Collection of sample

Biological samples were collected monthly from twelve stations (Table 1) using Hydrobios plankton net of 55µm mesh size. For quantitative analysis

5litres of the water was concentrated. Biological samples were preserved in 4% unbuffered formalin. Identification was done using Olympus BX51 photomicroscope. Water samples were collected into clean plastic containers for chemical analysis while *in situ* measurements of temperature, transparency, pH and depth were made.

TABLE 1 : AVERAGE DEPTH (M) AND GEOGRAPHICAL POSITION OF SAMPLING STATIONS

STATIONS	Average depth	Longitudes	Latitudes
Emina 1	2.77	4°5.080E	6°32.754N
Emina II Entrance of River Mosafejo	1.61	4°7.511E	6°34.07N
Entrance of River Oni	1.51	4°10.239E	6°35.344N
Iwopin 1	1.80	4°12.153E	6°35.090N
Iwopin 11	1.80	4°13.153E	6°32.309N
Imoki	2.69	4°9.651E	6°32.137N
Ise 1	2.17	4°10.048E	6°31.253N
Ise 11	1.81	4°13.413E	6°26.833N
Ebute lekki Entrance of Omu creek	2.41	4°9.788E	6°26.181N
Lagoon centre	1.29	4°5.353E	6°26.685N
	1.88	4°7.604E	6°28.867N
	2.23	4°3.348E	6°28.577N

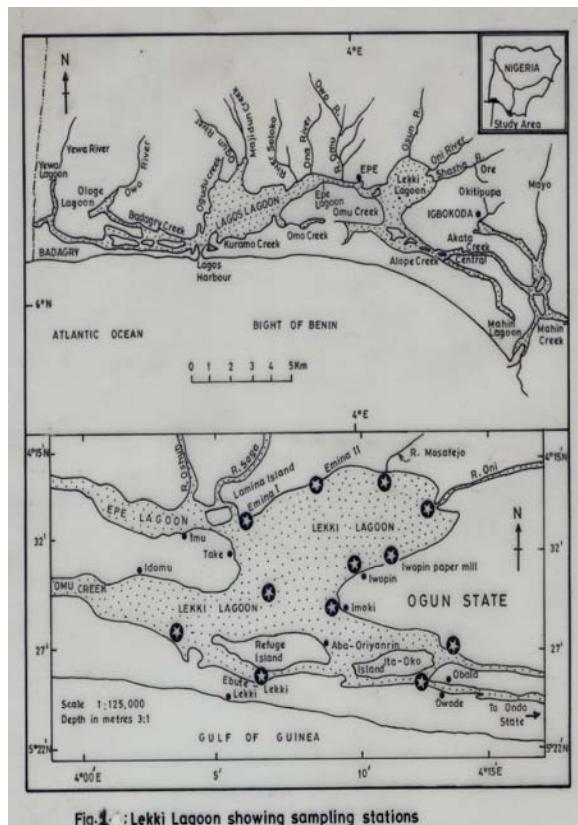


Fig.1.: Lekki Lagoon showing sampling stations

#### Physical and chemical analysis of water sample

The methods described by America Public Health Association (APHA 1998) were used for physical and chemical analysis. The air and surface water temperature were measured *in situ* with a simple mercury thermometer while the transparency was measured using a 20cm diameter Secchi disc. The depth was measured with a calibrated pole and the water pH determined using a Phillips pH meter (Model PW950). The chemical factors determined include Salinity, conductivity, dissolved oxygen (DO) and biological oxygen demand (BOD<sub>5</sub>). Salinity was determined using the Silver Nitrate Chromate titration method as described by Barnes (1980) while Dissolved oxygen content was determined using a Griffin oxygen meter. Oxygen saturation was recorded in percentage. Biological Oxygen Demand is the measure of the amount of dissolved oxygen that could be depleted from the water body during natural biological assimilation or degradation of organic compounds by the organisms present especially bacteria. This was done after the dissolved oxygen had been measured using the standard method of biochemical consumption of oxygen in 5 days at 20°C while conductivity was

determined using the HANNA instrument (H18733), a wide range conductivity meter that has salinometer range in  $\mu\text{S}$ . Conductivity values were recorded as  $\text{mScm}^{-1}$  at  $25^\circ\text{C}$  (APHA 1998). The department The Federal Meteorological Department, Oshodi, Lagos kindly provided rainfall and sunshine hours data for the period of investigation (Table 2).

