The Journal of American Science

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Cover Page, Introduction, Contents, Call for Papers, am0703

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2	**Antifungal Macrodiode Production By *Streptomyces albidoflavus*-143: Fermentation, Purification and Biological Activities** Houssam M. Atta [1], El-Sehrawi M.H.[2] and Bahobail A.S.[2] [1.] Botany and Microbiology Department, Faculty of Science (Boys), Al-Azhar University, Cairo, Egypt. The present address: Biotechnology Department. Faculty of Science and Education- Al-Khurmah, Taif University; KSA. [2.] Biology Dept. Faculty of Science - Taif University; KSA. houssamatta@yahoo.com **Abstract:** This work was carried out for the biosynthesis of antifungal substance that demonstrated inhibitory effects against pathogenic fungi from *Streptomyces albidoflavus,* 143. The active metabolite was extracted using ethyl acetate (1:1, v/v) at pH 7.0. The separation of the active ingredient of the antifungal agent and its purification was performed using both thin layer chromatography (TLC) and column	2	Full Text

	chromatography (CC) techniques. The physico-chemical characteristics of the purified antibiotic viz. color, melting point, solubility, elemental analysis, spectroscopic characteristics and chemical reactions have been investigated. This analysis indicates a suggested imperical formula of $C_{22}H_{36}O_6$. The minimum inhibition concentrations "MICs" of the purified antifungal agent were also determined. The purified antifungal agent was suggestive of being belonging to Macrodiode antibiotic produced by *Streptomyces albidoflavus,* 143. [Houssam M. Atta, El-Sehrawi M.H. and Bahobail A.S. Antifungal Macrodiode Production By *Streptomyces albidoflavus*-143: Fermentation, Purification and Biological Activities. Journal of American Science 2011;7(3):13-22]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Antifungal Macrodiode; *Streptomyces albidoflavus*; Fermentation; Purification; Biological Activities		
3	**Analyzing Attitude of Managers and Senior Experts of Jihad-e-Keshavarzi Organization Regarding Development of Knowledge Management (KM) in Khuzestan Province, Iran** Ahmad Reza Ommani[1], Saeed Rajabalipour[2], Mohammad Aghapour[1] [1]Assistant Professor Agricultural Management Department, Islamic Azad University, Shoushtar Branch, Iran Ommani75451@yahoo.com [2]Graduate Student Islamic Azad University Shoushtar Branch **Abstract:** The purpose of this study was analyzing attitudes of managers and senior experts regarding the development of knowledge management in Jihad-e-Keshavarzi Organization of Khuzestan province. Senior experts and managers of organization were considered as a statistical population (N=100). All individuals were investigated. After confirm the validity of the instrument by panel of experts, to determine the reliability coefficient using Cronbach alpha coefficients were obtained for all sections of the questionnaire over 0.7 were calculated. Method of research was descriptive and correlative. Based on the results, the correlation between job motivation and status of knowledge acquisition and absorption with dependent variable in 0.01 level, was significant. Also between organizational culture and attitudes of managers in 0.05 level, correlations was significant. The results also showed that organizational culture, leadership style and knowledge about IT can explain 37% of variance of attitude of managers and senior experts regarding the development of knowledge management in Jihad-e-Keshavarzi Organization of Khuzestan Province, Iran. [Ahmad Reza Ommani, Saeed Rajabalipour, Mohammad Aghapour. **Analyzing Attitude of Managers and Senior Experts of Jihad-e-Keshavarzi Organization Regarding Development of Knowledge Management (KM) in Khuzestan Province, Iran.** Journal of American Science 2011;7(3):23-27]. (ISSN: 1545-1003). http://www.americanscience.org. Keywords: Knowledge Management, Jihad-e-Keshavarzi Organization, Attitude	Full Text	3
4	**Influences of physiographic factors on growth of Alder stands (*Alnus subcordata*) in north forest of Iran** S. A. Rezaei Taleshi[1] and Esmaeil Yasari[2] [1]Corresponding author: Agricultural and Natural Resource Researches Center of Mazandaran, Sari, Iran. Cell: +98- 09111541476. email: rezaietaleshi@yahoo.com 2 Payame Noor University, Sari, Iran. e_yassari@yahoo.com **Abstract:** Generally in old forestry plans, estimation of growth and yield has been processed by static methods without considering effective environmental variables as slopes, aspect (direction of slops), etc. In fact these estimations without taking the dynamic characters of stands into the consideration couldn't interpret actual timber and growth volumes. This study carried out with analysis of Alder stands criteria in physiographic factors in north forest of Iran. The results of statistical analysis showed that altitude less than 400 m in natural stands have minimum dbh growth. Height growth of Alders in natural stands has significant relationship with altitudes. An increase in altitude results in decrease the total height growth, whereas basal area growth decreases when altitude increases. Volume growth in high altitude (1200-1600 m.s.l.) has minimum growth in both plantation and natural area. Maximum growth of dbh (cm) and total height growth (m) in natural stands were seen in west aspect and minimum growth of dbh in natural area	Full Text	4

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Abstract: Nowadays tourism is considered as an important basis for sustainable development. Therefore, rural tourism is regarded as a makeable and essential element of tourism. Consequently, it is evident that strategic planning for rural tourism and the identification of internal factors (weak points and strong points) and environmental factors (opportunities and threats) have an important role in the development of rural regions and also the tourism industry. In this article, by means of the survey method and field study, we have tried to design strategies to develop rural tourism in Meymand village of Shahr Babak, Kerman Province, Iran, through specifying weak points and strong points, opportunities and threats in the form of a SWOT table. The results delineated that the strategy of "*extensive utilization of native methods to repair, construct and renovate the internal architecture of Meymand village*" is the most important and premier strategy among all rural tourism development strategies.
[Hamid Taboli, Mehdi Yadollah. **Tourism Development Strategies for Meymand Village of Kerman, Iran (By SWOT model).** Journal of American Science 2011;7(3):59-73]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Rural tourism, rural development, Meymand of Kerman, strategy, strategic planning

	Review required activities before participatory rural appraisal (PRA)	Full Text	
9	Mohammad Abedi[1] and Sharareh Khodamoradi[2] [1]Department of Agricultural Management, Islamic Azad University, Qaemshahr Branch, Iran [2]Department of Agricultural Extension Education, Science and Research Branch, Islamic Azad University, Tehran, Iran *Corresponding author: skhodamoradi2007@yahoo.com **Abstract:** Much of the spread of participatory rural appraisal (PRA) as an emerging family of approaches and methods has been lateral, South-South, through experiential learning and changes in behavior, with different local applications. Rapid spread has made quality assurance a concern, with dangers from "instant fashion", rushing, formalism and ruts. Promising potentials include farmers' own farming systems research, alternatives to questionnaire surveys, monitoring, evaluation and lateral spread by local people, empowerment of the poorer and weaker, and policy review. Changes in personal behavior and attitudes, and in organizational cultures, are implied. PRA parallels and resonates with paradigm shifts in the social and natural sciences, business management, and development thinking, supporting decentralization, local diversity, and personal responsibility. [Mohammad Abedi and Sharareh Khodamoradi. **Review required activities before participatory rural appraisal (PRA).** Journal of American Science 2011;7(3):74-81]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Participatory Rural Appraisal (PRA)	9
10	**Effect of a Specific Combination of Mannan-Oligosaccharides and -Glucans Extracted from Yeast Cell Wall on the Health Status and Growth Performance of Ochratoxicated Broiler Chickens** M. H. H. Awaad[1], A. M. Atta[2], Wafaa A. Abd El-Ghany[1], M. Elmenawey[2], K. Ahmed[3]; A. A. Hassan[4], A. A. Nada[4] and G. A. Abdelaleem[1] [1]Poultry Diseases Department, Faculty of Veterinary Medicine, Cairo University, Egypt. [2]Animal Production Department, Faculty of Agriculture, Cairo University, Egypt. [3]Pathology Department, Faculty of Veterinary Medicine, Cairo University, Egypt. [4]Animal Health Research Institute, Cairo, Egypt. wafaa.ghany@yahoo.com **Abstract:** The effect of a specific combination of Mannan-oligosaccharides (MOS) and -glucans extracted form the cell wall of a specific strain of *Saccharomyces cerevisiae* (AGRIMOS[®]) was	Full Text	10

investigated on zootechnical performance, ochratoxicosis and immune dysfunction caused by ochratoxin in broiler chickens. Three hundred and sixty, one day-old chickens were randomly allocated in a 2x2 factorial design for 5 weeks: supplementation of 2kg/ton of MOS (presence or absence) and feed contamination (presence or absence) with 50 µg/kg of ochratoxin A (OTA) for the first 3 weeks of life was done. Obtained results revealed that OTA did affect bird's growth one week after the contamination, although the final weight gain after 5 weeks was not different from the control. The use of AGRIMOS® stimulated the overall daily gain compared to the OTA group. Feed intake and feed conversion were not affected by the dietary treatments. Cumulative mortality was similar between treatments and performance indexes significantly improved with AGRIMOS® for the OTA challenged regimes. AGRIMOS® supplementation reduced macroscopic and microscopic lesion scores associated with ochratoxicosis. Also, it corrected the depression in phagocytosis induced by ochratoxin intoxication and it had strong immunomodulation as it stimulated the immune response to vaccination. It could be concluded that administration of a specific combination of Mannan-oligosaccharides and -glucans extracted form yeast cell wall (AGRIMOS®) to chickens improved zootechnical parameters had a potent immunomodulatory effect, evoked immune response and enhanced vaccination effectiveness. It helps not only in controlling chicken ochratoxicosis but also can play a positive role in treating chicken immune dysfunction.

[M. H. H. Awaad, A. M. Atta, Wafaa A. Abd El-Ghany, M. Elmenawey, K. Ahmed; A. A. Hassan, A. A. Nada and G. A. Abdelaleem. **Effect of a Specific Combination of Mannan-Oligosaccharides and -Glucans Extracted from Yeast Cell Wall on the Health Status and Growth Performance of Ochratoxicated Broiler Chickens.** Journal of American Science 2011;7(3):82-96]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Mannan-oligosaccharides**,** AGRIMOS®**,** -glucan**,** Ochratoxins, Chickens.

| 11 | **Pathological Studies on Experimental Systemic Candidiasis induced by Candida albicans Isolated from Different Animals in Immunosuppressed Mice** | Full Text | 11 |

Nahla AG. Ahmed Refat

Department of Pathology , Faculty of Veterinary Medicine, Zagazig University, Egypt
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Abstract: Fifty male mice (6 weeks old) were used to evaluate the severity of the pathological lesions induced by *Candida albicans* isolated from different animals (goats, sheep, cattle and buffaloes). The mice were immune suppressed by subcutaneous injection of 0.5 mg cortisone/kg B wt for 5 successive days before the beginning of the experiment and extended to the first 5 days after *Candida albicans* inoculation. These mice were randomly assigned to five groups (n=10). These groups intravenously (via tail vein) inoculated with 0.5 ml suspension of candida albicans 1×10^{6} *blastospores* isolated from goats (gp 1), sheep (gp 2), cattle, (gp3) or buffaloes (gp 4), besides the gp (5) which inoculated with phosphate buffer solution (PBS) as a control group. The clinical signs, mortalities and the gross lesions were recorded before different specimens from lungs, heart, liver, kidneys, spleen and brain collected and were routinely processed for histopathological examination.Multiple granulomas were detected replacing the pulmonary tissue, pleura, myocardium, hepatic and renal parenchyma of gps (1 and 2). Such granulomas were represented by central basophilic structurless mass containing blastospores, pseudohyphae, hyphae and oval yeast cells, 3-8 µm in diameter, surrounded by a thick zone of mononuclears mostly of macrophages and lymphocytes besides few polymorphnuclear cells. Fibrinonecrotic pseudomembranes and multifocal suppurative areas were observed in the pleura and pericardium. Meanwhile, the gps (3 and 4) showed minimal lesions and poor fungal growth besides lowering in mortalities from 70-80%(gps 1 and 2) to 30-40% (gps 3 and 4). Finally, it could be concluded that the *Candida albicans*, isolated from goats and sheep, induced severe multiple lesions than that isolated from cattle and buffaloes.

[Nahla AG. Ahmed Refat. **Pathological Studies on Experimental Systemic Candidiasis induced by Candida albicans Isolated from Different Animals in Immunosuppressed Mice.** Journal of American Science 2011;7(3):97-107]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Pathology, Experimental Systemic Candidiasis, Candida albicans**,** Mice

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| 12 | **Selective Antimicrobial properties of Leaf extract of Samanea Saman against Candida albicans, Staphylococcus aureus and Escherichia coli using several microbial techniques** | Full Text | 12 |

Raymond .C. Jagessar*[a], Akini. .Mars[a], Subramaniam Gomathinayagam[b]
*[a]Lecturer and Supervisor, Department of Chemistry, Faculty of Natural Sciences, Turkeyen Campus, University of Guyana, South America; [b]Microbiologist, Faculty of Agriculture and Forestry, Berbice Campus, John's, University of Guyana, South America; [a]Final Year Research student, Department of Biology, University of Guyana, Turkeyen Campus, South America. raymondjagessar@yahoo.com

ABSTRACT: Antibacterial and antifungal activities of Samanea Saman were investigated against pathogenic microorganisms: S.aureus (gram+ve), E.coli (gram-ve) and C.albicans using the Stokes disc diffusion, Well diffusion, streak plate methods and a dilution technique. The solvent type extracts were obtained by three extractions each with hexane, CH_2Cl_2, EtOAc and CH_3CH_2OH respectively. Solvents were removed in vacuo to yield viscous oils and paste which were made up to a concentration of 0.035g in 0.01L(10 mL) of the respective solvents. These were tested in varying volumes of 0.2-0.6ml/plate (i.e. concentrations of 0.03-0.18 mg/10 mL agar). Solvents were used as control whereas ampicillin and nystatin were used as references for bacteria and fungal species respectively. The solvents had no effect on the microorganisms whereas ampicillin and nystatin inhibited microbial growth. Saman Samanea showed selective antimicrobial inhibitory activity, with activity most prominent for the CH_3CH_2OH and CH_2Cl_2 extracts and negligible with the hexane. Its the first time in our study that the CH_2Cl_2 extracts is found to be more potent antimicrobially than the EtOAc extract. This study suggests that the CH_3CH_2OH and CH_2Cl_2 extracts of Saman Samanea can be used as herbal medicines in the control of E.coli and S.aureus and C.albicans induced diseases, following clinical trials.
[Raymond .C. Jagessar, Akini. .Mars, Subramaniam Gomathinayagam. **Selective Antimicrobial properties of Leaf extract of Samanea Saman against Candida albicans, Staphylococcus aureus and Escherichia coli using several microbial techniques.** Journal of American Science 2011;7(3):108-119]. (ISSN: 1545-1003). http://www.americanscience.org.
 Key words: Antimicrobial; *Samanea Saman; S.aureus; E.Coli*; *C. albicans*, Stokes Disc diffusion; Well diffusion; Streak plate; Dilution Method; Selective; herbal medicines.

Diagnosis of Recurrent Pyoderma in Dogs by Traditional and Molecular Based Diagnostic Assays and Its Therapeutic Approach

Full Text

*Wael, M. Kelany[1] and Husein, M. Galal[2]
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Abstract: Canine recurrent pyoderma is a common skin problem encountered in small animal practice and also resistant staphylococci may cause hazards in contact human. The main objective of the present investigation was to study the underlying etiologies of recurrent pyoderma and antimicrobial resistance guidelines of staphylococci on traditional and molecular basis. Also, the present workup was aimed to select satisfactory antimicrobial prescriptions for cases of recurrent pyoderma on empirical and bacteriological basis. A total number of 44 dogs were thoroughly examined for dermatological lesions and classified into 32 empirically treated dogs and 12 treated dogs based on bacteriological results. Pyoderma were classified into surface (13.6%), superficial (66%) and deep pyoderma (20.5%) with main clinical signs of pruritus, skin lesions (papules and pustules), marked alopecia (specially in superficial and deep pyoderma) and epidermal collarettes. The common recurrent pyoderma was German Shepherd pyoderma (38.6%) and the common pathogen was *Staphylococcus intermedius* (100%). *S. intermedius* was isolated alone in 58.3% and 41.7% in combination with *Corynebacterium spp.* and *Staphylococcus aureus* from skin of 12 examined dogs. The present study was recorded multidrug resistance exhibited by 75% of the 12 *S. intermedius* isolates. Oxacillin MIC testing revealed 6 *S. intermedius* isolates (50%) to be resistant, which included 2 strains with the mecA gene. The mec A (Methicillin resistant *Staphylococcus intermedius*, MRSI) was detected by PCR in 5 isolates (41.7%). Amoxicillin-clavulinic acid, cephalosporines and flouroquinolones were achieved magic results on empirical and antibiogram basis in treatment of idiopathic recurrent pyoderma. It was concluded that our data provided the first Egyptian guidelines in companion animals for common bacterial pathogens with antibiogram for bacterial resistance

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Abstract: This paper presents many trials to repair a diesel engine cylinder head made of pearlitic grey cast iron, which was used in a truck. The cylinder head was repaired due to the existence of cracks at the junction between the valve seat and the spark plug seat. Shielded metal arc welding (SMAW) process using different electrodes was applied, the increase in preheating temperature resulted in a formation of a continuous carbide layer in the partial fusion zone and a decrease in the Martensite formed at the heat affected zone. However, the decrease in preheat temperature resulted in an increase of Martensite at the heat affected zone and a decrease in the carbide layer at the partial fusion zone. Most of the SMAW electrodes resulted in the formation of regions with high hardness values which imply that the repair welding of the cylinder head using these electrodes is inefficient. Application of the powder flame spray method in repair welding of the cylinder head resulted in partial fusion zone and heat affected zone with hardness values comparable to that of base metal. Preheating in furnace to 500 °C then immediately putting the specimen in the furnace at the same temperature for 1 hour after applying powder flame spray gave excellent hardness results for the heat affected zone (HAZ) and partial fusion zone (PFZ).

Keyword: Failure, Cylinder head, Pearlitic gray iron, SMAW, Flame spray method, Heat affected zone, Partial fusion zone

| 17 | **Improving gladiolus growth, flower keeping quality by using some vitamins application** [1]Bedour, A. Abo Leila and [2]*Rawia, A. Eid
Water Relations and Field Irrigation Dept., Ornamental Plant and Woody Trees Dept., National Research Centre, Dokki, Cairo, Egypt. *Corresponding author, emil,rawiaabdelhady@yahoo.com.

Abstract: Response of growth, flowering quality and active chemical constituents of gladiolus plants by using some vitamins such as, thiamin ascorbic acid and their combination during two seasons were studied .Plant which received the combined treatments of both vitamins recorded the highest growth, flowers quality and cormelets induction. Thiamine treatments had the lowest effect on photosynthetic pigments, while 200 ppm, thiamin+ ascorbic acid+ppm, improved growth, delayed flowering opening of vase life , stimulated accumulation of carbohydrate and increased photosynthetic pigments and macronutrients status. Photosynthetic pigments and macronutrients.

Keywords: gladiolus plant flower quality, vase life and chemical constituents | Full Text | 17 |
| 18 | **Productivity Changes of Hoteling Industry in Iran**
(Case study: Kadoos Hotel of Guilan)

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Department of Management, Islamic Azad University, Rasht Branch, Ira
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ABSTRACT - Productivity is the standard by which human power in using resources to achieve their desired goal is determined. In recent years this standard has seen an up most attention in calculating technologies in industrial and service productivity industries. Value added methodology is a comprehensive way of measuring producing of all producing factors in a particular unit. In this case study we have interested recent changes in the hoteling Industry in Iran, along with our investigation we have selected kadoos hotel. A 5 stars hotel located in Giulan of Iran as our case study we have analyzed the productivity of this hotel in the period of 5 years 2005 – 2009. Results have shown that the foreign rate of occupation of hotel rooms – has increased in this hotel. This in turn has resulted in an increase in hotel revenue. But still we have not seen any creativity and change in other parts of the hotel such as food and beverage. There has not been an optimal use in new capitals and equipments in this hotel. To see more | Full Text | 18 |

	improvement In the hoteling industry in Iran, we have to have new knowledge in the field of quality management and connections to the world net of hoteling. [Mohammad Taleghani. **Productivity Changes of Hoteling Industry in Iran (Case study: Kadoos Hotel of Guilan).** Journal of American Science 2011;7(3):175-178]. (ISSN: 1545-1003). http://www.americanscience.org. keywords: Productivity, Hoteling Industry, Tourism, Iran, Guilan.		
19	**siRNA Mediated-hTERT Knockdown Impedes Proliferation of Mammalian Cancer MCF7 and HepG2 Cells** Abeer Mostafa El-Sayed Ashmawy[*1], Essam Shawky A.El H. khattab[2], Wael Bakry Abd El-Aziz Anbar[3], Bakry Mohamed Haroun[3], Sabry Mohamed Shaarawy[1]. [1]Tumor Biology Department, National Cancer Institute, Cairo University,[2]Chemistry Department (Biochemistry Division), [3]Microbiology Department, faculty of science, Al-Azhar University *abeermanci@yahoo.com **Abstract:** Telomerase is an attractive molecular target for cancer therapy because it is present in most malignant cells but is undetectable in most normal somatic cells. Human telomerase consists of two subunits, an RNA component (hTR) and a human telomerase reverse transcriptase component (hTERT). Small interfering RNA (siRNA), one kind of RNA interferences, has been demonstrated to be an effective method to inhibit target gene expression in human cells. We investigated the effects of siRNA targeting both hTERT mRNA and protein expression on the inhibition of proliferation and growth of human breast carcinoma cells (MCF-7) and liver carcinoma cells (HEPG-2). Here we used two siRNAs sequences (siRNA#1 and siRNA#2) that differentially target hTERT. Our results revealed that treatment of MCF7 and HepG2 cells with either of hTERT siRNAs resulted in significant decrease in both mRNA ($p<0.05$) and hTERT protein expression ($p<0.05$). Summary, our results clearly demonstrate that siRNA mediated knockdown of telomerase has efficiently suppressed proliferation rate of MCF7andHepG2cells. From these findings, we propose that targeting telomerase using siRNA might be a rational approach in cancer therapy. [Abeer Mostafa El-Sayed Ashmawy, Essam Shawky A. El H. khattab, Wael Bakry Abd El-Aziz Anbar, Bakry Mohamed Haroun, Sabry Mohamed Shaarawy. **siRNA Mediated-hTERT Knockdown Impedes Proliferation of Mammalian Cancer MCF7 and HepG2 Cells.** Journal of American Science 2011;7(3):179-186]. (ISSN: 1545-1003). http://www.americanscience.org. **Key words:** telomerase knockdown, proliferation, siRNA, liver carcinoma, breast carcinoma	Full Text	19
20	**Agronomical and Biochemical Responses of White *Lupinus albus* L. Genotypes to Contrasting Water Regimes and Inoculation Treatments** Manal M. Hefny Agronomy Department, Faculty of Agriculture, Suez Canal University, Ismailia 41522, Egypt manhef@yahoo.com **Abstract:** Two field experiments were conducted over the growing season November 15 – April 15- 2008-2009 and 2009/ 2010 at the experimental farm of Suez Canal University, Ismailia .The purpose of this research was to study the effects of water stress and inoculation treatments on the yield, growth parameters and biochemical traits under field conditions and during two growing seasons. The experimental design for both seasons was randomized complete block in split-split plot arrangement with three replications. Where Irrigation treatments included normal (W0) and water stressed (Ws) were allocated to main-plots, two inoculation treatments: no-inoculation and inoculation with commercial inoculums were assigned to sub-plots. Five lupin genotypes including two cultivated varieties (Giza 1 and Giza 2) and three landraces (LR 1, LR 2 and LR 3) constituted the sub-sub-plots. Significant differences of irrigation, inoculation, genotype and their different interactions were detected for the most measured traits. Water stress reduced yield and growth parameters, whereas antioxidant enzyme activities were increased significantly as plants exposed to limited irrigation. Protein % was not affected by water limitation at both seasons, while 100-seeds weight was significantly affected in the first year only. There were potential beneficial effects of commercial	Full Text	20

inoculation, where it increased yield and growth parameters under water shortage condition and reduced enzyme activities. The landrace LR 1 is obviously, the best genotype in seeds yield, growth parameters over the two growing seasons and high activity of defense mechanism (activity of catalase and peroxidase enzymes) under water stress conditions and over all inoculation treatments. Thus it is considered a promising line under water limited environments.

[Manal M. Hefny. **Agronomical and Biochemical Responses of White *Lupinus albus* L. Genotypes to Contrasting Water Regimes and Inoculation Treatments.** Journal of American Science 2011;7(3):187-198]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: antioxidant enzymes; drought; inoculation; Lupine; yield

Vascular Endothelial Growth Factor (VEGF) Gene Insertion/Deletion Polymorphism and Diabetic Retinopathy in Patients with Type 2 Diabetes

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Abstract: Background: Vascular endothelial growth factor (VEGF) appears to play a central role in mediating microvascular pathology in diabetic retinopathy (DR). Aim of the study: To assess the possible association of the insertion/deletion (I/D) polymorphism of VEGF gene with diabetic retinopathy in Egyptian patients with type 2 diabetes mellitus. Subjects and Methods: This cross-sectional case-control study enrolled 87 unrelated subjects with type 2 diabetes mellitus, 43 diabetic patients without signs of retinopathy but did have type 2 diabetes for more than 10 years and 44 patients with diabetic retinopathy. The control group involved 44 normal subjects without diabetes. Total genomic DNA was isolated from peripheral blood leukocytes. PCR analysis was conducted to detect the insertion/deletion gene polymorphism of the 18 bp fragment at position 2549 of the promoter region of VEGF. The frequency of D and I VEGF alleles and genotype distribution were compared in diabetics with retinopathy, diabetics without retinopathy and the control subjects. Results: There was no significant difference in genotype distribution (D/D, I/D and I/I), (p=0.43) and in (D and I) allele frequency (p=0.093) between diabetic patients with retinopathy, diabetics without retinopathy and control subjects. The distribution of the VEGF, D/D genotype was higher in patients with diabetic retinopathy compared with diabetic group without retinopathy and healthy controls (40.9% *vs*. 27.9% and 22.7% respectively), however the difference was still not statistically significant with Chi-Square= 3.637 and p value = 0.162. Despite the insignificant results, this study adjusted OR of 2.25 (95% CI, 0.672- 7.538) for D/D genotype versus I/I genotype between diabetic patients with retinopathy and controls with p value = 0.185 and the OR of 1.6 (95% CI, 0.873- 2.891)   for the D allele versus I allele between diabetic patients with retinopathy and controls with p= 0.129, while the OR of the D allele versus I allele between diabetic patients without retinopathy and controls was only 1.2 and the p value was 0.539. In multivariate analysis only increased triglyceride level was the independent risk factor for diabetic retinopathy among Egyptian patients with type 2 diabetes. Conclusion: Our study suggested that I/D polymorphism in the promoter region of the VEGF gene was not significantly associated with retinopathy in Egyptian type 2 diabetic patients, however a moderate risk (i.e., OR, < 2 for D/D genotype and < 1.5 for D allele) could not be excluded. Only increased triglyceride level was the independent risk factor in the development of diabetic retinopathy detected in this study.

[Hanan Fouad; Mona A. Abdel Hamid; Amira A. Abdel Azeem; Hany M. Labib and Nervana A. Khalaf.

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	Vascular Endothelial Growth Factor (VEGF) Gene Insertion/Deletion Polymorphism and Diabetic Retinopathy in Patients with Type 2 Diabetes. Journal of American Science 2011;7(3):199-205]. (ISSN: 1545-1003). http://www.americanscience.org. **Key words:** Vascular endothelial growth factor (VEGF), insertion/deletion polymorphism, diabetic retinopathy, type 2 diabetes, Egyptian patients.		
22	**Ion Recombination Correction Factor Dependence on the Field Size, Depth, Nominal Dose Rate and Stem Length in Therapeutic Photon Beams (6 And 10 MV)** A. I. Abd El-Hafez[1], Hany A. Shousha[*1], M. S. Zaghloul[2] and M. A. AbouZeid[3] [1]Radiation Metrology Dept., National Institute for Standards (NIS), Giza, Egypt. [2] National Cancer Institute (NCI) - Cairo University, Cairo, Egypt. [3] Faculty of Science - Mansoura University, Al-Mansoura, Egypt. [*]drshousha@yahoo.com **Abstract:** The use of ionization chamber in linear accelerator radiotherapy photon dosimetry requires various corrections to the measured charges, one of these being the ion recombination correction factor (k_s). As stated by the IAEA (2000) TRS-398 dosimetry protocol, k_s was characterized for the available thimble ionization chamber PTW 30006 using two pulsed megavoltage photon beams 6 and 10 MV. The dependence of the k_s values against the changing of field size, water depth, nominal dose rate and stem length was studied. For photon energy 10 MV, k_s shows an increase with the field size and for photon energy 6 MV, k_s values decrease from field size 4x4 cm^2 to 10x10 cm^2 and increase at field sizes larger than 10x10 cm2. Also, k_s values are inversely proportional with the water depth and directly proportional with the nominal dose rate and stem length, for both photon energies. It is also recommended to determine the absorbed dose at lower (*p.r.f*) pulse repetition frequency or nominal dose rate and if the dose is determined at the highest (*p.r.f*), a correction must be introduced in the assessment of the dose related to the ion recombination correction factor k_s difference at different *p.r.fs*. These measurements help to correct k_s values at different dosimetry conditions and minimize the errors in the assessment of the radiotherapeutic dose calculations. [A. I. Abd El-Hafez, Hany A. Shousha, M. S. Zaghloul and M. A. AbouZeid. **Ion Recombination Correction Factor Dependence on the Field Size, Depth, Nominal Dose Rate and Stem Length in Therapeutic Photon Beams (6 And 10 MV).** Journal of American Science 2011;7(3):206-213]. (ISSN: 1545-1003). http://www.americanscience.org. **Key words:** Ion recombination correction factor, ionization chamber, megavoltage x-ray, pulsed photon beams and linear accelerator	Full Text	22
23	**Curative effect of basil on liver injury in experimental rats** Soha Mohamed Sameh El-safty Home Economics Dept, Faculty of Education, Suez University, Egypt Ma_saadany@yahoo.com Abstract: Forty two albino male rats were classified into six groups. Group I (n=7) served as control (-ve), and animals in groups II–VI CCl4 were induced liver injury. Group II served as control(+ve) and treated groups from III to VI rats received daily oral doses of ursofalk drug , basil ethanolic extract, basil aqua extract, and basil powder. The results revealed that control (+ve) rat group showed a significant decrease in final body weight, body weight gain, food intake & food efficiency ratio (FER); serum total protein, globulin, glutathione transferase (GST) & catalase and liver triglyceride, total lipid, superoxide dismutase (SOD), glutathione peroxidase (GPX) & GST but a significant increase in serum alanine and aspartate aminotransferase , alkaline phosphates, gamma glutamyle peptidase (ALT, AST, ALP & GT), total bilirubin & nitrite (NO); albumin/ globulin ratio and liver glycogen, cholesterol & malondialdehyde (MDA) compared with control (-ve) group. All treated groups showed a significant decrease in body weight gain; serum globulin, GST& catalase and liver glycogen but a significant increase in serum ALP,	Full Text	23

total bilirubin & NO and albumin/ globulin ratio compared with control (- ve) group. Drug group showed a significant increase in serum AST & total bilirubin and liver cholesterol and MDA but a significant decrease in liver triglyceride, total lipid, SOD, GPX, GST compared with control (- ve) group. Basil ethanol extract and basil aqua extract rat showed a significant increase in serum ALT albumin/ globulin ratio and liver cholesterol & MDA and a significant decrease in serum total protein, liver triglyceride while basil powder showed a significant increase in serum ALT, AST, GT and albumin/ globulin ratio and a significant decrease in serum total protein, albumin, liver total lipid and liver SOD compared with control (-ve).

[Soha Mohamed Sameh El-safty. Curative effect of basil on liver injury in experimental rats. Journal of American Science 2011;7(3):214-220]. (ISSN: 1545-1003). http://www.americanscience.org.

Key word: Basil- Liver injury- Rats

| 24 | Effect of thyme powder, extract and oil on carbon tetrachloride-induced liver injury

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Abstract: Forty -two albino male rats, Sprague Dawley strain were randomly classified into six groups (7 rats each). One served as control (-ve) group while the other groups were administered CCL4 to induce liver injury which were control (+ve), silymarin, thyme powder, thyme extract and thyme oil rat groups. The results showed that control (+ve) rat group showed a significant decrease in final body weight , body weight gain ,food efficiency ratio (FER) , blood hemoglobin, packed cell volume & glutathione (GSH) , serum total protein & globulin and liver GSH , superoxide dismutase (SOD), glutathione peroxidase (GPX) ,glycogen and triglyceride. Moreover , showed a significant increase in blood malondialdehyde (MDA) ,serum alanine and aspartate aminotransferase , alkaline phosphates, gamma glutamyle peptidase (ALT, AST, ALP & GT) enzymes activity ,total bilirubin ,A/G ratio and liver MDA, cholesterol and total lipid compared with control (-ve) group. Silymarin showed a significant decrease in final weight, hemoglobin, blood GSH, liver GSH, SOD & glycogen and a significant increase in serum AST& MDA and liver cholesterol. Thyme powder showed a significant decrease in final weight, blood GSH & MDA and liver GSH ,SOD and glycogen and a significant increase in serum ALT, AST, ALP and GT enzymes activity but thyme oil showed a significant decrease in hemoglobin ,liver glycogen and significant increase in the values of liver cholesterol compared with control (-ve) group. The all treated rat groups showed a significant increase in serum total bilirubin, A/G ratio and liver MDA, triglyceride & total lipid and a significant decrease in body weight gain ,FER, serum globulin and liver GPX compared with control (-ve) group.
[Nawal .A. Al Badr. Effect of thyme powder, extract and oil on carbon tetrachloride-induced liver injury. Journal of American Science 2011;7(3):221-227]. (ISSN: 1545-1003). http://www.americanscience.org.

Key words: thyme leaves; oil ,extract; liver; rats | Full Text | 24 |

| 25 | **Fruit physical and chemical characteristics at maturity stage of Tommy Atkins, Keitt and Kent mango cultivars grown under Nubariya conditions**

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Abstract: The present study was conducted at orchard located at the side of Alexandria desert road (Cairo - Alexandria, Km. 140), at Nubariya region during 2007 and 2008 seasons. The three experimented mango cultivars were Tommy Atkins, Kent and Keitt. The trees of the three studied cultivars are grafted on seeded rootstocks, attained nine years old and grown in sandy Soil .Trees of all mango cvs. Were planted at 3×5 meter apart and subjected to the same horticultural practices **.**The objective of the present study is | Full Text | 25 |

evaluating some fruit physical and chemical characteristics at maturity stage of Tommy Atkins, Kent and Keitt mango cultivars grown under Nubariya conditions. The obtained results can be summarized as follows: The observation besides the analysing of some physical and chemical characteristics of the fruits indicated that the physiological maturity was attained in Tommy Atkins cvs. at fruit age 113 day, Kent and Keitt cvs. at fruit age 122 day. Keitt cv. had the highest values of pulp percentage of weight followed by Kent cv. while, Tommy Atkins cv. had the lowest values of pulp percentage of weight. Meanwhile Tommy Atkins cv. had the highest percentage of peel of weight followed by Kent cv., while Keitt cv. had the lowest percentage of peel of weight. Tommy Atkins cv. had the highest percentage of seed of weight followed by Kent cv. while, Keitt cv. had the lowest percentage of seed of weight. Keitt cv. had the highest seed length, followed by Tommy Atkins cv. while, Kent cv. had the lowest values in the two seasons. The highest seed width was Tommy Atkins cv., followed by Kent cv. meanwhile; Keitt cv. had lowest values in the two seasons. Ascorbic acid (vitamin c) percentage was lowest in Kent cv. compared to Tommy Atkins cv. which had the highest value; Keitt cv. had intermediate values in the two seasons. Moisture percentage was almost the same with no significant difference among cvs. Tommy Atkins cv. had the highest moisture percentage, followed by Keitt cv. while, Kent cv. had the lowest moisture percentage in the two seasons. Kent cv. had the highest dry matter content percentage, followed by Tommy Atkins cv. while, Keitt cv. had the lowest value in the two seasons. Total sugars percentage was highest in Kent cv., followed by Keitt cv. while, Tommy Atkins cv. had the lowest total sugars percentage in the two seasons. Tommy Atkins cv. had the highest crude fiber percentage, followed by Keitt cv. while, Kent cv. had the lowest crude fiber percentage in the two seasons.

| 26 | **Effect of Aqueous Extract of Damsissa(*Ambrosia maritima*) on The Biochemical Changes Induced By Potassium Dichromate In Rats** | Full Text | 26 |

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Abstract: Chromium is a naturally occurring element found in volcanic dust, in earth crust and is widely distributed in air, water, rocks, soil, plants and animals**.** Humans are occupationally, environmentally, or intrinsically (Surgery implants), exposed to high Cr^{6+} concentrations (8.9 mg/m^3, 20mg/L, 890 mg/kg) respectively. The general population may be exposed to Cr^{6+} compounds through inhalation of ambient air , ingestion of water ,or dermal contact with products that contain chromium (VI) compounds such as pressure treated wood**.**The present study aims to evaluate the antioxidant effect of aqueous extract of Damsissa (Ambrosia maritima) against biochemical changes induced by potassium dichromate in rats. The study was conducted on 48 rats which were classified into four equal groups.Group I : untreated animals (control).Group II: Damsissa treated group: rats were orally supplemented with aqueous extract of damsissa at dose of 100 mg/ kg b.wt. for 14days using stomach tube. Group III: Potassium dichromate treated group, animals injected subcutaneously with potassium dichromate at dose of 10 mg/kg b.wt. for fourteen days , then the half number of the animals sacrificed and the remaining animal left without any treatment for seven days(recovery period).Group IV: Combined treatment group animals were orally administrated with aqueous extract of damsissa by means of stomach tube at dose of 100 mg/kg b.wt. and injected subcutaneous with potassium dichromate at dose of 10 mg/kg for two weeks and the half number of the animals sacrificed and the remaining left without any treatment for one week. Six rats from different groups were sacrificed after 14 days and the rest were left for 7 days as a recovery period. The obtained results revealed significant increase in TBARS concentration which accompanied with significant decrease in GSH content and CAT activity in renal tissue in treated group with potassium dichromate also,

significant increase in urea and creatinine was recorded. The serum levels of sodium significantly increased and level of potassium significantly decreased as a consequence decrease in aldosterone level. Calcium and estradiol (E2) levels significantly decreased. However, the levels of phosphorous (P), magnesium (Mg) and parathermone hormone (PTH) were significantly increased in animals injected with potassium dichromate. Consecutive administration of aqueous extract of damsissa with potassium dichromate for 14 days revealed significant improvement in the tested parameters. Also, animals injected with potassium dichromate and left without any treatment for one week as a recovery period showed significant improvement in some of the tested parameters. In conclusion, the results demonstrate the protective role of damsissa against oxidative stress and biochemical changes of potassium dichromate. [N. E. Mohamed and R. A. Saber. **Effect of Aqueous Extract of Damsissa (*Ambrosia maritima*) on The Biochemical Changes Induced By Potassium Dichromate In Rats.** Journal of American Science 2011;7(3):234-242]. (ISSN: 1545-1003). http://www.americanscience.org.

Key Words: *Ambrosia maritima*, Potassium dichromate, Kidney function , Radioimmunoassay, Oxidative stress

27	**Detection of Greening in Potatoes using Image Processing Techniques** Ebrahim Ebrahimi[1,*], Kaveh Mollazade[2], Arman Arefi[3] [1,*] Department of Mechanical Engineering of Agricultural Machinery, Faculty of Engineering, Islamic Azad University, Kermanshah Branch, Kermanshah, Iran. ebrahimi.kiu@gmail.com [2]Department of Agricultural Machinery Engineering, Faculty of Agricultural Engineering and Technology, University of Tehran, P.O. Box 4111, Karaj 31587-77871, Iran. [3]Department of Agricultural Machinery Engineering, College of Agriculture, Urmia University, Urmia, Iran. **Abstract:** Quality is one of the important factors in marketing of agricultural products. Grading machines have great importance in the quality inspection systems. Most of the current grading machines operate based on machine vision systems to detect blemishes and defects of products, where one image or more are taken for each individual object and the results of processing will decide the quality of the object. One of the major blemishes in potatoes is physiological skin greening, which has negative influence on human health. In this research, a simple machine vision algorithm has been developed in order to detect physiological skin greening of potato tubers rapidly and precisely. The experimental image acquisition setup was consisted of an image capturing box equipped with lighting system, a color CCD camera, and a capturing card. The data set consisted of 25 images of potatoes with physiological skin greening blemishes. Image pre-preprocessing has been carried out to modify the non-uniform distribution of background light intensity. Since potatoes have bright skin, the CCD was saturated in a small part of each image. These parts were eliminated from the images using a relation found between RGB and HSI spaces. The difference between red and green components of RGB space for green parts of potatoes was lower than that of other parts. Finally, the $1.02R - G$ relation was found to be suitable for detection of green parts of potato tubers. The average of error between actual green parts area and estimated green parts area for 25 images was 5.26%. [E. Ebrahimi**,** K. Mollazade**,** A. Arefi. Detection of Greening in Potatoes using Image Processing Techniques. Journal of American Science 2011;7(3):243-247]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Automation; machine vision; potato blemish.	Full Text	27
28	**Effects of irreversible different parameters on performance of air standard Otto cycle** Reza Masoudi Nejad [1], Iman Soleimani Marghmaleki [1], Rouhollah Hoseini [2], Pouyan Alaei [3] [1] School of Engineering, Shahrekord University, Shahrekord, Iran [2] MS student of Mechanical Engineering, Sharif University of Technology, Tehran, Iran [3] Mechanics laboratory, Hafez Avenue, Shahrekord, Iran	Full Text	28

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Abstract: An irreversible air standard Otto cycle model is proposed in this paper. The performance of an air-standard Otto cycle with heat transfer loss and variable specific heats of working fluid is analyzed by using finite-time thermodynamics. They are generalized formulas for internal combustion engines because they include the performance characteristic of special cases of Otto engines. The objective of this study is to analyze the effects of heat loss characterized by a percentage of the fuel's energy, friction and variable specific heats of working fluid on the performance of an air standard Otto cycle with a restriction of maximum cycle temperature. A more realistic and precise relationship between the fuel's chemical energy and the heat leakage that is based on a pair of inequalities is derived through the resulting temperature. The power output and the working range of the cycle increase with the increase of specific heats of the working fluid, while the efficiency decreases with the increase of specific heats of the working fluid. The friction loss has a negative effect on the performance. The results obtained in the present study are of importance to provide good guidance for performance evaluation and improvement of practical Otto engines.
[Reza Masoudi Nejad, Iman Soleimani Marghmaleki, Rouhollah Hoseini, Pouyan Alaei. Effects of irreversible different parameters on performance of air standard Otto cycle. Journal of American Science 2011;7(3):248-254]. (ISSN: 1545-1003). http://www.americanscience.org.

Key words: Otto cycle; Heat leakage; Friction; Irreversible; Variable specific heat

29	**The Perceptions of Graduate Students about Factors Influencing the Extension of Entrepreneurship Education in College of Agriculture and Natural Resources in Iran** Seyed Jamal F.Hosseini [1], Heidar Ahmadi [2], Maryam Omidi Najafabadi [1] [1.] Department of Agricultural Extension and Education, Science and Research Branch, Islamic Azad University, Tehran, Iran [2.] Department of Natural Resources Extension and Education, Science and Research Branch, Islamic Azad University, Tehran, Iran jamalfhosseini@srbiau.ac.ir **Abstract:** Graduate students at the college of agriculture and natural resources were surveyed in order to explore their perception about the factors influencing the extension of entrepreneurship education in the Science and Research Branch at Islamic Azad University. The methodology used in this study involved a combination of descriptive and quantitative research. The total population was 313 master and doctorate students majoring in agriculture. The results of regression analysis showed that 50% of the variance in the perception of respondents could be explained by tendency toward being successful, being innovative, entrepreneurship education in universities, role of instructor and educational contents. [Seyed Jamal F.Hosseini, Heidar Ahmadi, Maryam Omidi Najafabadi. **The perceptions of graduate students in the college of agriculture and natural resources about factors influencing the extension of entrepreneurship education in Islamic Azad University, Science and Research Branch**. Journal of American Science 2011;7(3):255-259]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** entrepreneurship, extension, college of agriculture, graduate students	Full Text	29
30	**Cigarette Smoking among University Students: Family- related & Personal risk factors** Ghada F. El-Sharkawy Public Health & Community Medicine Department, Faculty of Medicine, Zagazig University, Egypt Address: Department of community medicine and public health, Faculty of Medicine- Zagazig - Egypt. E-mail: ghadaelsharkawy@hotmail.com. Mobile: 002 0121407940 **ABSTRACT:** Smoking is considered as an increasing epidemic among youth. This threats with increasing	Full Text	30

epidemics of chronic non-communicable diseases. <u>Objective</u>: The objectives of this study were to have a recent estimate of smoking problem among university students in Egypt and to identify possible risk factors related to family life & personal aspects. <u>Methods</u>: This cross-sectional study included a representative sample of 1072 Egyptian University students. The students were interviewed and asked to complete a modified questionnaire derived from the standard questionnaire of National Center for Social and Criminal Research. <u>Results</u>: The prevalence of current smoking was (20.2%) while ever smoking was (22.1%). Friends were the first ranked motive for smoking (37.2%%) followed by family related derives (13.8 %) then sense of hopeless future (12.2 %). Personal risk factors for smoking were young age, males gender, studying in theoretical faculties and suffering a chronic health problem. The important family-related risk factors were large number of the family, late order among siblings and living away from the family. <u>Conclusion</u>: This study determined some personal& family- related factors contribute in smoking problem. Dealing with them by coordinated efforts of the family, school, university and government will alleviate smoking problem among youth.

[Ghada F. El-Sharkawy. **Cigarette Smoking among University Students: Family- related & Personal risk factors.** Journal of American Science 2011;7(3):260-268]. (ISSN: 1545-1003). http://www.americanscience.org.

Keyworks: Cigarette; Smoking; University; Student; risk factor

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Effects of educational program on Insight Into Illness and Attitudes Toward Medications Among Schizophrenic patients

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Abstract: This study assessed the impact of the constructed nursing educational program on insight and attitudes toward medications in a sample of schizophrenic patients who randomly selected. A quasi-experimental design was utilized in this study. The study was conducted in in-patient clinic at Benha governmental hospital for mental health. A total sample of 40 schizophrenic patients (experimental group 20 and control group 20) was selected randomly to participate in the study. Three tools were used for data collection, socio-demographic/ medical data sheet, Insight Scale , and Drug Attitude Inventory Scale. Findings of this study proved the effectiveness of the constructed educational program on schizophrenic patients' insight, whereas no effect on patients' attitude toward medications .The total insight and patients' attitude toward medications were not correlated significantly with number of hospital admission, duration of illness and age at onset of the disease. Patients' attitude toward medications didn't correlated significantly with insight at pre, post, and follow-up program. Therefore the study highlight there is a need to conduct educational sessions periodically for the schizophrenic patients, a matter which increase the schizophrenic insight into illness.

[Yosr Mohamed Elmasri. **Effects of educational program on Insight Into Illness and Attitudes Toward Medications Among Schizophrenic patients.** Journal of American Science 2011;7(3):269-277]. (ISSN: 1545-1003). http://www.americanscience.org.

Key words: schizophrenia- attitude toward medications - insight into illness

Full Text

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-Methyl Crotonitrile in Synthesis of Some New Compounds and Evaluation of Their Herbicidal Efficiency

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Abstract: In an effort to establish new candidates with improved antiherbal activities we report here the

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	synthesis and herbicidal evaluation of various series of -methyl crotonitrile benzoxazoles, -(benzoxazole-2-yl)- -cycloalkylidene crotonitrile (**3**) and 3-(benzoxazole-2-yl)-2-mercapto-4- methyl-6-pyridinethione (**10**) together with the synthesis of some substituted benzoxazolyl anilines(**5,7,9**).The herbicidal evaluation of these compounds was carried out on wheat as pattern for monocotyledonous plants under laboratory conditions. Three plant parameters, seed germination, root and shoot growth of wheat seeds were taken as indicators for the herbicidal efficiency of the newly synthesized compounds. The most active compounds that showed an observable inhibition effect on the process of germination, root and shoot growth or one of them were (**3**),(**5b**),(**5c**),(**9a**) and (**9c**) so that, they were rescreened by a serial of concentrations to stand on the most potent derivative. Their EC_{50} values were calculated and showed that compound (**9a**) was the most potent and greatly inhibited shoot growth (EC_{50}, 1.4mg/ml). [S.E.S. Hamouda, Nermeen. S. Abbas, S.M.A. Sherif, and A.M.A. Elkady. **-Methyl Crotonitrile in Synthesis of Some New Compounds and Evaluation of Their Herbicidal Efficiency.** Journal of American Science 2011;7(3):278-286]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords**: -Methylcrotonitriles, cycloalkylidenecrotonitrile pyridinethione, benzoxazolyl anilines, wheat, monocotyledonous plants, growth parameters and herbicidal efficiency.		
33	**Phenolic Metabolites from *Acacia nilotica* Flowers and Evaluation of its Free Radical Scavenging Activity** Sayed A. El-toumy[1,*], Samy M. Mohamed[2], Emad M.Hassan[2], Abdel-Tawab H. Mossa[3] [1]Chemistry of Tannins Department, [2]Medicinal and aromatic plants Department [3]Environmental Toxicology Research Unit (ETRU), Pesticide Chemistry Department, National Research Center, 12622 Dokki, Cairo, Egypt, [*] sayedeltomy@yahoo.com **Abstract:** The study of the chemical constituents of the flowers of *Acacia nilotica* has resulted in the isolation and characterization of nine compounds. These compounds were identified as catechin (**1**), catechin 7-*O*- gallate (**2**), gallic acid (**3**), naringenin 7-*O*- - glucopyranoside (**4**), quercetin 3-*O*- - glucoside (2 1) glucopyranoside (**5**), quercetin 3-*O*- -glucopyranoside (**6**), chalconaringenin 4 -*O*- - glucopyranoside (**7**), naringenin (**8**) and quercetin (**9**), which were isolated for the first time from *Acacia nilotica* flowers. The above compounds were individually identified by spectroscopic analyses and were compared with reported data. The total amount of phenolic compounds of the aqueous methanol extract and fractions was determined by ultraviolet (UV) spectrometry and calculated as gallic acid equivalents. The antioxidant potential of *Acacia nilotica* extract and fractions has been investigated by DPPH radical scavenging assay. [Sayed A. El-toumy, Samy M. Mohamed, Emad M.Hassan, Abdel-Tawab H. Mossa. **Phenolic Metabolites from *Acacia nilotica* Flowers and Evaluation of its Free Radical Scavenging Activity.** Journal of American Science 2011;7(3):287-295]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** *Acacia nilotica*; total phenolic compounds; antioxidant activity; DPPH	Full Text	33
34	**Effect of Hyperthermia at Different Ages and Mode of Recovery on the Chromosomal Aberrations and Biological Parameters in Female Rats.** Amal I Hassan[1] and Abeer H. Abd El-Rahim[2] [1]Radioisotope Department, Nuclear Research Centre, Atomic Energy Authority, Egypt. [2] Department of Cell Biology, National Research Center, Egypt. [*]aml_h@hotmail.com **Abstract:** The present study was designed to investigate the various biological changes induced by hyperthermia (at 42°C) in female rats and the mode of recovery at 1, 6 , 24 & 72 hrs at different ages (2, 6 , 12 & 24 months). Biological parameters studied were RBCs, WBCs, Hb count, B% & T% lymphocytes. IgG & IgA and serum activities of T3 & T4, the HSP70. Besides, the chromosomal aberrations test and micronucleus formation were investigated in female rats. In attempt to find out the interaction between age	Full Text	34

and hyperthermia in such parameters in normal female rats. Female Albino rats of four different ages 2,6,12 and 24 month .We have studied the thermal kinetics of whole body hyperthermia (WBH, at 42°C) and its thermal late effect at 1, 6, 24 and 72 hours post WBH in rats. The results revealed that highly significant increases of WBC, s, B%, IgG and HSP70 at 1 till 72hr post WBH in aged 2 and 6 months. On the other hand, WBH caused a significant decrease in each RBC's, T3 & T4 at 6 till 72 hrs post WBH. As well as, the count of Hb decreased in age 2 month at 1 till 72 hrs post the heat exposure but increased at 1 & 6 hr in 6 months aged post WBH then decreased at 24 hr & 72 hr post WBH. T% lymphocyte count significantly (p< 0.05) decreased at 1 hr post WBH and increased at 6 hr & 24 hr then decreased again at 72 hrs post WBH in ages 2 & 6 months. IgA level significantly increased in 6 aged rats at 1, 6 & 24 hrs post WBH then decreased at 72 hr below the control value post WBH. The results revealed that WBH caused a significant increase of B% lymphocyte, Hb and IgA at 1 & 72 hr post WBH in age 12 & 24 months, except Hb in 12 month decreased at 72 hr post heat exposure. On the other hand, T% lymphocyte, RBC's , IgG and serum T3 &T4 decreased at 1 & 72 hr post heat exposure except IgG level increased at 72 hr post WBH . The level of HSP70 increased significantly at 1 till 24 hr post WBH in 12 month and reached to the control value at 72 hr post WBH. On the contrary, HSP70 decreased significantly at 1hr in aged rats (24 month), then increased significantly at 6 and 24 hr post heat exposure then decreased below the control value at 72 hr post WBH. With respect to chromosomal aberrations positive responses were observed at all ages but in different frequencies and recover may occur at 72 h for the all except young ages (2 month) which needed more time to completely recover., in the micronucleus test, we observed positive responses in all ages at 24h only, while at 72h the mean frequencies of micronucleated poly chromatic erythrocytes (MNPCEs) were within the vehicle control group at all ages except 2 month which increased significantly than control group. The results suggest that hyperthermia can induce both chromosomal aberrations and micronucleus formation.

[Amal I Hassan and Abeer H. Abd El-Rahim. **Effect of Hyperthermia at Different Ages and Mode of Recovery on the Chromosomal Aberrations and Biological Parameters in Female Rats.** Journal of American Science 2011;7(3):296-307]. (ISSN: 1545-1003). http://www.americanscience.org.

Key words: Hyperthermia –HSP70, chromosomal aberration -micronucleus

	Health risk assessment of workers exposed to heavy metals in cement kiln dust (CKD)	Full Text

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Abstract: Cement kiln dust (CKD) like cement itself is not considered to be hazardous material under EP regulations. However, this does not mean that CKD does not contain anything that could pose a hazard to the environment. Therefore, the objectives of this paper were to: 1) evaluate the concentration of six hazardous metals; arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), nickel (Ni) and zinc (Zn) in CKD. 2) Carry out health risk screening analysis for occupational exposure in the cement plants. CKD samples were collected from the biggest three companies for Portland cement production, which are located at Helwan governorate south of Cairo, Egypt. In the present study concentrations of the six metals were measured using Atomic Absorption Spectrometry techniques. The obtained average concentrations were 35.95; 30.17; 15.4 12.49; 1.27; and 1.02 for Cr, Zn, Ni, Pb, As and Cd, respectively. The average daily and lifetime average daily doses for each metal were calculated to evaluate the health risk assessment (HRA) among workers exposed hazardous metals detected in CKD. Moreover, the results of the current work showed that Cr represents high risk in the three cement plants comparing to the others measured ones. It might be attributed to high content of this metal in CKD and its carcinogenicity characters.
[A.A. El-Abssawy; M.A. Hassanien Y.H. Ibrahim, and N.M. Abdel Latif. Health risk assessment of worke exposed to heavy metals in cement kiln dust (CKD). American Science 2011;7(3):308-316]. (ISSN: 154 1003). http://www.americanscience.org.

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	Keywords: cement kiln dust, carcinogenic metals, exposure, health risk assessment		
36	**The Direct Inversion of QUOTE λ/μ λ/μ from Elastic Impedance** Samba Charles Prisca, Jiangping Liu Institute of Geophysics and Geomatics, China University of Geosciences, Wuhan, Hubei 430074, PR China. *Corresponding author: Email: sambadebima@hotmail.com **Abstract:** Elastic impedance (EI) contains valuable information that can be used in reservoir rock as fluid and lithology identification. To get more understanding about the reservoir properties, EI can be reformulated according to the Gray's approximation, in which lamé parameters and density can be successfully extracted. QUOTE λ/μ λ/μ, the most sensitive parameter to variations in rocks properties going from shale to gas sand, is often derived indirectly from lamé parameters. On real seismic data often affected by noises, However, This procedure may poses the numerical computation that can introduce cumulative errors in the inverted results. To avoid these ambiguities, the gray's approximation is reformulated introducing the ratio QUOTE λ/μ QUOTE λ/μ λ/μ. the application of this equation to synthetic and real data show that the inverted results are more stable and less ambiguous than that from conventional procedure, and thus can recover reservoir information very well. **Keywords:** gray approximation; elastic impedance; inversion; lamé parameters	Full Text	36
37	**Relationship between coping styles and level of depression among depressed patients** [1] Magdala Habib Farid Maximos, [1]Fatma Hussein Ramadan and [2]Mohab Mahmoud Naeem [1] Department of Psychiatric Nursing and Mental Health , Faculty of Nursing, Alexandria University,Egypt [2]Department of Psychiatric Nursing and Mental Health, Faculty of Nursing, Tishreen University, Syria. **Abstract:** Depressed patients perceive stress more readily than non-depressed persons, and they struggle to cope with their problematic situations, and their depressive symptoms. Changing maladaptive coping style to adaptive one is a crucial aspect of nursing role through treatment of depression. The aim of this study is to identify relationship between depressed patients coping styles and their level of depression. Total sample of 150 depressed patients of both sexes aged between 21-60 years were selected conveniently from outpatient clinics in El Maamoura Hospital for Psychiatric Medicine and Ras El-Teen General Hospital in Alexandria. Two tools were used to collect the necessary data: the first one is the Ways of Coping Questionnaire" (WOC) to assess thoughts and actions that individuals use to cope with stressful encounters of every day living. Second tool is Beck Depression Inventory is (BDI). to identify or confirm the presence of depressive symptoms and measure their severity. The results showed positive correlation between level of depression and each of emotion focused coping, confrontive coping, accepting responsibility, and escape avoidance coping styles, while negative correlation was found between level of depression and each of problem focused coping, self controlling coping, planful problem solving, and positive reappraisal coping styles. **Keywords:** Relationship; coping; depression; patient	Full Text	37
38	**Bioremediation the Toxic Effects of Mercury-Exposure in Nile Tilapia (*Oreochromis Niloticus*) by using *Lemna gibba L***	Full Text	38

*1Hussein A. Kaoud and 2Mohey M. Mekawy
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Abstract: The effect of mercury (Hg) toxicity, its impact on histopathological changes, the median lethal concentration (LC_{50}-96 h) and the bioremediation effect of *Lemna gibba L* to Nile tilapia, *Oreochromis niloticus*, were investigated through semi-static acute toxicity test developed with mercury chloride ($HgCl_2$).). Fingerlings (2.76 ± 0.21 cm and 0.51 ± 0.12 g) were kept during 96 hours in 5-liter glass aquaria, according to the following mercury concentrations, set up in three replicates: 0.00 (control), 0.037, 0.185, 0.370, 0.740, 0.925 mg Hg L^{-1}. The value of $_{LC50-96h}$ was estimated in 0.220 mg Hg L^{-1}. This study indicated that:1) Hg poisoning caused structural damage in the fish organs ,2) *Lemna gibba L* (weed and extract) were effective in removing Hg from water and reducing Hg bioaccumulation in liver and muscular tissues of fish , 3) The addition of *Lemna gibba L*-extract reduced significantly ($P<0.05$) the Hg level uptake as compared to fish exposed to Hg alone and 4) Addition of *Lemna gibba L* remediated the toxic effect of Hg and provided protection against the degenerative action of Hg.
[Hussein A. Kaoud and Mohey M. Mekawy. **Bioremediation the Toxic Effects of Mercury-Exposure in Nile Tilapia (*Oreochromis Niloticus*) by using *Lemna gibba L*.** Journal of American Science 2011;7(3):336-343]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Mercury**;** Histopathology*; Lemna gibba L***;** Bioremediation; *Oreochromis niloticus*

39	**Evaluation of Quaternary aquifer for agricultural purposes in northwest Sinai, Egypt** Abd-Alrahman A.A. Embaby and Samah M.A. El-Barbary Geology Department, Faculty of Science, Damietta Branch, Mansoura University, Egypt embaby@mans.edu.eg **Abstract:** Northwest Sinai is characterized by a semiarid climate condition and due to insufficient surface water resources; Quaternary aquifer is the main water supply. The Quaternary groundwater exists under free water table conditions with water level ranges between -3.7 m and 12 m. The groundwater flow is concentric around El-Salam Canal as a result of its closure and dependence of the new reclaimed lands on the groundwater. In order to evaluate the suitability of Quaternary aquifer for irrigation purposes, the chemical characteristics have been investigated in fifty samples collected from tube wells and dug wells. The total dissolved solids (TDS) are ranged from 692 mg/l to 9384 mg/l; indicating fresh to saline water classes. Sodium, chloride and sulphate display a nearly linear increase with increasing salinity. The main groundwater genetic types are $CaCl_2$ and $MgCl_2$, reflecting the marine water affinity. Such waters are mostly unsuitable for irrigation under a normal condition and further action for salinity control is required in remediating such a problem. Also, the poor irrigation water can be managed by improving irrigation management technologies and using salt tolerance plants. [Abd-Alrahman A.A. Embaby and Samah M.A. El-Barbary. Evaluation of Quaternary aquifer for agricultural purposes in northwest Sinai, Egypt. Journal of American Science 2011;7(3):344-361]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Evaluation; Quaternary; aquifer; agriculture; Sinai; Egypt	Full Text	39
40	**A Review of some Ecto-and Endo Protozoan Parasites Infecting *Sarotherodon Galilaeus* and *Tilapia Zillii* from Damietta Branch of River Nile, Egypt** Enayat Salem Ahmed Reda Department of Zoology, Faculty of Science, Mansoura University, Mansoura, Egypt enayatsalem40@yahoo.com **Abstract:** The present study was carried out as a general survey searching for the possible protozoan	Full Text	40

parasites that can infect the Nile fishes *S. galilaeus* and *T. zillii*. A total of 125 live fish specimens were obtained from Damietta branch of River Nile and El-Sahel canal, Nile tributary. Examination of the investigated fish species revealed that, fishes were infected with eleven parasitic protozoan species belonging to eight genera. These species were: *Apiosoma piscicolum*, *A. conica*, *Scopulata epibranchialis*, *Vorticella* sp., *Ambiphrya ameiuri*, *Amphileptus* sp., *Chilodonella hexasticha*, *Tetrahymena corlissi*, *Trypanosoma mansouri*, *T. syanophilum* and *Trypanosoma* sp. Among the obtained parasites, the following were recovered for the first time in Egypt. *Apiosoma conica*, *Vorticella* sp., *Ambiphrya ameiuri*, *Amphileptus* sp., *Tetrahymena corlissi* and *Trypanosoma* sp. While *S. galilaeus* represent a new host for *Chilodonella hexasticha*. The recorded numerous parasites have pathological effects on the host fish with subsequent economic losses were discussed.

[Enayat Salem Ahmed Reda. **A Review of some Ecto-and Endo Protozoan Parasites Infecting *Sarotherodon Galilaeus* and *Tilapia Zillii* from Damietta Branch of River Nile, Egypt.** Journal of American Science 2011;7(3):362-373]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Endo; Protozoan; Parasites; *Sarotherodon Galilaeus*; *Tilapia Zillii*; Damietta; Nile; Egypt

	Political Parties: Extent and Nature Hossein Asayesh[1], Adlina Ab Halim[1], Seyedeh Nosrat Shojaei [1] [1.] Department of Politics & Government, Faculty of Human Ecology, University Putra Malaysia h.asayesh@yahoo.com **Abstract**: The political party is a means of connecting the political process with society and it helps to improve the political system. In addition, a central feature of any democracy is political party which serve as a vehicle through which citizens can come together freely to define their political and policy aspirations and campaign for public office. In fact political parties have developed alongside democracy, and it is commonly assumed that democracy cannot survive without them. This article is an overview from the extent and nature of the political party in any society. [Hossein Asayesh. Adlina Ab Halim Seyedeh Nosrat Shojaei, Department of Politics & Government, Faculty of Human Ecology, University Putra Malaysia. Journal of American Science 2011;7(3):374-379]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Political Party, democracy, political development	Full Text	

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	Pollution assessment of the aquatic resources in the Lagos lagoon system. [*1]Oshisanya, Khaphilah Ibironke; [2]Nubi, Olubunmi Ayoola; [3]Amusan, Esther Esak; [4]Salaudeen, Mutiat Motolani [1,2,3,4] Nigerian Institute for Oceanography and Marine Research, P.M.B.12729, Victoria Island, Lagos, Nigeria. Email: kharphioshi@yahoo.com *(Corresponding author). **Abstract:** Sediment, water samples and fish (*Oreochromis niloticus* and *Chrysichtys nigrodigitatus)from each of Unilag,Ikorodu and Iddo Lagoons in Lagos State, Nigeria were analysed for the presence* Zinc (Zn), Lead (Pb), Cadmium (Cd), Copper (Cu), Iron (Fe) and Chromium (Cr) using Buck Scientific 200A model, Atomic Absorption Spectrophotometer (AAS).Sediment contain highest concentration of Fe with a value of 113.02mg/kg against 0.96mg/L in water and 3.92mg/kg in fish, fish contain higher concentration of Zn 7.236mg/kg against 3.740mg/kg in sediment and 3.96mg/L in water. Cu is higher in fish 3.7mg/kg followed by water 2.96mg/L and sediment sample 1.163mg/kg.Cd,Cr and Pb were found not to be present in the water sample while these metals were found to be higher in the Sediment sample than fish tissue. Bioaccumulation was observed in tissues of *Oreochromis niloticus* and *Chrysichtys nigrodigitatus* as higher concentrations of metals were observed in fish tissues than in the water in which they live. The	Full Text	

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	concentration of Zn in the water is above the limits permitted by the Lagos State Environmental Protection Agency (LASEPA) of 1.0 mg/L Zn set for water, there should be need for continuous monitoring of these Lagoons for heavy metals/pollution status. [Oshisanya, Khaphilah Ibironke; Nubi, Olubunmi Ayoola; musan, Esther Esak; Salaudeen, Mutiat Motolani. **Pollution assessment of the aquatic resources in the Lagos lagoon system.** Journal of American Science 2011;7(3):380-383]. (ISSN: 1545-1003). http://www.americanscience.org. Key Words: Fish, Bioaccumulation, Sediment, *Oreochromis niloticus*, *Chrysichytis nigrodigitatus*.		
43	**Seasonal Variation Of Heavy Metals In Sediment And Water Of Lagos Lagoon** *Oshisanya, K.I[1]., Unyimadu J.P[2].,Shelle R.O.D[3]., Nubi A.O[4]., Ladigbolu, I.A.[5], Oguguah N.M.[6], Olumodeji,O.O.[7], Adeleye A.O.[8], Fashade, A.O[9]. [1-9]DEPARTMENT OF PHYSICAL AND CHEMICAL OCEANOGRAPHY NIGERIA INSTITUTE FOR OCEANOGRAPHY AND MARINE RESEARCH VICTORIA ISLAND LAGOS, NIGERIA. E-mail: kharphioshi@yahoo.com Phone: +2348035031722 **ABSTRACT:** The concentration of Chromium (Cr), Lead (Pb), Zinc (Zn), Cadmium (Cd) and physicochemical parameters were determined in Surface water and Sediment at different stations in Lagos lagoon during the wet season. The result showed that the concentration of Cr 0.039mg/l, Pb 0.043mg/l, Zn 0.107mg/l, Cd 0.17mg/l in surface water were generally low when compared to WHO standard 2004, The concentration of Zn in the water is within the limits permitted by the Lagos State Environmental Protection Agency (LASEPA) of 1.0 mg/L Zn set for water. The mean levels of heavy metals in the sediment of Lagos lagoon were generally low and fell within the acceptable limits described by WHO,2004 and FEPA.The average concentration for the heavy metals were Cr 0.046mg/g, Pb 0.054mg/g, Zn 0.730mg/g, Cd 0.523mg/g respectively. Comparism of these values with that of the surface water indicates that most metals were adsorbed to the sediment. None of the trace metals investigated were above the maximum permissible level set by world health organization (WHO).For the physicochemistry the salinity ranged from 0.0 ‰ to 3.1 ‰ indicating a typical freshwater condition, higher dissolved oxygen were also recorded this may be due to run-off during the wet season. [Oshisanya, K.I., Unyimadu J.P., Shelle R.O.D., Nubi A.O, Ladigbolu, I.A., Oguguah N.M., Olumodeji,O.O., Adeleye A.O., Fashade, A.O. **Seasonal Variation Of Heavy Metals In Sediment And Water Of Lagos Lagoon.** Journal of American Science 2011;7(3):384-387]. (ISSN: 1545-1003). http://www.americanscience.org. **Key words:** Sediment, heavy metals, Surface Water, Lagos Lagoon	Full Text	43
44	**The Response of *Galega officinalis* Plant to Different Nitrogen Sources and their Effect on Active Ingredients and Biological Activity** S. El-Gengaihi[*], Abeer Y. Ibrahim, S.F. Hendawy, and S. R. Abd El-hamid Medicinal & Aromatic plants Department National research centre Dokki 12311 Cairo Egypt [*]souadgengaihi@hotmail.co.uk **Abstract:** *Galega officinalis,* goat s rue, a plant native to Euro-Asia and North Africa was introducing to Egypt as an objective for introducing important medicinal plants. The plant claimed to be useful in diabetic treatment, reduce weight and as anti platelet aggregation. Plant density and different nitrogen sources (organic, bio and chemical fertilizers) were tried to evaluate their effect on growth, yield, active ingredient, and its biological activity as anti diabetic. The results revealed that wide propagation distance produced higher growth and yield than the narrower one. The mineral nitrogen proved to be the effective source followed by compost then cattle manure on growth and yield of leaves, whole plant, flower, and sucker number. Total alkaloid was determined in leaves and it fluctuated with no clear trend, however was higher in sample taken at June. The fertilization had no effect on alkaloid accumulation. Goat s rue total alkaloid showed the highest reduction percentage in blood glucose level after 2 hours in diabetic rats.	Full Text	44

	[S. El-Gengaihi, Abeer Y. Ibrahim, S.F. Hendawy, and S. R. Abd El-hamid. **The Response of *Galega officinalis* Plant to Different Nitrogen Sources and their Effect on Active Ingredients and Biological Activity.** Journal of American Science 2011;7(3):388-398]. (ISSN: 1545-1003). http://www.americanscience.org. **Key words:** Galega, Nitrogen sources, rates, planting distance, alkaloid, anti-diabetic		
45	**Effect of Putrescine and Uniconazole Treatments on Flower Characters and Photosynthetic Pigments of *Chrysanthemum indicum* L. Plant** [1] Kandil, M. Mahros; [2]El-Saady, M. Badawy; [*1]Mona, H. Mahgoub; [2]Afaf, M. Habib and[1]Iman, M. El-Sayed [1]Department of Ornamental Plants and Woody Trees, National Research Centre, Dokki, Cairo, Egypt [2]Department of Ornamental Horticulture, Faculty of Agric., Cairo University, Giza, Egypt [*]free2hamona@yahoo.com **Abstract:** The effect of Putrescine at the concentration of 100,200 and 300 ppm and Uniconazole at 20, 40 and 60 ppm in addition to control (distilled water) on flower characters, total carbohydrates and photosynthetic pigments in flowers of Chrysanthemum plant during 2004/2005 and 2005/2006 had been evaluated studied. The obtained data indicated that all flower characters and chemical composition were significantly increased by foliar application of Putrescine at the three concentrations. Uniconazole treatments delayed start of flowering after spraying, decreased pedicle length and length of flower stalk, while it increased yield of flowers, diameter of inflorescence, vase life, total carbohydrates in the flowers and photosynthetic pigments chl. (a),(b) and carotonoids . The highest values were found when plants were treated with 200 ppm Putrescine and 20 ppm Uniconazole. [Kandil, M. Mahros; El-Saady, M. Badawy; Mona, H. Mahgoub; Afaf, M. Habib and Iman, M. El-Sayed. **Effect of Putrescine and Uniconazole Treatments on Flower Characters and Photosynthetic Pigments of *Chrysanthemum indicum* L. Plant.** Journal of American Science 2011;7(3):399-408]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords**: Cut flowers, Polyamines, Growth retardant, chlorophylls, carbohydrates	Full Text	45
46	**The empowerment of rural women through Information and Communication Technologies (ICT)** Mohammad Abedi[1] and Sharareh Khodamoradi[2] [1]Department of Agricultural Management, Islamic Azad University, Qaemshahr Branch, Iran [2]Department of Agricultural Extension Education, Science and Research Branch, Islamic Azad University, Tehran, Iran *Corresponding author: skhodamoradi2007@yahoo.com **Abstract:** Rural women are among those major groups at society who previously were considered less by planners, due to specific reasons in the past. And this problem is more observable at developing countries. While, by looking at women's history of economic and social life, we can find that this great group, continuously have played basic role in forming economic condition of country. This great group consistent with men have had active role at areas of social-economic activities and always have had major part on economic production of society. ICT is now recognized as a technological tool which can serve as a catalytic intervention in respect of transforming the lives and livelihoods of rural families.The economic and income divides between urban and rural areas can be overcome only by the technological upgradation of rural professions. [Mohammad Abedi and Sharareh Khodamoradi. **The empowerment of rural women through Information and Communication Technologies (ICT).** Journal of American Science 2011;7(3):409-413]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** rural women, empowerment, Information and Communication Technologies (ICT)	Full Text	46
47	**The importance of Information and Communication Technologies (ICT) in agriculture development**	Full Text	47

in developing countries

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Abstract—In recent years, assistance from developed countries to developing countries has intensified. Information and Communication Technologies (ICTs) have also been widely deployed in developmental programmes, leading to the creation of a new field – ICT for development. This paper reviews a number of projects that introduce technically innovative ICTs that are intended for the development of marginalised rural areas.
[Mohammad Abedi and Sharareh Khodamoradi. **The importance of Information and Communication Technologies (ICT) in agriculture development in developing countries.** Journal of American Science 2011;7(3):414-420]. (ISSN: 1545-1003). http://www.americanscience.org.
 Keywords: Information and Communication Technologies (ICT)**,** agriculture development, developing countries

48	**Necessary of attention to indigenous knowledge toward improving agriculture**	Full Text	48

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Abstract: society. Indigenous knowledge is different with scientific knowledge that was established by universities and scientific communities. This knowledge is basis for decision making at field of agriculture, health, education, food and natural sources Indigenous knowledge is set of all knowledge and skills that people enjoy in one geographical area (in one environmental conditions) that most of their skills and knowledge be transmitted to next generation , and new generation would be adapted with them and add to it Since, each knowledge is consequent of individual interaction with environment, so indigenous knowledge is consequent of indigenous people interaction with their environment. Chambers with emphasis on people's role at development process believes that "rural people's knowledge" term is more eloquent than other terms for indigenous knowledge. Our purpose of rural people are producer farmers , input buyers , agriculture production sellers and etc. "people" in above phrase emphasis that this knowledge is more verbal and less has been written . This word also referred to whole knowledge system which contains concepts, beliefs, and attitudes and also contains gain, store and transmitting knowledge process.
[Mohammad Abedi and Sharareh Khodamoradi. **Necessary of attention to indigenous knowledge toward improving agriculture.** Journal of American Science 2011;7(3):421-425]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: agriculture, indigenous knowledge

49	**Magnetic resonance imaging of the brain in the diagnostic evaluation of microcephaly**	Full Text	49

Elham A, Nawar, * Laila A,Selim , Manal S. El-dafrawy, Marwa A, Hassan, **Ahmed F, Yousef
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Abstract: Microcephaly is defined as small head size characterized by occipito-frontal circumference (OFC) at least 2 standard deviations (SD) below the mean for age and sex. Microcephaly is associated with numerous disorders of diverse etiology. Radiology plays a fundamental role in determining the etiology. MRI is often the imaging modality of choice. **Aim of the work:** To assess the contribution of brain

magnetic resonance imaging (MRI) in establishing an etiological diagnosis in children presenting with microcephaly in the first two years of life. **Methods**: Nine hundred Egyptian patients attending the general pediatric and neurology clinic of Benha University Hospital (BUH) and clinic of inherited metabolic disorder at the centre of social and preventive medicine of Cairo University Children Hospital (CUCH) were screened for microcephaly. This was done by measuring the **(OFC),** and then MRI was performed to all microcephalic patients. Other investigations done according to the condition. **Results:** Fifty five patients out of 900 cases were microcephalic, below the 3rd percentile of Egyption charts. Male and female distribution was 31 (56.4%) and 24 (43.6%) with ratio of 3:2.The ages of presentation ranged from 2 months to 84 months with mean age of 20.6± 15.6 months. All patients were symptomatizing before 24 months with mean age of (6.5 ± 4.2 months). The patients were classified according to the final diagnosis into 3 groups: primary microcephaly 11cases (20%), secondary microcephaly 29 cases (52.72 and undiagnosed cases 15 cases (27.28%). The most frequent MRI finding is brain atrophy in 11(20%) cases followed by demylination in 10(18.18%) cases, leukomalecia &atrophy in 7(12.7%) cases, demylination &atrophy in 6(10.9%) cases, basal ganglia lesion in 5(9%) cases, congenital brain malformations in 4(7.3%) cases, microcephalic changes in 3(5.5%) cases and leukomalecia only in 2(3.6%) cases. **Conclusion:** MRI is considered as a golden standard in the evaluation of brain abnormalities in patients with microcephaly**.** It is diagnostic in congenital brain malformations and in combination with history & clinical findings, it can suspect the diagnosis, as in ARM, Leigh syndrome & HIE cases or point to specific test for diagnosis as in MLD & PKU.

	Pay-As-Bid versus Uniform Pricing Mechanism in Restructured Power Systems	Full Text

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Abstract: Energy markets have specifically different mechanism in quite varying countries. Even in one country, it might be that mechanism of electrical market is different from each other. What is similar in this markets is satisfying load or demand as a main target. In the worldwide electricity markets, ordinary mechanism of market clearing implied as a uniform price, while mechanism of payment in Iran's electricity market based on the model of pay-as-bid by energy generation companies. This paper is surveying these two mechanisms and introducing its weak and strong points.

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	The role of rural women's self-reliance in improving Livelihood	Full Text

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Abstract: Rural women are among those major groups at society who previously were considered less by planners, due to specific reasons in the past. And this problem is more observable at developing countries. If rural women can work through receiving credits, loan and others finance facilities at favorite jobs and

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live through earned income (as it called "self-reliance and independence"), so undoubtedly we would see changes in social, economic and cultural relations of village. Rural women's financial self-reliance has many social & economic influence as it made them self-sufficiency, it changes economic behavior and it makes women independent, it will be effective in economic development in family & society, it also improve the women's roles in society and it causes self-confidence in women, it builds family strength and it causes to respect the women rights more than before and women will become equal with men in all their rights, of course we won't have patriarchy in the family. The women's empowerment in the rural society will increase because of all the aspects of rural women's self-reliance and their position will be confirmed.

Decentralization in agricultural management in rural activities

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Abstract: Agricultural extension is a non-formal type of education that provides advisory services by the use of educational approach in acquiring knowledge and skills to deal with the growing needs of global world. Diverse agricultural extension funding and delivery arrangements have been undertaken since the mid-1980s by governments worldwide in the name of "privatization." When agricultural extension is discussed, privatization is used in the broadest sense – of introducing or increasing private sector participation, which does not necessarily imply a transfer of designated state-owned assets to the private sector. In fact, various cost-recovery, commercialization, and other so-called privatization alternatives have been adopted to improve agricultural extension. The form and content of decentralization has dominated development discourse and public sector reform agenda in Kenya in the last two decades. The case of agricultural extension service presents decentralization in a difficult context partly due to lack of information on its possible diverse impacts especially on resource poor farmers.

Full Text

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Proprietary of Total Intensity Magnetic Data to Detect the Subsurface Structures and Tectonics of Southern Sinai Peninsula, Egypt

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Abstract: This study presents the analysis and interpretation of magnetic data to map the subsurface structural framework of the present area. Structural interpretation of the magnetic data was achieved through applying advanced processing techniques that provide automatic delineation and depth estimation of the magnetic structures. Some structural elements could be deduced from the qualitative interpretation of such magnetic anomalies. Phase-shifts of magnetic anomalies due to the local direction of the geomagnetic field vector can be corrected using a reduction-to-pole filtering operation. At the interpretation stage, the analysis of the RTP magnetic data, which included low-pass/high-pass filtering by power spectrum and separation of the magnetic causatives of shallow sources from those of deeper sources through the matching band-pass filtering. The horizontal gradient and local wave number tools were used for locating the magnetic sources and their properties. In this case, peaks in those methods can be used to locate sources representing the edges of thin horizontal sheets and estimate their strike directions which,

Full Text

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	used to delineate the tectonic framework of the investigated area. The shallow structural depths located between 1.5 to 2.5 km (red color) dominate the majority of the southern part, as well as some parts in the northern part. While the deep depths 2.5 to 5.5 km (blue color) dominate the northcentral and westcentral parts. The mapped structures reveal that, the area is affected by a set of faults trending mainly in the NE-SW, NW-SE and N-S directions. Moreover, the area is dissected by a set of deep basement swells and troughs, as well as shallow anticlinal and synclinal trends controlled mainly by the predominant faults. [Ahmed A. El-Khafeef and Shadia T. El Khodary. **Proprietary of Total Intensity Magnetic Data to Detect the Subsurface Structures and Tectonics of Southern Sinai Peninsula, Egypt.** Journal of American Science 2011;7(3):453-463]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Proprietary; Magnetic Data; Subsurface Structure; Tectonics; Southern Sinai Peninsula; Egypt		
54	**Clinical value of transforming growth factor beta as a marker of Fibrosis in adolescents with Chronic Liver Diseases** *Elham A Nawar *Bahaa El-Din Hassanin, **Mona EL-Tokhy *Pediatric & ** Clinical Pathology Depart. Faculty of Medicine, Benha University elhamnawar2006@yahoo.ca **Abstract: Background:** Hepatic fibrosis is the final common path of liver injury in most chronic liver diseases and can lead to cirrhosis, which is responsible for the majority of clinical complications. Our aim is to asses the clinical value of serum transforming growth factor (TGF) as a fibrogenesis marker in adolescents with chronic Liver Diseases. **Methods:** We measured serum levels of TGF- in 25 adolescents with chronic liver disease and 25 healthy controls, and determined their relationship to frequently used liver function tests and liver biopsy findings. **Results:** Serum Transforming growth factor was significantly higher in patients than in controls ($P < 0.001$). Significant positive correlation between TGF and TSB ($r = 0.4682$ and $p< 0.05$). High significant positive correlation was noted between TGF and stage, grade of liver fibrosis, PT and duration of illness as p is $<0.001$and r is 0.9409, 0.7447, 0.5293 and 0.5952 respectively. Highly significant negative correlation was found between TGF and prothrombin concentration (PC) and serum albumin level as p is < 0.01 and r is -0.6460 and -0.5371 respectively. Sensitivity of TGF in diagnosis of fibrosis was 65%, specificity was 94% and area under curve (AUC) was 0.812. The cut-off value of TGF used to discriminate significant fibrosis was 22.6 ng/ml and it was a dependant predictor factor for diagnosis of fibrosis with positive predictive value 75.5% and negative predictive value 90.4 %. **Conclusions:** TGF- had the ability to discriminate patients with significant fibrosis and may be useful in reducing but not replacing the need for liver biopsy. [Elham A Nawar Bahaa El-Din Hassanin, Mona EL-Tokhy. **Clinical value of transforming growth factor beta as a marker of Fibrosis in adolescents with Chronic Liver Diseases.** Journal of American Science 2011;7(3):464-471]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Liver fibrosis; Hepatitis C virus; Hepatitis B virus; Liver fibrosis; TGF-	Full Text	54
55	**Comparing of yield and yield components of rice hybrid in different irrigation regimes and nitrogen levels** Ashouri, M and E, Amiri Islamic azad university Roudsar&Amlash branch, Iran. E-mail: mashouri48@yahoo.com and mashouri@iau-roudsar_amlash.ac.ir Tel: +981426212910; Fax: +981426212911 Corresponding Author: Ashouri, M. **Abstract:** In order to investigating the effect of different irrigation regimes and nitrogen fertilizer on yield and yield components of hybrid rice a biennial experiment was conducted at rice research institute of Iran during crop season 2008-9. experiment was arranged in split plot based on completely randomized block design with 3 replications in which water regimes were main factor included Continuous Submergence and Alternative Submergence conditions (irrigation intervals of 5, 8 and 11days) and nitrogen fertilizer levels were sub factor included 0, 90, 120 and 150 kg/ha. Grain yield in I1 to I4 were 7342, 7079, 7159 and 5168 kg/ha in 2008 and 4372, 4343, 4674 and 4208 kg/ha in 2009 respectively. Number of grains per panicle in	Full Text	55

I1 to I4 was 304,307,311 and 272 in 2008 and 183,180,181 and 179 in 2009 respectively. Weight of 1000 grain in I1 to I4 was 22.4, 22.2, 21.8 and 21.1 g in 2008 and was 22.8, 23, 23.1 and 23.2 g in 2009 respectively. Unfilled grain in I1 to I4 was 27.1, 32.9, 30.3 and 39.2 percentage in 2008 and was 55.2, 48.4, 50.4 and 46.8 percentage in 2009 respectively. Mean grain yield in irrigation regimes in 2009 compare to 2008 decreased 34% because mean number of grains per panicle in 2009 compare to 2008 decreased 40% and mean unfilled grain percentage increased 56%.

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Expression of Maspin, KI-67 and CD105 as Predictors of Postoperative Recurrence in Laryngeal Carcinoma: perioperative planning and proposed reconstructive tools

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Full Text

Abstract: Background: Maspin, a member of serpin super-family, has multifaceted biological functions and an unique tumor suppressing activity. Several studies showed that maspin suppresses tumor growth, angiogenesis, invasion and metastasis. The present study investigated the relationship between maspin expression, Ki-67 proliferative index (PI), CD105-assessed microvessel density (MVD) and postoperative recurrence in laryngeal squamous cell carcinoma (SCC). **Patients and methods:** Subcellular pattern of maspin expression was immunohistochemically evaluated in 28 cases of laryngeal SCC treated by total laryngectomy with reconstruction but without primary radiotherapy with a follow-up period from 10 to 36 months. The expression and interaction between Ki-67, CD105 and maspin were also studied. **Results:** Two patterns of positive maspin expression; cytoplasmic (n=9) and nuclear-cytoplasmic (n=16) were recognized. Significant inverse correlation between nuclear-cytoplasmic pattern and both Ki-67 PI (P=0.049) and CD105-assessed MVD (P=0.016) were disclosed. Comparing the two groups of patients with (pR+) and without (pR-) evidence of postoperative recurrence, none of the studied clinicopathological parameters (age, sex, pathological grade, tumor stage, and nodal stage) was significantly associated with recurrence (all p>0.05). The nuclear-cytoplasmic maspin expression was significantly higher in pR- patients (p=0.018), while higher Ki-67 PI and CD105-assesed MVD were significantly correlated with pR+ group (p= 0.007 & p = 0.004 respectively). **Conclusion:** The present results suggest that absence of nuclear localization of maspin and high Ki-67 PI and CD105-assessed MVD may predict a higher risk of recurrence in laryngeal SCC patients.