### Nutrient determination

For nitrate determination, Hach Cadmium reduction method was used (APHA 1998). Phosphate-phosphorus is known to be important in a number of ways, one being that it facilitates the uptake of nitrogen. It was determined by ascorbic acid method. The values obtained were recorded in milligrams per litre ( $\text{mgL}^{-1}$ ) (APHA 1998) (Table 2).

Biological Oxygen demand	0.23	0.22	0.26	0.23	0.22	0.25
Chemical oxygen demand	0.36	0.30	0.26	0.25	0.24	0.29
Oil and grease	0.03	0.02	0.02	0.02	0.00	0.01
Turbidity (FTU)	8.59	9.25	10.00	7.46	9.57	8.87
Total dissolved solids	9.48	10.29	10.65	8.51	11.29	11.50
Sulphate	0.03	0.02	0.03	0.04	0.03	0.03
Ca	10.46	11.11	11.92	12.07	11.57	10.71
Fe	0.30	0.23	0.24	0.26	0.22	0.25
Pb	0.01	0.08	0.25	0.08	0.08	0.01
Hg	0.01	0.00	0.00	0.00	0.00	0.00
Cu	0.05	0.02	0.02	0.02	0.03	0.06
Ni	0.02	0.01	0.02	0.01	0.01	0.01
Zn	0.02	0.01	0.01	0.01	0.01	0.02

### Water chemistry

The physical and chemical characteristics of the study area are presented in Table 2. The mean pH of the water with a range of 7.41-7.46 indicated that the system is highly buffered. Conductivity which is the numerical expression of the ability of a solution to carry an electric current represents the total ions of water ranged between 0.47-0.56  $\mu\text{Scm}^{-1}$  the lowest value for phosphate-phosphorus, nitrate-nitrogen and sulphate were 2.42, 2.70 and 0.002  $\text{mgL}^{-1}$ . Salinity recorded the least value of 0.40‰ while chloride ion had the highest value of 10.00  $\text{mgL}^{-1}$ .

Table 2: Mean physico-chemical values for Lekki lagoon (concentrations in  $\text{mg L}^{-1}$ ) (Stations A-L).

STATIONS	A	B	C	D	E	F
Surface Water tempemperature ( $^\circ\text{C}$ )	30.54	30.73	30.55	30.61	30.40	30.31
Air tempemperature ( $^\circ\text{C}$ )	29.68	29.60	29.60	29.71	29.51	29.44
Transparency (cm)	5.74	7.38	11.33	9.25	11.67	10.29
Total suspended solids	9.07	8.56	9.16	8.45	9.28	9.78
pH	7.42	7.43	7.41	7.43	7.44	7.46
Salinity ‰	0.47	0.47	0.45	0.45	0.40	0.55
Phosphate-phosphorus	2.53	2.70	2.42	2.50	2.56	2.61
Nitrate-nitrogen	2.73	2.93	3.42	2.98	2.54	3.85
Chloride	9.23	9.67	9.59	10.00	9.88	9.52
Conductivity ( $\mu\text{Scm}^{-1}$ )	0.56	0.52	0.56	0.47	0.47	0.48
Dissolved Oxygen	4.15	3.46	4.21	4.10	4.16	4.25
Biological Oxygen demand	0.23	0.22	0.26	0.23	0.22	0.25
Chemical oxygen demand	0.36	0.30	0.26	0.25	0.24	0.29
Oil and grease	0.03	0.02	0.02	0.02	0.00	0.01

STATIONS	G	H	I	J	K	L
Surface Water tempemperature ( $^\circ\text{C}$ )	30.35	30.23	30.38	30.63	30.70	30.55
Air tempemperature ( $^\circ\text{C}$ )	29.55	29.08	29.29	28.90	29.27	29.21
Transparency (cm)	11.08	10.42	14.42	7.30	10.04	12.33
Total suspended solids	11.76	12.71	8.39	11.50	8.87	8.87
pH	7.44	7.46	7.44	7.42	7.37	7.38
Salinity ‰	0.47	0.44	0.45	0.44	0.50	0.47
Phosphate-phosphorus	2.47	2.49	2.96	2.53	2.54	2.44
Nitrate-nitrogen	3.44	3.25	2.75	3.31	2.70	3.97
Chloride	9.40	9.17	9.31	9.31	9.54	9.99
Conductivity ( $\mu\text{Scm}^{-1}$ )	0.49	0.52	0.46	0.53	0.62	0.56
Dissolved Oxygen	4.25	4.19	4.17	4.16	4.18	4.17
Biological Oxygen demand	0.29	0.30	0.31	0.28	0.26	0.24
Chemical oxygen demand	0.34	0.32	0.30	0.31	0.31	0.25
Oil and grease	0.01	0.01	0.01	0.01	0.01	0.01

Turbidity (FTU)	8.37	7.99	8.42	10.29	9.15	8.69
Total dissolved solids	9.87	9.95	10.31	10.58	10.81	10.29
Sulphate	0.02	0.02	0.05	0.04	0.06	0.03
Ca	11.04	10.96	10.70	10.21	11.40	11.52
Fe	0.21	0.23	0.25	0.28	0.30	0.55
Pb	0.02	0.01	0.00	0.00	0.04	0.02
Hg	0.00	0.00	0.00	0.00	0.02	0.01
Cu	0.01	0.01	0.01	0.00	0.02	0.04
Ni	0.01	0.01	0.01	0.01	0.01	0.01
Zn	0.01	0.01	0.01	0.01	0.01	0.01

## Results

### Cyanobacteria analysis

In this study, cyanobacteria genera are arranged alphabetically within families and the species in alphabetical order within genera (Table 3).

**Table 3: Cyanobacteria checklist at Lekki lagoon, Nigeria.**

#### Division : Cyanophyta

#### Class : Cyanophyceae

#### Order: Chroococcales

##### Family 1: Chroococcaceae

*Chroococcus decorticans*

*C. dispersus* (V.Keiss) Lemm.

*C. limnecticus* Lemm.

\**C. limnecticus var. subsalsus* Lemm.

*C. minor*

*C. minutus* (Kutz.) Rabenh.

*C. palidus* Nageli

*C. prescottii* Dr. & Daily

*C. turgidus* (Kutz.) Lemm.

*C. turicensis* (Nag.) Hangirg

*C. varius* A. Braun

*Chroococcus* sp 1

*Chroococcus* sp 11

\**Cyanosarcina huebeliorum* Komarek & Anagnostids

*Dactylococcopsis raphidioides* Hansg.

*D. smithii* Chodat & Chodat

*Dactylococcopsis* sp.

##### Family 2: Merismopediaceae

*Agmenellum quadruplicatum*

*Agmenellum* sp.

*Aphanocapsa delicatissima* West & West

*A. elaschista* West & West

*A. elachista var conferta* West & West

\**A. nubilum* Nygaard

*A.pulvrea*

*A. rivularis*

#### *Aphanocapsa* sp.

\**Aphanothece bullosa* var *major* Geitler

*Aphanothece* sp.

*Merismopedia angularis* Geitler

*M. convoluta* Breb.

*M. elegans* A.Br.

*M. glauca* (Ehr.) Nag.

*M. major* G.M.Smith

*M. marsonii* Lemm.

*M. punctata* Meyen.

*M. tenuisima* Lemm.

#### Family 3: Chaemasiphonaceae

*Clastidium setigerum*

*Clastidium* sp.