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Environmental Studies of Domestic Wastewater Treatment Using Integrated Anaerobic/Aerobic System

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Full Text

Abstract: Conventional aerobic technologies based on activated sludge processes are dominantly applied for the treatment of domestic wastewater due to the high efficiency achieved, the possibility for nutrient removal and the high operational flexibility. Anaerobic pre-treatment of domestic wastewater can serve a viable and cost-effective alternative due to its relatively low construction and operational cost, operational

	simplicity, low production of excess sludge, production of energy in form of biogas and applicability in small and large scales. A viable alternative is the sequential anaerobic–aerobic systems. The performance of the integrated anaerobic/aerobic wastewater treatment system (AAWTS) for domestic wastewater treatment has been investigated. The domestic wastewater and activated sludge were collected from Ras El-Bar wastewater treatment plant. The overall removal efficiency of the suggested system, is in the order $TSS < TN < BOD < Cl^- = TDS < COD < NH_3$.The deficiency of the applied AAWTS may be due to the limiting effect of salts on the biological treatment of saline influents. [Talaat A. Hegazy, M. A. Abdel-Magied, A. Al-Asmar, and M. S. Ibrahim. **Environmental Studies of Domestic Wastewater Treatment Using Integrated Anaerobic/Aerobic System.** Journal of American Science 2011;7(3):485-492]. (ISSN: 1545-1003). http://www.americanscience.org. Keywords: Biological treatment, domestic wastewater, sludge, anaerobic/aerobic system, BOD and COD		
58	**H₂/H Controller Design for Singular Perturbation Systems** Fatemeh Jamshidi [1], Afshin Shaabany [1] [1] Islamic Azad University, Fars Science and Research Branch, Shiraz, Iran Fjamshidi59@yahoo. com, afshinshy@yahoo.com Abstract: In this paper the synthesis of logic-based switching H_2/H state-feedback controller for singular perturbation systems is considered that achieves a minimum bound on the H_2 performance level, while satisfying the prescribed H performance. The proposed hybrid control scheme is based on a fuzzy supervisor which manages the combination of two controllers. A convex LMI- based formulation of the two fast and slow subsystem controllers leads to a structure that ensures a good performance in both the transient and the steady state phase. It is shown that the system with the proposed controller remains globally stable despite the configuration (controller) changing. [Fatemeh Jamshidi, Afshin Shaabany. H_2/H Controller Design for Singular Perturbation. Journal of American Science 2011;7(3):493-499]. (ISSN: 1545-1003). http://www.americanscience.org. Keywords: Singular perturbation system; Fuzzy supervisor; Linear Matrix Inequality (LMI); Switching H_2/H control.		58
59	**Politics and Education: A case study of political participation of women in Iran** Seyedeh Nosrat Shojaei[1], Ku Hasnita Ku Samsu (**Corresponding Author**), Hossein Asayesh Department of Politics & Government, Faculty of Human Ecology, University Putra Malaysia. nosrat2007@yahoo.com Abstract: This article studies the structural obstacles of women's political participation in Iran. The objective of the study has been achieved by answering the question; how structural factors (Education and knowledge) act as obstacles to women's participation in top political positions in Iran? A qualitative case study method is used in the paper and the primary data are collected mainly through in-depth interviews with five informants by using purposive technique. The analysis of the study is supported by resources theory. The findings of the article suggested that inadequate intellectual resources act as the structural obstacles of Iranian women in politics. [Seyedeh Nosrat Shojaei, Ku Hasnita Ku Samsu, Hossein Asayesh Department of Politics & Government, Faculty of Human Ecology, University Putra Malaysia. Journal of American Science 2011;7(3):500-505]. (ISSN: 1545-1003). http://www.americanscience.org. Keywords: political participation, women, Iran, educational obstacles, resources theory		59
60	**Effect of Social Capital on welfare of Rural Households in South-western States, Nigeria** Authors: **Balogun**, Olubunmi. Lawrence and Suliamon. Adesina Yusuf Address: Department of Agricultural Economics, University of Ibadan, Nigeria. Correspondence E-mail: blarrybunmi@yahoo.com Telephone number: +23480238441788		60

Abstract: This study conducted an empirical investigation on the effects of social capital on welfare of rural households in the southwestern, Nigeria. Multistage sampling technique was employed. The data for the study were collected with the aid of structured questionnaires from three hundred and ninety nine households in Ekiti and Osun states. The data were analyzed using descriptive and regression techniques. The average age of the households head in the study areas was 41.3 years. Households belong to at least two associations and the most important one is religion association. Average household size is 5.0 members and has about 66.7 percent index of participation. However, the level of heterogeneity index is 54.7 percent while meeting attendance index of the households represents halves of the maximum recorded. Cash contribution index is surprisingly low with value of 16.8 percent while labour contribution index is 66.3 percent and with mean social capital value of 15.21. The result of regression show that location, marital status, household size, primary occupation cash contribution index and heterogeneity index of households significantly impacted welfare. The use of instrumental variable lead to an increase in the value of adjustment R^2 from 0.2302 to 0.2564 compared with the use of the actual social capital index. Policy that enhances better strong social ties of poor households is recommended for poverty alleviation.

61	**Identifying Contexts of Application of Electronic Education in Training of Youth Rural in Ilam Province, Iran** S.Jamal Farajollah Hosseini [1], Farhad Lashgarara [2], Azam Sanjabi [3] [1, 2, 3.] Department of Agricultural Extension, Science and Research Branch, Islamic Azad University, Tehran, Iran f_lashgarara@srbiau.ac.ir **Abstract:** Rural youth are only producer of future in providing the raw materials and food and industrial, productions in society. Youth are most number in society in terms of unfavorable living and less of suitable state of Training and improvement naturally; they could not advanced producer to progress agriculture sector. Rural youth immigrated to cities in result of unemployment and loss of training the cities have problem with rural emigration. The new technologies in rural area causes that youth can provide education needs. For this purpose, this research is accomplished with The Feasibility of E- learning Application for Training rural youth in the City of Ilam. It is applied research and research method is correlation. Questionnaire is main instrument in research and to study the observing research instrument, guide professor and experts and advisors got questionnaire. For the measure of questionnaire constant we take primary test with 20 questions and Cronbach's Alpha coefficient is calculated 87 percent. Statistical population of research is 7950 people of Ilam rural youth. According to Cochran formula 150 people are sample number and with helping of class accidental sampling method is selected best descent statistical analysis is done with Spssversion12 computer software. Descriptive conclusion of this research is presented that social and economic factors are most effective factor in performance of E-learning. Training and searching factors are less effect in performance of e-learning. Results of regression analysis presented that social and training factor are most effects in E-learning for rural youth.	Full Text	61
62	**Determining Causal Model Role of ICTs in Improving Food Security of Iran's Rural Households** Farhad Lashgarara [1], S. Mehdi Mirdamadi [2], S.Jamal Farajollah Hosseini [3] [2, 3.] Department of Agricultural Extension, Science and Research Branch, Islamic Azad University, Tehran, I f_lashgarara@srbiau.ac.ir **Abstract:** Access to desirable, sufficient, safe and nutritious food is one of the basic components of the development and health of a society. Information and communications technologies (ICTs) represent an	Full Text	62

important strategy that can be used in attaining food security. The main purpose of this research, performed in 2006-2007, was to identify the effectiveness of ICTs in improving food security of Iran's rural households. A descriptive methodology was applied in this research, through questionnaires. The statistical population for the study included 253 agricultural extension experts; from this population, 170 persons were selected. The results showed that, according to the experts   point of view, the situation of food security in Iran's rural households was unfavorable, but that ICTs could play an important role in improving this situation. The results of stepwise regressions showed that providing information about food, increasing food production, helping to market agricultural products, considering clientele needs, improving interactions and communications, ensuring appropriate ICTs, providing access to old technology and accessing the content of this type of technology, were determined to account for 78% of the variance of the food security of Iran's rural households. Moreover, the path analysis technique demonstrated that the improvement of interactions and communications had the greatest influence on determining the causal model of improving food security of Iran's rural households (=0.992).

| 63 | **Predicting manpower productivity promotion factors in Guilan University of Medical Sciences using Structural Equation Modeling (Iran)**

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 Abstract: Manpower productivity issues have attracted increasing interest among researchers during the last decade. There are various factors affecting human resources productivity. This study elaborated the human resources productivity promotion factors in Guilan University of Medical Sciences using structural equation modeling. The research was cross-sectional, descriptive and analytical. The study was carried out in two stages during three month of fall season in 2009. In quality stages of research, 45 specialists in management were involved. In the quantity stage, 321 members of the faculty, educational and human resources experts affiliated to Guilan University of Medical Sciences were selected and the data has been collected using the questionnaires. Expert panel has been used for content validity and exploratory factor analysis and confirmatory factor analysis were performed for construct validity. Finally, path analysis carried out in order to identify human productivity promotion factors. Manpower productivity promotion factors identified in path analysis were included organizational culture with 0.51 path coefficient, motivational factors with 0.25 path coefficients, environmental status with 0.17 path coefficient, faculty member's empowerment with 0.11 path coefficient and leadership style with 0.08 path coefficient. The results indicated that organizational culture, motivational factors, environmental conditions, empowerment and leadership style were the most important human productivity factors for Guilan University of Medical Sciences.
| Full Text | 63 |
| 64 | **Bit-Interleaved Turbo-Coded over Wireless Channels**

 Afshin Shaabany [1], Fatemeh Jamshidi [1]
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 Abstract: This paper presents an improved version of bit-interleaved turbo-coded modulation (BITCM) scheme designed for bandwidth efficient transmission over wireless channels. The proposed scheme consists to apply signal space diversity (SSD) to conventional BITCM and a rotated modulation. At the | Full Text | 64 |

	receiver side, an iterative demapping and decoding is proposed in order to optimize the error performance. Simulation results carried out on 2 bit/s/Hz 64-QAM BITCM indicate that is possible to obtain a gain exceeding 0.5 dB at a BER = 10^{-7} compared to the classical 64-QAM BITCM scheme. It is also shown that, the error floor can be significantly lowered using SSD technique at a little cost in terms of system's complexity. [Afshin Shaabany, Fatemeh Jamshidi. Bit-Interleaved Turbo-Coded over Wireless Channels. Journal of American Science 2011;7(3):533-537]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** BICM, DVB-RCS turbo code, signal space diversity, iterative demapping, error floor, wireless channels		
65	**Analysis of P53 Gene Mutations in Peripheral Blood Lymphocytes of Egyptian Spray Workers Exposed to Multiple Pesticides** Mohamed A. Noaishi [1], Mostafa M.M. Afify[2] and Samir H. Haggag[3] [1]Department of Mammalian Toxicology, Central Agricultural Pesticides Lab (CAPL), Ministry of Agriculture [2]Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Bani Sweif University, Egypt [3]Department of Medicinal Chemistry, National Research Center (NRC) **Abstract:** The advanced research of the molecular genetic toxicology is focused on the fundamental molecular mechanisms involved the risk of mutations in genes related tumor. Mutations of the p53 tumor suppressor gene plays an important role in the development of common human malignancies. Previous reports revealed that the tumor suppressor gene p53 is considered to be the most frequently mutated gene in human tumors. The present study was designed to investigate the association among three factors, which focusing on occupational exposure to pesticides, aging and smoking habit and their effect on p53 gene mutation in fresh blood lymphocytes of workers occupationally exposed to a mixture of pesticides in outdoor fields in El-Fayoum governorate, Egypt. Because p53 gene mutation is associated with many factors not one factor effects on the result but may be many factors affect on this result. So we used a multiple Linear Regression statistical test to give the effect of each factor individual as well as the interaction among these factors and the results showed that there was a significant Linear Regression of p53 mutations with age and smoking factors with ($P = 0.005$) and ($P = 0.002$) respectively but there is no significant with pesticide exposure factor ($P = 0.528$). P53 genetic mutation occurs relatively with age after 40 years old and the workers in any occupation when they are smokers will be prone to P53 gene mutations than other environmental factors. In conclusion, PCR-"cold" SSCP is a rapid and sensitive method for identifying p53 genetic mutation and useful as biomarker but at least should be used with many other biomarker tests to give a clear picture about environmental genotoxicity. [Mohamed A. Noaishi, Mostafa M.M. Afify and Samir H. Haggag. **Analysis of P53 Gene Mutations in Peripheral Blood Lymphocytes of Egyptian Spray Workers Exposed to Multiple Pesticides.** Journal of American Science 2011;7(3):538-543]. (ISSN: 1545-1003). http://www.americanscience.org. **Key Words:** Occupationally exposed, Pesticides, Age, Smoking, p53, SSCP	Full Text	65
66	**Assessment of Sport Practice among Adolescent School Students and Its Effect on Perceived Health in Sharkia Governorate –Egypt** **Shereen Eassa[*1], Sohair A Hagag[1] and Ghada Sanad Nageeb[2]** Community Medicine and Public Health[1] &rheumatology and physiotherapy[2] Departments, Faculty of Medicine, Zagazig University, Zagazig, Egypt [*]shereeneassa@yahoo.com **Abstract:** Sedentary lifestyle is a major contributing factor to increasing health problems among adolescents. Inactive youth have a high probability of becoming obese adults with increased risk for coronary heart disease, hypertension, and diabetes. This study aimed to assess the frequency of practice of adolescent students towards sport practice, to study some of the risk factors that affect sport practice and to measure the subjective direct and indirect effects of sports participation on perceived health. The sample was selected by multistage simple random sample technique from students of preparatory and secondary	Full Text	66

schools. The tool in our study was questionnaire which was included data about practice of sports and its association to socioeconomic condition, some lifestyle factors, knowledge, attitude, , the direct and indirect effect on perceived health, then we measured body weight and height of the students and calculated their Body Mass Index. Obtained data revealed that 81.8% our sample practice sports but the majority of them with low level of practice 75.2%, study work were the most barriers against practicing sports 29.4% while fun and social role were the most common motives (30.5%& 23.5%respectively). Low levels of knowledge, attitude as well as negative perception of health & high level of anxiety, feeling depression and psych-physiological score were significant among those not practice sports. In addition; smoking, obesity, female sex were significant associated with non practice of sports. In conclusion; feeling anxiety, depression, negative attitude, low level of father and mother education were the most common predictor factors of not practice sport, so we recommend health education programs, social mobilization to eliminate barriers and increase motives toward sport practice and incorporated more students into sports programs into schools or community.

[Shereen Eassa, Sohair A Hagag and Ghada Sanad Nageeb . **Assessment of Sport Practice among Adolescent School Students and Its Effect on Perceived Health in Sharkia Governorate –Egypt.** Journal of American Science 2011;7(3):544-551]. (ISSN: 1545-1003). http://www.americanscience.org.
Keywords: sport practice-physical activity-risk factors-perception of health.

| 67 | **Relationship between Impulsivity and Coping Strategies among Psychiatric Outpatients at Assiut University Hospital** | Full Text | 67 |

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Abstract: Routine assessment of impulsivity and accompanying coping skills is essential for planning care and appropriate management of patients identified as impulsive. Impulsivity is one of the defining characteristics of many adult psychiatric disorders and is also a key component in the clinical risk assessment of anger and aggression. This study aimed to assess the impulsivity and coping skills among psychiatric patients and to determine the relationship between impulsivity and coping skills. The study was carried out in psychiatric outpatient clinic at Assiut University Hospital. The study sample comprised 120 psychiatric patients (70 males) and (50 females), diagnosed with schizophrenia, mood disorders and delusional disorder. Three tools were used for data collection, namely: Patient's assessment structured interview schedule, Impulsivity scale, and Coping scale. the main results yielded by the study proved that, concerning the level of impulsivity , the majority sample had moderate impulsivity, highest among patient's aged from 30–39 years, males than females (51%), married, illiterate (33%), and farmers (32%). The study recommended teaching nurses to understanding the effect of impulsivity on personality, behavior and coping strategies is essential for the accurate assessment and appropriate management of impulsive individuals.

[Samia Abed Dayem, Naglaa Abd El megied Mohamed and Nadia Abd Elghanay Abd El Hammed **Relationship between Impulsivity and Coping Strategies among Psychiatric Outpatients at Assiut University Hospital.** Journal of American Science 2011;7(3):552-557]. (ISSN: 1545-1003). http://www.americanscience.org.
Key Words: Impulsivity, Coping Strategies, Psychiatric Patients

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| 68 | **Control of Construction - Associated Nosocomial Invasive Aspergillosis Outbreak at Kuwait Cancer Control Centre** | Full Text | 68 |

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Abstract: Outbreaks of nosocomial invasive aspergillosis may occur in association with construction/renovation activities. Outbreak of Nosocomial invasive aspergillosis had been declared at July 2010 at Kuwait Cancer Control Centre coinciding with different construction and renovation activities. A total of four cases of aspergillosis were identified .An urgent meeting of a multidisciplinary team comprising infection control staff, clinicians, hospital deputy director, engineering department staff and the director of nursing staff was established with subsequent implementation of different control measures including: Sealing of construction sites with impermeable barriers , face-masking of patients with N95 mask, frequent wet cleaning around construction area and posaconazole antifungal prophylaxis for high risk patients.

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Full Text

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EVALUATION OF SOME ESSENTIAL OILS AGAINST *SESAMIA CRETICA* LED. UNDER FIELD CONDTIONS

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Abstract: Efficacy of some volatile plant oils against the corn borer, *S. cretica* Led. was investigated under field conditions throughout 2010 early summer corn season . Oils of four plants, namely; Cinnamon, Clove, Marjoram and Ginger Essential oils were used at concentrations 2.5 and 5%. Also, Eugenol (aromatic fragment) which was found in all the essential oils was used in 4 concentrations. The recommended pesticide Diazinox ® was used in addition to the control treatment. It was found that Cinnamon at 5% achieved the highest percentage reduction in egg masses, larvae and dead heart being 95.2, 85.5 and 92.1, respectively. The heaviest yield of maize ears was obtained as a result of treating plants with cinnamon 5% and eugenol 0.4% being 89.9 and 86.2% increase than control, respectively.

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Full Text

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Study of Resistin and Leptin in patients with Thyroid Dysfunction

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Abstract: Background: Leptin and resistin are adipocytokines associated with body mass, insulin resistance and inflammation. Data linking adipokines with thyroid hormones are confusing. **Aim:** Evaluation of leptin and resistin in patients with thyroid dysfunction. **Subjects and methods**: 28 patients with hyperthyroidism, 26 patients with hypothyroidism and 24 age and gender matched control subjects were included in the study. BMI was calculated. Serum concentrations of TT3, FT4, TSH, resistin and leptin were measured by ELISA. **Results**: A higher BMI (29.4 ± 2.1)kg/m^2, TSH (21.7 ± 2.4) Mu/L and leptin (34.9 ± 2.8) ng/ml were found in the hypothyroid group compared with the hyperthyroid group BMI (23.7 ± 2.7)kg/m^2, TSH (0.07 ± 0.03) Mu/L and leptin (9.7 ± 1.8)ng/ml. The hyperthyroid group exhibited a significant increased TT3 (6.6 ± 1.6)nmol/L, FT4 (2.6 ± 0.1)Pmol/L and resistin $(13.8+3.7)$ng/ml compared with the hypothyroid group TT3 (0.3 ± 0.1)nmol/L, FT4 (0.68 ± 0.04)Pmol/L and resistin (6.3 ± 3.4)ng/ml. Resistin correlated significantly and negatively with TSH $(P<0.01)$ and BMI $(P< 0.01)$ and positively with TT3 $(P<0.01)$ and FT4 $(P< 0.05)$. Leptin correlated positively with TSH $(P< 0.01)$ and BMI $(P<0.01)$ and

negatively with TT3 (P<0.01) and FT4 (P<0.05). Factors affecting resistin level in a multivariate logistic regression analysis were sex, TT3 and FT4. Leptin is affected only by sex and TSH. The cutoff level of leptin associated with hyperthyroidism is 15.3 ng/ml with sensitivity of 100%, and specificity of 60%. **Conclusion:** Thyroid hormones have direct effect on resistin but not leptin. Leptin may affect the thyroid function indirectly through its central action on TSH independent of the BMI. Leptin level of 15.3 ng/ml is associated with hyperthyroidism.

Full Text

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IT IS NOT ENDOCANNABINOIDS BUT THE TYPE AND AMOUNT OF FOOD ARE THE MAIN CAUSE OF METABOLIC DISTURBANCES IN RATS

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Abstract: Obesity has been identified as a major global health problem. A major cause of the obesity is the changes in feeding behaviour. Many controversy data concerning the role of endocannabinoid system in regulation /or disturbing of the metabolic parameters. The aim of this research is to identify the effect of methanandamide (as a one of CB1 selective agonist) on some metabolic parameters in rats fed by different types of food to clarify which is the cause of metabolic abnormality in obese ?.Design: A total number of 56 healthy adult male albino rats were used to study the effect of different types of diet and daily i.p injection methanandamide (CB1 agonist) in a dose of 0.5 mg/kg BW for 6 weeks on some metabolic parameters using pair feeding paradigm.Results: a significant increase in final body weights and a significant dyslipidemia and hyperglycemia with insulin resistance was in both HFD and HFrD fed groups when compared with that of standard chow diet fed group. Moreover, a significant dyslipidemia and hyperglycemia with insulin resistance was observed in methanandamide treated ad libitum group. In addition, our study revealed an insignificant change in all parameters measured between HFD and HFrD fed groups except for TG and VLDL parameters which are significantly higher in HFrD-fed group in comparison with that of HFD fed group. Interestingly, an insignificant change in serum levels of all previously mention parameters in the three different methanandamide treated pair fed groups in comparison with that of the three different fed control groups respectivelyConclusion: we can conclude that endocannabinoid system is not the main responsible for metabolic disturbance in obese rats.

Full Text

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The role of information and communication technologies (ICT) in agricultural development

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Abstract: Policy makers and service providers have increasingly come to view information and communication technologies (ICT), and particularly the Internet, as an important tool in providing disadvantaged groups and areas with access to information, services and markets that would otherwise be inaccessible. The concept of development of the rural, today, is not just project initiatives and governance; it is much more beyond that. This paper uncovers a whole plethora of ICT emergence as a technology of the new millennium. Against the backdrop of the ongoing ICT boom, this paper makes an attempt towards studying its applications and usage planning process and policy making for the rural communities focusing

Abstract: Gender and sex role stereotyping are recognized as having the potential to limit the professional development of males within the nursing profession. Male nurses have been a minority group within the nursing profession; nevertheless, the nursing community nowadays aims to increase the number of male nursing students and practicing male nurses lately. This study aimed to determine the undergraduate male nursing students' perception about the image of the nursing profession. The study subjects included 370 male nursing students who were enrolled in the four academic years and internship year within the academic 2008-2009. *Nursing Image –as a profession questionnaire (NIPQ)* was used for data collection. It was developed by the researcher after thorough review of literature. The current study revealed that (62.16%) of the subjects had positive image toward nursing as a profession. The subjects' perceived nursing as women's` profession were 37.8%, while the majority (91.4%) of them agreed that both males and females can be a good nurse. There was significance difference between information received about nursing before joining the faculty of nursing and nursing image .It is concluded that the highest percentage of the male students had a positive image towards nursing as a profession. It was recommended to conduct a longitudinal study to examine male nursing students regarding nursing image.

Serum Levels of Vaspin and Osteoprotegerin in Premenopausal Women with the Polycystic Ovary Syndrome

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Abstract: Polycystic ovary syndrome (PCOS) is a common endocrine disorder that affects 5-10 % of reproductive-age women. It is characterized by menstrual dysfunction and hyperandrogenism and is associated with insulin resistance, impaired glucose tolerance, dyslipidemia and visceral obesity. Vaspin (visceral adipose tissue-derived serine protease inhibitor) levels increase with hyperinsulimenia and obesity. Osteoprotegerin (OPG) is a member of the tumour necrosis factor receptor superfamily. Recent data showed that obesity and insulin resistance result in decrease in serum OPG concentrations. The present study aimed to measure serum vaspin and osteoprotegerin levels in women with PCOS to show possible involvement in the pathogenesis of PCOS. Forty eight women with PCOS, 25 non-obese [body mass index (BMI) less than 30 kg/m^2] and 23 obese (BMI >30 kg/m^2) were enrolled for the study. Each group of them is compared to apparently healthy women as a control group matched for each in age and BMI. Clinical history, anthropometric measurements and biochemical and hormonal analysis were determined. The mean serum level of fasting blood sugar (FBS), insulin, homeostasis model assessment (HOMA-IR), triglyceride (TRIG) and high density lipoprotein-cholesterol (HDL-C) showed statistically significant difference between PCOS patients (non-obese and obese) when compared to control women (non-obese and obese) respectively. In both PCOS non-obese and obese patients groups as compared to the non-obese and obese control groups, the mean serum level of vaspin showed a statistically significant increase ($P<0.001$) in both PCOS groups, while the mean serum level of OPG showed a statistically significant decrease ($P<0.001$) in the same PCOS groups. Also, the levels of both previous two parameters (vaspin and OPG) showed significant differences between PCOS obese patients and PCOS non-obese ones. It is concluded that serum vaspin level increased in PCOS women particularly the obese, whereas serum OPG concentration reduced in the same patients group. These data suggest their involvement in the pathogenesis of PCOS.

Full Text

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77	**Evaluation of immediately loaded dental implants placed in healed bony sites with or without addition of autologous platelet-rich plasma** Lama El-marssafy [1] ; Omnia Abo Ul-Dahab [2] ; Amr Zahran [3] and Mona Shoeib [4] [1]. PhD. student, Oral Medicine and Periodontal Department, Faculty of Oral and Dental Medicine, Cairo University. [2]. Professor, Oral medicine and Periodontal Department, Faculty of Oral and Dental Medicine, Cairo University. [3]. Professor, Oral medicine and Periodontal Department, Faculty of Oral and Dental Medicine, Cairo University. [4]. Professor, Oral medicine and Periodontal Department, Faculty of Oral and Dental Medicine, Cairo University. **Corresponding author:** lama.marssafy@gmail.com **Abstract:** The concept of immediate loading by using titanium one-piece implant can be preferred to the two stage technique due to the ability of the immediate loading to eliminate the need for the healing period to restore the implant. The aim of the present study was to evaluate the effect of adding platelet-rich plasma with immediately loaded self-tapping dental implant (OsteoCare™ Maxi-Z one piece) placed in healed bony sites (posterior maxillary area) on accelerating the rate of osseointegration or reducing the crestal bone resorption around these implants through the first three months follow-up period. **Materials and Methods:** The present study was conducted on 12 patients; 9 males (75%) and 3 females (25%) with a mean age of 37.5 years (28-55). Twenty four Maxi Z implants were used; each patient received two implants placed bilaterally in healed bony sites in the posterior maxillary area after the addition of platelet-rich plasma in one side while the other side was used as a positive control. All implants were immediately loaded after implant placement. **Results:** Complete soft tissue healing had occurred in all patients and all the implants were successfully osseointegrated over the twelve months follow-up period with a success rate of 100%. The results of the present study showed that there was no statistical difference between the two sides (test + control) regarding PD, MBI, MPI, implant mobility, crestal bone resorption and bone density through the twelve months. **Conclusion:** The Osteocare's Maxi Z one-piece, self-tapping self-drilling implant has shown high success rate regarding initial stability and successful osseointegration. However, within the limitations of the present study, local application of autologous platelet-rich plasma into the prepared drill holes immediately before implant placement didn't accelerate the rate of osseointegration or decrease the crestal bone resorption "through first three months period" in immediately loaded dental implant placed in posterior maxillary area. 	Full Text	77
78	**Adjectival Phrases as the Sentiment Carriers in the Urdu Text** Afraz Z. Syed[1], Aslam Muhammad[2], Martinez-Enriquez A. M.[3] [1,2]Department of CS & E, U. E. T. Lahore, Pakistan [3]Department of CS, CINVESTAV-IPN, D.F. Mexico ([1]afrazsyed@uet.edu.pk, [2]maslam@uet.edu.pk, [3]ammrtin@cinvestav.mx) **Abstract.** In this paper we present a comprehensive overview of the structures of the adjectival phrases in the Urdu language with respect to the task of sentiment analysis. Urdu is a widely spoken but one of the least explored languages by the computational linguistics community. After a detailed analysis of adjectival phrases in Urdu text we conclude that this language is orthographically, morphologically and grammatically different from other well established languages, like English and hence, it requires updated or different approaches and algorithms for the task of sentiment analysis. We present our approach in which the adjectival phrases are combined with polarity shifters, and conjunctions to make sentiment expressions in the opinionated sentences. We label these sentiment expressions as the SentiUnits. We apply	Full Text	78

	shallow parsing based chunking to extract the SentiUnits. The overall polarity of a sentence in a given review can be determined by computing the polarity of these expressions. Adjectives are the head words, which appear with modifiers and postpositions. The experimentation based evaluation of the model with a sentiment-annotated lexicon of Urdu words and two corpuses of reviews as test-beds, shows encouraging achievement in terms of sentimental analysis and accuracy. **[Afraz Z. Syed, Aslam Muhammad, Martinez-Enriquez A. M. Adjectival Phrases as the Sentiment Carriers in the Urdu Text. Journal of American Science 2011;7(3):644-652]. (ISSN: 1545-1003). http://www.americanscience.org.** **Keywords:** Natural language processing, computational linguistics, sentiment analysis, opinion mining, shallow parsing, Urdu text processing.		
79	**Isolation and Biotyping of *Brucella melitensis* from Upper Egypt** Affi, M. M.,[1&2] Abdul-Raouf, U. M.,[1] El-Bayoumy, E. M.,[3] Montasser, A. M.[3] and Mohamad, H. A.[1] [1.]Department of Microbiology, Faculty of Science, Al-Azhar University, Assuit 71524, Egypt [2.]Department of Applied Medical Science, Faculty of Science and Arts, King Khalid University, Bisha 551, Saudia Arabia [3.]Department of Brucellosis Research, Animal Health Research Institute, Dokki, Giza, Egypt magdy_afifi@yahoo.com **Abstract:** A total 106 seropositive samples from sheep, cattle and goats were collected from May 2009 to May 2010. Species of *Brucella* were isolated from, 9 (28.13%) of 32 in cattle, 25 (36.23%) out of 69 in sheep and of 5(100%) out of 5 in goats, from lymph nodes and spleen tissues. the south province of Egypt. The species examined by biochemical characteristics and had identical reactions with the standard strain. Oxidative metabolic tests performed, by substrate specific tetrazolium reduction (SSTR) test on the species, confirmed them as *B. melitensis*. Based on the biochemical, oxidative metabolic, and biotyping tests (CO_2 requirement, H_2S production, growth in the presence of thionin and basic fuchsin dyes, and agglutination test with monospecific A and M anti-sera) the strains were determined as *B. melitensis* biotype 3. [Affi, M. M., Abdul-Raouf, U. M., El-Bayoumy, E. M., Montasser, A. M. and Mohamad, H. A. **Isolation and Biotyping of *Brucella melitensis* from Upper Egypt.** Journal of American Science 2011;7(3):653-659]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** *Brucella melitensis*; Isolation; Biotyping; Upper Egypt.	Full Text	79
80	**Effects of Some Cations on Dissolution Rate of Calcium Phosphate.** **N. S. yehia ,A. M. EL Gamal** Department of chemistry, Faculty of science, Menofia University. Egypt waelgcc@yahoo.com **Abstract:** Dissolution of HAP crystals studied at =0.6, temperature 37^oc and at I= 0.15 mal dm-3 using NaCl as electrolyte at pH= 7.4 using constant composition method. The HAP crystals prepared and confirmed using XRD, SEM, IR and chemical analysis .it was found that n 2 suggesting surface mechanism. The rate of dissolution increased with increasing PH and ionic strength of medicine. The Mg^{2+}, Zn^{2+} ,and Cd^{2+} rates of dissolution were studied . The order of inhibited the dissolution of HAP at the same conditions. the order of inhibition was; $Mg^2+ > Cu^{2+} > Zn^{2+} > Mn^{2+} > Cd^{2+}$ the cations inhibited the dissolution by blocking the active sites on the surface of HAP crystal .From langmuir isotherm , K_L were found $12x10^{5,}$ $4.7x10^5$, 4.38 $x10^5$, 3.85 $x10^5$ and 1.8 $x10^5$ for Mg^{2+} , Cu^{2+} ,Zn^{2+} , Mn^{2+} and Cd^{2+} respectively inhibition of the dissolution rates of HAP in the presence of Cd^{2+} was found increase with increasing ionic strength and PH of the medium . The inhibition was found to change the morphology of HAP crystals depending on the order of mixing the reagents at the begging of the dissolution process. **[N. S. yehia, A. M. EL Gamal. Effects of Some Cations on Dissolution Rate of Calcium Phosphate.** Journal of American Science 2011;7(3):660-669]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Cations, calcium phosphate, HAP crystals, ionic strength	Full Text	80
81	**Assessing Major obstacles to rural women's participation** Sharareh Khodamoradi [1] and Mohammad Abedi[2]	Full Text	81

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Abstract Development along with economic growth and income increase is an important goal for most cou
Recently the growth of awareness about destructive effects of poverty has made countries believe that th
way to achieve sustainable development is to eradicate poverty; therefore most development programs have
oriented towards poverty eradication by micro-credit services. Supporting poor to raise their life standards
be based on the belief that the poor are able to help themselves. Explicitly, this proves that among a vari
deprivation they do consider their survival. Overall, the goal of all credit plans is to increase the poor's in
through creating self-employment opportunities and providing educational services to make the best
resources.

	Improving empowerment of rural women through micro- credit	Full Text

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Abstract: One of the raised strategy , in order to accelerate investment process and reinforcing financial
foundations , and saving , at deprived and rural areas , has been empowering and eradicating poverty of
rural societies through efficiency with emphasize on applying micro-credits. Micro-loans as useful tool to
fight against poverty and starvation, has proven its capabilities and values to develop these areas. These
tools have ability to change and improve human's life, especially poor peoples**.** Supplying credits and
analyzing credits approaches cause opportunity to activate poor men's working power , establishing field
for sustainable production and income , prevent usurers and pre shoppers of agriculture productions to
plunder poor rural men and finally empowering poor people especially women who can work but were
deprived to have capital and work tools , and extension accordance to their activities such as needs
assessment , identifying target group , organizing poor people , giving needed specialized and public
training and … have important role on effectiveness and make effective activities of these credits.

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83	**Preparation and Characterization of Sulphated Zirconia Catalyst Precipitated in Acidic Medium**	Full Text

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Abstract: Recently, sulphated zirconia has been widely studied, particularly its potential application as
solid catalysts in acid catalyzed reactions. In addition, sulphated zirconia has oxidizing properties which
may play a role in synthesis of organic compounds. The aim of this work is to prepare a series of
SO_4/ZrO_2 catalysts precipitated by different sulphuric acid concentrations using sol gel preparation
method. The prepared catalysts were calcined at 450, 550, 650, 800 ^{o}C. The surface acidity of the
prepared catalysts was measured by n-butylamine method, and by pyridine adsorption. Sulphated zirconia
tetragonal phase was successfully prepared in acidic medium using 2N H_2SO_4. It was observed that

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	drawn via suction tube. The amount of removed tissue is easily visually online controlled, which increases the safety factors for adjacent structure/tissues such as; The Eustachian tube (orifice) and pre-vertebral muscles. Hence the adenoidectomy operation becomes more accurate. Theatrical model was made to calculate the tool parts which was obtained by the existent experimental work to get the blade velocity and target tissue standards. For a better visualization and precise control a built-in suction orifice added to the tool to clean the bloody field during the operation. A small lamp is located backward of visualization sensor to allows perfect vision during the operation. As a result the adenoidectomy performance becomes faster and safer than before while the surgeon's hand used to carry the endoscope becomes free. The new tool added aggregation cavity which is Used to aggregation fluid during adenoidectomy and small lamp which located backward of visualization sensor that allows perfect vision during the operation. As a result the adenoidectomy performance becomes faster and safer than before while the surgeon's hand used. [S. Badawy, M. Koutb, G. GouId, Y. Nour, S. Akamy. **New Device for Controlled Resection of Nasopharyngeal Swellings.** Journal of American Science 2011;7(3):708-712]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Curettey, adenoidectomy, nasopharynx, endoscope, microdebrider, DC motor, Camera	
86	**Isolation and Characterization of Chitosanase Enzyme from Different Parts of Some Higher Plants** **EL-Sayed, M. El-Sayed[1], Sanaa T. El-Sayed[*2], Wafaa, G. Shousha[1], Abeer, N. Shehata[2] and Nagwa, I. Omar[2]** [1]Biochemistry, Chemistry Department, Faculty of Science, Helwan University, Helwan, Egypt [2] Biochemistry Departments, National Research Center, Dokki, Giza, Egypt [*]futtur@yahoo.com **Abstract:** Isolation and characterization of a chitosanase enzyme with high activity from different parts of some higher plants were studied. Different vegetative plant parts (leaves, fruit peels and dried seeds) representing nine families were screened in order to select the best source for extraction of the chitosanase enzyme. Results of screening experiments indicated that the enzymatic activity levels varied not only according to differences in plant species, but also to their morphological parts. In general, pepper, cabbage and purslane were the best leaves of chitosanases extractions and green bean was the best peels of chitosanase extraction while green bean, cabbage and purslane seeds were the best seeds of chitosanases extractions in this study. Results showed that leaves have high chitosanase activity more than seeds by three times. Seeds have high chitosanase activity more than peels of fruits by two times. Green bean peels, pepper leaves and opuntia peels were chosen for further study because of their high chitosanase activity. The activities of these three sources were measured by two methods. The two sources of chitosanase namely pepper leaves (C_1) and opuntia peels (C_2) were chosen for further studies. The optimal chitosanase activities of C_1 and C_2 enzymes on chitosan were obtained in 0.05 M acetate buffer, pH 5.8 and 5.4 at $40°C$ and $60°C$, respectively. The isolated C_1 and C_2 chitosanase enzymes were stable on storage for more than three months at -20°C. Chitosanase C_1 and C_2 were stable for 60 minutes at $50°C$ and $60°C$, respectively. The yields of the crude chitosanases C_1 and C_2 with optimum conditions were 47.616 and 59.146 U/g dry tissue, respectively. [EL-Sayed, M. El-Sayed, Sanaa T. El-Sayed, Wafaa, G. Shousha, Abeer, N. Shehata and Nagwa, I. Omar. **Isolation and Characterization of Chitosanase Enzyme from Different Parts of Some Higher Plants.** Journal of American Science 2011;7(3):713-721]. (ISSN: 1545-1003). http://www.americanscience.org. **Key words:** chitosanase - chitosan - pepper leaves – opuntia peels – physicochemical properties.	Full Text 86
87	**Field studies on Prevailing Internal Parasitic Diseases in Male and hybrid tilapia relation to Monosex Tilapia at Kafr El-Sheikh Governorate Fish Farms** **Eissa, I. A.M.[1]; Gado, M. S.[2]; laila, A.M[3].; Mona S. Zaki[*3] and Noor El-Deen,A. E[3]** [1]Dept. of Fish Diseases and Management, Fac. of Vet. Med. Suez Canal University [2]Dept. of Fish Diseases and Management, Fac. of Vet. Med., kafr El -Sheikh University [3]Dept.of Hydrobiology, Vet. Division, National Research Centre. [*]dr_mona_zaki@uk.com **Abstract:** The present study was carried out on 1800 specimens of *Oreochromis niloticus* (*phenotypic ,*	Full Text 87

hybrids and monosex of different lengths and body weights. They were randomly collected at different seasons from Kafr El- Sheikh Governorate cultured fish farms. The clinical signs of infested fish revealed no pathognomonic abnormalities on the external body surface .Such fish were shown emaciation. The postmortem showed that the internal organs were appeared anemic with enlargement and congestion. As well as, haemorrhage and ulceration of intestine and stomach mucous membrane. Monogenetic trematode (*Enterogyrus* cichlidarum*),* Adult flukes including *(Orientocreadium batrochoides, Afromacroderoides sp, Astiotrema reniferum* and *Eumasenia egypticus*), Nematodes including*(Procamallanas laeviconchus* and *Paracamallanas cyathopharynx),* Cestodes including *Polyonchobothrium sp and* Acanthocephalan including *Acanthocentis tilapiae* were investigated and identified. The highest prevalence possessed in hybrids of *O. niloticus* while monosex *O.niloticus* occupied the last position.. Also some physico-chemical parameters of pond waters represented as alkalinity pH, salinity, ammonia and sulphates were examined in relation to the infestation rate with internal parasites.

Hepatopulmonary Syndrome Evaluation in Egyptian Patients with Portal Hypertension and Hepatitis C Virus Cirrhosis

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Abstract: Background: Hepatopulmonary syndrome HPS was defined as a triad of portal hypertension with or without hepatic dysfunction, intrapulmonary vascular dilatation or shunting, and hypoxemia. HPS was known as an independent predictor of survival in end-stage liver disease patients after hepatic transplantation. Egypt ranked among the highest countries in prevalence and incidence of portal hypertension caused by bilharizial peri-portal fibrosis and/or post- hepatitis HCV induced liver cirrhosis, or both. The frequency of occurrence of HPS clinical and laboratory criteria showed wide variability in the different studies. Therefore detection of clinical and laboratory criteria of HPS in a sample of Egyptian patients was of utmost importance. Design and participants: In a cross-sectional comparative observational hospital based study sixty Egyptian patients with portal hypertension in comparison with age matched control group were subjected to: (1) History taking and physical examination to detect manifestations of portal hypertension, hepatopulmonary syndrome and liver cirrhosis. (2) Laboratory investigations including estimation of 1- liver functions including alanine aminotransferase [ALT], aspartate aminotrasferase [AST], alkaline phosphatase, serum albumin, total and direct bilirubin, and prothrombin time and concentration, and international normalized ratio [INR].2- chronic hepatitis viral markers: anti-hepatitis C virus antibodies, hepatitis B surface antigen, hepatitis B surface antibodies, and hepatitis B core antibodies. 3- HCV RNA- PCR qualitative assessment. (3) Arterial blood gases ABG determination in the recumbent and standing position. (4) Chest x-ray. (5) Abdominal ultrasound to detect splenomegaly, ascites, liver cirrhosis, or portal vein dilatation or abnormal flow. (6) Upper endoscopy UE to detect evidence of portal hypertension. (7) Upright trans-thoracic contrast enhanced echocardiography UTCEE to detect intrapulmonary right to left shunting. Objective of the current study was to determine the percentage of occurrence of HPS in a sample of Egyptian patients [with a mean age of 50 ± 4 years] with portal hypertension and HCV induced cirrhosis based on clinical, ultrasound, and laboratory findings, ABG abnormalities, UE and UTCEE. Results: In a total of 60 Egyptian patients with portal hypertension and HCV induced cirrhosis clinical and laboratory evidence of HPS was positive in 6 patients [10%]. All of them belonged to Child-Pugh C class with serum albumin level below 3 mg/dl and prothrombin concentration of less than 50%. Among clinical features dyspnea showed the maximum sensitivity (100%), followed by cyanosis (83.33 %), spider naevi (83.33 %) and palmer erythema (83.33 %), while platypnea (100 %) and clubbing (94.4 %) were the most specific. Partial pressure of oxygen PO2 was less than 70 mmHg in (100%) of HPS cases and was less than 60 mmHg in (50%) of them. Orthodeoxia was present in (66.66 %) of HPS versus (0 %) of non HPS patients with 66.66 % sensitivity and 100% specificity. All

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	Child C patients, who were all HPS positive, showed oesophageal varices and congestive gastropathy, denoting severe portal hypertension. UTCEE was a useful non-invasive diagnostic tool for detection of trans-pulmonary abnormal blood shunting characteristic for HPS. Conclusion: The severity of HPS was clearly correlated with the degree of portal hypertension and liver dysfunction. Dyspnea had the maximum sensitivity followed by cyanosis, spider naevi and palmer erythema. Platypnea and clubbing were the most specific clinical features. Orthodeoxia strongly suggested the diagnosis of HPS with 100% specificity. Trans-thoracic contrast enhanced echocardiography in the upright position was a safe, useful semi-quantitative bed-side tool for assessment of shunting evidence to select cases for further quantitative lung scintigraphy based evaluation. [Abir Zakaria, Ahmed El-Mazny, and Tarek Heshmat **Hepatopulmonary Syndrome Evaluation in Egyptian Patients with Portal Hypertension and Hepatitis C Virus Cirrhosis,** Journal of American Science 2011;7(3):729-737]. (ISSN: 1545-1003). http://www.americanscience.org. **Key Words:** portal hypertension, liver cirrhosis, hepatopulmonary syndrome, upright trans-thoracic contrast-enhanced echocardiography, hypoxia, dyspnea, platypnea, clubbing, orthodeoxia		
89	**A Study of the Application of Ergonomics in Ready-made Garments Factories in Egypt** Z. M. Abdel Megeid[1] and A beer Hamdi[2] [1] National Research Center, Textile Division, Dokki, Cairo, Egypt, [2] Faculty of Applied Arts, Industrial Design Department. z_algory@yahoo.com **Abstract:** Garment industry is one of the most important strategic industries which constitute about 7% of total industrial production in the world and 8.3% of the total trade in industrial materials. Also, occupies more than 14% of the total labor force in the world. It employs about 40 million people in various countries of the world. The garment industry suffers from poor efficiency of workers performance due to stress as a result of the following factors: (1) The inappropriate Design of equipment and tools used by the Group. (2) The inappropriate Design of the workplace. (3) The absence of a suitable work environment. The International Organization for Occupational Safety and Health Administration (OSHA) is concerned about providing protection, safety and occupational health of workers. For that their must be application of human Ergonomic which studies the working environment where there is a mismatch between the materials needed of equipments, tools and the physical capacities of workers, thereby reducing or limiting the Musculoskeletal disorder (stresses on the group during the performance of the work), which had not been applied ideally, till now in the garment industry in Egypt. Garment industry passes through numerous stages represented in the (Design - Action Pattern - cut - sewing of all kinds - Finishing – Ironing. etc.) and this research focuses on the stage of sewing and knowledge of occupational diseases resulting from it. By considering ready- made Garment industry and address the problems faced by the employees of poor organization of the workplace and the provision of (a appropriate chair, sewing Tables with standard specifications, comfortable pedals, and appropriate work place, good handling) we could achieve high added values in which we can develop the structure of the industry in Egypt and so increase the size of Egyptian exports. The research aimed at raising the efficiency of the performance of the garment industry, by examining the application of human ergonomics engineering which is interested in improving the employment and upgrading the conditions of the appropriate environmental factor (which prevent the stress-causing diseases). A field study was used in this method where a survey form was prepared covered five different garments factories in different places in Egypt in the governorates of Cairo and Alexandria and El-Gharbia in addition to the observations and interviews. Through the data and information collected by the computers in the extraction of statistical indicators which give indications can be used in a comprehensive picture of strengths and weakness points in the garment industry, particularly in the stage of sewing, and through this step we reach the research results. [Z. M. Abdel Megeid and A beer Hamdi. **A Study of the Application of Ergonomics in Ready-made Garments Factories in Egypt.** Journal of American Science 2011;7(3):738-747]. (ISSN: 1545-1003). http://www.americanscience.org. Keywords: **Occupational safety & health administration (OSHA), musculoskeletal disorders (MSDs , Handling**	Full Text	89
90	IT IS NOT ENDOCANNABINOIDS BUT THE TYPE AND AMOUNT OF FOOD ARE THE MAIN CAUSE OF METABOLIC DISTURBANCES IN RATS	Full Text	90

Kariman E. Slim[*], Mostafa H. AbdSalam, [*]Abeer A. A. Khalefa and Eman R. H. Abozid
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Abstract: Obesity has been identified as a major global health problem. A major cause of the obesity is the changes in feeding behaviour. Many controversy data concerning the role of endocannabinoid system in regulation /or disturbing of the metabolic parameters. The aim of this research is to identify the effect of methanandamide (as a one of CB1 selective agonist) on some metabolic parameters in rats fed by different types of food to clarify which is the cause of metabolic abnormality in obese?. Design: A total number of 56 healthy adult male albino rats were used to study the effect of different types of diet and daily i.p injection methanandamide (CB1 agonist) in a dose of 0.5 mg/kg BW for 6 weeks on some metabolic parameters using pair feeding paradigm. Results: a significant increase in final body weights and a significant dyslipidemia and hyperglycemia with insulin resistance was in both HFD and HFrD fed groups when compared with that of standard chow diet fed group. Moreover, a significant dyslipidemia and hyperglycemia with insulin resistance was observed in methanandamide treated ad libitum group. In addition, our study revealed an insignificant change in all parameters measured between HFD and HFrD fed groups except for TG and VLDL parameters which are significantly higher in HFrD-fed group in comparison with that of HFD fed group. Interestingly, an insignificant change in serum levels of all previously mention parameters in the three different methanandamide treated pair fed groups in comparison with that of the three different fed control groups respectively. Conclusion: we can conclude that endocannabinoid system is not the main responsible for metabolic disturbance in obese rats.
[Kariman E. Slim, Mostafa H. AbdSalam, Abeer A. A. Khalefa and Eman R. H. Abozid. IT IS NOT ENDOCANNABINOIDS BUT THE TYPE AND AMOUNT OF FOOD ARE THE MAIN CAUSE OF METABOLIC DISTURBANCES IN RATS. Journal of American Science 2011;7(3):748-757]. (ISSN: 1545-1003). http://www.americanscience.org.
Key Words: high fat, high fructose, endocannabinoid, insulin resistance, dyslipidemia.

	Evaluation of Correction Factors Applied in Photon Calibration of NIS TE Neutron Ionization Chambers	Full Text

Evaluation of Correction Factors Applied in Photon Calibration of NIS TE Neutron Ionization Chambers

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Abstract: Calibrations of two tissue equivalent (TE) ionization chamber were made in five photon beams (100 kV, 180 kV, 250 kV, ^{137}Cs and ^{60}Co) with two different pure gases namely acetylene C_2H_2 and carbon dioxide CO_2. The different calibration factors were compared both for in-air and in-water phantom, the measurements was performed according to the international atomic energy agency (IAEA) recommendations. For ionization chamber the total absorbed dose can be derived from the charge produced within its cavity employing a number of physical parameters. To discuss the charge produced in the cavity several correction factors have to be introduced which are related to the operational characteristics of the chambers. Information on the operational characteristics of two TE neutron ionization chambers were studied as a function of the effects of the warm-up to 3 hours, polarity, stem scattering, ion recombination, leakage current. Six different caps 1, 2, 3, 4, 6 and 8 mm were used to investigate wall thickness effect. Also, gas flow rate up to 31 ml/min and the radial & axial uniformity were investigated.
[A. I. Abd El-Hafez and M. Ezzat **Evaluation of Correction Factors Applied in Photon Calibration of NIS TE Neutron Ionization Chambers** Journal of American Science 2011;7(3):758-772]. (ISSN: 1545-1003). http://www.americanscience.org.
Key words: Tissue Equivalent Neutron Ionization Chambers- Photon Calibration- Correction Factors

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92	**Pushover Analysis for Estimating Resonance Factor of Tall RC Frames with Steel Eccentric Bracing**	Full Text

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Abstract: Since the commonplace designs are directed on linear analyses, a practical estimation on actual displacements and deformations which are taken out by linear analyses could be handled by adding a factor. On the other word, displacement resonance factor (DRF) supplemented to linear analysis responses may be held superior in order to determine actual displacements concerning time and cost concerns. According to provision of Iranian Code for seismic design, displacement resonance factor (DRF) receives same values for all structural systems. To make sure for required modifications on seismic design codes, one hundred concrete moment resisting frames with eccentric braces, designed based on the Iranian National Seismic Standard, has been considered to capture seismic parameters by performing two-dimensional nonlinear pushover analyses. Pushover Analyses have been conducted using SAP-2000 program, which can consider material nonlinearities almost near reality. In this case the applied forces have been considered as the lateral forces of the Seismic Standard. Seismic parameters including overstrength, ductility and behavior factors are excerpted by following Young Theory. Also studies based on Newmark and Hall practice has been pursued to withdraw coefficient of force reduction due to ductility. Concentrating on tall buildings, variation of DRF has been illustrated concerning bracing kind of spans, length of link beam and height of structure. Analytical results show that in the case of reminded frames the value of DRF can be much higher than that recommended by Iranian Code. On the suggestion side, this problem can be devised by multiplying a coefficient of 1.54 to the former resonance factor.

Full Text

Assessment of the susceptibility of polyculture reared African Catfish and Nile tilapia to *Edwardsiella tarda*

M. D. Ibrahem[1], Iman, B. Shaheed [2*], H. Abo El-Yazeed[3], and H. Korani[3]
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Abstract: The study aimed to clarify the relative susceptibility of polyculture rearing African sharptooth catfish (*Clarias gariepnus*) and Nile tilapia (*Oreochromis niloticus*) , the two main reared species in Egypt to Edwardsiella tarda. Experimental infection of catfish and Nile tilapia with *E. tarda* was carried out after determination of the Mean lethal dose in African catfish (LC50). Infectivity pattern and pathology of *E. tarda* in catfish and Nile tilapia were tested via intra-peritoneal inoculation of 0.2ml of 10^4 CFU/ml of the bacteria at 25 °C. The mortality rates were 70% and 60% in catfish and Nile tilapia respectively. Congestion and hemorrhages in fish body were detected in both species. African catfish showed abdominal distention together with ulcers and excessive mucus in the skin. Internally; pinpoint white nodules in the liver was the main lesions observed. Histopathological examination of organs of both species revealed presence of myositis, and degenerative changes in liver and kidneys. Establishment of infection was confirmed with the laboratory diagnosis; culture characters; biochemical reactions; API -20E test kits in addition to molecular studies based on detection of the 1106-bp PCR product in tissue samples from experimentally infected fishes at 24 hr post experimental infection. In conclusion: *E. tarda* can express a potential role in polyculture fish farming. The African catfish exhibited severe pathological lesion and histopathological changes in comparison to Nile tilapia which show moderate to mild lesions. Direct probing for the presence of *E. tarda* in infected fish by PCR is reliable and helpful in diagnosis, anticipating and rapid interference.
[M. D. Ibrahem, Iman, B. Shaheed, H. Abo El-Yazeed, and H. Korani**. Assessment of the**

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	Susceptibility of polyculture reared African Catfish and Nile tilapia to *Edwardsiella tarda*]. Journal of American Science 2011;7(3):779-786]. (ISSN: 1545-1003). http://www.americanscience.org. **Key words:** African sharptooth catfish, *Edwardsiella tarda*, experimental infection, histopathology, Nile tilapia, PCR		
94	**The General Equation Of The Pipe To Soil Potential At All Humidity Conditions By The Use Of Both Soil Factor and Stray Potential Of The Pipe-Soil-Earthing Grid System** Ashraf Abdel Raouf, Mohamed Fouad Ahmed Ashrafahmed9000@yahoo.com **Abstract:** For pipe-soil-earth system, the buried pipe line segment with soil surrounding medium could be simulated electrically by an electric circuit where the system is subjected to the law: charge = capacitance $\times$ voltage between the pipe surface and earth. This is where each of circuit electric parameter (electrolytic stray capacitor between pipe & earth, the stray potential across the stray capacitor, surface charge and the protection current of the cathodic protection system passed through the pipe segment) could be obtained by an equation which is function of the measured electrochemical properties of the soil (soil factor), 4th degree polynomial at room temperature but the A's constants are different for each electric quantity .The constants of each equation (A's) considered to be as a print of such pipe-soil-earth system . The useful of these prints is to obtain complete electrical data correlated with many cathodic protection levels**.** One of the most critical problems in CP systems is the presence of the earthing network beside the protected pipe line**.** The behavior of the stray potential between the external surface of the pipe and earth could be plotted as stray potential print which will be always valid in all times as the pipe-soil-earth system is maintained and without any external interference. This paper tries to calculate pipe to soil potential along the pipe line without the need of $Cu/CuSO_4$ half cell by the deduction of a general equation of the pipe to soil potential which is function of an electric quantities and system's print. In other words, the aim is to deduce a correlation between pipe to soil potential and both of the measured stray potential of the pipe segment and the measured soil factor around it in the presence of an earthing grid. [Ashraf Abdel Raouf, Mohamed Fouad Ahmed. **The General Equation Of The Pipe To Soil Potential At All Humidity Conditions By The Use Of Both Soil Factor and Stray Potential Of The Pipe-Soil-Earthing Grid System.** Journal of American Science 2011;7(3):787-795]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Electrical study of pipe – soil – earth system	Full Text	94
95	**In Defense Of Thermoluminescence Dosimeter Zero Dose Readouts** **A.I. Abd El-Hafez[*1] A. Maghraby[1]and N. A El-Faramawy [2].** [1] Ionizing Radiation Metrology Laboratory, National Institute for Standards (NIS), Giza, Egypt. [2] Physics Department, Faculty of Science, Ain shams University, Cairo, Egypt [*]nis_arafa@yahoo.com **Abstract:** Zero dose readout of thermoluminescence dosimeters is a very important parameter which is considered in all accurate dosimetry procedures in order to correct for the additive doses arising from other sources than irradiation processes, however, in many cases this parameter is neglected. In this paper, effects of zero-dose readings for three different thermoluminescence dosimeters glow curves were investigated. Dosimeters included in this study are: sensitized TLD-700, sensitized TLD-600, and CaF_2:Tm (TLD-300). Deconvolution of glow curves was performed in order to investigate individual behavior of each glow peak using a GCAFIT glow curve analysis software. It was found that readout of zero doses usually accompanied by changes in glow curves quantitatively (i.e. area under the curve increases), and qualitatively (relative changes in glow curve peaks intensities and their maxima positions). It is recommended that, even if the zero dose value is to be neglected as an added value to be subtracted, zero dose readouts should be performed for enhancements arise in thermoluminescence glow curves and hence better performance. This behavior is verified even LiF detectors were sensitized or not. In contrary, for CaF_2:Tm (TLD-300), a little effect is noticed because there is no thermal quenching effect and competing deep trap in this material. [A. I. Abd El-Hafez, A. Maghraby and N. A El-Faramawy**, In Defense Of Thermoluminescence**	Full Text	95

96	**Early Post-Percutaneous Coronary Stent Intervention Period: Is The Tooth Extraction Safe?** **Mohamed Zaghlool Amer** [*1], **Maged Zaghlool Amer** [2] * Oral and Maxillofacial Surgery Department- Faculty of Dentistry-Mansoura University, Mansoura, Egypt ** Cardiology Department - Faculty of Medicine- Mansoura University, Mansoura, Egypt [*]norhanmohammed910@yahoo.com **Abstract:** Objectives: Several risk factors can affect the cardiovascular outcome resulted from minor dental surgical procedures in patients received Percutaneous Coronary Stent Intervention especially in the early postoperative phase. So, the aim of this study was directed to evaluate the cardiovascular changes and post-operative bleeding occurred during the early post- PCI period (early six months) for patients subjected to tooth extraction and received L.A drug with or without vasoconstrictors in the presence or absence of preoperative sedation. Patients & Methods: Fourty four patients included within this study were subjected to tooth extraction during the early 6 months following percutaneous coronary stent intervention (PCI). Patients were divided equally into four groups. In 1st group, patients received L.A with vasoconstrictor under preoperative sedation. While, 2nd group was similar but without the presence of preoperative sedation. In 3rd group, patients received L.A without vasoconstrictor under the presence of preoperative sedation. The 4th group was similar to 3rd group but without the presence of preoperative sedation. Systolic, diastolic blood pressure, heart rate and ST segment deviation were recorded for patient assessment. Results: No significant difference between the 1st and 4th group regarding to Mean Bp (P=0.130), Mean HR (P=0080) and Mean ST segment deviation (P=0.205)and Sys Bp.(P=0.417). A significant difference between the 2nd group and 3rd group regarding to Sys. BP. (P=0.000) Diastolic Bp (P=0.004), Mean HR (P=0.000) and Mean ST segment deviation (P=0.000). Conclusion :Combined role of presence or absence of presedation and vasoconstrictor in PCI patients subjected to tooth extraction can play a dramatic effect on cardiovascular parameters rather than each of them separately for the same type of patients. 	Full Text	96
97	**Evaluation of Abrasion Behaviour of Knitted Fabrics under Different Paths of Martindale Tester** N. A. Kotb[1], Z. M. Abdel Megeid[2] 1. Faculty of Education, Department of Technical education, Helwan, University, Egypt 2 .National Research Center, Textile Research Division, Dokki, Cairo, Egypt nabihakotb@yahoo.com **Abstract:** The Martindale tester is used for both the abrasion, pilling resistance of fabrics, and straight line test by adjusting three moving parts, each one has three setting levels; making twenty seven paths possibilities. According to the standards there are only three types of motion to perform different tests. Therefore the aim of this study is to evaluate the effect of other setting possibilities on abrasion behaviour. The Lissajous patterns which consist of the Path traced by the fabric over the abradent in Martindale tester have been drawn in continuously changing directions at different setting. The total numbers of working conditions are sixteen patterns, since some adjustments did not work or gave the same path or lines. Then the areas of all patterns have been calculated and analyzed. Three samples of knitted fabrics produced from three counts have been tested at the combinations of different path of the Martindale tester. Therefore forty eight results of abrasion resistance for all fabrics at different settings have been measured and analyzed. Using Martindale standard testing setting is not enough to determine the actual abrasion behaviour of knitted fabrics. Other probabilities of setting, producing other different patterns in area and shape, could be	Full Text	97

	simulated to the actual abrasion behaviour of fabrics during the end use. It could help the textile designer and producer to understand and improve their products according to the actual performance requirements. [N. A. Kotb, Z. M. Abdel Megeid. **Evaluation of Abrasion Behaviour of Knitted Fabrics under Different Paths of Martindale Tester.** Journal of American Science 2011;7(3):812-817]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords**: Lissajous curve figures – Abrasion resistance- Martindale-weft knitted fabric		
98	**The relation between fabric construction, treatments and sewability** F. Fathy Saied [1], Z. m.abdel-megeid [2] and L.K. El Gabry [2*] Academy of Specific Studies, Worker University [1] and National Research Centre [2], Textile Research Division, Dokki, Cairo, Egypt. lamiaa_gabry@yahoo.com **Abstract:** Cotton/polyester blend fabric at construction is produced. Fabric mechanical tests are measured using FAST (Fabric Assurance by Simple Testing) system, for assessing aspects of the performance in garment manufacture and garment appearance after wear. The optimization construction are used to carry out treatments impart to improve pilling and antimicrobial activity. The effect of treatments on sewing needle penetration fabrics untreated and treated is measure for determined any damage which appears in garment. It was found that formability, bending rigidity and shear rigidity decrease with decrease the weft count, but extensibility increase consistently. Also, the construction of plain has count No.40/1 gave low force penetration.[Fathy Saied, Z. m.abdel-megeid and L.K. El Gabry. **The relation between fabric construction, treatments and sewability.** Journal of American Science 2011;7(3):818-826]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Construction, FAST, Cotton/polyester blend fabric, sewability, ant pilling and antimicrobial	Full Text	98
99	**Performance Optimization of Field Oriented Vector Control Drive using PSO and GA Techniques** *M.M.A. Mahfouz and Mohamed .M .Ismail Faculty of Engineering, Helwan University, Egypt *mohamed.mahfouz@yahoo.co.uk **Abstract:** In this paper optimization of flux vector control (FOC) drives performance are studied. Genatic algorithm (GA) and Particale swarm optimization (PSO) are used for this purpose. Optimuim flux referance identifection by using GA and PSO are used to minimize the motor input power to have the optimuim motor efficiency. Selecting of the optimal gains using both methods are done to improve the motor response and behavior. A comparison between the simulation results were illustrated to evaluate the Performance for the developed controller adopting (GA) and (PSO) algorithms.The results show that, the proposed PSO controller algorithm has better optimization performance more than the proposed GA in both for gain tunning and also for the selection flux set point.[M.M.A. Mahfouz and Mohamed .M. Ismail. **Performance Optimization of Field Oriented Vector Control Drive using PSO and GA Techniques.** Journal of American Science 2011;7(3):827-834]. (ISSN: 1545-1003). http://www.americanscience.org. Keywords**: Induction motors, Filed Oriented Control drives, Flux Observer Controller, Genetic Algorithm, Particle Swarm Optimization.**	Full Text	99
100	**The effects of antioxidants supplementation on haemostatic parameters and lipid profiles in diabetic rats**	Full Text	100

Zeinab Abdel-Rahman

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Abstract: Diabetes mellitus is a complex, progressive disease, which is accompanied by multiple cardiovascular complications. Oxidative stress is significantly increased in diabetic patients and may lead to great haemostatic disturbances existing in these patients. Antioxidants have been reported to reduce oxidative and haemostatic variables by arresting free radical damage .The aim of this study was to assess the role of antioxidants (vitamin E and C) in modulation of the haemostatic parameters and lipid profiles in experimentally-induced diabetic rats. Blood samples are obtained from control rats (no=24) and diabetic rats (no=24) to estimate haemostatic status by platelets aggregation, fibrinogen levels and prothrombin time. Oxidative status was assessed by estimation of the lipid profiles {Triglycerides (TG), low density lipoprotein (LDL), high density lipoprotein (HDL) cholesterol} and plasma uric acid. Diabetics rats were divided into two sub-groups. The first sub-group (no=12) was orally supplemented with Vitamin E (7mg/rat) daily for 4 weeks and the second sub-group (no=12) was co-administrated Vitamin C (7mg/rat) and Vitamin E daily for 4 weeks. Blood samples are withdrawn from the two sub-groups and the previous parameters were assessed. .Increased levels of TG and LDL cholesterol and reduced levels of HDL cholesterol and plasma uric acid were recorded in the rats after induction of diabetes, compared to prediabetic values. Hypercoagulability state was observed in diabetic rats through percentage increase in platelet aggregation and fibrinogen level. Oral supplementation of Vitamin E to diabetic rats resulted in a significant inhibitory effect on the oxidative stress and partial reduction of the hypercoagulability state, which were more observed by co-administration of vitamin C. It is concluded that hyperglycemia in rats increased oxidative stress which may play a role in induction of hypercoagulable state. Dietary co-administration of vitamin E and C induced protective effects to diabetic rats.
[Zeinab Abdel-Rahman. **The effects of antioxidants supplementation on haemostatic parameters and lipid profiles in diabetic rats.** Journal of American Science 2011;7(3):835-840]. (ISSN: 1545-1003). http://www.americanscience.org.

Key words: Diabetes, oxidative stress ,lipid profile , platelet aggregation, antioxidants

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In vivo and *in vitro* studies on *Thevetia* species Growing in Egypt
II. Establishment of *in vitro* tissue culture system and production of cardiac glycosides

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Abstract: Applicable protocol for enhancement of calli production, shootlets regeneration, evaluation and determination of cardiac glycosides from growing *Thevetia spp.* was established. Calli and regenerated shootlets were induced from immature seeds (IS), leaf, stem and root explants of *Thevetia neriifolia* Jussieu. and *T. thevetioides* Kunth. (Apocynaceae). MS medium supplemented with 1mg/l 2,4-D + 3mg/l Kin showed the best results of mass calli production. Seed explants gave the highest value of calli formation under either light or dark condition. The 3rd week of cultivation for 5 weeks recorded the significant growth dynamic of mass calli production from seed explants. *T. neriifolia* exhibited high degree of multiple shootlets proliferation on MS + 1mg/l 2,4-D + 3mg/l BAp from stem explants as compared with *T. thevetioides*. The best result of *in vitro* rootlets/ shoot formation recorded with MS + 1mg/l NAA. Qualitative and quantative determinations of thevetin B, digitoxigenin, neriifolin and peruvoside contents were carried out using HPLC technique.
[Taha H. S., Farag.S.H., Shams K. A., Abdel-Azim N. S., and Seif El-Nasr M. M**. In vivo and *in vitro* studies on *Thevetia* species Growing in Egypt Establishment of *in vitro* tissue culture system and production of cardiac glycosides.** Journal of American Science 2011;7(3):1-12]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: *Thevetia spp.* callus, regeneration, Cardiac glycosides, HPLC

1. Introduction

        Thevetia neriifolia Juss. and *T. thevetioides* Kunth. are small tree commonly used as an ornamental plant. It is belongs to the Apocynaceae family and it can be found in South and Central America, Asia, and Africa. This plant species produces several compounds with industrial application as pharmaceutical compounds, such as cardiac glucosides neriifolin, thevetoxin, peruvoside, and thevetin A and B (Arnold *et al.* 1935; Omino and Kokwaro*,* 1993). Among these compounds, peruvoside is particularly attractive because it uses as a digoxin substitute in allergic patients, and it commercially distributed in Germany for that purpose (Kumar 1992, Abe *et al.* 1995). In addition, these compounds usually found in low concentrations in the plant, therefore, making the direct extraction is difficult and expensive. Additionally, successful procedures for its chemical synthesis have not been estimated, probably because for their complex structures. As such, the strategy of plant cell culture is attractive for the production of these metabolites. Plant cell culture has several advantages over the traditional cultivation, such as the control of the production conditions, weather independency, and continuous production. It is still necessary to overcome some difficulties such as low productivity and low specific growth rate (Sajc *et al*. 2000).

        In vitro culture of plants has gained in importance during recent years because, besides other applications, this technique used for the rapid multiplication of some plants (Tisserat 1987). The establishment of *in vitro* calli cultures were initiated from young leaves of *T. neriifolia* on MS medium supplemented with 1mg/l 2,4-D (Anjani and Pramod 1990). Anjani (1992) reported that calli cultures were induced within 30 days by culturing young leaf discs of *T. neriifoia* on MS medium supplemented with 9 µM 2,4-D and 4.6 µM Kin. Sub-culturing calli at lower 2,4-D and Kin concentrations (4.5 µM 2,4-D and 0.46 µM Kin) increased calli growth and produced numerous highly organized structures on calli surface. Furthermore, Anjani (1995) established *in-vitro* regeneration system from embryonic axis of *T. neriifolia*. He found that more than 85% of the *in vitro* derived shoots were rooted to give complete plantlets with an 80% survival rate on half-strength of MS medium containing 5.37 µM of NAA under glasshouse conditions.

        Moreover, Dantas *et al.* (1993) reported that the highest cardiac glycoside contents were recorded in cell suspension cultures of *T. neriifolia* cultured on MS medium supplemented with kin + NAA, Kin+2,4-D, and IAA, respectively.

        Consequently, Dantas *et al.* (1994) studied six strains of *T. neriifolia* cell cultures for cardenolides production. Even after two years of subculture, cardiac glycosides proved to be present in all these strains. The cardiac glycosides content varied from one species to another according to the nature of the original explants. Further, Lopes *et al.* (2001) reported that some compounds of cardiac glycosides found in the intact plant could be accumulate in cultured cells, cardiac

glycosides of *T. neriifolia*, accumulated in cultured cells during one year of cultivation.

This present work aim to described efficient protocol for; calli production; shootlets regeneration and, enhancement the accumulation rate of cardiac glycosides in different cultures of Egyptian *Thevetia spp*.

2. Materials and Methods
In vitro Immature seeds (IS) germination.

Immature seeds of both *Thevetia neriifolia* Juss. and *Thevetia 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 National Research Centre, Dokki, Cairo, Egypt. Then they were surface sterilized by immersion in 70 % ethanol for 15 sec., followed by washing with sterile distilled water for 3 times. Subsequently, they were immersed in 50 % of Clorox solution (5.25 Cl_2) containing a drop of Twin 20 for 15 min, and rinsed several times with sterile distilled water. The sterilized seeds were separated and aseptically cultured on MS basal medium (Murashige and Skoog, 1962) containing 3 % sucrose and 0.7 % agar added prior autoclaving at a pressure of 1.5 psi (1.2 Kg/cm^2) for 20 min. The pH of the culture medium adjusted to 5.8 by addition of 0.1 N HCl or 0.1 N KOH. Within 30 days, seeds germinated.

Establishment of calli

One-month-old plantlets at the physiological age of 4-7 cm of shoot length, 15- 26 cm length of root and the number of leaves 4-10, were used as a source of explants (Fig.1). Leaf, stem and root explants as well as IS were cultured on MS medium supplemented with different plant growth regulators as follow:- Basal MS medium (free of PGRs) (MS_0) ; MS+1mg/l 2,4-D +1mg/l Kin (MS_1); MS +1mg/l 2,4-D + 3mg/l Kin (MS_2); MS +3mg/l 2,4-D + 1mg/l Kin(MS_3); MS +5mg/l 2,4-D + 1mg/l Kin(MS_4);MS +1mg/l 2,4-D + 5mg/l Kin(MS_5);MS +5mg/l 2,4-D + 5mg/l Kin(MS_6) ;MS+ 1mg/l 2,4-D + 3mg/l BA(MS_7) ;MS + 1 mg/l NAA (MS_8); MS + 1 mg/l IAA (MS_9);MS + 1mg/l IBA (MS_{10}).

Cultures of all treatments were maintained under photoperiod of 16 hrs/day photoperiod at intensity of 1400 Lux (80 μ mol $m^{-2}S^{-1}$) using cool light fluorescent lamps (Philips). All cultures were incubated at 26 ±1 °C for 4 weeks.

Determination of calli growth parameters

The percentage of callus formation, fresh & dry weights and dry matter contents (%) were determined for each treatment after 4 weeks of cultivation.

Shootlets regeneration

Three pieces about 250 mg/jar of leaf, stem, root and IS derived calli from MS-medium supplemented with 1mg/l 2,4-D + 3mg/l Kin were cultured onto the following regeneration media: MS basal medium (free of PGRs) (MS_0);MS + 1 mg/l 2,4-D + 1 mg/l BA (MS_1); MS + 1 mg/l 2,4-D + 3 mg/l BA (MS_2); MS + 3 mg/l 2,4-D + 1 mg/l BA (MS_3); MS + 5 mg/l 2,4-D + 1mg/l BA (MS_4); MS + 5 mg/l 2,4-D + 1mg/l BA (MS_5) ;MS + 5 mg/l 2,4-D + 1mg/l BA (MS_6); Water+ Agar medium (free of salt and PGRs) (WA)

All cultures were incubated at 26°C and 1400 Lux (80-μ mol $m^{-2}S^{-1}$) using cool light fluorescent lamps (Philips) in a controlled growth room. After one month of culturing, percentage and number of formed shootlets recorded.

Adaptation and acclimatization

In vitro rooted shoots were carefully taken out of the tissue culture jars and gently washed under tap water to remove the residual agar and medium sticking to it. Then, the obtained plantlets were dipped in 1% aqueous solution of bavistin, a systemic fungicide for 10-15 min and then washed with tap water. Subsequently, the treated plantlets were transferred in (8 cm) pots filled with peatmoss or a mixture of peatmuss: sand in (1:1) or peatmoss: sand: perlit (1:1:1) or peatmuss: sand: perlit: vermiculite (1:1:1:1). Cultures were covered with polypropelene bags and kept in green house. The cultured plantlets were watered once in a week. The top corners of polypropelene bags were cut after two weeks to gradually expose the plants to the outside environment. After 3-4 weeks, the polypropelene bags were completely removed.

Preparation of cardiac glycosides forHPLC analyses

Extraction of cardiac glycosides based on the method described by Abe *et al*. (1994). One mg of the total cardiac glycosides was re-dissolved in 1ml of methanol HPLC grade, then filtered through 0.2-0.4 mM nylon filter and subjected to HPLC techniques.

Authentic compounds

The reference cardiac glycosides (peruvoside and neriifolin) were purchased from Sigma Chemicals Co., St. louis Mo. USA. Thevetin B and digitoxigenin were chemically separated and identified throughout this work according to the described method by Bisset *et al*. (1962) and Decosterd *et al*. (1994).

General procedures

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$_2$O (1:1) under gradient conditions. This method was carried out according to Kathleen *et al.* (2007). Column chromatography was carried out on silica gel 60 (Merck; 230 - 400 mesh). TLC: pre-coated silica gel 60F$_{254}$ plates (Merck); CC: silica gel type 60 (Merck). MS: Murashige and Skoog medium (Duchefa Biochemie The Netherlands).
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Statistical analysis

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).

3. Results and Discussion
Calli production

Data tabulated in Table (1) show that the effect of different combinations of 2,4-D, NAA, Kin or BA at 1mg/l on fresh weight frequency of calli production from IS, leaf, stem and root explants of *T. neriifolia* and *T. thevetioides*. In this experiment MS$_2$

gave the highest value of calli production (Fig. 2 A and B) from IS, leaf, stem and root explants, respectively. Moreover, the incubation under light condition was preferred as compared with incubation under dark condition. Concerning, calli production from *Thevetia spp.* on MS$_2$ was in agreement with Anjani (1992) who reported that the presence of 2,4-D and Kin increases the biomass of different calli culture production from leaf explants of *T. neriifolia*. Similar effect was observed with calli culture production from *Solanum aviculare* (Kittipongpatana *et al.* 1998). Further, Preece (1995) reported that when the nutrient salts are optimized for *in vitro* tissues of a plant, the nutrient level and their balance in the medium makes tissues to be under less stress and *in vitro* performance is dramatically improved. This evidence was accordance to our observation that; calli cultures of *Thevetia spp.* which cultured on MS$_2$ medium do not exude any phenolics, with high calli biomass. From the obtained results, it may speculate that 2, 4-D might be less antagonistic hormone interplay with combination of Kin and resulted in higher calli biomass production. The pervious results are in consistent with Dasgupta and Datta (1980); Dhru *et al.* (1990) and Anjani (1992) who reported that the presence of 2,4-D and Kin increased the biomass of different calli production from leaf explants of *T. neriifolia*..

Table(1). Effect of augmented MS-medium with different combinations and concentrations of auxins and cytokinins on frequencies of callus formation from IS, leaf, stem and root explants of *T. neriifolia* and *T. thevetioides* cultured under light condition at 26 ± 1 °C.

Type of MS media	Calli production (g/jar)							
	T. neriifolia explants				*T. thevetioides* explants			
	IS	Leaf	Stem	Root	IS	Leaf	Stem	Root
MS0	-	-	-	-	-	-	-	-
MS1	-	++	++	++	++	+++	++	++
MS2	++++	++++	++++	++++	+++	+++	+++	+++
MS3	-	-	-	-	-	-	-	-
MS4	++	+++	+++	++	++	+++	+++	++
MS5	+	+	+	+	+	++	++	++
MS6	+	+	+	+	+	+++	++	++
MS7	+++	+++	+++	+++	++	+++	+++	++
MS8	++	++	++	++	+	++	++	+
MS9	++	++	++	++	+	+	+	+
MS10	++	++	++	++	+	++	++	+

Where: - =No calli induction, + =Calli FW 0.25~ 0.5 g/jar, ++ = Calli FW 0.5~ 1.5 g/jar, **+++**= Calli FW 1.5~ 2.0 g/jar and ++++ =Calli FW 2.0 ~ 3.0 g/jar.

Calli growth parameters

The obtained healthy calli (~250 mg/jar) from IS, leaf, stem and root of *Thevetia* spp. were sub-cultured on MS$_2$ to follow their growth pattern for five weeks. Data in Tables (2 and 3) show that, the highest fresh (FW), dry weights (DW) and dry matter content (%) (DMC) were recorded with IS explants of *T. neriifolia* and *T. thevetioides,* respectively.
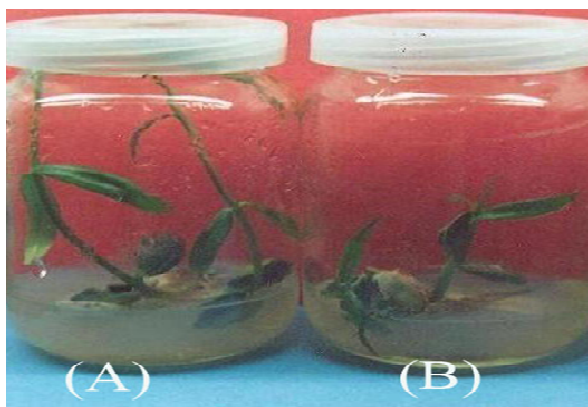
Fig. (1). One-month old seedlings of *T. neriifolia* (A) and *T. thevetioides* (B) were established on free of plant growth regulators MS medium

Fig.(2). Calli production from IS of *T. neriifolia* (A), and *T. thevetioides*, cultured on MS +1mg/l 2,4-D+3mg/l BA medium and incubated under light condition at 26±1°C for 3 weeks.

Concerning, *T.neriifolia* and *T. thevetioides* calli growth dynamics, the obtained results clearly showed that fresh & dry weights were weekly increased gradually up to the 3rd week of cultivation. Subsequently, the fresh & dry weights decreased in the 4th and 5th weeks of cultivation. Similar effect was observed with calli culture of *Solanum aviculare* (Kittipongpatana *et al.*, 1998). At the early stage of growth, the calli tissues were actively growing through cell multiplication and enlargement. During growth, synthesis of protein, nucleic acid, phospholipids, multiplication of organelles and utilization of energy in the form of ATP occurs (Kumar, 1999). Regarding, the morphological characters, it is interesting to mentioned that during 1st to 3rd week of cultivation, calli derived from IS, leaf, stem and root explants of the two investigated *Thevetia* spp. were healthy, friable and yellowish. However, the decline phase recorded after the log phase (i.e. 4-5th week), the growth slightly or even sharply was decreased. De and Roy (1985) attributed the decrease in the calli weights to the degradation of compounds over synthetic processes and/or the production and release of extracellular materials, which accumulated in the medium and not recovered in the cells of the senescent calli. Accordingly, the routine production of calli it was manifested by sub-culturing the initial calli on the same new fresh MS medium at the end of the 1st week to the end of the 5th week for the first month of calli cycle production.

Table (2). Effect of fortified MS medium with 1 mg/ l 2,4-D + 3 mg/ l Kin on calli fresh, dry weights (g/jar) and dry matter content (%). Calli were derived from IS, leaf, stem and root explants of *T. neriifolia* and incubated under light conditions at 26 ± 1°C.

Expl ts	*T. neriifolia* calli growth parameters incubated under light condition														
	1st week			2nd week			3rd week			4th week			5th week		
	FW	DW	DMC	FW	DW	DMC	FW	DW	DMC	FW	DW	DMC	FW	DW	DMC
IS	1.25±0.011	0.056±0.2	4.48	1.63±0.04	0.095±0.01	5.83	2.95±0.2	0.211±0.06	7.15	2.83±0.04	0.187±0.04	0.187	2.75±0.02	0.175±0.03	6.36
Leaf	1.25±0.11	0.053±0.6	4.24	1.51±0.21	0.073±0.01	4.83	2.73±0.7	0.185±0.2	6.78	2.65±0.05	0.175±0.03	0.175	2.54±0.01	0.154±0.2	6.06
Stem	1.26±0.02	0.055±0.03	4.37	1.43±0.05	0.052±0.06	3.64	2.47±0.09	0.153±0.32	6.19	2.35±0.01	0.143±0.01	0.143	2.27±0.6	0.127±0.02	5.59
Root	1.27±0.03	0.050±0.07	3.94	1.35±0.3	0.041±0.07	3.04	2.34±0.03	0.142±0.3	6.09	2.25±0.03	0.125±0.1	0.125	219±0.09	0.109±0.04	4.98

Table (3). Effect of fortified MS medium with 1 mg/ l 2,4-D + 3 mg/ l Kin on calli fresh, dry weights (g/jar) and dry matter content (%). Calli were derived from IS, leaf, stem and root explants of *T. thevetioides* incubated under light conditions at 26 ± 1°C.

Explants	Calli growth parameters incubated under light conditions														
	1st week			2nd week			3rd week			4th week			5th week		
	FW	DW	DMC	FW	DW	DMC	FW	DW	DMC	FW	DW	DMC	FW	DW	DMC
IS	1.25±0.03	0.054±0.09	4.32	1.54±0.02	0.083±0.01	5.85	2.85±0.03	0.193±0.06	6.77	2.49±0.01	0.165±0.02	6.63	2.34±0.03	0.154±0.03	6.58
Leaf	1.26±0.07	0.053±0.02	4.21	1.48±0.03	0.070±0.08	2.60	2.60±0.02	0.175±0.02	6.73	2.43±0.03	0.153±0.03	6.29	2.31±0.3	0.143±0.01	6.19
Stem	1.25±0.05	0.049±0.06	3.92	1.35±0.05	0.041±0.03	2.40	2.40±0.05	0.143±0.08	5.96	2.25±0.05	0.138±0.02	6.13	2.19±0.1	0.125±0.01	5.71
Root	1.28±0.02	0.048±0.03	3.75	1.30±0.03	0.029±0.05	2.29	2.29±0.02	0.135±0.03	5.89	2.18±0.02	0.125±0.04	5.73	2.12±0.1	0.120±0.01	5.66

Shootlets regeneration

Data tabulated in Table (4) show that the best culture medium for shootlets regeneration was MS$_2$ as compared with other media (Figs. 3 and 4). The maximum numbers of regenerated shootlets 10.3, 6.7 and 4.0 shootlets were recorded with stem, leaf and seed calli cultures, respectively of *T. neriifolia*. However, they recorded 4.5, 3.1 and 2.2 shootlets for stem, leaf and seed calli cultures of *T. thevetioides*. The maximum value of direct shootlets regeneration was noted with shoot tip, leaf, seed, stem and root cultures, respectively. Concerning, the derived shootlets length 5.6, 3.5 and 2.6 (cm) were recorded with stem, leaf and seed calli cultures of *T. neriifolia*. However, they recorded 4.3, 3.1 and 2.5 (cm) for stem, leaf and seed cultures of *T. thevetioides* (Fig.4 A and B).

Regarding the maximum numbers of shootlets regeneration from different calli-derived explants were recorded with MS$_2$ as compared with other treatments. The obtained results are in consistent with the obtained results by Ratna and Misra (2005). They reported that shoot tips derived calli of mature plants of *Carissa carandas*; when cultured on MS basal medium supplemented with 0.8 mg /l IBA and 0.2 mg /l NAA produced the maximum sprouting rate.

Table (4). Effect of supplementation of MS-medium with different combinations and concentrations of auxins and cytokinins on percentage of sootlets formation from IS, leaf, stem and root explants of *T. neriifolia* and *T. thevetioides* cultured under light condition at 26 ± 1 °C.

Type of MS media	Calli production (g/jar)							
	T. neriifolia explants				*T. thevetioides* explants			
	IS	Leaf	Stem	Root	IS	Leaf	Stem	Root
MS0	-	-	-	-	-	-	-	-
MS1	-	++	++	++	++	+++	++	++
MS2	++++	++++	++++	++++	+++	+++	+++	+++
MS3	-	-	-	-	-	-	-	-
MS4	++	+++	+++	++	++	+++	+++	++
MS5	+	+	+	+	+	++	++	++
MS6	+	+	+	+	+	+++	++	++
WA	-	-	-	-	-	-	-	-

where (*): **–** = No response, **+** = Low regeneration value (0~20%), **++** = Medium regeneration value (20~60%), **+++** =High regeneration value (60~100%). Each treatment is the average of 5 replicates.
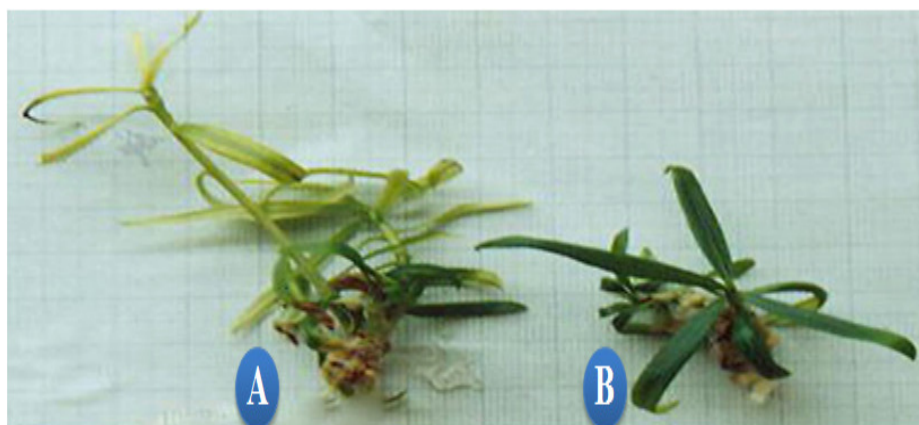
Fig.(3). *In vitro* shootlets regeneration from stem *derived* calli of *T. neriifolia* (A) and *T. thevetioides* (B) on MS$_2$ after 4 weeks of cultivation under light condition at 26±1ºC.