#### Family 4: Microscystaceae

\**Gleocapsa alpicola* (Lyng.) Bornet

\**G. arenaria* (Hass.) Rabenh.

*G. biformis* Novacek

*G. compacta* Kutz.

*G. conglomerata* Kutz.

\**G. decorticans* (A.Br.) P.Richter

*G. delicatissima*

*G. magma* (Breb.) Kutz.

*G. quarternata* (Breb.) Kutz

*Gleocapsa* sp.

*Gleothece heufleri*

*Gleothece linearis* Nag

*Gleothece* sp.

*Microcystis aeruginosa* Kutz.

*M. aeruginosa* var *elongata* Rao,C.B

\**M. aeruginosa* var *major* (Witt.) Smith

*M. elongata* sp.nov.

\**M. firma* (Kutz.) Dr. & Daily

*M. flos-aquae* (Witt.) Kirchner

*M. paludosus*

\**M. pulvrea* (Wood) Forti

\**M. ramosa* Bharadwaja

\**M. robusta* (Clack) Nygaard

*M. viridis* (A. Br.) Lemm.

*M wesenbergii* Kosinskaja

#### Order 2: Nostocales

##### Family: Nostocaceae

*Anabaena azollae* Strasburger

*A. circinalis* (Kutz.) Rabh.

\**A. confervoides* Reinsch

*A. constricta* Lauter b.

*A. cycadeae* J.Reinsch

*A. cylindrica* Lemmermann

*A. fircinalis*

*A. flos-aquae* (Lyng.) Breb.

- A. limnectica* G.M.Smith  
*A. spiroides* Lemm.  
*\*A. torulosa* (Carm.) Lagerh.  
*Anabaena* sp.1  
*Anabaena* sp 11  
*Aphanabaena* sp  
*Calothrix* sp  
*Cylindrospermum catenatum* Ralfs  
*Cylindrospermum majus* Kutz.  
*Cylindrospermum* sp 1  
*Nostoc carneum*  
*Nostoc linkia*  
*N. muscorum* Agardh  
*N. peltigerae* Letellier  
*N. sphaericum* Vauch.  
*Nostoc* sp.1  
*Nostoc* sp 11  
**Order 3: Oscillatoriales**  
**Family 1: Oscillatoriaceae**  
*Lyngbya birgei* G.M.Smith  
*L. contorta* Lemm.  
*\*L. lagerheimia* (Mobius) Gom.  
*L. limnectica* Lemmermann  
*\*L. martensiana* Menegh.  
*L. versicolor* (Wattman)Gomont  
*Lyngbya* sp  
*Oscillatoria acuminata* Gomont  
*O. acutissima* Kufferath  
*O. agardii* Gomont.  
*O. angustissima* West & West  
*O. articulata*  
*O. brevis* Kutz.  
*O. curviceps* Agardh  
*O. formosa* Bory.  
*\*O. formosa f. edaghica* Novickova  
*O. germinata* Meneghini  
*O. lacustris*  
*\*O. lemmermanni* Wolosz  
*O. limnectica* Lemm.  
*O. limosa* (Roth) Ag.  
*O. margaritifera* Kutzing (Gomont)  
*O. minima*  
*O. planctonica* Wolosz  
*\*O. rubescens* DC ex Gomont  
*O. sancta* (Kutz.)Gom  
*\*O. simplissima* Gomont.  
*O. subrevs* Schmidle  
*O. tenuis* Ag.  
*Oscillatoria* sp.  
**Family 2:Phormidiaceae**
- Arthrospira fusiformis* Fott & Karim  
*Arthrospira* sp.  
*Microcoleus codii* Fremy  
*M. subtorulosus*  
*M. willeana*  
*Microcoleus* sp.1  
*Microcoleus* sp 11  
*Phormidium angustissimum* West & West  
*\*P. caerulescens* Geitler  
*\*P. chlorinum* Komarek  
*P. cortianum*  
*P. crouanii* Gomont  
*P. foveolarum* (Mont.) Gomont  
*\*P. insigne* Skuja  
*\*P. laetevirens* Skuja  
*P. luridum* (Kutz.) Gomont  
*\*P. luteum* Kosinskaja  
*\*P. molle* Palik  
*P. nigro-viride* Gomont  
*\*P. papyraceum* (Ag.) Gom.  
*P. retzii* (Ag.) Gomont  
*P. tenue* (Menegh.)Gom.  
*P. tinctorium* Kutz.  
*Phormidium* sp 1  
*Phormidium* sp 11  
*\*Plantothrix clavarata* Skuja  
*\*P. cryptovaginata* Skacelova & Komarek  
*\*P. isothrix* Komarek  
*P. minor*  
*P. planctonica*  
*Plantothrix* sp1  
*Plantothrix* sp 11  
*Trichodesmium laucustre* Klebahn  
*Trichodesmium* sp.  
**Family 3:Pseudanabaenaceae**  
*\*Limnothrix planctonica* Geitler  
*Limnothrix* sp.  
*\*Pseudoanabaena curta* Hollerbach  
*\*P. moniliformis* Komarek & Kling  
*\*P. thermalis* Anagnostidis  
*Pseudoanabaena* sp  
*Spirulina filiformis*  
*S. princeps* W.et G.S.West  
*S. major* Geitler  
*S. meneghiniana* Anagnostidis  
*S. tenerima*  
*Spirulina* sp 1  
*Spirulina* sp 11  
*\*Leptolyngbya hypolimnectica*  
*\*Leptolyngbya ocricana* Cardo