Rootlets shoot formation

The effect of MS$_0$ or MS$_8$ or MS$_9$ or MS$_{10}$ on achievement of rootlets formation on *in vitro* derived regenerated shootlets of stem explants of *T. neriifolia* and *T. thevetioides* is illustrated in Fig 5 (A and B). The highest percentages of root formation 63% and 52% were recorded with *T. neriifolia* and *T. thevetioides*, respectively. The maximum numbers of rootlets/shootlets formation 32 and 10 were recorded with *T. neriifolia* and *T. thevetioides* respectively. Furthermore, length of rootlets formation 17.6 and 14 (cm/shoot) were recorded with *T. neriifolia* and *T. thevetioides*, respectively. MS medium supplemented with 1mg/l NAA gave the favorable percentages of rootlets formation, number of rootlets/shootlet and length of rootlet (cm) as compared with other supplementations, as well as compared with MS basal medium.
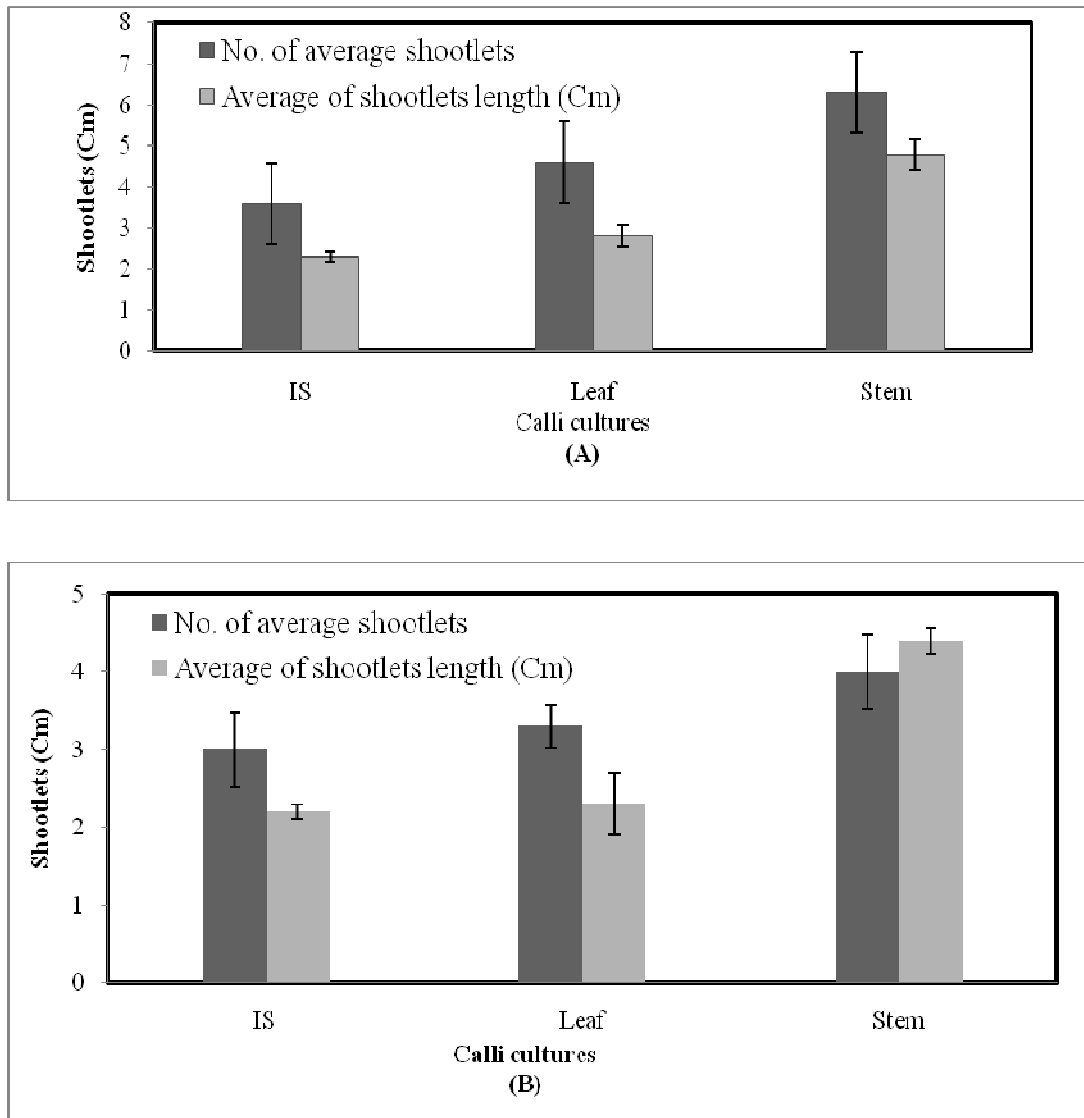
Fig. (4). Effect of MS$_2$ on number and length (cm) of regenerated shootlets derived from IS, leaf and stem calli-cultures of *T. neriifolia* (A) and *T. thevetioides* (B) cultured under light conditions at 26±1ºC for4 weeks.

The obtained results are in agreement with the obtained results with Anjani and Abha (1994) they reported that shoots of *Catharanthus roseus* and *T. neriifolia* were rooted on MS basal medium containing 1mg/l NAA. In addition, the obtained results are in harmony with the obtained results by Soo *et al.* (2003) they reported that, MS augmented with 1mg/l NAA was proved to be the best concentration of NAA for induction of adventitious roots from base of regenerated shoots of *T. neriifolia*.
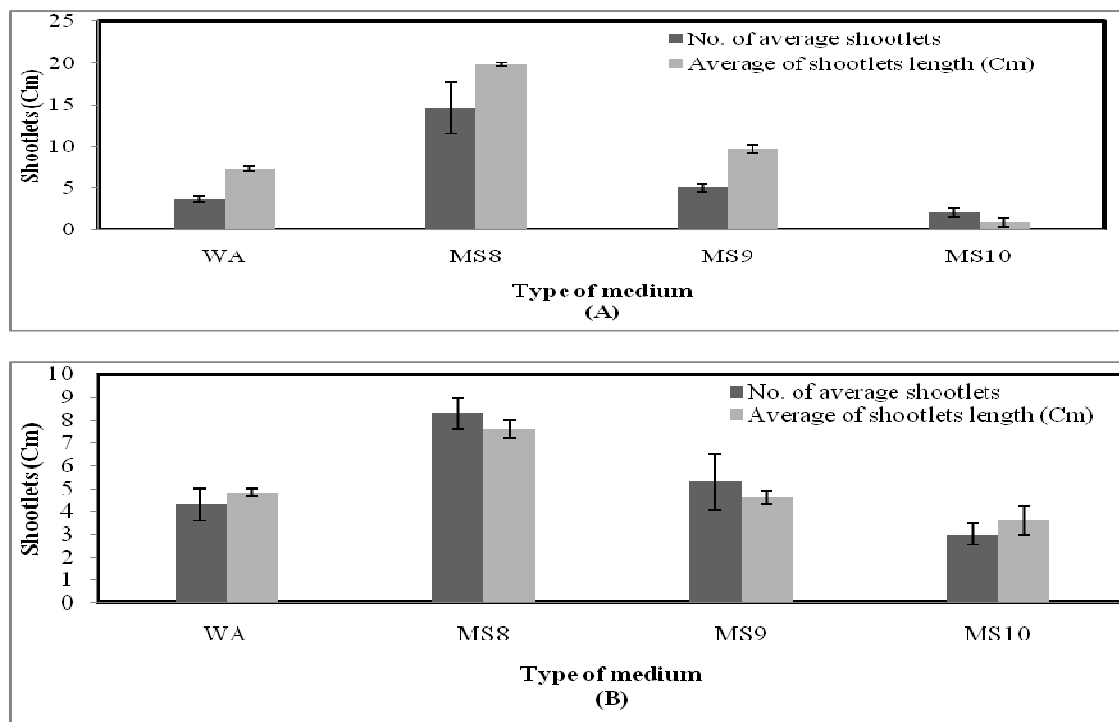
Fig. (5). Effect of WA; MS$_8$; MS$_9$ or MS$_{10}$ media on rootlets shoots formation parameters of *T. neriifolia* (A) and *T. thevetosides* (B).

Fig.(6). In vitro rootlets/shootlets formation of *T. neriifolia* (A) and *T. thevetosides* (B) leaf explants and cultured on MS medium supplemented with 1 mg/l NAA

Adaptation and acclimatization

As shown in Table (5) the highest percentages of survival plantlets 70% and 63% were recorded with *T. neriifolia* and *T. thevetioides*, respectively. The best potting mixture medium for *in vitro Thevetia* spp. plantlets acclimatization was peatmuss, sand, perlit and vermiculite (1:1:1:1) as compared with other media structure. Furthermore, the best number of shootlets formation 6 and 5 (cm) and length of root 10.5 and 6.3 (cm) as a growth parameters were recorded with *T. neriifolia* and *T. thevetioides*, respectively, when cultured on peatmuss, sand, perlit and vermiculite (1:1:1:1) as compared with other potting mixture media (Fig. 7).

The obtained results are in close with the recorded results by Ratna and Misra (2005). They reported that shoot tips derived from calli of mature plants of *Carissa carandas*; when cultured on MS basal medium supplemented with 0.8 mg /l IBA and 0.2 mg /l NAA produced maximum sprouting rate. The obtained shoots were

rooted on MS medium supplemented with 1mg/l NAA. Moreover, in agreement with the obtained results; Anjani and Abha (1994) reported that shoots of *Catharanthus roseus* and *T. neriifolia* were rooted on MS basal medium supplemented with 1mg/l NAA. Furthermore, the use of NAA, as a potential root hormone, is in agreement with those reported for rooting in microshoots of *Nerium oleander* (Hatzilazarou *et al.* 2003). The rootlet plantlets of *T. neriifolia* and *T. thevetosides* were successfully acclimatized in potting mixture peatmuss, sand, perlit and vermiculite (1:1:1:1). It was found to be in consistent with the obtained results by Ratna and Misra (2005). Moreover, Debergh and Maene (1981) reported that the induction and development of root system at the bases of *in vitro* grown shoots of *Nerium oleander* is an essential and indispensable step to establish tissue culture derived plantlets to the soil.

Qualitative determination of cardiac glycosides using HPLC technique

Standard curves corresponding to the compounds thevetin B, digitoxigenin, peruvoside and neriifolin were carried out using the described protocol by Scott (1996). The concentrations of referred compounds in the examined samples were detected using its retention time and peak area.

Table(5). Effect of different nutrient media composition on root parameters formation on isolated shootlets of *T. neriifolia* and *T. thevetioides*.

Media construct	*T. neriifolia*			*T. thevetosides*		
	(%) of survival plantlets	No. of shoot /plantlet	Length of root (cm)/plantlet	(%) of shoots formation	No. of survival plantlets	Length of root (cm)/plantlet
1.	20± 0.7	1± 0.1	2.1± 0.3	14± 1.3	2± 0.3	2± 0.3
2.	35 ± 5.3	2 ± 0.4	3.4± 0.2	25 ± 1.2	3± 0.1	2.1±0.1
3.	49± 3.3	4± 0.9	4.2± 0.4	33± 2.7	4± 0.2	4.1± 0.5
4.	70± 4.1	6± 0.1	10.5± 0.1	63± 1.3	5± 0.7	6.3± 0.2

Where: 1- Peatmoss (1); 2- Peatmoss + sand (1:1); 3- Peatmoss+ sand+ perlit (1:1:1); 4- Peatmoss+ sand+ perlit+ vermiculite (1:1:1:1).
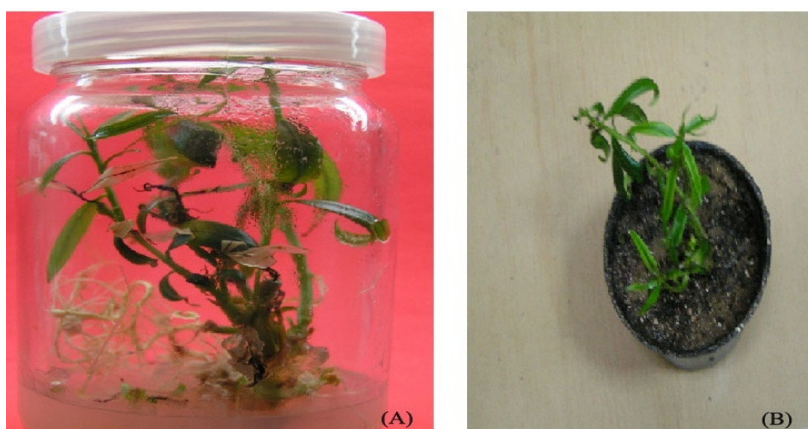
Fig. (7). Rootlets shootlets formation (derived from leaf calli cultures) on MS medium supplemented with NAA (1 mg/l), after 4 subcultures (A). Hardened *in vitro* derived plantlets of *T. neriifolia* in pots containing peatmuss, sand, perlit and vermiculite (1:1:1:1) (B).

As shown in Table (6) the percentages of thevetin B, digitoxigenin, peruvoside and neriifolin of *in vivo* IS of *T. neriifolia* plant were found to be 19, 12, 39 and 25 % , respectively. Moreover, the percentage of those compounds in *in vivo* IS of *T. thevetioides* plant were found to be 99, 15, 24 and 39 %, respectively. In addition, the maximum relative percentage of accumulated cardiac glycosides in different types of calli and regenerated shootlets cultures was recorded after 4 weeks of cultivation for IS of *T. neriifolia* and *T. thevetioides*, respectively. The highest relative percentage of thevetin B (85.7%) was found in *T. neriifolia* IS calli cultures. However the highest value of digitoxigenin (52.8 %) was found in *T. thevetioides* stem calli cultures. The maximum percentage of peruvoside (33.1%) was found in root calli cultures of *T. neriifolia*. While the maximum percentage of neriifolin (62.2 %) was found in *T. thevetioides* root calli cultures.

Moreover, the maximum percentages of thevetin B and neriifolin were found in shootlets (I) of IS calli cultures of *T. neriifolia* to be 29 % and 15.5%, respectively. While, the maximum percentage of digitoxigenin and peruvoside were found in shootlets (II) leaf calli cultures of *T. neriifolia* to be 42 % and 33%, respectively compared with *T. thevetioides*. The detail relative percentages were tabulated in Tables (7 and 8). The obtained calli from IS, leaf, stem and root explants of *Thevetia* spp. under dark condition clearly showed no cardiac glycosides formation.

In agreement, with our obtained results Gopa and Datta (1981) reported that the highest accumulation of thevetin B was detected in seed calli cultures of *T. neriifolia*. Dasgupta *et al.* (1987) reported that HPLC analysis of *in vitro* mass propagation of *T. neriifolia* revealed the presence of cardiac glycosides at different values. Furthermore, Dantas *et al.* (1993) noted that cardenolide formation was recorded in cell suspension culture of *T. neriifolia* cultures. In addition, Lopes *et al.* (2001) reported that some compounds of cardiac glycosides were accumulated in cultured cells of *T. neriifolia*. In close with our obtained results, Dantas *et al.* (1994) studied six cell suspension cultures that established from different organs of *T. neriifolia* under various growth conditions. Further, they reported that after two years of sub-culturing, cardiac glycosides was a present in all of the studied cultured organs. Moreover, Anjani and Pramod (1990) reported that the quantity of the glycoside of *T. peruviana* was increased with the age of culturing on MS medium supplemented with 1 mg /l 2,4-D, for more than 200 days. Whereas, in the present study we clearly showed that the cardiac glycosides content was decreased after 4 weeks of cultivation on supplemented of MS medium with 1mg/l 2,4-D+3mg/l Kin.

Table (6). Percentage of thevetin B, digitoxigenin, peruvoside and neriifolin in *in vivo* IS of *T. neriifolia* and *T. thevetioides*.

	Percentage cardiac glycosides in *in vivo* IS			
	Thevetin B	**Digitoxigenin**	**Peruvoside**	**Neriifolin**
T. neriifolia	19	12	39	25
T. thevetosides	99	15	24	39

Accumulation rate of cardiac glycosides during 4 weeks of cultivation

It was noted that, the 2nd week of IS of both *Thevetia* spp recorded the best time for thevetin B and neriifolin production. In addition, the 3rd week gave the maximum rate of peruvoside formation. However, the 4th week of cultivation recorded the best result of digitoxigenin accumulation under light condition (Fig. 8 A and B).

Concerning, the accumulation rates of cardiac glycosides in *T. neriifolia* calli cultures at different times were in close with Marina *et al.* (1999), they reported that the regenerated shoot cultures of *Digitalis lanata* produced cardiac glycosides reached up to 0.6 μ mol/g DW when cultured under continuous white light for 3 weeks.. Moreover they reported that the accumulation rate of cardiac glycosides was gradually decreased and reached non-detectable levels after 12 weeks of cultivation. These results are in agreement with our obtained results that clearly showed that the incubation of the different explants of *Thevetia* spp. under dark condition is not favorable for biosynthesis and accumulation of cardiac glycosides.

Table (7). Determination of cardiac glycosides (%) in different calli cultures of *T. neriifolia* and *T. thevetoside* relative to IS of intact plant using HPLC technique.

Type of *Thevetia* species	Percentage of cardiac glycosides in calli cultures relative to IS of intact plant															
	IS				Leaf				Stem				Root			
	The	Dig	Per	Ner	The	Dig	Per	Ner	The	Dig	Per	Ner	The	Dig	Per	Ner
T. neriifolia	85.7	28.9	13.2	19.2	34.7	24.9	27.5	13.71	30.01	29.43	21.56	20.3	14.3	12.33	33.1	56.4
T. thevetosides	47	27.9	17.8	32.3	42.3	17.81	6.8	5.6	42.4	52.8	6.03	60.2	37.2	23.6	26.4	62.2

Where: Not detected (-), thevetin B (R_t = 9.48) (The), digitoxigenin (R_t = 11.01) (Dig), peruvoside (R_t = 11.32) (Per) and neriifolin (R_t = 12.72) (Ner)

Table (8). Determination of cardiac glycosides (%) in different type of regenerated shootlets as compared with corresponding content of IS of *T. neriifolia* and *T. thevetosides* using HPLC technique.

	Shootlets (I)				Shootlets (II)				Shootlets (III)			
	The	Dig	Per	Ner	The	Dig	Per	Ner	The	Dig	Per	Ner
T. neriifolia	57.29	18.5	17.15	15.5	26.6	42	33	12.4	32	21.8	5.8	7.9
T. thevetosides	-	19.2	16.8	5.23	12.3	22.1	27.3	8.9	31.1	24.4	53.2	19.9

Where: Not detected (-), thevetin B (R_t = 9.48) (The), digitoxigenin (R_t = 11.01) (Dig), peruvoside (R_t = 11.32) (Per) and neriifolin (R_t = 12.72) (Ner), shootlets (I) from seed, shootlets (II) from leaf and shootlets (III) from stem calli cultures

(A)   **(B)**
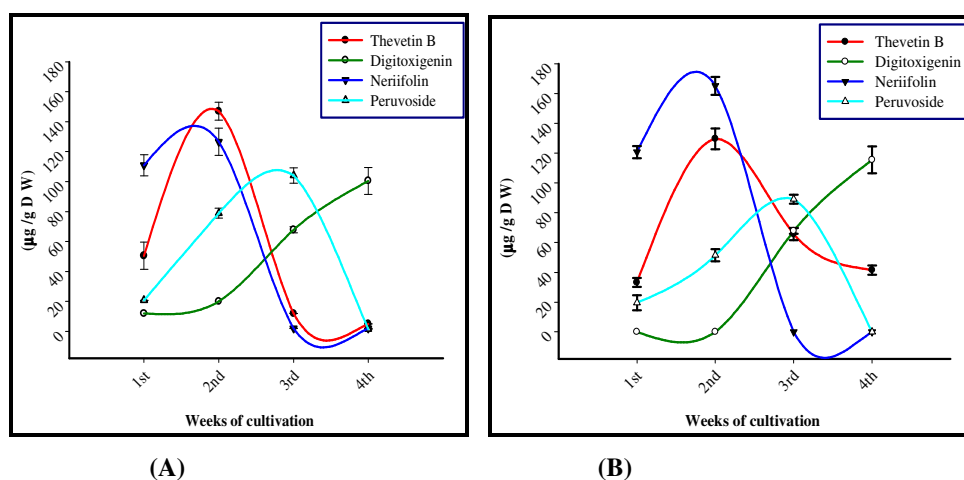
Fig. (8). Production dynamic of thevetin B, neriifolin, peruvoside and digitoxigenin (µg/g DW) of seeds derived calli *of T. neriifolia* (A) and *T. thevetioides* (B) during 4 weeks of growth stages.

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Antifungal Macrodiode Production By *Streptomyces albidoflavus*-143: Fermentation, Purification and Biological Activities

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Abstract: This work was carried out for the biosynthesis of antifungal substance that demonstrated inhibitory effects against pathogenic fungi from *Streptomyces albidoflavus,* 143. The active metabolite was extracted using ethyl acetate (1:1, v/v) at pH 7.0. The separation of the active ingredient of the antifungal agent and its purification was performed using both thin layer chromatography (TLC) and column chromatography (CC) techniques. The physico-chemical characteristics of the purified antibiotic viz. color, melting point, solubility, elemental analysis, spectroscopic characteristics and chemical reactions have been investigated. This analysis indicates a suggested imperical formula of $C_{22}H_{36}O_6$. The minimum inhibition concentrations "MICs" of the purified antifungal agent were also determined. The purified antifungal agent was suggestive of being belonging to Macrodiode antibiotic produced by *Streptomyces albidoflavus,* 143.
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Keywords: Antifungal Macrodiode; *Streptomyces albidoflavus*; Fermentation; Purification; Biological Activities

1. Introduction

Many species of actinomycetes, particularly those belonging to the genus *Streptomyces,* are well known as antifungal biocontrol agents that inhibit several plant pathogenic fungi (Joo, 2005). The antagonistic activity of *Streptomyces* to fungal pathogens is usually related to the production of antifungal compounds (Fguira *et al.,* 2005) and extracellular hydrolytic enzymes (Taechowisan *et al.,* 2005). Chitinase and -1,3-glucanase are considered to be important hydrolytic enzymes in the lysis of fungal cell walls, as for example, cell walls of *Fusarium oxysporum, Sclerotinia minor*, and *S. rolfsii* (Mukherjee and Sen, 2006)

The macrotetrolides are a family of cyclic polyethers produced by a number of *Streptomyces* species (Birch and Robinson, 1995 and Fleming and Ghosh, 1996). Nonactin (NON), the smallest homolog and a symmetric member of the family, was first isolated in 1955 (Corbaz *et al.,* 1955). Its structure was initially deduced from spectroscopic analysis and was later confirmed by X-ray crystallography (Dobler, 1972, Kilbourn *et al.,* 1967), revealing that the intriguing molecular topology of NON consists of the (+)(-)(+)(-)-ester linkage of the enantiomeric nonactic acid (NA) building blocks. NA-type building blocks have also been identified in several macrodiolides (Jois *et al.,* 1986), including the pamamycins (Natsume *et al.,* 1991). The macrotetrolides exert a broad spectrum of biological activities (Zizka, 1998), ranging from antifungal, antitumor (Borrel *et al.,* 1994), antiprotozoan, antiparasitic, and insecticidal activities to immunosuppressive activities (Callewaert *et al.,* 1988). In fact, comparative studies on the immunosuppressive activities of tetranactin and cyclosporin, the latter being the most widely used immunosuppressant agent, showed that these two compounds were approximately equally effective and that tetranactin has the advantage of low toxicity (Teunissen *et al.,* 1992). The biological activities of the macrotetrolides are generally traced to their ionophoric properties (Marrone and Merz, 1992.), and the potencies of these activities appear to parallel the size of the alkyl substituent's of the macrotetrolides: tetranactin is often the most potent member of the family, while NON is generally inactive. The biosynthesis of NON has been extensively studied by in vivo feeding experiments with ^{13}C-, ^{2}H-, and ^{18}O-labeled precursors and biosynthetic intermediates (Ashworth *et al.,* 1989) and by isolation of both enantiomers of NA and the dimeric NA (Fleck *et al.,* 1996). These results established unambiguously the polyketide origin of NON, the assembly of which from one molecule each of propionate and succinate and two molecules of acetate must have invoked (i) the rare use of succinate as an intact four-carbon fragment (C-3 to C-6) and (ii) the derivation of a three-carbon unit (C-7 to C-9) from two molecules of acetate (one of which is activated in the form of malonate). Feeding experiments with ^{13}C- and ^{18}O-doubly-labeled

precursors indicated that the C-3-O bond is formed during closure of the tetrahydrofuran ring, presumably by an intramolecular Michael addition of the 6-hydroxy group onto the enone moiety of 2-methyl-6,8-dihydroxynon-2E-enoic acid (NEA) (Ashworth and Robinson, 1988). The involvement of the latter step in NON biosynthesis was further substantiated by the efficient and enantiospecific incorporation of both (6R,8R)-NEA into NON (Spavold and Robinson, 1988) and by the drastic reduction of NON production upon the addition of an NEA analog into the fermentation medium, which presumably acts as a suicide inhibitor for this enzymatic step (Priestley and Earle, 1997).

The Macrodiode has molecular weight 396 and empirical formula $C_{22}H_{36}O_6$, and (U.V) strong end absorption spectrum (Jois and Gurusiddaiah, 1986).

In the present study, the productio of the bioactive substances that demonstrated inhibitory affects against microbial pathogenic, from *Streptomyces albidoflavus,* 143 were reported, along with some physico-chemical properties of secondary metabolites with high biological activities.

2. Material and Methods
2.1. Test organisms
2.1.1. Unicellular fungi
Saccharomyces cerevisiae, ATCC 9763, *Candida albicans* IMRU 3669.

2.1.2.-Filamentous fungi
Aspergillus niger, IMI 31276.*; Aspergillus flavus*, IMI 111023, *Aspergillus fumigatous*, ATCC 16424; *Aspergillus terreus; Fusarium solani; Fusarium oxysporum, Fusarium moniliform, Alternaria alternata, Botrytis cinerea, Penicillium chrysogenum* and *Rhizoctonia solani*.

2.2. Fermentation
A loopful of the, *Streptomyces albidoflavus,* 143 from the 5-day culture age was inoculated into 250 ml Erlenmeyer flasks containing 75 ml of liquid starch nitrate medium (seven flasks). The flasks were incubated on a rotary shaker (200 rpm) at 30 ^{0}C for 5 days.

Twenty-liter total volume was filtered through Whatman No.1 filter paper, followed by centrifugation at 5000 r.p.m for 20 minutes. The clear filtrates were tested for their activities against the test organisms (Sathi *etal.,* 2001).

2.3. Extraction
The clear filtrate was adjusted at different pH values (4 to 9) and extraction process was carried out using different solvents separately at the level of

1:1 (v/v). The organic phase was concentrated to dryness under vacuum using a rotary evaporator (Atta, 2010).

2.4. Precipitation
The precipitation process of the crude compound dissolved in the least amount of the solvent carried out using petroleum ether (b.p 60-80 °C) followed by centrifugation at 5000 r.p.m for 15 min. The precipitate was tested for its antifungal activities (Atta *et al.,* 2009).

2.5. Separation
Separation of the antifungal agent(s) into its individual components was conducted by thin layer chromatography using chloroform and methanol (24:1, v/v) as a solvent system (Atta *et al.,* 2009).

2.6. Purification
The purification of the antimicrobial agent(s) was carried out using silica gel column (2 X 25) chromatography. Chloroform and Methanol 9**:**1 (v/v), was used as an eluting solvent. The column was left for overnight until the silica gel (Prolabo) was completely settled. One-ml crude precipitate to be fractionated was added on the silica gel column surface and the extract was adsorbed on top of silica gel. Fifty fractions were collected (each of 5 ml) and tested for their antimicrobial activities (Atta *et al.,* 2009).

2.7. Physico-chemical properties of the antifungal agent
2.7.1. Elemental analysis
The elemental analysis C, H, O, N, and S was carried out at the micro analytical center, Cairo University, Egypt.

2.7.2. Spectroscopic analysis
The IR, UV, Mass spectrum, and NMR spectrum were determined at the micro analytical center of Cairo University, Egypt.

2.7.3. Reaction of the antifungal agent with certain chemical test
For this purpose, the following reactions were carried out: Molish's, Fehling, Sakaguchi, Ninhydrin, Ehrlish, Nitroprusside, Ferric chloride, and Mayer reactions (Atta *et al.,* 2011).

2.7.4. Biological activity
The minimum inhibitory concentration (MIC) could be determined by the cup assay method (Kavanagh, 1972).

2.7.5. Characterization of the antifungal agent

The antifungal agent produced by *Streptomyces albidoflavus,* 143 was identified according to the recommended international references of (Umezawa, 1977; Berdy, 1974; Berdy, 1980a b & c; Jois and Gurusiddaiah, 1986).

3. Results

3.1. Fermentation and Separation of the antifungal agent

The fermentation process was carried out for three days at 30°C using liquid starch nitrate medium as production medium. Filtration was conducted followed by centrifugation at 5000 r.p.m. for 15 minutes. The clear filtrates containing the active metabolite (20 liters), was adjusted to pH 7.0 then the extraction process was carried out using Ethyl acetate at the level of 1:1 (v/v). The organic phase was collected, and evaporated under reduced pressure using rotary evaporator. The residual material was dissolved in the least amount of DMSO and filtered. The filtrates were test for their antifungal activities. The antifungal agent was precipitated by petroleum ether (b.p. 60-80°C) and centrifuged at 5000 r.p.m for 15 minute where a yellowish brown oil precipitate could be obtained. Separation of the antifungal agent(s) into individual components was carried out by thin-layer chromatography using a solvent system composed of chloroform and methanol (24:1, v/v). Among three bands developed, only one band at R_f 0.9 showed antifungal activity. The purification process through column chromatography packed with silica gel indicated that the most active fractions against the tested organisms ranged between 20 to 31 Fig. (1).

3.2. Physicochemical characteristics of the antifungal agent

The purified antifungal agent produces characteristic odour, their melting point is 180°C. The compound is freely soluble in chloroform, ethyl acetate, n-butanol, acetone, ethyl alcohol, methanol and 10 % isopropyl alcohol, but insoluble in water, petroleum ether, hexane and benzene.

3.3. Elemental analysis

The elemental analytical data of the antifungal agent(s) revealed the following: **C**=66.17; H=9.70; N= 0.0, O = 25.3 and S=0.0. This analysis indicates a suggested imperical formula of $C_{22}H_{36}O_6$.

3.4. Spectroscopic characteristics

The infrared (IR) spectrum of the antifungal agent showed characteristic band corresponding to 17 peaks, 810.21, 1060.19, 1201.17, 1268.12, 1331.21, 1426.10, 1512.10, 1604.08, 1715.24 (lactone), 1998.20, 2880.21, 2940.05 (C-H stretching), 2320.12, 3278.23, 3601.11, 3624.07 and 3788.13 (Fig.2).The ultraviolet (UV) absorption spectrum of the antifungal agent recorded a maximum absorption peak region at 223.30, 260.7 and 271.58 (Fig. 3). The Mass spectrum revealed that the molecular weight is 396 (Fig. 4). The NMR-Spectrum exhibited the multiple at 5.0 to 4.85 was due to the methine proton-bearing ester bonded oxygen (R-CH-O-COR)., the doublet at 1.24 was due to the methylene group of homononactic acid moieties attached to a carbon-bearing ester-bonded oxygen (R-CH$_2$-CHOCOR). Multiplets at 4.1 to 3.95 and 3.95 to 3.75 are characteristics of tetrahydrofuran methine protons (-CH-O-CH-). Peaks at 2.1 to 1.7 were assigned to methylene protons of the tetrahydrofuran moiety (-CH$_2$-CH$_2$-) (Fig.5).

3.5. Biochemical reaction of the antimicrobial agent

The reactions revealed the detection of certain groups in the investigated agent. The antifungal agent exhibited positive results with ninhydrin, ferric chloride and Mayer tests and negative results with nitroprusside, Molish's, Fehling Sakaguchi, and Ehrlish reactions (Table 1).

3.6. Biological activities of the antifungal agent

Data of the antifungal agent spectrum indicated that the agent is active against unicellular and filamentous fungi (Table 2). The MIC of antifungal antibiotic was determined and the results showed that the minimum inhibitory concentration (MIC) of the compound against unicellular fungi *Saccharomyces cervisiae* ATCC 9763 (31.25 μg/ ml) and *Candida albicans,* IMRU 3669 (25.25 μg/ ml) and maximum inhibitory activity was observed against filamentous fungi *Aspergillus niger* IMI 31276 (46.9 μg/ ml), *Aspergillus flavus* (46.9), *Botrytis fabae* (46.9 μg/ ml), *Fusarium oxysporum* (52.7 μg/ ml), *Rhizoctonia solani* (52.7 μg/ ml), *Alternaria alternate* (62.5 μg/ ml), *Aspergillus fumigatus* ATCC 16424 (62.5 μg/ ml)*,* and *Penicillium chrysogenium* (62.5 μg/ ml).

3.7. Identification of the antifungal agent

On the basis of the recommended keys for the identification of antibiotics and in view of the comparative study of the recorded properties of the antifungal agent, it could be stated that the antifungal agent is suggestive of being belonging to Macrodiode antibiotic (Table 3).

Table 1. Summarizes the response of the antifungal agent to certain biochemical reactions.

Chemical test	Result	Remark
Molish's reaction	-	Absence of sugar moiety
Fehling test	-	Absence of free aldehyde or keto sugar
Ninhydrin test	+	Present of free-NH_2 group
Sakaguchi reaction	-	Arginin is Absence
Nitroprusside reaction	-	Absence of Sulfur
Ferric chloride reaction	+	Absent of Di-ketons group
Ehrlish rection	-	Absence of indolic acid
Mayer reaction	+	Presence of nitro group

Table 2. Biological activities (MIC) of the antifungal agent by paper method assay.

Test organisms	MIC (µg/ml) concentration
1-Unicellular fungi:	
Candida albicans, IMRU 3669	25.25
Saccharomyces cervisiae, ATCC 9763	31.25
2-Filamentous fungi:	
Aspergillus niger, IMI 31276	46.9
Aspergillus fumigatus, ATCC 16424	62.5
Aspergillus flavus, IMI 111023	46.9
Fusarium oxysporum	52.7
Rhizoctonia solani	52.7
Alternaria alternata	62.5
Botrytis fabae	46.9
Penicillium chrysogenium	62.5

Table 3. A comparative study of the characteristic properties of the antifungal agent in relation to reference antifungal Macrodiode

Characteristic	Purified antibiotic	Antifungal Macrodiode
1- Melting point	180°C	ND
2- Molecular weight	396	396
3- Chemical analysis:		
C	66.17	66.17
H	9.70	9.73
N	0.0	0.0
O	25.3	25.0
S	0.0	0.0
4- Ultra violet	240	240
5- Formula	$C_{22}H_{36}O_6$	$C_{22}H_{36}O_6$
6- Active against	Unicellular and filamentous fungi	Unicellular and filamentous fungi

ND=No data
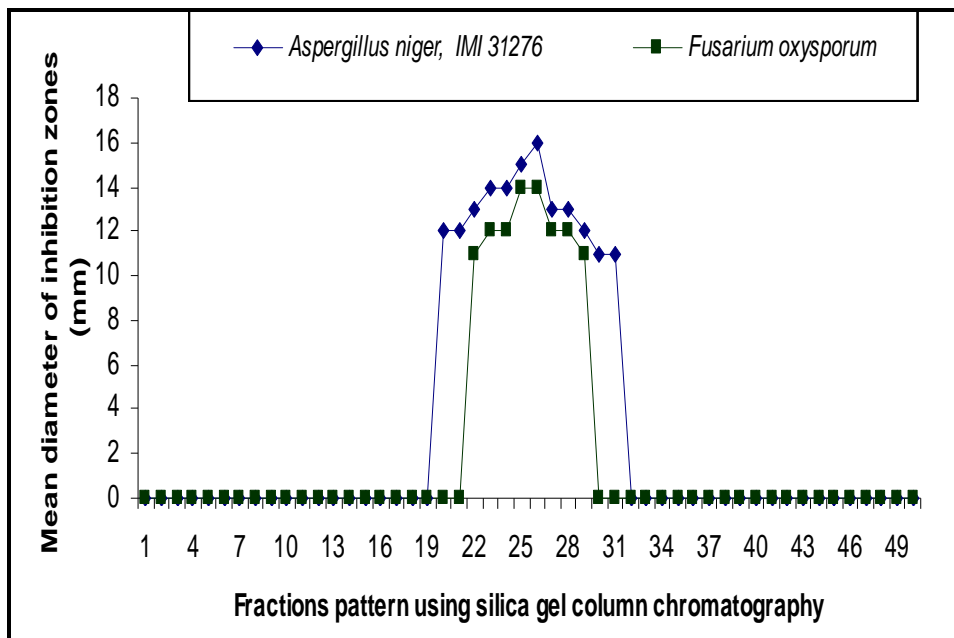
Figure 1. Antifungal activity of fractions obtained using silica gel column chromatography technique for antifungal agent produced by *Streptomyces albidoflavus,* 143.
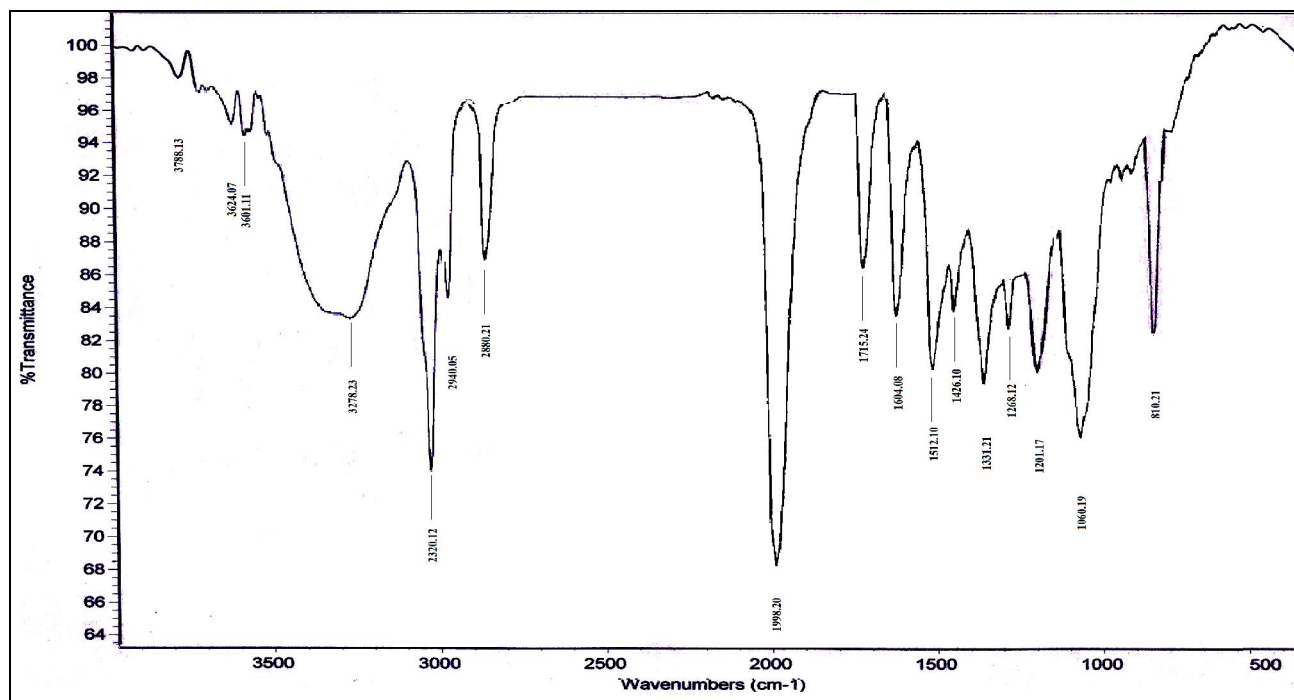
Figure 2. I.R spectrum of antifungal agent produced by *Streptomyces albidoflavus,* 143.
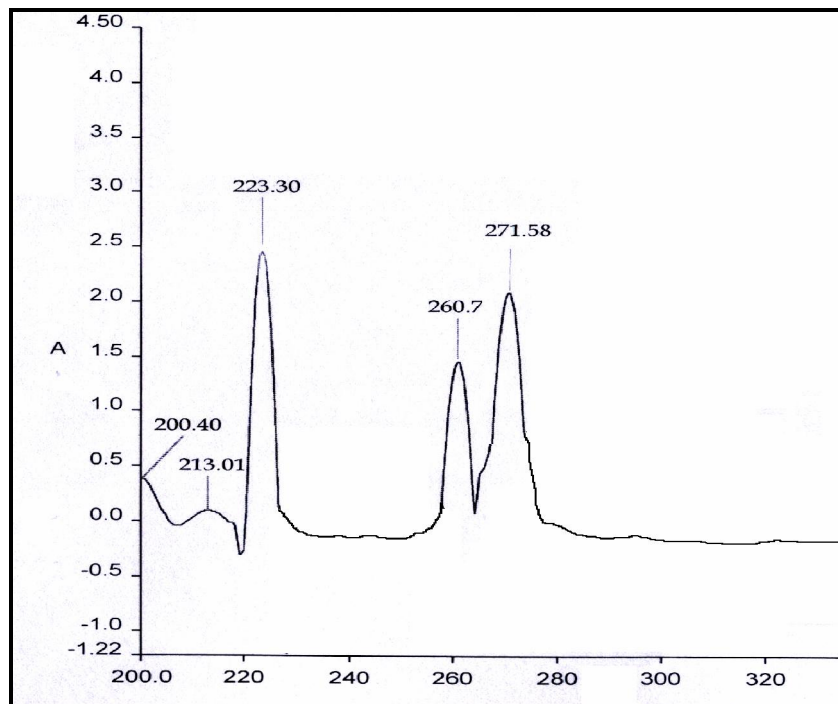
Figure 3. Ultraviolet absorbance of antifungal agent produced by *Streptomyces albidoflavus,* 143.
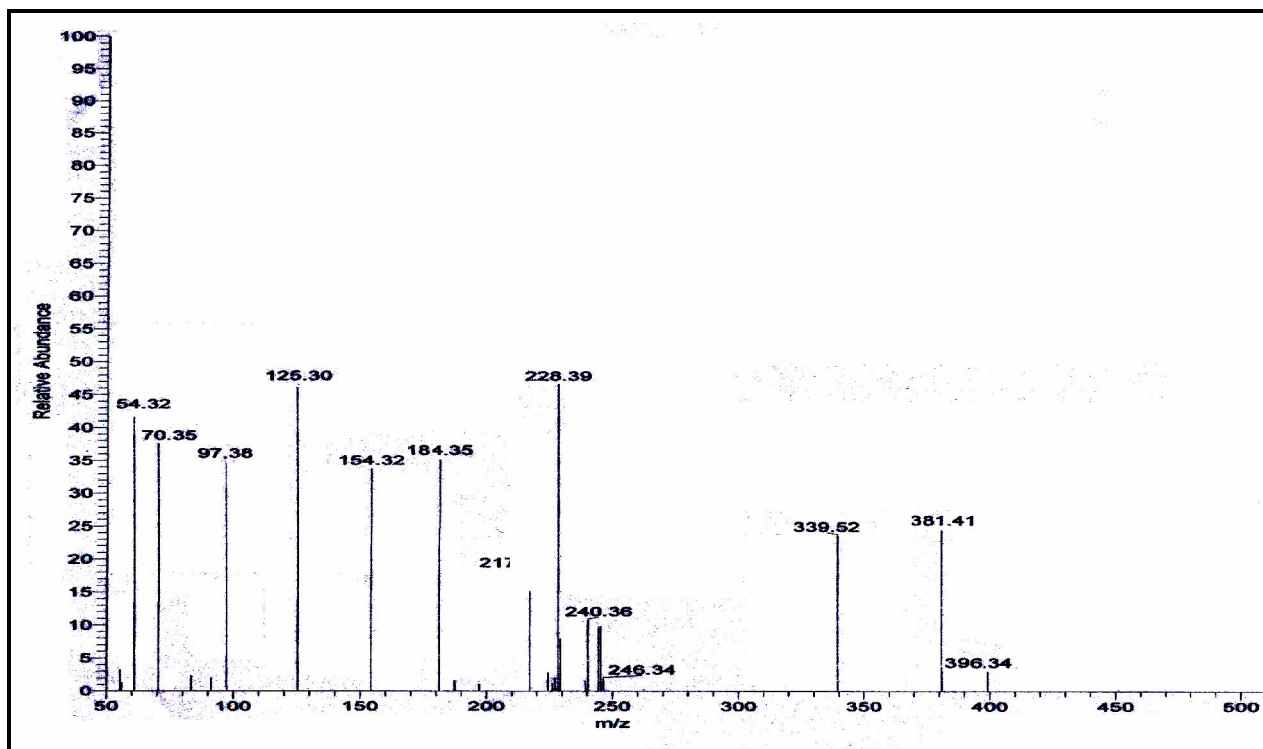
Figure 4. Mass-Spectrum of antifungal agent produced by *Streptomyces albidoflavus,* 143.
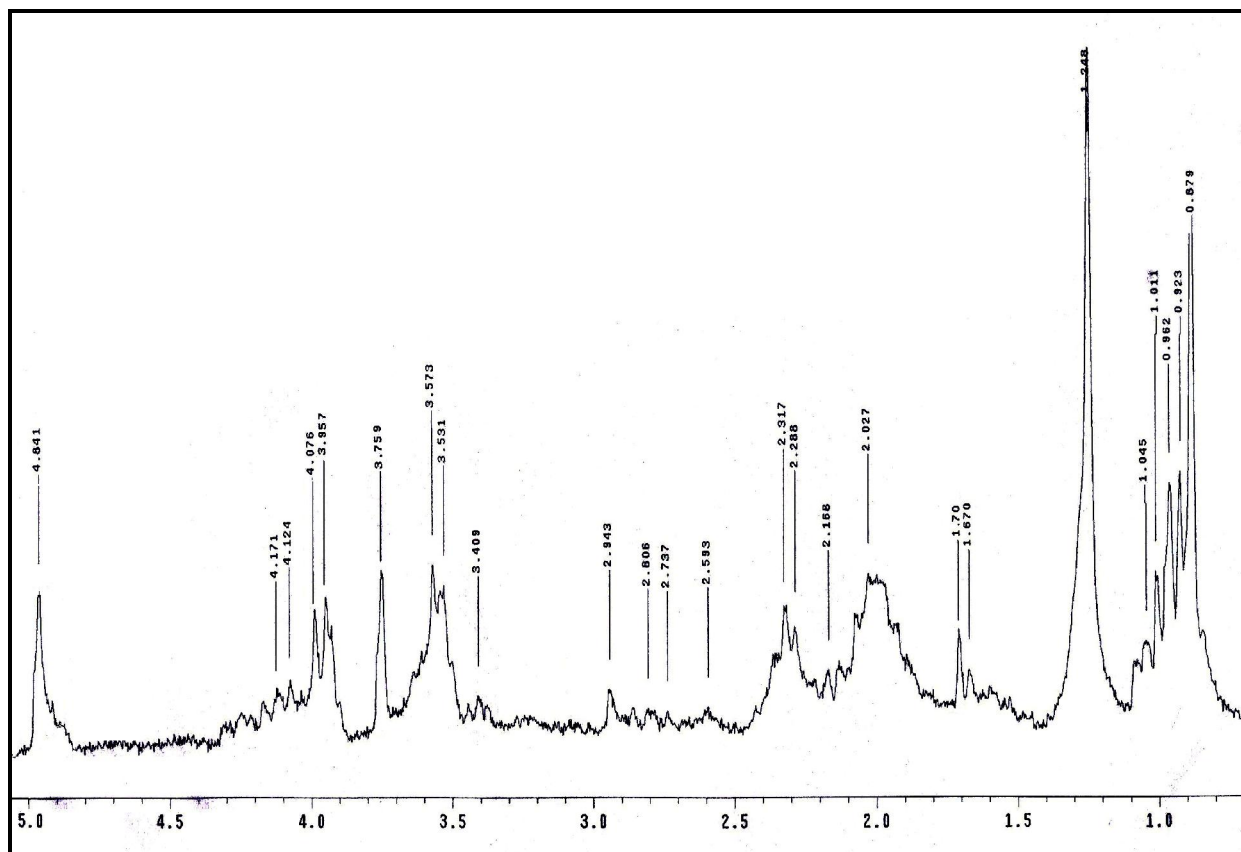
Figure 5. NMR-Spectrum of antifungal agent produced by *Streptomyces albidoflavus,* 143.