*L. tenuis*

*Leptolyngbya* sp.

\**Plantolyngbya brevicellularis* Cronberg

&Komarek

\**P. minor* Komarek&Cronberg

\**P. tallingii* Komarek & Kling

\**P. minor*

*Planktolyngbya* sp.

#### Family:Schizotrichaceae

*Schizothrix pulvinata*

*S. friesii* (Ag.) Gomont

*Schizothrix* sp.

*Scytonema* sp.

#### Discussion

The cyanobacteria checklist reflects the influence of hydrological conditions of this area. The dominance of *Oscillatoria* throughout the season could be a pointer that the hydrology and salinity of the studied area favours its growth. The particular high diversity of blue-green observed in the lagoon could also be that the water chemistry favours growth of cyanophytes. Five bloom forming cyanophytes identified in this study include *Microcystis aeruginos*, *M. wesenbergii*, , *Anabaena flos-aquae*, *A. spiroides* and *Oscillatoria formosa*.

The variation in physical and chemical parameters observed during the study period may be as a result of the influence of weather conditions. For instance, the rainy season occurring between June and October, characterized by low transparency and pH; increased total suspended solids, higher turbidity and increased flood water condition which might have initiated stressful environmental condition and these conform with Dart and Stretton (1980) who stated that variations in water temperature could cause alterations in the pH due to changes in ionization and increased solubility or precipitation of bottom deposits. Nwankwo and Onitiri (1992) also pointed out that it is possible that rainfall triggers off flood situations which usually increases total solids, reduces transparency and consequently light penetration and also dislodges attached algal forms. The phytoplankton community and the physico-chemical parameters exhibited seasonal changes closely related to the pattern of rainfall. The presence or absence of any blue-green species may be due to the changing physical environment other than pollution (Nwankwo 1994).

#### Acknowledgements

Adesalu T.A. is grateful to Fulbright exchange program scholarship,University of Lagos, Nigeria; Central Michigan and Bowling Green state

Universities U.S.A.. Thanks are also due to Dr Akinsoji, O. my co-supervisor.

#### Corresponding author:

Adesalu, Taofikat Abosede

Department of Botany and Microbiology

University of Lagos, Akoka, Nigeria

E-mail: [boseadesalu@yahoo.com](mailto:boseadesalu@yahoo.com).

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9/9/2009

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