4. Discussions

The active metabolites were extracted by ethyl acetate at pH 7.0. Similar results were obtained by (Criswell *et al.,* 2006; Sekiguchi *et al.,* 2007 and Atta *et al.,* 2011). The organic phase was collected and evaporated under reduced pressure using rotary evaporator. The extract was concentrated and treated with petroleum ether (b.p. 60-80°C) for precipitation process, where only one active fraction was obtained in the form of yellowish brown oil. The purification process through a column chromatography packed with silica gel and an eluting solvents composed of chloroform and methanol (9:1, v/v), indicated that fractions activities was recorded from fraction Nos. 20 to 31. Many workers used a column chromatography packed with silica gel. Similar results were obtained by (Jois and Gurusiddaiah, 1986; Hitchens and Kell, 2003; El-Naggar, 2007 and Atta *et al.,* 2009). The physico-chemical characteristics of the purified antibiotic revealed that, their melting point is 180°C. The compound is freely soluble in chloroform, ethyl acetate, n-butanol, acetone, ethyl alcohol, methanol and 10 % isopropyl alcohol, but insoluble in water, petroleum ether,

hexane and benzene. Similar results were recorded by (Yanai, 2004; Yoram *et al.,* 2006 and Wenli *et al.,* 2008). A study of the elemental analysis of the antifungal agent C=66.17; H=9.70; N= 0.0, O = 25.3 and S=0.0 lead to an imperical formula of $C_{22}H_{36}O_6$. The spectroscopic characteristics of the antifungal agent under study revealed the presence of a maximum absorption peak in UV. at 223.30, 260.7 and 271.58 nm, infra-red absorption spectrum represented by 17 peaks, 810.21, 1060.19, 1201.17, 1268.12, 1331.21, 1426.10, 1512.10, 1604.08, 1715.24 (lactone), 1998.20, 2880.21, 2940.05 (C-H stretching), 2320.12, 3278.23, 3601.11, 3624.07 and 3788.13. The spectral characteristics of the hydrolysis product were as follows: IR spectrum 3278 (broad, OH), 2940 and 2880 (C-H stretching), 3601 to 2880 (broad nature of peak, COOH), 1715 (COOH), 1426, 1331, 1268, 1201 and 1060 cm[-1](Jois and Gurusiddaiah, 1986).

The Mass spectrum revealed that the molecular weight is 396 and NMR-spectrum exhibited the multiple at 5.0 to 4.85 was due to the methine proton-bearing ester bonded oxygen (R-CH-O-COR)., the doublet at 1.24 was due to the

methylene group of homononactic acid moieties attached to a carbon-bearing ester-bonded oxygen (R-CH_2-CHOCOR). Multiplets at   4.1 to 3.95 and 3.95 to 3.75 are characteristics of tetrahydrofuran methine protons (-CH-O-CH-). Peaks at   2.1 to 1.7 were assigned to methylene protons of the tetrahydrofuran moiety (-CH_2-CH_2-) (Kumar and Kannabiran, 2010). The biochemical tests of the antifungal agent gave positive reaction ninhydrin, ferric chloride and Mayer tests and negative results with nitroprusside, Molish's, Fehling Sakaguchi and Ehrlish reactions. Similar results were recorded by (Pamboukian and Facciotti, 2004 and Atta *et al.,* 2011).

The MIC of antifungal antibiotic was determined and the results showed that the minimum inhibitory concentration (MIC) of the compound against unicellular fungi *Saccharomyces cervisiae* ATCC 9763 (31.25 μg/ ml) and *Candida albicans,* IMRU 3669 (25.25 μg/ ml) and maximum inhibitory activity was observed against filamentous fungi *Aspergillus niger* IMI 31276 (46.9 μg/ ml), *Aspergillus flavus* (46.9), *Botrytis fabae* (46.9 μg/ ml), *Fusarium oxysporum* (52.7 μg/ ml), *Rhizoctonia solani* (52.7 μg/ ml), *Alternaria alternate* (62.5 μg/ ml), *Aspergillus fumigatus* ATCC 16424 (62.5 μg/ ml)*,* and *Penicillium chrysogenium* (62.5 μg/ ml), similar investigations and results were attained by (Kavitha and Vijayalakshmi, 2007 and Atta, 2010).

Identification of the antifungal agent according to recommended international keys indicated that the antibiotic is suggestive of being Macrodiolide antibiotic (macrotetrolide antibiotic) (Jois and Gurusiddaiah, 1986).

5. Conclusion

It could be concluded that: The Macrodiolide antibiotic produced by *Streptomyces albidoflavus,* 143 demonstrated obvious inhibitory affects against pathogenic fungi.

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1/21/2011

Analyzing Attitude of Managers and Senior Experts of Jihad-e-Keshavarzi Organization Regarding Development of Knowledge Management (KM) in Khuzestan Province, Iran

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Abstract: The purpose of this study was analyzing attitudes of managers and senior experts regarding the development of knowledge management in Jihad-e-Keshavarzi Organization of Khuzestan province. Senior experts and managers of organization were considered as a statistical population (N=100). All individuals were investigated. After confirm the validity of the instrument by panel of experts, to determine the reliability coefficient using Cronbach alpha coefficients were obtained for all sections of the questionnaire over 0.7 were calculated. Method of research was descriptive and correlative. Based on the results, the correlation between job motivation and status of knowledge acquisition and absorption with dependent variable in 0.01 level, was significant. Also between organizational culture and attitudes of managers in 0.05 level, correlations was significant. The results also showed that organizational culture, leadership style and knowledge about IT can explain 37% of variance of attitude of managers and senior experts regarding the development of knowledge management in Jihad-e-Keshavarzi Organization of Khuzestan Province, Iran.

1. Introduction

In recent years one of the many topics that views managers of public and private organizations to have attracted is the topic "Knowledge Management (KM)". KM history dates back to ancient Greece. But in recent years this issue has been raised with more attention. Growth of knowledge management as a strategy for managing the organization is considered as a new approach. So far, several approaches have appeared in the field of management but have been gradually change shortly after. (Mehr Ali Zadeh, 2002).

Organizational knowledge in the contemporary world of rapid, is an opportunity for organizations that do not know it well and they manage and yet a serious threat to organizations that reduce the environmental changes and do not know it. Now, knowledge management is a new concept and is considered popular and is a process that helps organizations to information and critical specials that are part of the memory organization, usually there are no structures in the organization, identify, select, organize, distribut and deliver (Lajvardi and Khanbabaei, 2005).

Present time is fast changing era of knowledge. Every five years and a half size of knowledge are doubling, while the average life of less than four years. In such circumstances,

knowledge as a "valuable strategic resource" and "property" is considered to require management. If the above conditions "intense competition in world markets," we added the importance of organizational knowledge management as a competitive advantage in knowledge-based economy is now double (Jalali et al, 2005).

Lack of trust among employees is one of the main barriers to knowledge transfer. Increase the exchange of knowledge by mutual trust cause knowledge creation. There trust between organizational systems is essential. (Lee and Choi, 2003). Informal interactions help to create and transfer of knowledge. Recognized cause loss of communication and interaction are essential for knowledge creation (Farhangi et al, 2008).

Prubst et al (2001) during the study, specific conceptual models for knowledge management have developed. This model consists of two dimensions. The first, the main operational processes of knowledge management (knowledge identification, knowledge acquisition, knowledge development and dissemination and knowledge sharing) and a second later, the main strategic knowledge management processes (knowledge goals, knowledge assessment) are (Prubst et al, 2001).

Purpose of research is analyzing attitude of managers and senior experts regarding the development of knowledge management in Jihad-e-Keshavarzi Organization of Khuzestan Province.

2. Material and Methods

From the viewpoint of classification of research based on objective, type of research is applied research. The research method is descriptive and correlative.

Through this method, a field study of library methods, data collection is done and the results obtained by percentage frequency, mean and variance are described. Researcher in the study investigates the correlation relationship between variables are explored. In this study Jihad-e-Keshavarzi Organization of Khuzestan Province as a case study organizations were selected. Managers and senior experts in this organization, as the statistical community have been considered that the whole community, including N=100 in the target audience as a community is considered.

In order to validate research tools, panel of expert's method was used. Then collect the questionnaires and using the results of the opinions of experts and specialists, we have modified the questionnaire. A number of questionnaires in the next step modified using a number of contacts and complete research about the content and length of the questionnaire.

To determine the reliability of the questionnaire, 30 copies of the questionnaire in the Jihad-e- Keshavarzi Organization Chahar Mahal and Bakhtiari province has completed, then SPSS 16 software using Cronbach's coefficient alpha levels through a questionnaire reliability was investigated. According to the results of questionnaire reliability levels are acceptable, and in all items over than 0.70.

3. Results and discussion

First, managers characteristics described and then discussed the inferential statistics are presented.

This study showed that based on level of education 76 percent of managers and senior experts had B.Sc degree and 24 percent had M.Sc (Table 1).

Based on the results the average income of participants was 6.21 million Rial in month (Table 1).

In this study for analyzing attitudes of managers regarding the development of knowledge management in Jihad-e-Keshavarzi organization of Khuzestan province , 9 items designed and to their responses on a five-level (high agree, agree, unsure, disagree and high disagree) expression did (Table 2). Based on the viewpoint of respondents, we classified them in to 5 groups. 37% of managers had moderate level of attitudes regarding the development of knowledge management (Table 3).

A. Correlation Study:

To investigate the relationship between attitude of managers and senior experts regarding the development of knowledge management in Jihad-e-Keshavarzi Organization of Khuzestan Province as dependent variables with independents variables, Spearman correlation coefficient was used. Based on the results presented in table 4, between job motivation and status of knowledge acquisition and absorption with dependent variable in 0.01 level correlations was significant. Also between organizational culture and attitudes of managers 0.05 level correlations was significant.

B. Multiple Regression Analysis

According to the regression coefficients and the constant value obtained from multiple regression analysis stepwise method, regression equation under investigation form was obtained:

$$Y = 15.576 + 0.425X_1 + 0.196X_2 + 0.359X_3$$

The results also showed that organizational culture, leadership style and knowledge IT can explain 37% of variance of attitude of managers and senior experts regarding the development of knowledge management in Jihad-e-Keshavarzi Organization of Khuzestan Province (Table 5).

Table 1 Frequency distribution of senior managers and experts studied by personal characteristics

Education Level	Frequency	Percent	Cum percent
BSc	76	76	76
MSc	24	24	100
Income (Million Rials)			
4-6	54	54	54
6-8	41	41	95
8-10	5	5	100
Mean=6.21 sd=1.023			

Table 2. The mean attitude of managers regarding knowledge management items
(5=high agree, 4=agree, 3=unsure, 2=disagree and 1=high disagree)

Items	Mean	sd
Record of experience	2.71	1.20
Easily using of past experience	2.47	1.15
Awareness of managers of up-to-date knowledge	2.46	1.15
Reduce cost of mistakes	2.38	1.17
Reduce Risk of decisions	2.31	1.16
Availability of knowledge sources	2.25	1.27
Increase motivations in organization	2.25	1.22
Increase productivity in organization	2.56	1.28
Increase teamwork activity	2.23	1.27

Table 3. The frequency distribution of managers regarding level of attitude

Attitude in terms of knowledge management development	Frequency	Percent	Cumulative percent
Very High	1	1	1
High	42	42	43
Moderate	37	37	80
Low	15	15	95
Very low	3	3	97
No response	2	2	100
Sum	100	100	

Table 4 Correlation with level of knowledge management variables

First variable		Second variable		Spearman correlation coefficient	Significance
Variable	scale	Variable	scale		
Job motivation	Ordinal	Attitude	Ordinal	0.304^{**}	0.002
Organizational Culture	Ordinal	Attitude	Ordinal	0.253^{*}	0.012
Status of knowledge acquisition and absorption	Ordinal	Attitude	Ordinal	0.309^{**}	0.002
Income	Ordinal	Attitude	Ordinal	0.092	0.390
Knowledge IT	Ordinal	Attitude	Ordinal	0.251^{*}	0.013
Service	Ordinal	Attitude	Ordinal	0.031	0.764
Literacy	Ordinal	Attitude	Ordinal	0.154	0.129

*: Significant level of 0.05 ,   **: Significant level of 0.01

Table 5. Results of multiple regression analysis step by step style

Independent variables	B	SE B	Beta	t	sig
Organizational culture (X_1)	0.425	0.179	0.269	2.375	0.020
Leadership style (X_2)	0.196	0.097	0.237	2.021	0.047
Knowledge IT (X_3)	0.359	0.136	0.272	2.639	0.010
Constant	15.576	4.696	----	3.317	0.001
R= 0.609 ,R2=0.371, Signif F=0.020 F= 5.652					
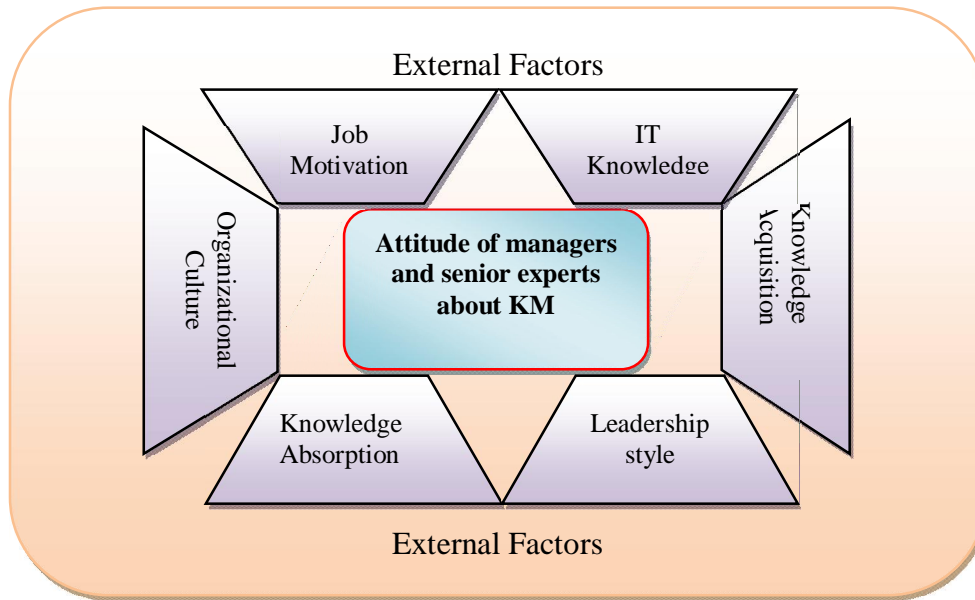
Figure 1: Filed Frame work of Research

Recommendation

In this section, based on the results, recommendations needed in this field are presented:

Based on results, between job motivation and attitudes of managers in the development of knowledge management in the organization was a significant relationship. Therefore it is recommended to those involved with the organization necessary measures of motivation for managers to change their attitude to enhance the development of knowledge management.

Also between organizational culture and attitudes of managers was a significant relationship. Accordingly, the organization must correct toward making organizational culture changes for create conditions in developing knowledge management efforts.

In addition between acquisition and absorption of knowledge and attitudes of managers was a significant relationship. In this regard, the organization must provide a suitable platform for the acquisition and absorption of tacit knowledge and experiences of people in organizations to create positive attitudes of managers in the field of knowledge management to take action.

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2/2/2011

Influences of physiographic factors on growth of Alder stands (*Alnus subcordata*) in north forest of Iran

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Abstract: Generally in old forestry plans, estimation of growth and yield has been processed by static methods without considering effective environmental variables as slopes, aspect (direction of slops), etc. In fact these estimations without taking the dynamic characters of stands into the consideration couldn't interpret actual timber and growth volumes. This study carried out with analysis of Alder stands criteria in physiographic factors in north forest of Iran. The results of statistical analysis showed that altitude less than 400 m in natural stands have minimum dbh growth. Height growth of Alders in natural stands has significant relationship with altitudes. An increase in altitude results in decrease the total height growth, whereas basal area growth decreases when altitude increases. Volume growth in high altitude (1200-1600 m.s.l.) has minimum growth in both plantation and natural area. Maximum growth of dbh (cm) and total height growth (m) in natural stands were seen in west aspect and minimum growth of dbh in natural area related to north, northeast and east and in plantations related to west and south aspects. Maximum volume growth per hectare in natural and plantation area was in northwest and southeast aspects, respectively. Results of growth in different slop showed that in natural stands slop ranged 0 to 15 percent have maximum growth of total height and basal area. Mean comparison of volume growth per hectare indicated that in natural area in low slope (0 -15%) is significantly more than areas with 15 -45% slope.

1. Introduction

Growth and yield models constricting with consideration of effective ecological variables is useful in the exact estimations of tree's biomass and forest productivity which is effective for harvesting. Harvesting rationalization needs the preparation of growth and yield models base on site proportions, soil and climatic factors, completion in stands, geographical position, elevation from free sea level, and so many other ecological variables. At this way harvesting and silvicultural practices such as thinning, lighting, etc, will be done according to forest reproducibility under sustainable forest management.

Barnes et al. (1998) realized that the groups are used to help distinguish and map landscape ecosystems in the field by their presence or absence and by the relative coverage of plants in each group. They are never used alone, but always with attributes of physiographic, soil, microclimate, and the composition and vigor of overstory trees. Slope position is often used to account for environmental effects associated with landform, such as differences in soil moisture (Helvey et al. 1972). Twenty-three natural red alder stands in western Washington and northwestern Oregon were selected to provide a range

in geographic location, site quality, and soil conditions. The information collected on each stand included: (1) elevation, (2) slope, (3) aspect, (4) soil drainage class, and (5) estimated Douglas-fir (Pseudotsuga menziesii (Mirb) Franco) site class (King, 1966).

Comparing distribution of plant ecological groups with topographical factors (i.e. slope, aspect and elevation) revealed that there was significant relationship between aspect and plant ecological group distribution and in some ecological groups with slope, while elevation had no remarkable influence on plant ecological group distribution. In some ecological groups were strongly affected by slope and aspect. For example, in tree and shrub layer some species such as *Ruscus hyrcanus, Alnus subcordata ,Crataegus microphylla* and *Ilxe spinigera* were more frequently distributed in the slopes of 40-70 % and in northeastern aspect in this research, the distribution of different ecological groups was manually compared based on topographical factors (i.e. slope, aspect and elevation). Topography may be sufficient to mark changes in temperature and humidity with increasing altitude. The presences of highland induce orographic rainfall so ecosystem at high altitudes receives more precipitation than low ones (Emberlin, 1984).

Physiographic measurements included elevation, azimuth (defined here as the departure in degrees from due south exposure), slope, position on the slope, profile of the horizon, and proximity of the study tree to a local water supply. Position on slope was rated numerically from 0 (top of slope) to 9 (bottom of slope) based on topographic maps.

In the southern Appalachians, the distribution and growth of trees are highly correlated with local topography, but the relationships have been difficult to describe quantitatively. A quantitative expression of the geometric shape of the land surface (terrain shape index) is described and correlated with overstory tree heights and site quality. Application of the index in three even-aged stands of yellow-popular (*Liriodendron tulipifera* L.) on high-quality uniform sites shows that it is highly correlated with total height of trees in a stand, with r^2 ranging from 0.45 to 0.74. In comparisons among stands, the index accounted for an average of 51% of the variation in site index. The relationship was validated in two supplementary stands and accounted for about 49% of variation in site index (McNab, 1989).

Plot mean relative deviations were plotted against elevation, aspect, slope, and drainage class (Harrington and Curtis, 1986). Multiple regressions were used to identify variables that appeared to influence tree growth. These regression equations are not presented as predictive equations, but rather as summaries of the data and an indication of the amount of variability in tree volume growth that can be accounted for by tree, stand, and physiographic factors (Merrill and Michael, 1986).

2.   Materials and Methods

Locations:

This study was carried out in central north forest of Iran in Mazandaran province, south of Caspian Sea. Survey area consists of forest region in south of Sari, Ghaemshahr and Neka. Geographical positions was latitude from 36° 09 32˝, to 36° 26 14˝ N, and longitude 52° 49 05˝, to 53° 28 29˝ E with area about 500, 000 hectares and distribute. Sari forest region is about 650.000 ha area and there are on 100 to 2800 m altitude but distribution of *Alnus subcordata* is rang of 100 to 1700 meter from free sea level. The dividing line between lowland forests and mountain forest is the presence of thermophyllous species like *Pterocarya fraxinifolia*, *Parrotia persica*, *Diospyros lotus*, *Albizia julibrissin* and *Gleditsia caspica* in the lowland forests. Other characteristic lowland species are *Alnus glutinosa*, *Quercus castaneifolia*, *Zelkova carpinifolia, Carpinus betulus, Acer velutinum* and

Populus caspica. There are also some characteristic evergreen species like *Buxus hyrcana*, *Hedera pastuchovii*, *Danaë racemosa* and *Ruscus hyrcanus.* All these evergreens are more or less restricted to the hyrcanian forests.

Survey area is including sub humid and humid areas with cold to temperate winters. Mean precipitation of survey area ranged 600 to 1240 mm. An average precipitation also is 907mm. Mean temperature of entire area is about 14.6°C and absolute minimums and maximum temperature ranged -8.5 to 40°C. Relative humidity in entire survey areas is about 80% and average minimum and maximum of humidity varied between 69 and 90%.

As geological data, survey area was emerged in cretaceous of second geological period as Eocene of Paleocene and Neogan of third geological period including Miocene and Pliocene. This parent materials are sediments of calcareous, siltstone, argillite with accompanied sea sediment as lomashals and some Conglomerate stones.

Usually soil is neutral with pH 6.8 to 8.09, in some part acidic, and mostly mature soil with profile A (B) C until ABC as type of brown forest. The soil texture in the most part of Sari forest region emphasized influence of parent materials (eg. Existence of Marl, calcareous, siltstone and argillite) on soil formation to semi heavy texture (clay loam) to heavy (clay) with 30 to 60 percent clay.

Methods:

The approach of sampling and collecting data of this study is the type of randomize systematic design which number of sample plot calculated according to objectives, time, facilities, budget and accuracy.

At first base on information of Sari Natural Resources Service (e.g. forestry plans notebooks details in parcels and series) we identified the natural and plantation area of *Alnus subcordata* stands. Pilot stag of study was done with visiting of alder stands area and had marked these stands on contour line (25_m) map with 1:50000 scale. Base on pilot survey was shown which alder stands distributions in deferent elevation from 100 to 1700 meter (from free sea level). Next stag of work was done for make the normal distribution of sample in alder stands. Forest area divided in map by rang of 200 meter altitude. These selected stands had covered the most of aspect and distribute homogenous in all area. In next stage in each alder stands was selected one sample plot randomly and forest inventory was done by measuring and filing special forms. Overall 80 sample plot used for collecting data of

Alder stands. Sample plot of survey area is circle shape with area about 500 m^2 and nested form (definition to nested plot) with two parts of macro plot and micro plot (sub-plot). Macro plot is whole part of sample plot with area about 500 m^2 and about 12.60 meters radial. Micro plots are about 50 m^2 area in center of macro plot with 4 meter radial for collecting special data. Measurement of 80 SP, each about 500 m2 areas, in natural and plantation stands of alder was done. Data collections include quality and quantity is shown as mentioned below:

1-Alder trees specification: (total height, diameter at breast height, quality and health of trunk and canopy cover of trees, natural regeneration, etc.)

2-Site's specification was noted (Geographic data such as altitude, soil data, aspect, and slope)

3-Other data (type of forest, land use in neighboring region) was recorded carefully.

Tree and stand parameters calculated by standard formula of volume and SPSS software. Data in each experiment was anal**y**zed by MSTATC software (1999, Ver 2.1) separately by split plot method which had been conducted in Randomized Complete Block Design (RCBD). The means of treatments which had significant differences have been compared by using Duncan's method with 0.95 % accuracy (= 0.05%).

3.   Results and Discussion

Elevation:

Average growth of dbh (cm) in different elevations of natural and forestation stands haven't confidence significant (a=0.05) in different altitude. Results in table 1 showed which means of growth of diameter in all area is about 0.9 cm per years.

Average growths of total height of alders (cm) in different elevations of natural stands have shown that increase of altitude due to decrease of mean of total height growth. Rang of average total height growth is 0.35 to 0.56 m 1600 to 0 above m.s.l. respectively. Altitude was deduced from the topographic maps of the area and categorized into three classes; low (210-399), mid (400- 699) and high (700 a.s.l.). In general, tree growth varies in space and time depending on the prevailing climatic conditions and the growing stage of the subject trees (Dawkins 1956, Dickinson et al., 2000). In plantation area there aren't confidence significant (a=0.05) among mean of total height growth in different altitude.

Comparisons of average growth basal area at breast height (m^2) per hectare of Alders in different elevations of natural stands have shown that maximum growth of basal area there in altitude 400 to 800 above m.s.l. about 0.62 m^2 and minimum basal area related to elevation rang 1200 to 1600 above m.s.l. about 0.1 m^2. in plantation stands maximum basal area there in altitude 800 to 1200 above m.s.l. about 2.12 m^2 and minimum basal area related to elevation rang 1200 to 1600 above m.s.l. about 0.85 m^2 per hectare.

Volume growth (m^3) of individual Alders in different elevations of natural stands has shown that increase of altitude due to increase of average growth volume. The most individual tree volume growth recorded in Rang of altitude 1200 -1600 above m.s.l. equal to 0.057 m^3. In plantation area maximum average individual tree volume growth was 0.024 m^3 have observed in ringed of altitude 800 to 1200 above m.s.l.

Volume growth of Alders per hectare (m^3/ha) in different elevations of natural stands have shown that rang of altitude 400 to 800 have maximum annual growth volume about 6.04 and minimum annual growth volume about 0.43 related to elevations rang 1200 to 1600 m.s.l. Mania et al. (2005) showed that tree growth increased with altitude on a large scale across regions, and with disturbance intensity on a small scale at the plot (stand) level. In plantation area maximum average of that volume growth was 19.9 m^3 have observed in rang of altitude 800 to 1200 m.s.l and minimums average of that volume growth was 4.54 m^3 had observed in rang of altitude 1200 to 1600 m.s.l.

Aspects (Direction of slope):

Study on different aspects of natural stands has shown that maximum diameter growth of tree observed in west aspect and minimum diameter growth had seen in east aspect. Despite in plantation stands average growth of dbh was confidence significant (a=0.05) among different aspects. Minimum growth observed in west and south aspect about 7 mm and maximum growth is north aspects about one cm.

Results showed that means of growth of tree heightments in different aspects of natural stands which have shown that maximum diameter growth of tree observed in west aspect (0.42 m) and minimum diameter growth had seen in southwest aspect (0.34 m). Despite in plantation stands maximum average growth related to north aspect (0.92 m). Results of basal area per hectare in different aspects of natural was shown the maximum growth of basal area there northwest aspect (0.67 m^2) per years and minimum related to southwest aspect (0.12 m^2). Maximum average growth of basal area in plantation stand related to east aspect

about 2.4 m^2 and minimum basal area growth per hectare related to south aspect with about 0.14 m^2.

Volume growth of individual Alders in different aspects of natural stands was shown in table 4-2 and maximum growth volume there are in southwest and northwest aspects (0.077 and 0.074 m^3 respectively) and minimum mean of volume growth related to south aspect about 0.026 m^3. In plantation stands maximum average volume growth of individual alders related to southeast (0.027 m^3) and minimum of that average related to south aspect (0.006 m^3). Maximum growth volume there are in northwest aspects equal 8.17 m^3 /ha and minimum mean of volume growth related to southwest aspect about 0.44 m^3. In plantation stands maximum average volume growth of alders related to southeast (19.2 m^3/ha) and east (18.9 m^3/ha). Minimum of that average related to south aspect about 0.65 m^3/ha.

Slopes:

Analysis of diameter growth in natural and plantation stands and different slopes showed there aren't confidences significant (a=0.05) in different aspects. Analysis mean of alder total height growth in natural stands and different slopes were shown in areas with low slope (0 to 15 percent) have minimum mean of growth (about 0.39 m). In plantation stands height growth means weren't confidence significant (a=0.05) among slopes (table 3).

Analysis means of basal area growth per hectare in different slopes of natural area shown that maximum amount related to rang of slope 0-15 percent about 0.77 m^2 and minimum basal area there ranges to 30 to 45 percent about 0.1 m^2. In plantation stands weren't shown confidence significant (a=0.05) among slopes. The spatial heterogeneity of ecological conditions was represented using simple environmental information such as altitude, aspect, slope, topographical position in relation to the top and bottom of the ridge, and disturbance level (Vanclay 1989b, Vanclay, 1992).

Also analysis means of volume growth of individual alders in different slop of natural stands were shown high volume of individual alders found about 0.057 m^3 in slopes rang 45 to 60 percent. Minimum average volume growth of individual alders found 0.041 m^3 in slope rang 15 to 30 percents. Means of volume in deferent slopes of plantation stands weren't shown confidence significant (a=0.05) among slopes. Analysis means of volume growth alders per hectare in deferent slop of natural stands shown high growth volume of alders calculated about 9 m^3 in slopes rang 0 to 15 percent. Minimum average volume growth had found 1.65 m^3 per hectare in slope rang 30 to 45 percents. Means of volume in deferent slopes of plantation stands weren't shown confidence significant (a=0.05) among slopes. Gunatilleke et al. (1996) and Veenendaal et al. (1996) in experimental studies of seedling growth also demonstrated that topographic gradients in soil fertility can cause differences in growth of individual species.

Table1: Growth parameters of Alders in different elevations.

		Natural			Plantation		
	Elevation	Mean	Duncan's Group	Number of Sample plot	Mean	Duncan's Group	Number of Sample plot
Annual diameter growth (cm)	0– 400	0.879	B	6	1.053	A	6
	401– 800	0.95	A	6	1.008	A	6
	801–1200	0.953	A	6	1.064	A	6
	1201–1600	0.995	A	6	0.839	A	6
Annual total height growth (m)	0– 400	0.56	A	6	0.76	A	6
	401– 800	0.5	B	6	0.81	A	6

	801–1200	0.44	C	6	0.84	A	6
	1201–1600	0.35	D	6	0.66	A	6
Basal area growth (m^2) per hectare	0– 400	0.166	BC	6	1.36	AB	6
	401– 800	0.62	A	6	1.845	A	6
	801–1200	0.327	B	6	2.12	A	6
	1201–1600	0.101	C	6	0.855	B	6
Volume growth of individual alder trees (m^3)	0– 400	0.031	B	6	0.014	AB	6
	401– 800	0.046	A	6	0.016	AB	6
	801–1200	0.049	A	6	0.024	A	6
	1201–1600	0.057	A	6	0.006	B	6
Average volume growth (m^3) per hectare	0– 400	1.6	C	6	10.3	AB	6
	401– 800	6.04	A	6	14.3	AB	6
	801–1200	3.77	B	6	19.9	A	6
	1201–1600	0.43	C	6	4.55	B	6

Table 2: Growth parameters of Alders in different Aspects.

		Natural			Plantation		
	Aspects	Mean	Duncan's Group	Number of Sample plot	Mean	Duncan's Group	Number of Sample plot
Annual diameter growth (cm)	North	0.92	C	3	1.09	A	3
	Northeast	0.93	C	3	1.09	A	3
	Northwest	1	BC	3	1.05	A	3
	East	0.94	C	3	0.98	A	3
	West	1.13	A	3	0.75	B	3
	South	0.8	D	3	0.82	AB	3
	Southeast	0.99	BC	3	1.07	A	3
	Southwest	1.07	AB	3	1.03	A	3
	North	0.42	BC	3	0.92	A	3

Annual total height growth (m)	Northeast	0.51	AB	3	0.84	B	3
	Northwest	0.42	BC	3	0.8	B	3
	East	0.47	AB	3	0.68	C	3
	West	0.52	A	3	0.45	D	3
	South	0.44	BC	3	0.48	D	3
	Southeast	0.43	BC	3	0.94	A	3
	Southwest	0.34	C	3	-	-	-
Basal area growth (m^2) per hectare	North	0.167	CD	3	1.539	D	3
	Northeast	0.066	D	3	1.832	C	3
	Northwest	0.675	A	3	2.086	B	3
	East	0.215	C	3	2.465	A	3
	West	0.094	D	3	1.693	CD	3
	South	0.472	B	3	0.14	E	3
	Southeast	0.33	BC	3	1.792	C	3
	Southwest	0.126	D	3	-	-	-
Volume growth of individual alder trees (m^3)	North	0.044	BC	3	0.016	AB	3
	Northeast	0.031	D	3	0.015	AB	3
	Northwest	0.074	A	3	0.021	AB	3
	East	0.037	CD	3	0.021	AB	3
	West	0.054	BC	3	0.007	B	3
	South	0.026	D	3	0.006	B	3
	Southeast	0.061	AB	3	0.027	A	3
	Southwest	0.077	A	3	-	-	3
Average volume growth (m^3) per hectare	North	1.66	CD	3	13.1	B	3
	Northeast	0.47	D	3	14.2	B	3
	Northwest	8.17	A	3	18	AB	3
	East	1.27	CD	3	18.9	AB	3
	West	0.65	D	3	9.34	C	3

South	5.06	B	3	0.65	D	3
Southeast	3.24	C	3	19.2	A	3
Southwest	0.44	D	3	14.2	B	3

Table 3: Growth parameters of Alders in different slopes.

		Natural			Plantation		
	Slopes	Mean	Duncan's Group	Number of Sample plot	Mean	Duncan's Group	Number of Sample plot
Annual diameter growth (cm)	0 – 14	0.924	A	6	1.041	A	6
	15 – 29	0.910	A	6	0.996	A	6
	30 – 44	0.943	A	6	1.053	A	6
	45 – 60	1.008	A	6	-	-	-
Annual total height growth (m)	0 – 14	0.395	C	6	0.812	A	6
	15 – 29	0.532	A	6	0.801	A	6
	30 – 44	0.390	C	6	0.770	A	6
	45 – 60	0.463	B	6	-	-	-
Basal area growth (m^2) per hectare	0 – 14	0.772	A	6	1.827	A	6
	15 – 29	0.175	C	6	1.58	A	6
	30 – 44	0.104	C	6	1.917	A	6
	45 – 60	0.525	B	6	-	-	-
Volume growth of individual alder trees (m^3)	0 – 14	0.0479	AB	6	0.0157	A	6
	15 – 29	0.0410	B	6	0.0170	A	6
	30 – 44	0.0453	AB	6	0.0177	A	6
	45 – 60	0.0569	A	6	-	-	6
Average volume growth (m^3) per hectare	0 – 14	9.16	A	6	15.15	A	6
	15 – 29	1.71	C	6	13.99	A	6
	30 – 44	1.65	C	6	16.77	A	6
	45 – 60	5.60	B	6	_-	___-	-

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Education for Rural Development in Iran

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Abstract: Most of the human capital literature pertaining to developing countries focuses on the returns to education in rural development. In developed countries education has an important role in the processes of rural development. But in third world countries there are some important barriers in face of education for rural development. This paper looks at the barriers of education for rural development in rural communities of Iran. The objective of this study is, through reviewing the available evidences, analyses and experiences in the role of education in rural development, to identify weaknesses pertinent to basic education achieving rural development and to come out with some conclusions that can be taken into consideration in policy making or planning successful basic education and training for rural development.

1. Introduction

The greater proportion of people in the world lives in rural environments. The World Bank (1975) defined rural development as a strategy designed to improve the economic and social life of a specific group of people- the rural poor. The contribution of rural areas to conomic development is usually lirnited; however, the future potential for fire conti'ibution is great, especially in developing countries. Therefore, rural development will become a key factor during the next decade or so in the overall economic development of many developing countries (Navaratnam, 1986). Rural development aims at improving rural people's livelihoods in an equitable and sustainable manner, both socially and environmentally, through better access to natural, physical, human, technological, and social capital, and services, and control over productive capital, that enable them to improve their livelihoods on a sustainable and equitable basis (Atchoarena & Gasperini, 2003). Rural development becomes a major focal issue in national and international development agendas. One of the strategies set to poverty reduction is provision of equitable and quality basic education and for this targets have been set as education for All (Abdulahi, 2008).

Education is one of the mechanisms to empower people to take part in rural development. It was launched as a key strategy of rural development. Increased the education is a means to achieve development to resolve the rural problems (Lasker, Weiss, & Miller, 2001). Education may directly influence rural agricultural productivity via one or more of the routes described above. Education may also indirectly increase output through its interaction with other institutional variables (Weir, 1999). Governments and their development partners have recognized the importance of education for rural people and placed great emphasis and adopted policies and strategies to increase or improve access to quality basic education (Abdulahi, 2008). Education and training are two of the most powerful weapons in the fight against rural poverty and for rural development. Unfortunately, these are also among the most neglected aspects of rural development interventions by national governments and by donors. In most countries public policies fail to integrate rural development and basic education (Atchoarena & Gasperini, 2003).

Education in rural development processes can support and uphold local culture, tradition, knowledge and skill, and create pride in community heritage (Lacy, Battig, Moore, & Noakes, 2002). The paper illustrates from the macro standpoint the contribution of education in rural areas. It stresses that education in rural areas is the foundation for both macroeconomic trends, and other educational activities contributing to rural development. The paper also emphasizes that mere primary education in rural areas is insufficient to cope with the needs of rural development in the present world. Although, education has economic and noneconomic benefits to educated individuals and to the social as a whole, this study intended to focuses on the aspect of economic benefit of education to rural areas. It reviews some critical issues that are related to education and

training in the context of rural development in developing countries especially in Iran. This study begins with an overview of educational issues and economic benefits in rural context. For this study, pertinent articles and reports on critical issues of education in rural development are reviewed. Despite effort to improve access, equity and quality of basic education in rural areas, they still remain problematic (Abdulahi, 2008).

2. Methods

The research was performed as a qualitative library in which the researcher had to refer to relevant and related sources. I have used a number of articles and official websites of the various Iran known organizations. For existing analyses, I looked at the research literature on rural education in developing countries and the World Bank Education.

3. Education for Rural development

There is little dispute that education-often characterized as the reading, math, and other skills and knowledge taught in the first four years of primary school- is critical to economic and social development. Without basic skills, according to one definition, a person cannot comprehend the instructions on a bottle of medicine or a bag of fertilizer or read a government notice. Neither can the person compute a bill or write a letter. Without basic skills, it is impossible to develop one's potential or to contribute in anything more than a rudimentary manner to society (Moulton, 2001).

Education is widely accepted as a key factor in achieving poverty reduction and sustainable development. However, the vast majorities of world's poor, depending on small-holed fanning, live in developing countries mainly in rural areas with insufficient or lack of access to basic education. The discussion about education in rural areas is closely related to the broader rural development concept. Education has emerged as an essential prerequisite for improving agriculture, reducing poverty and living conditions of rural people (Abdulahi, 2008). Education may enhance farm productivity directly by improving the quality of labour, by increasing the ability to adjust to disequilibria, and through its effect upon the propensity to successfully adopt innovations. Education is thought to be most important to farm production in a rapidly changing technological or economic environment (Shultz, 1964, 1975; Weir, 1999).

Recent research shows that improvements in education boost local development prospects. Higher educational levels lead to faster income and employment growth, and better schools can produce

higher academic achievements and improve long run economic prospects for students. According to a study of rural South Carolina in the 1990s by researchers at Clemson University, a small but significant link occurs between school quality and employment growth in the local community (Gibbs, 2005). Education has a desirable controlling influence over development of the rual individual, community, and society, leading to reduced poverty, income and controlled unemployment (Navaratnam, 1986). Education is a phenomenon of affluent contemporary societies is a particularly difficult concept in communities in developing countries to grasp. Education improves the individuals choices available to peoples as well as an educated population provides the type of labor force necessary for industrial development and economic growth (Fägerlind & Saha, 1986). Much of the theoretical debate about the role of education in rural development and economic growth has focused upon whether education is productive in an economic sense. There is much evidence that levels of schooling amongst the population are highly correlated with levels of economic development (Oxaal, 1997).

In attempts to understand factors that prevent communities from being involved in formal education, Shaeffer (1992) found that the degree of community participation is particularly low in socially and economically marginal regions. This is because such regions tend to have the following elements: (a) a lack of appreciation of the overall objectives of education; (b) a mismatch between what parents expect of education and what the school is seen as providing; (c) the belief that education is essentially the task of the State; (d) the length of time required to realize the benefits of better schooling; and (e) ignorance of the structure, functions, and constraints of the school (Uemura, 1999).

Education that contributes to rural development is includes basic education, rural adult education, vocational education and higher education (UNESCO, 2002). The impact of education on rural development and correlation between education and development have been analyzed by social scientists for a long time. Helliwell and Putnam (1999) also find that education is correlated with typical measures of social capital: trust and social participation. However, only recently have studies attempted to determine whether education exerts a causal influence on rural development, or whether the correlation arises because both education and civic participation are jointly influenced by unobserved factors (Riddell, 2006). Education may increase the probability of success in each of these endeavours and, in so doing, diversify household income sources to reduce risk

and improve economic security. Since farming is the primary activity in rural Iran, this paper will focus on the part played by eudcation in rural development.

World Bank studies demonstrate education raises the production of farmers. Four years of schooling on the average appears to increase the output of farmers by about 8%. The rate of return to rural education in Korea, Malaysia and Thailand was at least 20%. The study also reveals farmers with four years of primary schooling had higher crop yield than those had never been to school (Abdulahi, 2008; Psacharopoulos & Woodhall, 1985). Education has long been recognized as a potential means for rural development. In many developing countries education has been seen as a panacea for national development (Hegtvedt-Willson, 1984). However, the movement of young adults from rural to urban areas for college means that much of the potential benefit to earnings from improving schools will be lost to the local community. This effect weakens the rationale for supporting good schools, especially if these improvements are perceived to encourage outmigration (Gibbs, 2005).

4. Barriers of Education in Iran

Education has an essential role to play in rural development. Understanding barriers of education for rural development is important when a community is getting organized for involvement in development planning. This understanding can help community and organizations more effectively impact the educational policy-making process (F. Aref, 2010). Further, it is important for government to understand that educational system also face barriers that can hinder its progress in responding and recognizing the priorities of local communities in Iran. Overcoming the barriers to education will serve to facilitate the policy making process. There are several literatures that directly deal with the barriers of communities particularly in third world countries, especially in Iran. Following are the main barriers:

-Inability to analyze the changing socio-cultural dimensions of educational system
-Lack of understanding of the educational policy
-Lack of access to new information

Involving rural communities in the education planning requires facing and tackling a number of challenges. While the evidence for links between basic education, economic development, and poverty alleviation is abundant, and funding agencies are investing significantly in basic education, it remains more difficult to provide good-quality basic education to children in rural areas than to those in urban areas. One set of factors lies in the rural's perception of

education, and the other is that facing the ministry of education (Moulton, 2001)

Rural barreirs
Because rural areas are less densely populated than urban areas, rural schools are farther apart, requiring many children to walk long distances or pay for transportation and to lose valuable time in walking that could otherwise be spent helping at home. Some families are unwilling to send their children down long roads alone.
-Rural children, more than urban children, are required by their parents to supply labor on the farm and in the home.
-As many incidences of household poverty in most countries are likely to occur in rural areas, children who attend school often suffer from poverty ailments.
-Some parents see school as a funnel to urban areas where there are jobs.
-Even where a primary school is accessible, there may be no secondary school within commuting distance.
-Finally, in spite of the poor conditions of schools, parents are usually asked to pay fees, official and unofficial, in addition to other costs (Moulton, 2001).

Organizational barreris:
 The ministry of education faces physical, social, and economic limitations in rurals.
-Far fewer teachers want to serve in rural schools. Most individuals who have the education credentials that would qualify them as teachers have had some urban or quasi-urban experience, if only in teacher training school. Many are reluctant to be posted to remote rural areas.
-Long distances, poor roads, and inadequate shipping vehicles make it difficult to get building materials, furniture, equipment, and textbooks to remote rural schools.
-While in many cases, building materials and furniture can be locally supplied, instructional materials are not available. These include not only textbooks but also the visual materials that decorate classrooms and stimulate learning, as well as simple scientific lab equipment, radios, and other audio-visual equipment that has become a standard part of many classrooms.
-Communication between ministry offices—even provincial and/or district offices—and schools is difficult, so school principals and teachers get little if any guidance from a professional support network. It is difficult to bring teachers, principals, parent groups, and other school supporters, together for training and information centers.

-The curriculum may not be relevant to rural communities. When the curriculum goes beyond basic math, reading, and writing, teachers use little discretion in adapting it to what students know and what their needs and interests are.

-While urban parents and communities sometimes play an active oversight role in their schools, this rarely happens in rural communities, where parents are less skilled at holding officials accountable, reviewing financial statements, and even feeling confident that they can ask questions.

-Support services for remote rural schools are not always fully institutionalized. Unlike systems of agricultural extension, most systems of school supervision merely attempt to link rural schools through the bureaucratic structure to central ministry offices. The ministry often lacks the resources to help these links function as channels of support (Moulton, 2001).

5. Conclusion and Recommendation

Education is a critical part of rural development. Individuals who have had some education are better farmers and more capable of finding off-farm employment. The rural sector also benefits from the overall development of the national economy and the alleviation of poverty, in which basic education is essential (Moulton, 2001). In any effort to promote education for rural development, it is necessary to assess the communities' capacity to carry out what they are expected to achieve in a long run. It is a process that facilitates the realization of improving educational quality and the promotion of democracy within society. This paper suggests that there are strong justifications for government of support for rural education. It outlines several important and broad issues, including the need for and challenges of broad approaches to provide formal and non-formal education in rural areas. Hence, Education contributing to rural development must be locally controlled, practical, applied, problem-posing, and focused on functional specialization. The following is the ways which education can contribute to the development.

-Boosting morale of school teachers

-Raising budget for rural schools

-Constructing, repairing, and improving school facilities

-Recruiting and supporting teachers

-Monitoring and following up on teacher attendance and performance

-Actively attending school meetings to learn about children's learning progress

-Providing skill instruction and local culture information

-Garnering more resources from and solving problems through the education

-Providing security for teachers by preparing adequate housing for them

-Identifying factors contributing to educational problems (Uemura, 1999; Aref, 2011).

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Effectiveness of the Training Program for the Production of Trousers Clothes to Serve the Small-Scale Industries

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Abstract: The research aims to formulate the content of the training program in a scientific manner and determine the time required for training. In addition to determine the effectiveness of a training program based on scientific bases to give graduates the knowledge and skills for the production of trousers blouse. Throughout, the prepared and qualified graduates and equip them with knowledge and skills required by the labor market, to support the small-scale industries and to contribute to solve the unemployment problem. In order to achieve that the study used these tools as follows: the training program, a test knowledge (me / me), a practical skill test (me / me) and measure of appreciation. The research found that the presence of statistically significant differences between the average scores before and after in both the knowledge and skills included in the program for drawing Pattern and enforcement techniques for the post test, indicating the success of the training program and raise the efficiency of the trainees.
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1. Introduction:

The world is the era of accelerating the pace of change and development, and no doubt that the most important tools for change and development is the effective training of manpower as the most important element of energy development and the most important institutions, which are paid to achieve its objectives in all countries of the world (Abdel-Moneim, 1996).

The manpower is the most important elements of the economic process and social development. Where researches tend to the necessity of activating the human power through a proper planning and to accommodate changes and adapt administrative systems and rehabilitation programs with the requirements of development. (www.hrdf.org.sa)

For human capacity development, must support the development process through all the organs of state and private educational institutions to develop services in line with the needs of industry, especially the garment industry which has many characteristics and attributes that qualify it to be one of the drivers behind the wheel of human development in Egypt. (Mohammad, 2002).

The training of human resources is considered as effective trends that contribute to increase production efficiency because it is in response to changes in the external environment. As a result of the current world of rapid global changes and the application of the convention on the global trade liberalization and technology revolution, was

necessary to promote small businesses as a way to solve many economic problems.

Therefore, it is necessary to develop trained technical staff can contribute to the wheels of development, because the human element is the basis of the development process particularly in the garment industry, which is considered as a fundamental pillar for building the future. Where is represents the most important economic sectors, which attract great attention from all over the world.

The training is defined as those efforts that aim to provide the trainer with information and knowledge to gain skills in the performance of work or development and the development of his skills, knowledge and experience as to increase the efficiency in the performance of his current or be prepared to perform the action with a higher level.

Training is a strategic choice to anyone looking forward to the preparation of human cadres capable of meeting the needs of work and keep abreast of developments and rapid changes that occur in different areas.

Training creates trainee knowledge and new skills required by the profession for up to a level that aspire to any party seeking to advance the progress. Where that training is essential for the learner and development of the human race and then the progress and build of society will be done. (www.suwaidan.com)

Training Objectives:

Training objectives are divided into three main groups:

1 - Traditional goals:

- Training new employees in their fields and keep pace with scientific and technological progress, by providing trainees with information during their work or skills when you enter certain modifications in the methods of work.

2 - The goals of solving problems:

- Search for the best ways to help workers to overcome the problems they face.

3 - Creative objectives:

- It aimed to achieve high levels of business, through the use of sophisticated scientific methods, and distinctive elements capable of innovation and creativity.

The training system consists of five basic elements, namely:

1 - Goal of the training: means the specific results in time, space, quality, and training system, which seeks to achieve them.

2 - Inputs to the training process: It consists of the human elements (trainers, trainees); physical elements (equipment and funds), and elements of the significance (studies, facts and problems).

3 - The processes necessary for implementation: includes the preparatory processes for the train to get to know the possibilities, then the operational processes of transfer of knowledge, skill and experience and, finally, a follow-up complementary processes and performance appraisal training.

4 - Output the training process: It means the trainees to acquire skills and new directions, and then increased performance and improved methods of work and the spirit of productivity and profitability.

5 - Returns impact: a comparison of the output resulting from the training and pre-defined objectives as well as the statement of deviations with adjusted constantly.

Principles of training:

1 - Objective: the aim must be clear and specific in accordance with the actual needs of the trainees, taking into account that the target is realistic and applicable.

2 - Continuity: The training continues step-by-step with the individual to promote and develop in line with the requirements of the career development of the individual.

3 - Inclusiveness: it must be given to all levels of functional and includes all the classes in the hierarchy.

4 - Rank: training begins with simple issues to address, and then cascade down to the more difficult and so on up to address the most difficult and complex problems.

5 - Keep pace with development: training in order to be an inexhaustible source of it all to avail itself of all that is new and modern in the various areas of work, you must adopt the latest methods and technology training.

6 - Realism: that meets the actual needs of the trainees and commensurate with the level of them, (www.alhassan.owno.com).

7 - Change and renewal: training reacts with variables and then freezes may not be in the templates, but must be characterized by change and renewal. (Ali and Aida 2002)

7 - selfness: Although the training process are joint and mutual, but the trainee is primarily responsible for the development of himself, trying to give him benefit of training, since the behavioral aspects complement the technical aspects, and both complements the mental aspect. (Mohammad 2007)

Training systems:

Training system to include:

1 - Identification of training needs.

2 - Design the training program.

3 - The implementation of the training program.

4-Evaluation of the trainee. (www.hrdiscussion.com)

5 - The training programs are evaluated on the steps:

- Prior to implementation: to measure the success of the preparation.

- During implementation: in terms of time allocated to training, and training techniques used to ascertain the suitability of the implementation process with the plan.

- After implementation: to stand on its training program objectives and their success in meeting training needs.

The role of universities is the greatest common denominator in any operations for the overall development as it is primarily concerned with the development of trained human resources to boost production, (Hatem, 1999).

Based on the above mentioned the author prepared a training program for the production of clothes trousers on a scientific basis for graduates wishing to set up small businesses in an effort to advance human development.

Research problem:

There is clearly the research problem in the following questions:
- What is the scientific basis upon which the program to give graduates the knowledge and skills for the production of trousers and blouse for the establishment of small projects?
- What is the content of the training program?
- To what extent the program's effectiveness in giving the trainees the knowledge and skills necessary to produce the shirt, trousers?

Research Objectives:
- Formulation of the content of the training program in a scientific manner and determine the time required for training.
- Determine the effectiveness of a training program based on scientific bases to give graduates the knowledge and skills for the production of trousers blouse.
- Prepare and qualify graduates and equip them with knowledge and skills required by the labor market.
- Support for small-scale industries and to contribute to solving the unemployment problem.

The importance of research:
    The importance of this research lies in the preparation of qualified technical staff to cope with the incident in the garment industry and the opportunity for them to work and improve the level of performance as well as to the establishment of small projects have a positive impact on national income.

Hypotheses:
1 - There are significant differences between the average scores of trainees in the knowledge gained on forging the model before and after training for the post test
2 - There are significant differences between the average scores of trainees in the knowledge gained special implementation techniques before and after training for the post test
3 - There are significant differences between the average scores of trainees in the knowledge acquired before and after training for the post test
4 - There are significant differences between the average scores of trainees in the skills acquired on forging the model before and after training for the post test
5 - There are significant differences between the average scores of trainees acquired skills in the implementation of special techniques before and after training for the post test
6 - There are significant differences between the average scores of trainees in the skills acquired before and after training for the post test

7 - There are significant differences between the average scores of trainees program before and after training for the benefit of the post

2. Search procedures:
Research Methodology:
    These experimental methods follow the experimental issues, for their suitability in achieving the objectives of the research and verification of hypotheses.

Sample of the research:
    The application program (30) of qualified graduates' girls with high and medium at the Higher Institute of Applied Arts from 15/08/2009 for a period of five weeks (three times/week /three hours / day).

Search Tools:
- The training program.
- A test knowledge (before / post).
- A practical skill test (before / post).
- Measure of appreciation.

Search limits:
- The search is limited on the study (external blouse trousers) in terms of preparation Pattern flat and study of the techniques for implementation.
- The training of graduates of higher and middle education. The researcher has a training in the clothing factory at the Higher Institute of Applied Arts.

Search Terms:
Training program: is the activity of a renewed and sustained planning begins and ends with monitoring and evaluation. Which aimed at developing knowledge and skills of individual and collective by its effect on behavior with a positive impact and effectiveness is measured as much as can be applied, (Ali and Aida, 2002).

Small Industries: there are many definitions and concepts, and usually the definition of small-scale industry by the employment size or the amount of capital invested in fixed assets.
Moreover, the small-scale industries can be defined as in the modern sense as (those industry that accept the development and improvement in line with the wishes of the arts of production and the level of consumer with an average in capital intensity),
(http://www.alhadag.com/column.php?id=422)

Search procedures:

First: the preparation of the training program:

The author set up a training program to give graduates the knowledge and skills-building and implementation of the Pattern of blouse and trousers in accordance with sound methodological steps.

A - Determine the program's topic:

The program has chosen in line with the trends, which calls for the development of human resources and support for small industries.

Procedural goals of the program:

After the training the trainee will be able to:
- Take body measurements in a scientifically sound.
- Know the Pattern and trends
- Known as the scientific basis for divisions on sizes taken for the preparation of Pattern.
- Defines forms pockets.
- Mastered the work of the Panda Pocket
- Recognize the concept of sjav.
- Recognize the forms of refrain.
- Compares the **coller** (Oovise - Sport - Chimisais - flatbed).
- Specify the types of sleeves.
- The types of bracelets.

Kinetic targets of the program:

The trainee after completion of the program is able to lead the following skills:
- Raise the measurements properly.
- Draw the Pattern a sound scientific manner (in front of - behind - sleeve).
- Writes the data necessary on Pattern parts.
- The cutting and installation of sjav.
- The weaving refrain
- Pastes stuffing **coller**
- The installation of **coller**
- The installation of sleeves
- Mastered the installation bracelets

Content of the training program:

The program included training on the following topics:
- Identify the concepts related to the preparation Pattern.
- Identify the concepts related to garment industry.
- Identify the steps to be followed for the construction of Pattern statute.
- Preparation of blouse trousers Patern (Korsag).
- Preparation of Patern **coller** trousers.
- Preparation of blouse trousers pattern (sleeve).
- Identify the methods interleave (sleeve) model on the canvas.
- To identify the different folds of the tail.
- Sample preparation ruffle.
- Understand the concept of fragment.
- The implementation of the ruffle samples with different sizes.
- Sample preparation Nerverat.
- Installation of decorative zipper.

b- Determine program objectives

The general objectives of the program:

After completion of the training that the trainee can:
- Recognize the concepts of the garment industry.
- Recognize the foundations to be followed when building the form trousers.
- Draw the trousers form.
- Works factories, garment trousers.

- Know the Pattern and trends.
- Recognize the pied
- Write data on the parts of the Pattern.
- Determines the direction of the fabric.
- Recognize the different folds of the tail.
- Recognize the concept of ruffles.
- Specifies the sizes of fragments.
- Recognize the concept of sjav (fragments minute).
- Identifies the various tools closure.

- Cut Pattern parts and put it on the cloth properly.
- The process of being clutch parts Pattern correctly.
- Flex the tail.
- Mastered the process of ruffles.
- Weaving fragments.
- Mastered the nerver
- The installation of the appropriate tools sealed (zippers - buttons).
- Performs sample pocket with Band.
- Cutting and installation of sjav.

- Prepare pied bar.
- The implementation of the pied pied sample.
- The concept of panda.
- Sample preparation of pocket with panda.
- The concept of sjav.
- Sample preparation sjav.
- Refrain concept.
- The implementation of the refrain sample.
- The concept of coller.
- Forms coller.
- Implementation of samples coller (Oovise - Sport - Chimisais).
- The concept of sleeve.
- Forms of the sleeve.
- Installation of sleeve.
- The concept of bracelets.
- Types of bracelets

- Preparation of a sample installation of the buttons.   - Installation of bracelets.

Timeline for Training

Content of the training program	First week			Second week			Third week			Fourth week			Fifth week		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1-Understand the basic concept of Pattern -Understand the concept of the clothing industry - Steps to be followed to obtain Basic Pattern	ü														
2- Drawing the basic model of the blouse (front - back -sleeve - collar)		ü													
3- Identify the different folds - Sample numbers pagan tail - Sample preparation ruffles			ü												
The concept of Kasra (shards) - Sample preparation shards of various sizes - The number of sample Nerver (micro shards)				ü											
- To identify the different forms of tools closure - Installation of decorative zipper					ü										
Concept pied Preparation of pied - The implementation of the pied sample						ü									
- The concept of pocket - panda concept - Sample preparation external pocket with panda							ü								
-Sjav concept -Preparation of sjav sample (straight-curve)								ü							
- The concept of Refrain - The implementation of the sample due (online - separately) - Installation of buttons									ü						
- coller concept - coller forms - Implementation of coller samples										ü					
The concept of sleeve Sleeve forms Sleeve installation											ü				
- The concept of bracelets - Types of Bracelets - Installation of bracelets												ü			
- The implementation of the Women's Blouse													ü	ü	ü

Assess and evaluate the program: The program has been demonstrated on a group of specialized professors to make sure the integrity of

the scientific and technical aspects. The specialized professors agreed on the validity of the whole program after making minor amendments which, have been done.

To gauge the validity of the program has been prepared:

- Cognitive achievement test, which included a series of questions about setting Pattern and enforcement techniques. The questions are about thirty multiple choice questions, which were patch according to the key and select the correct question for each one degree only.

- Skill test, the test contains two questions one of each for preparing blouse trousers Patern and other techniques for the implementation of the Women's blouse. The test has been corrected according to the scale skills assessment of each part of the skill test.

- Measure of appreciation, appreciation of the scale contains over twenty points of model, and twenty points, especially techniques used to implement the blouse.

The correction was done by three specialists, by mark in front of each point (exact, exact to some extent, is not set) by (two degrees –one degree - zero), respectively.

Validity and reliability of the achievement test of knowledge:

1 - Honesty:
Logical validity:

• The achievement test has been demonstrated to a jury of specialized professors on a purpose of ascertaining the extent of ease and clarity of testing, and a link questions to the test goals. The arbitrators were unanimous on the validity of the achievement test with the application to make some proposals; the modification has been done based on the following proposals:

• Reduce the number of questions.

• taking into account the ease and clarity of the wording.

2 - Stability:

Stability is intended to be coordinated with the test gives the results, were calculated reliability coefficient achievement test in the following ways:

A - Using the retail mid-term stability:

The stability of achievement knowledge test is confirmed using the retail mid-term, and the values of the correlation coefficient were 0.715 - 0.834 on the portion of fee model while, 0.757 - 0.861 techniques for implementation of the values of a function at a level of 0.01 approaching to one, which

indicates the persistence of the achievement test of knowledge.

b - Constant alpha coefficient:

The alpha coefficient is found equal to 0.808 for part of Pattern drawing, and 0.847 for the implementation techniques. Theses are high values and this is proof the stability of the achievement test at the 0.01 level for approaching the values from the correct one, and the following table shows the values of stability

Table (1) test the stability of cognitive

stability of cognitive test	Mid-term retail		Alpha coefficient	
Graphic form	– 0.715 0.834	0.01	0.808	0.01
Enforcement techniques	– 0.757 0.861	0.01	0.847	0.01

Validity and reliability of the applied skills test:
1 - Honesty:

Logical validity: the test is displayed on a group of professors which, recognized the validity of all of the application.

2 - Stability:
Stability graders:

The reliability coefficient correctors can be obtained by calculates the correlation coefficient between the scores given by the two Correctors or more of the same individuals or for the same tests. In other words, each examined, gets two degrees or more of the correct one test, the applied stability test was calculated by evaluating the Pattern of the individuals trainees.

The correction was done by three professors of arbitrators, using a scale appreciation in the evaluation process and the corrector has done the each evaluation process alone.

The correlation coefficient was calculated between the three grades established by the correctors (x, y, z) of the applied test, using the dimensional correlation coefficient level for each model separately and the following table illustrates this:

Table (2) the correlation coefficient between the correctors of the parts of the skill test

correctors	Graphic form	Enforcement techniques	Skill test as a whole
x , y	0.881	0.829	0.798
x, z	0.743	0.736	0.928

| y, z | 0.909 | 0.776 | 0.801 |

Is clear from the preceding table, the high values of correlation coefficients between the correctors, which range between (0.736 - 0.928), the values of a function at a level of 0.01 approaching to one, which indicates the stability of the practical test, which measures the performance skills, and also

demonstrates the stability of the scale of assessment which is a tool Patch of testing skills.

3. Results and Discussion

First hypothesis: the first hypothesis is as follows:

"There are significant differences between the average scores of trainees program before and after training for the benefit of the post".

To investigate this hypothesis "T" test is applied, the following table illustrates this:

Table (3) significant differences between the mean scores of the trainees program before and after training

Total "Knowledge - Skill"	Mean "m"	Standard Deviation "sd"	Number of respondents "n"	Degree of freedom "df"	T value	Significance level and direction
before	7.371	1.966	30	29	29.625	0.01 for dimensional
after	54.771	8.482				

As seen from the table (3), the "T" value is equal to "29.625" which is statistically significant at 0.01 levels, where the average scores of trainees in the post "54.771" while the average scores of trainees in the application of before "7.371", which indicates to the existence of real differences between the two applications for the post. This means that the training program in this study is successful in achieving the goal of it and already knew the lines contained in respect of knowledge and skills.

To find out the size of the impact of the training program ETA equation was implemented: (T) value = 29.625, and degrees of freedom (df) = 29

$$n^2 = \frac{t^2}{t^2 + df} = 0.968$$

Calculating the effect size, it was found to be $n^2 = 0.968$

$$d = \frac{2\ n^2}{1 - n^2} = 11.01$$

The impact magnitude is determined as follows:

0.2 = small effect size

0.5 = medium effect size

0.8 = large effect size

This means that the size of the impact of the training program is significantly large, and thus achieved the first hypothesis. This finding corresponds with studies carried out in various areas, which addressed the effectiveness of training as emphasizes by "Abdel Fattah, 1997" The training program aims to improve the skills and enrich the individual information.

The second hypothesis: the second hypothesis is as follows:

"There are significant differences between the average scores of trainees in the knowledge gained on forging the model before and after training for the post test."

To verify the validity of this hypothesis, "T" test has been applied and the following table illustrates this:

Table (4) significant differences between the mean scores of the trainees in the knowledge gained special with drawing the Pattern before and after training

Graphic form	Mean "m"	Standard Deviation "sd"	Number of respondents "n"	Degree of freedom "df"	T value	Significance level and direction
before	1.817	1.117	30	29	40.251	0.01 for dimensional
after	13.808	1.049				

As seen from the table (4) that the "T" value is equal to "40.251", a value statistically significant at 0.01 level for the post test, where the average scores of trainees in the post "13.808", while the average scores of trainees in the application of before is

"1.817", which shows the benefit of trainees from training and knowledge of drawing Pattern, which contains the training program are clear, and thus achieved the second hypothesis.

The third hypothesis: is as follows:

"There are significant differences between the average scores of trainees in the knowledge gained with special implementation techniques before and after training for the post test."

To verify the validity of this hypothesis "T" test has been applied and the following table illustrates this:

Table (5) significant differences between the mean scores of the trainees in the knowledge gained special Implementation techniques before and after training

Enforcement techniques	Mean "m"	Standard Deviation "sd"	Number of respondents "n"	Degree of freedom "df"	T value	Significance level and direction
before	2.342	1.160	30	29	38.750	0.01 for dimensional
after	14.409	0.787				

As seen from the table (5) that the "T" value is equal to "38.750", a value statistically significant at 0.01 levels for the post test, where the average scores of trainees in the post "14.409" while the average scores of trainees in the application before is "2.342", which shows the benefit of trainees from training and knowledge of techniques and implementation contained in the training program are clear, and thus achieved the third hypothesis.

Hypothesis IV: the fourth hypothesis states as follows:

"There are significant differences between the average scores of trainees in the gained knowledge before and after training for the post test" and to verify the validity of this hypothesis "T" test has been applied and the following table illustrates this:

Table (6) significant differences between the mean scores of the trainees in the knowledge acquired before and after training

Total cognitive test	Mean "m"	Standard Deviation "sd"	Number of respondents "n"	Degree of freedom "df"	T value	Significance level and direction
before	4.159	1.163	30	29	20.724	0.01 for dimensional
after	28.217	6.229				

As seen from the table (6) that the value of "T" is equal to "20.724", a value statistically significant at 0.01 level for the post test, where the average scores of trainees in the post "28.217", while the average scores of trainees in the application before "4.159 ", which shows the benefit of trainees from training and knowledge contained in the training program are clear. Thereby achieving a hypothesis IV, are consistent result with all the studies that addressed the impact of training to increase knowledge and information of individuals. As emphasizes by " Ali, 1992" that training   an important step in the development plan to correct, amend and develop the overall knowledge and information about the individual and methods of work and requirements.

Hypothesis V: the fifth hypothesis states as follows:

"There are significant differences between the average scores of trainees in the skills acquired on forging the Pattern before and after training for the post test".

To verify the validity of this hypothesis "T" test has been applied, and the following table illustrates this:

Table (7) significant differences between the mean scores of the trainees in the acquired skills on draw a sample before and after training

Graphic form	Mean "m"	Standard Deviation "sd"	Number of respondents "n"	Degree of freedom "df"	T value	Significance level and direction
before	1.300	1.004	30	29	30.150	0.01 for dimensional
after	13.537	2.134				

As seen from the table (7) that the value of "T" is equal to "30.150", a value statistically significant at 0.01 level for the post test, where the average scores of trainees in the post "13.537", while the average scores of trainees in the application before is "1.300", which shows the benefit of trainees from the special skills of the pattern and included in the training program, thereby achieving the fifth hypothesis.

Terminals VI: the sixth hypothesis states as follows:
"There are significant differences between the average scores of trainees in the skills gained in the implementation of techniques before and after training for the post test".

To verify the validity of this hypothesis "T" test has been applied and the following table illustrates this:

Table (8) significant differences between the mean scores of the trainees in the acquired skills of the implementation techniques before and after training

Enforcement techniques	Mean "m"	Standard Deviation "sd"	Number of respondents "n"	Degree of freedom "df"	T value	Significance level and direction
before	1.912	0.880	30	29	35.211	0.01 for dimensional
after	13.017	1.262				

As seen from the table (8) that the value of "T" is equal to "35.211", a value statistically significant at 0.01 level for the post test, where the average scores of trainees in the post "13.017", while the average scores of trainees in the application before "1.912", which shows the benefit of trainees of the skills for implementation and included in the training program, thereby achieving the sixth hypothesis.

Terminals VII: the seventh hypothesis states as follows:
"There are significant differences between the average scores of trainees in the acquired skills before and after training for the post test"

To verify the validity of this hypothesis "T" test has been applied and the following table illustrates this:

Table (9) Significance of the differences between the mean scores of the trainees in the acquired skills before and after training

Total skills test	Mean "m"	Standard Deviation "sd"	Number of respondents "n"	Degree of freedom "df"	T value	Significance level and direction
before	3.212	1.531	30	29	34.015	0.01 for dimensional
after	26.554	3.530				

As seen from the table (9) that the value of "T" is equal to "34.015", a value statistically significant at 0.01 level for the post test, where the average scores of trainees in the post "26.554", while the average scores of trainees in the application before "3.212", which shows the benefit of trainees of the skills included in the training program. Thereby achieving the seventh hypothesis, this result agrees with studies that have been used in the training of individuals raising the efficiency of the process. As emphasizes by "Ahmed, 1990" that training aimed at development, technical and behavioral skills which are necessary for individuals to enable them to achieve themselves.

From the abovementioned, the training raises the efficiency of personnel, knowledge and leads to increased production and reduces spoilage and costs.

Research Recommendations:
From the results it is recommended that:

1 - The process of training should be at intervals not to exceed five years to cope with the rapid developments in the garment industry.
2 - Building training programs for various products to raise the efficiency of the trainees.
3 - Use of computers for preparing special programs for training planning and follow-up within the garment factories.

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Implication of private extension in developing countries

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Abstract: The evolution of public agricultural extension arrived at a worldwide turning point in the 1980s, one that represented the end of a major phase in the growth of publicly funded extension in both the developed and developing world. Agricultural extension increasingly has become defined as one or other of (apparently) differentiated activities of technology transfer or rural development. In many situations, the transfer of technology, heretofore considered the purview of public sector systems, has been reconceived. Such changes suggest a refocussing of paradigms for the delivery of public sector extension. In developed industrialized countries, which often provide models for extension service delivery elsewhere, the declining relative importance of agriculture for economic growth, the increasing education and affluence of smaller populations of rural producers, and the increasing use of externally purchased inputs have changed the nature of publicly funded extension services and led to a questioning of the means of delivery of extension services by governments . In developing countries, where publicly funded extension is often more important, there has been considerable questioning of the structure and forms of extension delivery.

Keywords: private extension, developing countries,

Introduction

Agricultural extension in many countries has come to encompass a wide range of activities in both the public and private sectors, yet the exchange of information continues to be the primary focus of all extension activities. The traditional concept of public agricultural extension involves a professional body of agricultural experts (generally government employees) who teach improved methods of farming, demonstrate innovations, and organise farmer meetings and field days on a wide range of topics. Public extension is sometimes used as a channel to introduce – and sometimes enforce – agricultural policies. Extension also functions informally as farmers transfer their best practices to each other. In addition, extension activities are carried out by a wide range of organisations in the private business and non-profit sectors.

Extension services may be loosely defined as including all activities involved in the exchange of information relevant to agricultural and livestock production, processing and marketing. The word "extension" has been criticized as inherently emphasizing the "top-down" dissemination of information while ignoring other types of information flow between farmers, extension and research – particularly activities that involve farmers as equal partners in the process. This paper uses the term "extension" while recognizing that extension functions are multi-faceted and go beyond "top-

down" dissemination of new technologies. For example, the information flow through extension channels may include anything from advice from a consultant on refrigerated flower shipping to the feedback to researchers of results from farmer-managed varietal trials.

Private firms provide services in accordance with their specialized incentives and farmers respond in terms of what they see as most beneficial to them. As each type of extension (public and private) has limitations, the objective for farmers, and agricultural development organisations of all types (local and international) is to attain the best mixture of public, private and NGO services. As Roth (1987) asserts, the public sector in general is over-burdened by numerous activities and moving some of them to the private sector might allow more effective implementation of essential services. While extension services cannot, and should not, be totally privatized, there is room for both some privatization of public extension activities and active promotion of private and NGO extension activities which complement rather than replace existing public extension services. The analysis in this paper draws on cases in which information exchange, feedback to research, and all other major extension functions form only one part of a larger agribusiness operation or agricultural project. This paper focuses on Implication of private extension in developing countries .

1- Privatization:

The debate on the role of the public sector is not limited to the context of agricultural extension, but encompasses the larger concerns of public policy and institutional and organizational development. Indeed, the degree of government versus private involvement in an economy is an enduring philosophically and politically vexing question. The move toward privatization and efforts to decentralize government functions relate to this theme.

There are two themes in the broader privatization debate: first, a "political economy" consideration of the role and size of government in an economy, which focusses on whether or not there is a failure of private markets; and, secondly, an expressed need to reduce government outlays. While many reassessments of publicly funded extension have reflected the second theme, it is worth considering the rationale for public versus private activity in an economy. In mixed economies, the prevailing economic justification for government involvement in an activity such as agricultural extension is market failure, whereby the market mechanism alone cannot perform all economic functions for appropriate resource allocation. Market failure may arise because some goods or services are public goods (such as publicly funded agricultural research knowledge) which can be consumed in a nonrival fashion by all members of society without any individual's consumption reducing the amount available for other individuals. Because the benefit of providing such goods cannot be appropriated by individuals, individuals generally will not provide such goods in a society even though there may be significant gains for producers and consumers. Some extension activities are clearly concerned with public goods subject to market failure. Other activities (such as individually tailored advice) confer appropriable private benefits which could be adequately supplied by private markets. Private goods sometimes are subject to market failure, whereby the operation of private markets does not provide certain services at a socially optimal level or where external costs or benefits are accrued by others rather than the provider of the goods. Market failure also may arise when current generations place insufficient value on preservation of resources for future generations. These latter circumstances are particularly characteristic of land and water degradation (Cary, 1983). Publicly funded conservation extension is often directed to overcoming such market failures (Barr & Cary, 1992). Government support for the provision of extension services may reflect that such services would be inadequately provided without intervention or, for reasons of equity, because

services would not be available to the extent thought socially desirable. Some situations for agricultural extension clearly reflect private goods; other situations clearly are characterized as public goods. There is a lot of fuzzy ground in the middle where it is not particularly clear that an extension activity is conferring a public or private good. In such situations, the extent of publicly funded extension is likely to be determined by the political influence brought to bear by relevant interest groups (Cary, 1993).

The philosophical thrust of the general privatization debate has centred, on the one hand, on whether certain government activities could be performed more efficiently by private agencies. operating in private markets and, on the other hand, on whether inequities may arise because not all individuals have access to resources to purchase privately supplied services.

1-1- Implications of extension "privatization"

In general, a more commercialized approach broadens the focus of extension personnel and makes an extension service more responsive to client needs and changing economic and social conditions. But other immediate implications of privatization appear to include
(1) the tendency toward a reduction of linkages both among organizations and among farmers in the exchange of agricultural and other relevant information;
(2) the tendency to enhance large-scale farm enterprise to the detriment of small-scale farming;
(3) the diminishing emphasis on public-good information and the advancement of knowledge as a saleable commodity; and
(4) the trend toward agricultural development services that cater primarily to large-scale farming.
The Netherlands' experience in moving to a partially privatized system highlights some of the implications for agricultural extension, particularly in developed countries. The Netherlands' approach reduced government outlays as well as the government agency role conflict between concern for farmers' interests and the implementation of increasingly stringent environmental policies. With farmers paying for an increasing share of the extension services, their representatives have more influence on the direction of the extension service. New organizational structures and linkages have had to be established to link the "privatized" and private extension services with the research institutes, experiment stations, and regional experiment farms. Consequent upon, or in parallel with, the changed Dutch arrangements, other changes have taken place in the Netherlands' extension system. There is some

evidence, at least for the vegetable greenhouse sector, that the high level of cooperation among extension information organizations in both the public and private sectors no longer exists (Huang, 1992). The more commercial orientation of the system appears to be creating tensions between extension workers and their clients in a less "open" knowledge and information system, with farmers who used to share information during study-group meetings now being more reluctant to do so.

The New Zealand Ministry of Agriculture and Fisheries advisory service, now fully commercialized and receiving no direct government funding, if sold will be the first extension service fully privatized from government ownership. In 1994 the number of consultants employed in this agency was about half of the peak number of advisers employed in 1987. Some of these advisers will have retired or departed voluntarily; others have established private consulting businesses. The consequence of the changes in New Zealand has been an increase in fee-for-service consulting (the number of farm and horticultural consultants has approximately doubled), with the traditional "advisory" extension no longer existing on a large scale. While, in most cases, the changes seem to have been readily accepted, there remains concern over the effective transfer of scientific findings to agriculture (Walker, 1993). Wider structural changes have sharpened the focus and efficiency of research agencies and advisory consulting work. Traditional technology transfer extension is now largely confined to agricultural commodity boards. Agriculture New Zealand engages in some specific "public good" technology transfer projects on a contract basis to commodity research agencies and the national Foundation for Research, Science and Technology. There has been no formal assessment of the impact of the New Zealand changes. However, there does appear to be less interaction among organizations, reduced feedback from farmers to science providers, and more limited information distribution, particularly to less well-off and poorer performing farmers (Walker, 1993).

Those extension services that have adopted a commercialization or privatization strategy most vigorously have traditionally employed an advisory approach to extension delivery. The advice given is more likely to be a private good. As well, the extension advisers are more likely to be able to adapt to providing services commercially. However, some staff will not make such a transition easily, new commercial skills will be required by newly commercialized advisers, and the dynamics of any change will have to be planned carefully. Le Gouis (1991) has noted that government "commercial" fees should be set at the market rate so as not to compete unfairly with existing private consultants.

1-2- Institutional Implications

The new developments highlight greater institutional pluralism. Extension, interpreted broadly, now is often a mixed system or a "complex" where services are provided by private and public sector entities. The larger context in which a mix of public and private services operates presents a new challenge with new potential roles and responsibilities for the public sector. A major premise of this chapter is that policy makers must consider the entire agricultural extension complex when planning to allocate funds or seeking alternative funding arrangements for the public sector.

By taking into account the complex of extension services, their purposes, and audiences, governments can better reconsider the public sector role. In particular, to what extent should the public sector:

1. Attend targeted audiences unserved by the private sector?
2. Coordinate multiple extension providers?
3. Serve as the final reference or arbitrator of conflicting information?
4. Assure accountability of both public and private extension services to the public?
5. Facilitate the operation of the complex through regulation and information provision?

Considering public sector extension as only one or one set among many providers of extension services facilitates analysis and informed decisions on how best to provide extension within the complex of diffused responsibility.

2- Strategies for change

Public sector extension, facing criticism for its cost and its lack of efficiency and for not pursuing programmes that foster equity, is confronted with a number of possibilities for change. There has been a trend, perceptible throughout various extension systems undergoing adjustment, of greater flexibility and multiple partners in funding agricultural advisory services (OECD, 1989). Le Gouis observed three major policies adopted by government and farm organizations regarding privatization of extension:

1. Public financing by the taxpayer only for the kinds of services that are of direct concern to the general public
2. Direct charging for some individual services with direct return (in the form of improved income)
3. Mixed funding shared between public and private professional association contributions for some services where the benefits are shared. A pervading development in new forms of financial support for

extension is the trend to mixed sources of funding, reflecting strategies to gain access to additional sources of funding. In several developing countries, public-private extension coordination is already established. Alternative patterns indicate a fostering of private corporate initiative, encouraging cooperative ventures by farmers, coordinating public-private extension services, and privatizing the public system (Wilson, 1991).

The need for improved and expanded extension activities, together with a strengthening philosophical view of less government involvement in national economies, has led to a number of strategies for changing the way extension services are delivered.

2-1- Revitalization

The United States Cooperative Extension Service, when criticized for lack of relevance and vision (Dillman, 1986), regrouped and reviewed the criticisms. Its Extension Committee on Organization and Policy (ECOP) organized a Futures Task Force to review issues and put forward recommendations with a view to revitalizing the system (ECOP, 1987), which has led to various alterations structurally and programmatically.1 Meanwhile, the advancement ofelectronic information systems is resulting in increased privatization, with important implications for the future structure of U.S. agriculture (Goe & Kenney, 1988).

2-2- Commercialization

New Zealand's Ministry of Agriculture and Fisheries' (MAF) agricultural advisory service now operates under user-pay, commercial criteria (Hercus, 1991). The MAF advisory service, renamed MAF Consulting and, subsequently. Agriculture New Zealand, has remained (temporarily) a public agency, although its employees have given up a number of public employment benefits and now receive commissions for consulting work undertaken. The agency depends for its annual budget on consulting fees received from farmers and contractual arrangements with government for the supply of policy information and rural intelligence to government.

2-3- Cost Recovery

Other public extension systems have moved toward cost-recovery approaches. Mexico has developed a fee-based system among large-scale farmers in the northwest region and plans the development of a similar arrangement among small-scale farmers in the south central region (Wilson, 1991). The Agricultural Development and Advisory Service (ADAS) in England and Wales, notionally "commercialized," operates on a partial cost-recovery basis. Clients of ADAS pay a fee for advice which formerly was free of charge. This process of cost recovery, introduced in 1987, was directed towards the agency receiving 50 per cent of its income from commercial fees by 1993-94 (Bunney & Bawcutt, 1991; Harter, 1992).

2-4- Voucher Systems

Some countries have replaced public extension delivery systems with vouchers, distributed by government services, for farmers to use in hiring private extension consultants (as in Chile). Coupons attached to agricultural bank loans, committing a certain percentage of the loan for extension services, have been used in Colombia.

3- Reassessment of Public Extension

While "modern" extension has existed since the nineteenth century, agricultural extension is quite young worldwide as a formal institution, with the majority of countries initiating such services since the 1950s and 1960s. Even in high-income countries where extension began at earlier dates, fiscal commitment took significant upswings following World War II when a backlog of science and technology had accumulated. In an FAO survey of 207 agricultural extension organizations in 115 countries (Swanson, Farner, & Bahal, 1990), 50 per cent of these organizations had been established or were reorganized in the previous two decades. Against this background, governments in recent times have found that they are less able to continue providing all the services previously provided. With costs rising, limited resources available, and changes in the prevailing philosophy of the appropriate extent of government intervention, governments have been slow to increase appropriations for many publicly funded activities. Some functions of government have been curtailed, and others have been privatized. Such changes have been particularly significant in the formerly centrally managed economies. Because extension worldwide has large numbers of staff, the recurrent costs of extension are of significant magnitude.

Swanson et al. (1990) reported that there were approximately 600,000 agricultural extension personnel worldwide, with 95 per cent of these working in public agricultural extension systems. In the United States, there are about 9,000 extension agents, 4,000 subject-matter specialists, and 1,000 directors and administrative support personnel (USDA, 1993 data). While the unit cost of extension staff in many countries is low, large staff sizes translate into large government outlays. As a result of financial concerns, many countries have examined

alternative structural arrangements, including the feasibility of reducing public sector extension expenditures (with associated staff reductions), changes in tax raising, charges for government extension services, and commercialization and privatization (Howell, 1985). A number of countries have moved towards reducing, recovering, or shifting the burden of the costs associated with provision of public sector agricultural extension, particularly transferring "private good" functions to private industry. Concerns about the costs of extension need to be judged against the economic and social returns associated with successful extension. While more research is needed on measuring the economic payoff from investment in public sector extension services, available research tends to indicate, in contrast to some current criticisms, that extension in many instances provides high rates of return and is, therefore, a profitable public investment (plus Evenson, 1987; Birkhauser, Evenson, & Feder, 1988). In addition, not all extension expenditure can be measured by benefits from technology transfer; the benefits of extension concerned with human development are difficult to quantify in the short term.

4- Gradual "Privatization"

In 1990 The Netherlands "privatized" approximately one-half of its public extension service by transferring field extension personnel, with initial government financial support, to the farmer associations. The elements of the extension service responsible for linking research and the privatized extension services, policy preparation, implementation, and promotion and regulatory tasks remained under the aegis of the Ministry of Agriculture (Le Gouis, 1991). The "privatized" extension service is governed by a board on which farmers' organizations and the government are equally represented (Proost & Röling, 1991). Dutch farmers make a partial contribution to the cost of the new organization through membership subscriptions to farmer associations, as well as through direct payment for individual analyses. Farmers will eventually contribute 50 per cent of the cost of the service: special services such as individual analyses will be fully paid for by the farmer clients. The Dutch government has established new government-funded structures for integrating subjectmatter specialists into extension teams to facilitate the transfer of information and knowledge and for the provision of information on government policy (Bos, Proost, & Kuiper, 1991; Proost & Röling, 1991).

A gentler form of "privatization" has been proposed for the delivery of government extension services in the Australian state of Victoria. A review

of extension services determined that, for government-provided services conferring essentially private benefits to individuals, rather than cost recovery by government fee charging, it is more desirable and more efficient that private advisers deliver such services. However, because of the complexities of extension service delivery and the varying nature and levels of development of different agricultural sectors, a number of constraints were identified which precluded universal application of such a principle (Cary, 1993).

In order for rural industry organizations to take a greater responsibility for technology transfer, the Victorian government has proposed "outsourcing" for delivery of future extension programs. Outsourcing means that the government extension agency will retain a core pool of extension project staff and "buy in" private sector professional services with skills that the agency considers unnecessary to maintain. Agricultural consultants and contract staff will be employed to help deliver services in specific projects funded by rural industry and the federal government. Such projects are likely to be broad and industry wide and not tailored to individual farm circumstances.

In most cases, governments have not actually "privatized" their agricultural extension services. In its pure sense, privatization implies a full transfer of ownership (usually by way of sale) from government to a private entity, with that entity meeting all costs and receiving any profits. In the case of extension, governments have followed a number of distinct pathways such as commercializing the service while retaining it as a public agency, shifting public sector delivery services to private sector delivery of the service while maintaining oversight and basic funding of delivery, or pursuing cost-recovery measures to pay for the service. Thus the phrase "privatization of agricultural extension" generally is misleading.

Other Arrangements

Some countries have never developed public sector agricultural extension services, leaving the function of agricultural extension to private sector commodity enterprises or industry agencies, albeit often with some government financial subsidy. In France, while chambers of agriculture and private sector companies provide extension services, the former are substantially supported financially by public funds. In New Zealand, extension services to the dairy industry for many years have been delivered by the Dairy Board consulting service, financed by the dairy industry. In other cases, nongovernmental organizations have been used to supplement public

sector extension services, especially in the area of rural development (Amanor & Farrington, 1991).

This arrangement has certain advantages for increasing extension coverage and encouraging farmer participation in technology systems, but it also has certain inherent limitations. In most countries, private sector companies are already important contributors to technology transfer and the advancement of agricultural development through, mainly, contract arrangements with farmers. Rightfully, the private sector has come to be acknowledged as a major information provider to both large and small farmers involved in monocropping (Cary & Vilkinson, 1992). The characteristic of "privatized" extension systems is a focus on commercial farms. It is salutary to state the obvious in relation to decisions regarding private and public provision of extension: when extension is delivered privately, it represents a commercial decision; when extension is delivered publicly, it is a political or bureaucratic decision. In determining whether to privatize, it is important, in the first instance, to establish whether an extension programme is designed to help commercial enterprises or small-scale farming and rural development.

Conclusion:

Privatization may have some attendant disadvantages because of unequal access to resources and because of a diversity of "agencies" and the associated difficulty of coordinating external groups and other government departments. Private delivery agents will be less responsive to government policy direction, and there may be linkage problems with public applied research organizations. While the process of information transfer amongst farmers traditionally has been characterized by a cooperative, free exchange of information, industrial information traditionally has been a private good characterized by patent rights, process licensing, the use of paid consultants, and differentiated production and marketing processes. In developed economies with commercialized agriculture sectors, many of these features of industrial information transfer are becoming more common in agriculture. The trend to privatization will be stronger the more such circumstances exist. The range of different circumstances prevailing in agricultural extension worldwide suggests that a wide variety of approaches should prevail.

The rationale for private sector provision of agricultural extension services is generally based on an expectation of increased efficiency with the operation of private markets and with the resulting efficiencies contributing to the growth of a country's GNP. In contrast, the rationale for public provision of agricultural extension services is based on the following points: (1) much agricultural information is a public good; (2) only government extension services are likely to promote concern for natural resources management; (3) public sector extension may enhance the education of farmers who often lack adequate access to educational institutions; (4) the public service often provides information that reduces risk to farmers; (5) the service may provide information that reduces transaction costs; and (6) an extension service may be concerned with community health issues related to possible human hazards such as accidents and poisonings linked to agricultural chemicals. The argument for privatization is based upon:

- More efficient delivery of services
- Lowered government expenditures
- Higher quality of services

The diverse financial arrangements adopted in the last two decades by governments worldwide to fund agricultural extension services provide a valuable menu of options for consideration by other countries confronting the "privatizing" of public sector services. Still, several countries have resisted the trend toward privatization of agricultural extension, concerned perhaps by the implications reviewed in this chapter. In both developed and developing countries, renewed debate and experimentation around extension is certainly needed, but not only around allocation decisions and how best to develop cooperative arrangements with the private sector.

In most countries, government-funded extension is likely to focus its activities more selectively on public-good activities which exist and on areas where the marketplace is unlikely to provide services at a socially optimal level. Such areas will include "broad" rather than "specific" technology transfer, dissemination of environmental and resource technology, and human resource development. The move in the public sector toward privatization and efforts to decentralize government functions can serve to highlight the continuing and key role of the public sector and focus the operative question on its responsibility as a coordinating agent. Its roles of regulation and providing service for priority audiences unserved by the private sector will be undiminished. Some suggestions are as follows:

- Governments in developing countries should take a quick and serious step to take on maximum number of agricultural extension professional in the Agriculture Department (extension wing) and also designed a proper policy for it.

- Governments in developing countries should impart extensive in-service agricultural trainings to train the extension personnel to cope with the growing needs of rural people.
- Governments in developing countries should impart trainings and refresher courses to train the extension field staff (EFS) about the philosophy and methodology of Decentralization of agricultural extension reforms.
- Effective and efficient evaluation mechanism should be launched to monitor and evaluate the activities of EFS and also their performance.
- Service structure for agriculture extension department should be revised like other departments so that young, talented and energetic staff can join the department.

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Tourism Development Strategies for Meymand Village of Kerman, Iran (By SWOT model)

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Abstract: Nowadays tourism is considered as an important basis for sustainable development. Therefore, rural tourism is regarded as a makeable and essential element of tourism. Consequently, it is evident that strategic planning for rural tourism and the identification of internal factors (weak points and strong points) and environmental factors (opportunities and threats) have an important role in the development of rural regions and also the tourism industry. In this article, by means of the survey method and field study, we have tried to design strategies to develop rural tourism in Meymand village of Shahr Babak, Kerman Province, Iran, through specifying weak points and strong points, opportunities and threats in the form of a SWOT table. The results delineated that the strategy of "*extensive utilization of native methods to repair, construct and renovate the internal architecture of Meymand village*" is the most important and premier strategy among all rural tourism development strategies.

INTRODUCTION:

Rural tourism

Nowadays tourism is one of the bases of economic development and most countries earn a remarkable income through it annually. The concept of rural tourism can revitalize the conventional concepts and views on tourism, and bring in a new dimension in sustainable development (Aref et al., 2009). Therefore, rural tourism and related issues such as poverty, occupation, hygiene, and food security are among the noticeable problems that strategic planning can address.

According to the World Tourism Organization (WTO), rural tourism can include a wide range of activities like: climbing, riding, adventure tourism, educational travel, sport and health tourism, arts and heritage tourism (Negrusa et al., 2007). Negrusa et al. (2007) defined rural tourism as that form of tourism offered by people from rural areas, with an accommodation on small-scale and with the implication of important components of their rural activities and customs. Tourism appears to be

developing an elitist bias as participation from all sections of society is clearly not visible.

According to the Organization of Economic Co-Operation and Development (OECD), rural tourism is defined as tourism taking place in the countryside (Reichel., 2000). Rural tourism is located in agricultural landscapes and is characterized by enjoyment of a tamed nature or highly modified landscape. It is about the land uses and human cultures that the interactions between humans and land have created. It positions agriculture and farms as the foundation upon which the attraction is built (Knowd, 2001).

Any form of Tourism that showcases the rural life, art, culture and heritage at rural locations, thereby benefiting the local community economically and socially as well as enabling interaction between tourists and locals for a more enriching tourism experience can be termed as rural tourism. Rural tourism is essentially an activity that takes place in the countryside (Aref & Sarjit, 2009).

According to the discussions for and against development, rural tourism is increasingly considered

as a panacea, economic power increaser, increaser of liveability in remote areas, stimulator of reliving and also improver of life conditions in rural societies (Brieden & Wicken, 2004). This issue is in relation to agricultural policies in many countries and often it is a tool to support the environment and rural culture. Thus, it can play a vital and fundamental role in the development and maintenance of villages (Sharifzadeh & Moradnejad, 2002).

Regarding what was mentioned above, in order to improve the development process in rural areas, especially the one studied and mentioned above, it is increasingly necessary to utilize suitable strategies and guidelines for rural tourism development considering environmental characteristics and conditions, tourism sites and the ecological (natural and human) products of each area.

In this research, regarding tourism the potential and limitations of Meymand historical village, and also considering environmental threats and opportunities of the area, strategic planning was started and the related strategies presented to remove the problems and optimize the usage of capacities.

In addition to answering these questions "*what is the potential for rural tourism development and its limitations? And what are the strategies and guidelines to develop tourism which leads to rural and national development?*" weak points, strong points, opportunities and threats from the viewpoint of participants and beneficiaries related to rural tourism development are analysed and studied. In addition, regarding the above opinions and attitudes, suitable strategies and guidelines are presented by means of the SWOT method in order to utilize strong points and opportunities, and remove weak points and threats optimally and purposefully to achieve tourism and rural development.

Generally, rural tourism is important in two aspects: one as a vast worldwide activity and the other is its effect on the development of regional and local policies. Thus, it is logical to admit that there is not any acceptable and general definition of rural tourism (Sharply & Sharply, 1997). Moreover, although it should be admitted that different characteristics such as special activities and situations separate rural tourism from other tourism parts or types, it is noticeable that there is not any public-acceptable definition or

characteristics of rural tourism (Sharply & Sharply, 1997). While at first glance, it seems easy to define rural tourism, as stated by Lane: the tourism that happens in suburbs is called rural tourism; however, such a simple definition contains many ambiguities. For example, apart from the different definitions of tourism and especially in a suburban framework in which the difference between activities that are forms of tourism, and spending leisure time, exercising and recreation, might be blurred; notwithstanding the fact that the definition of a suburb may vary. Therefore, it seems simple conceptually to define rural tourism as a trip to the suburbs; however, this definition cannot include the sets of activities, various managerial forms and developed institutions in different countries, which act in relation to the tourism industry. From the more comprehensive concept point of view, rural tourism can be considered as a range of activities and services related to recreation and the calmness of the tourists, which are organised by farmers and villagers to attract tourists to their areas for income. If the above mentioned is accepted, it includes rural, farming and agricultural tourism, generally, and, as a result, it will include services to tourists, such as residences, lodgings, recreation and entertainment equipment, holding local festivals, production and supplying of handicrafts and agricultural products (Sharifzadeh & Moradnejad, 2002). Therefore, regarding the mentioned definitions of tourism and rural tourism, rural tourism can be defined as follows (Eftekhari, 1994):

Rural tourism can be defined as all activities and services done by farmers, people, and governments for tourists' recreation, rest and attraction, and also activities undertaken by tourists in rural areas, which can include agricultural, farm, natural and cultural tourism.

As mentioned and based on the above definitions, rural tourism does not include agricultural tourism alone, but also all activities undertaken by tourists in rural areas. Therefore, it can be said that tourists of villages visit rural areas with different motives such as ecological uniqueness, special adventuring opportunities, visiting qualitative cultural attractions and rural areas (Brieden & Wicken, 2004). Therefore, regarding tourists' purposes and motives for travelling, rural tourism can be divided into five groups, as shown in Table 1.

Table 1 – types of rural tourism (Mehrjerdi, 2004)

Mostly interacts with ecological attractions	Natural Tourism
Related to culture, history, cultural and ancient heritage of villagers	Cultural Tourism
A type of tourism in addition to interacting with natural attractions (river, mountains, …) is related to people life and their social norms, which in itself is in interaction with the above mentioned natural attractions	Native Tourism
In this type, tourists live with rural families and participate in the economical and social activities of the village	Rural Tourism
Without causing any negative results in the ecosystem of the host area, tourists participate in or interact with traditional agricultural activities	Agricultural Tourism

Also, according to Jenny Holland et al. (2003), tourism can include farm tourism or agricultural tourism, however, both of them are popular among tourists in rural areas (Holland., 2003).

There are different viewpoints about rural tourism development. Some accept it as a part of the tourism market and believe it can be compared with other forms of tourism like tourism under sunshine, tourism on the beach, and tourism on sandy beaches (Sharply & Sharply, 1997). According to other aspects, rural tourism was introduced as a philosophy of rural development, which has three important attitudes:

One opinion is that tourism is a philosophy for rural development, which is a reflection of the rural environment.

According to others, tourism is a tool and policy for the reconstruction of the rural economy (Sharifzadeh & Moradnejad, 2002). Some also believe that tourism is a policy and tool for sustainable rural development (Jenkins, 1998). It is noticeable rural tourism industry in Iran requires attention to the following aspects: satisfaction of citizens' needs, development of regional-local policies, and attraction of tourists and absorption of foreign investment (Shahabiyan, 2003). Geological, morphological, and climatic equipment, mineral waters, plant cover, etc. in studies and policymaking of rural economic development are among the most important factors in the tourism industry (Ghareh, 1999), even though supervising them is difficult. Also, villages are a multi-purpose resource and tourism is just one of the village requirements, and, sometimes, tourism can even be considered a threat to the village.

Rural tourism in Iran

The rural cooperatives in Iran in recent years have diversified themselves into various areas of socio-economic activities. The failure of the government sector and various limitations of the private sector have compelled the policymakers to pin their faith on the cooperative system. For certain activities/areas, success is based on the ability of the grassroots institutions to tackle them with their participatory and people-based approach, the cooperatives are considered to have an advantage over other organizations. For example, in Iran, the rural cooperatives are considered the most effective organizations in the field of rural agriculture. Similarly, because of their vast network and reach, the rural cooperatives are considered to be the best promoters for agriculture in Iran. Rural tourism is often considered an economic alternative for rural areas facing decreasing profits and requiring a second or third economic footing (Verma, 2008). However, like other tourism activities, rural tourism has a full range of environmental impacts (Kuo, 2008). Rural tourism in Iran does not have a long history because of the inadequate foundations and preparation. Iran has a wonderful potential for tourism, particularly rural and ecotourism. The main problem and difficulty is in attracting tourists. Unspoilt nature, varied picturesque landscapes, a thousand-year-old cultural and architectural heritage, a profusion of leisure opportunities and reactions, closeness to the urban centres as well as the authentic character and rural charm, all these constitute the important factors for the development of rural tourism in Iran (Aref & Sarjit, 2009). However there are some other steps that should be taken, because Iran is not ready to welcome rural tourists yet – attraction (for example development of rural tourism around a heritage site), rural infrastructure accessibility (roads, transportation) and building rural capacity for tourism development.

Additional budget is required for rural areas. Rural tourism has some advantages in the rural areas of Iran, for example, it provides employment for local residents and prevents their immigration to the cities. Currently young people leave the countryside and go to cities to study or work. Usually they never come back

to their homelands. Some of the reasons for the failure of these efforts are as follows: The role of the rural cooperatives in this industry is not defined, socio-cultural and political barriers, and lack of human and economic resources.

Barriers to Rural Tourism

Understanding the barriers to rural tourism is important when a community is trying to organize itself for involvement in tourism activities. This understanding can help individuals, the community and organizations impact the tourism policymakers process more effectively.

Furthermore, it is important for the government to understand that rural areas face barriers that can hinder their progress in responding and recognizing the priorities of local communities in Iran.

Overcoming the barriers to tourism development presents a challenge to both communities and government, and will serve to facilitate the policymaking process. Various literatures address the barriers of tourism development through local communities, particularly in third world countries. Rural tourism in Iran has several barriers to development. Roads and accommodation infrastructure were cited as the two main barriers for growing rural tourism. In the long-term, developing accommodation, sealing the roads, and providing other services like cafes and shops are essential to fulfil the tourism potential of Iran, and attract a broader range of visitors to stay in the region overnight. In addition, the rural cooperatives in Iran still have to recognize the importance of tourism despite the rapid growth of the tourism sector around the world. The following are the main barriers:

1-Inability to analyse the changing socio-economic dimensions of rural tourism in Iran, and demarcate the areas in which rural cooperatives have a strategic advantage over other forms of organization.

2-Lack of policy research in this field, which can provide definite indicators for the future.

3-Inability to strategically link the rural cooperatives with the rural tourism in those cities in which tourism is booming. For example, in Esfahan and Shiraz, tourism has emerged as a major force. However, the rural cooperatives have not yet progressed to this level.

4-Inability of the cooperatives to externalise their areas of operation or activity to the field of rural tourism.

5-Weak advocacy for rural tourism development is also a big hindrance. Holding advocacy conferences by the cooperatives in the area of cooperative tourism can set the ball rolling in a big way and create a conducive atmosphere for rural tourism development (Verma, 2008).

Bushell & Engles (2007, p.154) also state that tourism, as a phenomenon of affluent contemporary societies, is a particularly difficult concept in local communities in developing countries to grasp. In this sense tourism development may be more difficult than other activities. Shortcomings are similar to those of local communities; however, a few factors tend to be more pronounced in local areas:

Lack of formal education and appropriate managerial training

Lack of foreign language skills

Different ways of dealing with hygiene, litter, maintenance of infrastructure

Limited knowledge of food preparation for foreigners, including catering for dietary, nutritional and culinary tastes

Lack of decision making and planning skills concerning the possible consequences of tourism, coupled with limited ability to control tourism, unpredictable political climates, and long-term funding uncertainty (Bushell & Engles, 2007, p.154).

Consequently, rural tourism facilities and services may be unacceptable for international tourists. Hence, building capacity through rural cooperatives is necessary for stakeholders involved in tourism in local communities (Bushell & Eagles, 2007).

However, due to a lack of awareness this is not being done at present. Similarly, lack of development of cooperatives in the field of cooperative tourism is also a sign of weak advocacy. There is also a lack of documentation of successful models of cooperative tourism in the region.

Research Method:

In this article both descriptive-analytic and survey methods were applied. Initially, attractions, equipment, tourism services, tourism situation and their effect on development of the studied society were surveyed and then a strategy and guideline for rural tourism development was introduced through a SWOT analysis.

First, the internal and external environment of the area was studied and a list of weak points, strong points, opportunities and threats were identified. Then by means of a questionnaire along with opinion polling among three levels: people, tourists and reporters, each one of these internal and external factors was weighed and ranked, and, finally, a table of internal factors analysis summary (IFAS) and table of external factors analysis summary (EFAS) were extracted to design a SWOT model and specify the strategies.

Weak Points (W)	Strong Points (S)	
WO Strategies	SO Strategies	Opportunities (O)
WT Strategies	ST Strategies	Threats (T)

Reference: David, 1999

The Sharp-Cochran method was used to specify the sample volume and then the questionnaires in the universe were filled out by means of random sampling.

Sharp-Cochran formula:

$$n = \frac{Nt^2pq}{Nd^2 + t^2pq}$$

N = the universe volume (100 families)   P = probability

n = required sample volume   q = No-characteristic probability

t = 1/96   d = sampling error (0.05)

Three groups form the universe regarding the above formula – 40 persons for head of household's (people), 100 for tourists and 20 for reporters. It is noticeable that the experts group includes reporters, technicians of cultural heritage organizations and tourism management teachers.

Findings:
In order to prepare the strategy in the SWOT model, first, the bases of the internal factors (weak and strong points) and also external factors (opportunities and threats) were identified and then the importance of each index and also rank of each was specified by testers. It should be noted that in this study the subjects were studied in four groups of economic, socio-cultural, ecological, institutional factors and with five degrees of intensity (very much, much, medium, low, very low), which is used throughout the entire research.

Analysis of internal factors effective for Meymand tourism development:
In this part and regarding the studies done concerning the Meymand area and factors effective for its tourism development, some indexes in the form of weak and strong points in four economic, socio-cultural, ecological and institutional dimensions were mentioned, and their importance (index) and rank were studied by sample volume.

Regarding the above table, the most important strong points of Meymand tourism are:
Rare tourism capacities and historical attractions through the world with an index of 0.07 and rank of 3.61, cultural heritage organization planning to register Meymand village internationally with an index

of 0.07 and rank of 3.60, residential houses in heart of rocks (stone engravings) with an index of 0.07 and rank of 3.52, mineral and sweet springs, castles and petroglyphs with an index of 0.07 and rank of 3.27.

Also other tourism strong points of Meymand village are as follows:
Handicrafts and active handicrafts workshops in village with an index of 0.05 and rank of 3.15, local and traditional culture and customs with an index of 0.04 and rank of 3.20, adjacent to populated and urban centres with an index of 0.05 and rank of 2.95, availability for tourists with an index of 0.04 and rank of 2.86, calm and still environment for citizens with an index of 0.03 and rank of 3.10.

Also, based on the above table, the most important tourism weak points of the Meymand village are as follows:
Lack of NGO investment in tourist parts of this area with an index of 0.07 and rank of 3.25, insufficient and inappropriate residential and welfare equipment with an index of 0.07 and rank of 3.27.

Moreover, other tourism weak points of Meymand village include:
Insufficient hygienic and service facilities with an index of 0.06 and rank of 3.12, inappropriate environmental and non-environmental infrastructure (road and sewage) with an index of 0.06 and rank of 3.35, inappropriate sport and recreation equipment and facilities with an index of 0.06 and rank of 3.34, lack of governmental investment in this area with an index of 0.05 and rank of 3.50, lack of skilled and trained persons in this area to guide and inform tourists with an

index of 0.05 and rank of 3.25, inappropriate distribution of tourists in different seasons of the year with an index of 0.05 and rank of 2.86.

Regarding the total score (3.34) it can be concluded that the reaction of Meymand tourism authorities was more than the average level.

Analysis of external factors effective for Meymand tourism development:

The purpose of this study is to identify environmental factors effective for the tourism development of Meymand village. Identification of all opportunities and threats effective for Meymand tourism, then study them in form of rural development dimensions (economic, socio-cultural, ecologically and institutionally). Index and rank each criterion presented based on the following table.

Regarding the EFAS table, the most important tourism development opportunities for Meymand village are:

Continuance of living in Meymand with an index of 0.067 and rank of 3.12, unique architecture index of 0.065 and rank of 3.90, increase in travel and tourism motive among people index of 0.060 and rank of 3.23.

Also other tourism development opportunities of Meymand village are:

Unique tourism attraction with no competitors in country index of 0.058 and rank of 3.65, government attention to tourism index of 0.030 and rank of 3.80, semi-migration style of living index of 0.050 and rank of 3.80, increase in country authorities' attention and support of rural development with job-creation approach and income acquirement index of 0.040 and rank of 3.95, mapping of Meymand oases index of 0.042 and rank of 3.15, specifying possession of the oases index of 0.035 and rank of 3.20, hygienic water, soil and climate resources of the village in comparison to other areas index of 0.035 and rank of 3.45.

Table 2 – Internal factors analysis summary effective for Meymand tourism development

Index + Rank (Score)	Rank	Index	Index	
0.16	3.15	0.05	- handicrafts and active handicraft workshops in village	Strong Points
0.13	3.20	0.04	- local and traditional culture and customs	
0.25	3.52	0.07	- residential houses in heart of rocks	
0.26	3.61	0.07	- rare tourism capacities and historical attractions over the world	
0.15	2.95	0.05	- adjacent to populated and urban centres	
0.11	2.86	0.04	- availability for tourists	
0.09	3.10	0.03	- calm and still environment for citizens	
0.23	3.27	0.07	- mineral and sweet springs, castles and petroglyphs	
0.25	3.6	0.07	- cultural heritage organization planning to register Meymand village internationally	
0.26	3.25	0.07	- lack of NGO investment in tourism parts of this area	Weak points
0.18	3.50	0.05	- lack of governmental investment in this area	
0.19	3.12	0.06	- insufficient hygienic and service facilities	
0.26	3.72	0.07	- insufficient and inappropriate residential and welfare equipment	
0.16	3.25	0.05	- lack of skilled and trained persons in this area to guide and help tourists	
			- inappropriate distribution of tourists in different seasons of the year	
0.14	2.86	0.05	- inappropriate environmental and non-environmental infrastructure (road & sewage system)	
0.20	3.35	0.06		
			- inappropriate sport and recreation equipment and facilities	
0.20	3.34	0.06	- lack of acquaintance and training among villagers in how to act/behave with tourists	
0.12	3.11	0.04		
3.34	--	1	Total	

Table 3 – External factors analysis summary effective on Meymand tourism development (EFAS)

Score (Index*Rank)	Rank	Index	Index		
0.114	3.80	0.030	1- government attention to tourism	Economic	opportunities
0.211	3.65	0.058	2- unique tourism attraction with no competitors in country		
0.193	3.23	0.060	3- increase in travel and tourism motive among people	Socio-cultural	
0.209	3.12	0.067	4- continuance of living in Meymand		
0.190	3.80	0.050	5- semi-migration style of living with three stages of migration		
0.253	3.90	0.065	6- unique architecture		
0.158	3.95	0.040	7- increase in country authorities' attention and support of rural development with job-creation approach and income acquirement	Institutional	
0.132	3.15	0.042	8- mapping of Meymand oases		
0.112	3.20	0.035	9- specification of the oases possession		
0.120	3.45	0.035	10- hygienic water, soil and climate resources of the village in comparison to other areas	Ecological	
0.146	3.12	0.047	1- weakness in NGO investment facilities	Economic	Threats
0.097	3.25	0.030	2- insufficient development credits		
0.092	3.30	0.028	3- selling non-native and factory products disparate to environmental values		
0.155	3.45	0.45	4- deficiency in informing about the village tourism attractions all over the province and country	Socio-cultural	
0.109	3.14	0.035	5- fading of the traditional and local culture (language, cloths, …) following tourists increase		
0.171	3.80	0.45	6- increase in social crimes following tourists entrance		
0.103	3.70	0.028	7- relative remoteness of Meymand from capital and big cities		
0.153	3.65	0.042	8- insufficient policies and goals of rural tourism		
0.170	3.34	0.051	9- migration of villages to city		
0.126	3.25	0.029	10- decrease in governmental services in the village due to migration and the village population decrease		
0.078	3.12	0.025	11- destruction of plants and plant covering	Ecological	
0.094	3.16	0.030	12- erosion of the ceiling in traditional houses due to dampness and traffic		
0.1	3.76	0.033	13- lack of development in tourism services, equipment and facilities	Institutional	
0.149	3.65	0.041	14- weak equipment for protection and maintenance of historical and religious places and buildings		
3.347	--	1	Total		

Moreover, the most important tourism development threats of Meymand village are:

Migration of villages to city index 0.051 and rank 3.34, weakness in NGO investment facilities index of 0.047 and rank of 3.12, deficiency in informing about the village tourism attractions all over the province and country index of 0.045 and rank of 3.45, increase in social crimes following tourists entrance index of 0.045 and rank of 3.80.

Regarding the total score (3.347) it can be concluded that the reaction of Meymand tourism and planning authorities to tourism environmental opportunities and threats of Meymand village was above the average level.

Designing tourism development strategies of Meymand village by SWOT model:
Considering the internal factors analysis table and identification of weak and strong points and also regarding the external factors analysis table and identification of environmental threats and opportunities effective on Meymand tourism, the tourism development strategies of Meymand village can be introduced in the form of a SWOT table and the following four strategies:

A) Aggressive strategies (SO)

B) Conservative strategies (WO)

C) Competitive strategies (ST)

D) Defensive strategies (WT)

Table 4 – SWOT matrix (weak and strong points, threats and opportunities) for rural tourism development of Meymand in Kerman

Weak points – W	Strong points – S	Internal factors
lack of NGO investment in tourism parts of this area - lack of governmental investment in this area - insufficient hygienic and service facilities - insufficient and inappropriate residential and welfare equipment - lack of skilled and trained persons in this area to guide and inform tourists - inappropriate distribution of tourists in different seasons of the year - inappropriate environmental and ……… infrastructure (road & sewage) - inappropriate sport and recreation equipment and facilities - lack of acquaintance and training among villagers to in how to act/behave with tourists	1- active handicraft workshops 2- local and traditional culture and customs 3- residential houses in heart of rocks (stone engravings) 4- rare tourism capacities and historical attractions through the world 5- adjacent to populated and urban centres 6- availability for tourists 7- calm and still environment for citizens 8- mineral and sweet springs, castles and petroglyphs 9- cultural heritage organization planning to register Meymand village internationally	External factors
WO Strategies (Conservative) * Review and development of	SO Strategies (aggressive) * Utilizing travel motive to	Opportunities - O 1- government attention to

related organizations and institutes in villages to train people and tourists in order to have optimized utilization of tourism benefits. (W3,W4,O1,O2) * Training and informing people about behaving with tourists. (W5,W9,O1,O7) * Review the way of distribution of equipment, services and tourism facilities and categorizing and devoting the equipment to rural areas. (W7,W8,O1,O2,O5)	encourage people to exploit rural tourism attractions. (S4,S5,S7,O3) * Establishment of Green Associations (support rural environment) among different levels of people to utilize rural potential. (S3,S4,S5,S6,O1,O2) * Introduction of rural tourism management field of study in Kerman universities. (S4,S5,S7,O1,O2,O7) * Popularize rural tourism attractions by related authorities through media and participate in festivals. (S2,S3,S4,S8,O7,O8) * Study, relive, enrich and update handicrafts. (S1,S2,01,O3,O7)	tourism 2- unique tourism attraction and no competitors in country 3- increase in travel and tourism motive among people 4- continuance of living in Meymand 5- semi-migration style of living with 3 stages of migration 6- unique architecture 7- increase in country authorities' attention and support of rural development with job-creation approach and income acquirement 8- Mapping of Meymand oases 9- Specification of the oases possession 10- Hygienic water, soil and climate resources of the village in comparison to other areas
WT strategies (Defensive) * Compiling special rules and regulations to utilize attractions optimally and preventing destruction and pollution. (W5,W7,W9,T1,T6,T11) * Using technology to protect the environment, strengthening, repair, etc. (W1,W2,W8,T1,T2) * Making constructive interaction between technicians, natives, environment and authorities. (W5,W9,T8,T9) * Reliving behaviours and customs (clothes, local games, foods, language and dialect, using horse, ...). (W3,W4,W6,T2,T13,T14) * Develop and build modern places with traditional style (parking, green space, landscape). (W2,W3,T1,T2,T13,T14)	ST strategies (Competitive) * Extensive and innovative utilization of native methods in repairing, building, reconstructing, internal architecture etc. (S1,S2,S4,T1,T2,T13) * Develop and equip the tourism paths of Meymand village. (S3,S4,T13,T14) * Development of tourism services, equipment and facilities. (S3,S4,S5,T13,T14) * Attraction of NGO investment for rural tourism. (S4,S5,S6,S7,T1) * Compilation of repairing and designing charter for rural part of Meymand. (S3,S4,S5,T4,T5,T14) * Use environmental resources related to capacity. (S3,S4,S5,S6,T1,T2,T11,T13) Use environmental resources as tourism goods. (S3,S4,S5,T11,T12) * Holding permanent exhibitions of handicrafts. (S1,S2,T1,T2,T3) * Directing rural purposeless capitals to develop and extend informational and communicational technologies	Threats - T 1- weakness in NGO investment facilities 2- insufficient development credits 3- selling non-native and factory products and disparate to environmental values 4- deficiency in informing about the village tourism attractions all over the province and country 5- fading of the traditional and local culture (language, cloths,…) following tourists increase 6- increase in social crimes following tourists entrance 7- relative remoteness of Meymand from capital and big cities 8- insufficient policies and goals of rural tourism 9- migration of villages to city 10- decrease in governmental services in the village due to migration and the village population decrease 11- destruction of plants and plant covering 12- erosion of the ceiling in traditional houses due to dampness and traffic 13- lack of development in

	(ICT). (S4,S5,S6,S9,T1,T2,T13)	tourism services, equipment and facilities 14- weak equipment for protection and maintenance of historical and religious places and buildings

As seen in the SWOT table, rural tourism development strategies for Meymand village are as follows:

A) SO Strategies (aggressive)

These strategies are applied using weak and strong points, which are:

- Utilizing travel motive to encourage people to exploit rural tourism attractions.

- Establishment of Green Associations (support rural environment) among different levels of people to utilize rural potential.

- Introduction of rural tourism management field of study in Kerman universities.

- Popularize rural tourism attractions by related authorities through media and participate in festivals.

- Study, relive, enrich and update handicrafts.

B) ST Strategies (Competitive)

These strategies using strong points to control environmental threats:

- Extensive and innovative utilization of native methods in repairing, building, reconstructing, internal architecture, etc.

- Develop and equip tourism paths of Meymand village.

- Development of tourism services, equipment and facilities.

- Attraction of NGO investments for rural tourism.

- Compilation of repairing and designing charter for rural part of Meymand.

- Use environmental resources related to capacity.

- Use environmental resources as tourism goods.

- Holding permanent exhibitions of handicrafts.

- Directing rural purposeless capitals to develop and extend informational and communicational technologies (ICT).

C) WO Strategies (Conservative)

In these strategies the weak points are decreased utilizing opportunities:

- Review and development of related organizations and institutes in villages to train people and tourists in order to have optimized utilization of tourism benefits.

- Training and informing people in how to act/behave with tourists.

- Review the way of distribution of equipment, services and tourism facilities and categorizing and devoting the equipment to rural areas.

D) WT Strategies (Defensive)

- Compiling special rules and regulations to utilize attractions optimally and preventing destruction and pollution.

- Using technology to protect environment, strengthening, repair, etc.

- Making constructive interaction between technicians, natives, environment and authorities.

- Reliving behaviours and customs (clothes, local games, foods, language and dialect, using horse, ...).

- Develop and build modern places with traditional style (parking, green space, landscape).

- Validity (effectiveness rate) of designed strategies from the view of elites.

Regarding the variants of "effectiveness of designed strategies", which include 22 indexes, the average of these indexes is calculated as the variant amount. Among 30 studied elites, the effectiveness of the designed strategies was medium, much and very much from the viewpoints of 5 (16.7%), 16 (53.3%) and 9 (30%), respectively. Generally, according to the calculated average of the variant (4.08), the validity of designed strategies was considerably different from the viewpoint of the elites (Table 5).

Table 5 – frequency distribution of designed strategies effectiveness from the viewpoint of elites

Frequency percentage	Frequency	Rate
0	0	Very low
0	0	Low
16.7	5	Medium
53.3	16	Much
30	9	Very much
100	30	Total

Primacy of designed strategies to develop rural tourism:

Based on the calculated averages and primacy of the designed strategies, it was specified by elites that the first primacy related to the strategy of "extensive and innovative utilization of native methods to repair, construct, reconstruct, internal architecture ..." and last primacy was related to the strategy of "establishment of rural management field of study in Kerman universities".

Table 6 – Average score of rural tourism development strategies

Score average	Strategy	primac

4.65	Utilizing travel motive to encourage people to exploit rural tourism attractions.	1
4.60	Establishment of Green Associations (support rural environment) among different casts of people to utilize rural potential.	2
4.52	Introduction of rural tourism management field of study in Kerman universities.	3
4.48	Popularize rural tourism attractions by related authorities through media and	4
4.45	participate in festivals.	5
4.44	Study, relive, enrich and update handicrafts.	6
	Vast and innovative utilization of native methods in repairing, building,	
4.32	reconstructing, internal architecture etc.	7
4.28	Develop and equip tourism paths of Meymand village.	8
4.20	Development of tourism services, equipment and facilities.	9
4.14	Attraction of NGO investment for rural tourism.	1
4.02	Compilation of repairing and designing charter for rural part of Meymand.	0
4.01	Use environmental resources related to capacity.	1
3.90	Use environmental resources as tourism goods.	1
3.81	Holding permanent exhibitions of handicrafts	1
	Directing rural purposeless capitals to develop and extend informational and	2
3.75	communicational technologies (ICT).	1
	Review and development of related organizations and institutes in villages to train	3
3.68	people and tourists in order to have optimized utilization of tourism benefits.	1
3.64	Training and informing people about behaving with tourists	4
	Review and way of distribution for equipment, services and tourism facilities and	
3.50	categorizing and devoting these equipment to rural areas.	1
	Compiling special rules and regulations to utilize attractions optimally and	5
3.40	preventing destruction and pollution.	
3.38	Using technology to protect the environment, strengthening, repair, etc.	1
3.35	Making constructive interaction between technicians, natives, environment and	6
3.34	authorities.	1
	Reliving behaviours and customs (cloths, local games, foods, language and dialect, using horse, ...)	7
	Develop and build modern places with traditional style (parking, green space, landscape)	1 8
		1 9 2 0 2 1 2 2

Matrix of evaluation of situation and strategic act (Space) for tourism industry of Meymand village:

A) Specifying factors of financial support

Score	Factor	
2	Capital yield	1
3	Pressure (financial tool)	2
1	Capability of converting to money	3
4	Capital in circulation	4
3	Cash	5
1	Easiness in existing from the system	6
1	Existing risks	7
1	Money flow	8
16.8=2	Average	

B) Factors specifying environment stability (ES)

Score	Factor	
-2	Change in technology of rural tourism industry	1
-4	Inflation rate in rural tourism industry	2
-4	Change in demand	3
-3	Competition degree in rural tourism	4
-4	Existing obstacles to enter into industry	5
-2	Completion pressure in industry	6
-2	Demand flexibility against price	7
-21.7=3	Average	

C) Factors specifying the strength of rural tourism industry (IS)

Score	Factor	
2	Potential growth of industry	1
3	Potential interest of industry	2
4	Financial stability of industry	3
5	Required skill	4
5	Utilizing resources	5
4	Capital density	6
3	Easiness of entering the industry	7
5	Utilization & using of resources	8
4	Others (flexibility against market variations)	9
36.9=4	Average	

D) Factors specifying the competitive advantage of rural tourism (CA)

Score	Factor	
-4	Rural tourism share among general tourism	1
-3	Services quality	2
-2	Training courses cycle	3
-2	Substitution cycle in industry process	4
-3	Faithfulness to the customers	5
-3	Use the competition capacity	6
-3	Technology private knowledge	7
-4	Vertical integration	8
-24.8=3	Average	

Results:

- the point on the X axis

$$CA+IS=4+ (-3) =1$$

- the point on Y axis

$$ES+FS= -3+2=-1$$

Matrix of evaluating situation and strategic act for rural tourism industry

```
                    ┌─────────────────┐
                    │ Financial power │
                    └────────┬────────┘
                             │
                             │
┌──────────────┐         +1  │    ┌──────────┐
│ Competitive  │─────────────┤    │ Industry │
│ Advantage    │         │   │    │ Power    │
└──────────────┘      -1 │───┘    └──────────┘
                         │
                             Competitive
                    ┌─────────────────┐
                    │  Environment    │
                    │  Stability      │
                    └─────────────────┘
```
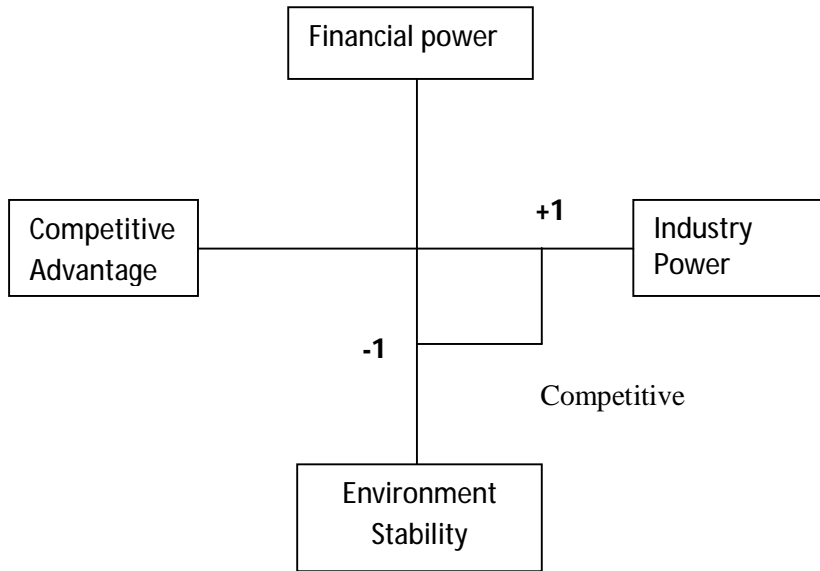
Rural tourism industry should implement a competitive strategy.
Rural tourism industry in such a position (competitive) requires financial resources to increase its share, increase the strength of the industry, and extend and improve the processes.

Conclusion and recommendations:

Considering the Meymand village, especially the Dastkand oasis, which is one of the rare attractions of the world and regarding other strong points mentioned through the research and also based on the results in which threats like villagers' migration, insufficient NGO investment facilities are the most important threats, it should be tried by means of the mentioned strategies to utilize the weak and strong points – optimise the opportunities and reduce the threats.

Furthermore, the research results delineated that the strategy "*extensive utilization of native methods to repair, construct, reconstruct and internal architecture of Meymand village*" devotes the most important primacy of rural tourism development strategic planning of Meymand to itself.

Moreover, the matrix of evaluating situation and strategic act of rural tourism industry delineates that this industry should implement a competitive strategy.

In conclusion, regarding the research results the following is recommended:
A) Present a report of SWOT analysis related to all touristic villages throughout the country

and the related strategies should be introduced and implemented.

B) Design rural tourism industry within future 20 years.

C) Design and implement moral charter for rural tourism.

D) Regarding effectiveness of designed strategies, it is recommended that organizations and executives of the tourism industry attempt to implement the designed strategies regarding presented primacies.

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Review required activities before participatory rural appraisal (PRA)

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Abstract: Much of the spread of participatory rural appraisal (PRA) as an emerging family of approaches and methods has been lateral, South-South, through experiential learning and changes in behavior, with different local applications. Rapid spread has made quality assurance a concern, with dangers from "instant fashion", rushing, formalism and ruts. Promising potentials include farmers' own farming systems research, alternatives to questionnaire surveys, monitoring, evaluation and lateral spread by local people, empowerment of the poorer and weaker, and policy review. Changes in personal behavior and attitudes, and in organizational cultures, are implied. PRA parallels and resonates with paradigm shifts in the social and natural sciences, business management, and development thinking, supporting decentralization, local diversity, and personal responsibility.
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Keywords: Participatory Rural Appraisal (PRA)

Introduction:

Participatory Rural Appraisal (PRA) is one of the participatory research techniques in the class of qualitative research. Robert Chambers (2004) describes PRA as "a growing family of approaches, methods, attitudes and behaviours to enable and empower people to share, analyze and enhance their knowledge of life and conditions, and to plan, act, monitor, evaluate and reflect".

There exist different methods of data collection and analysis, each with its own strengths and weaknesses. Through time, more appropriate and refined methods have been developed. In the context of rural development, information regarding the communities, their livelihoods, their beliefs, the physical environment in which they live, and their resource endowments need to be gathered and interpreted in a manner that identifies their priorities with a view of developing better understanding of their status and designing appropriate intervention projects directed at resolving their problems. The different ways of data collection and interpretation can be seen under two perspectives(IUCN, 2001): qualitative versus quantitative, and participatory versus top down. While the quantitative methods generate information that can be captured numerically, the qualitative methods generally do not generate specific numbers. Qualitative methods are concerned with exploring meanings, processes, reasons, and explanations(lnglis, 1992).

RRA was criticized for being extractive and highly dependent on expert interpretation. It was thus found useful to replace it with PRA which involves a process of learning from, with and by rural people about rural conditions. PRA shares much with its parent, RRA, but is distinguished from it in practice by correcting two common errors: roles of investigation are reversed; and rushing is replaced by relaxation and rapport. At the heart of all these developments was Robert Chambers, although Paulo Friere has also had strong influence especially in similar developments in education circles (Provention Concertium).

PRA has evolved and spread from beginnings in Ethiopia, India, Kenya, Sudan and elsewhere, and in early 1994 is known to be being quite widely practiced in parts of Bangladesh, Botswana, Ethiopia, francophone West Africa, India, Indonesia, Kenya, Nepal, Nigeria, Pakistan, the Philippines, Sri Lanka, Sudan, Uganda, Vietnam, and Zimbabwe, while starts have been made in at least a score of other countries in Latin America, Africa and Asia. Hundreds of nongovernment organizations (NGOs) have adopted PRA and developed applications, as have a number of government departments. The use of PRA methods is being increasingly explored by students and faculty in universities for research, and by training institutes for fieldwork. Spread appears to be accelerating.

PRA TECHNIQUES:

There are six popular techniques/methods that are used to facilitate PRA exercise that enables the community to develop and compile a detailed profile of themselves and their situation.

• **Venn Diagram**

Venn Diagrams are drawn to help understand the current formal and informal institutions in the area under study and the nature of relationship between the communities and these existing institutions and structures. The community is led to identify their needs, analyze these needs and assess the **cause and effect** relationship. This process provides an opportunity for the community to arrive at the most pressing or priority need utilizing a logical format and this often culminates into a problems tree(Clayton, 1997).

• **Time line**

This technique describes chronologies of events, listing major remembered events in a village with approximate dates. The process involves elderly people in a village to narrate their life history, summarizing major events and changes that have taken place over a period of time. Major events and political regimes including their significance and influence to the change in the lives of the community over time are recorded. Time line shows a broad movement of different aspects in a village during the community's lifetime(Chambers, 1994).

• **Time trend**

This is a technique where people given an opportunity to account about their past and discuss how things close to them have changed. Issues such as ecological history, changes in land-use, cropping patterns, changes in customs, practices & trends in population, migration, education, health, prices, yields, etc. This technique is more precise in giving indication of change (increase or decrease) about a particular item/activity(KGVK, 1991).

• **Mapping**

This is where people use ground, floor or flip charts to map and draw the different aspects of their village e.g. social issues, demographic, resources, health, wealth, literacy, livestock, economic activities, water resources, trees, housing layout etc. This technique portrays the image dwellings in a village(Hollandand and Blackburn, 1998).

• **Transect Walk**

This is a systematical walk with the Community members through the village observing, discussing, identifying different forms, local techniques, introduced technologies, seeking their uses, problems, solutions and opportunities. It is done to ensure that the team fully explores the spatial differences in the community, assessing the infrastructure that exists and any possible activities that might be taking place within the village.

• **Matrix**

Matrix is a ranking & scoring technique that is used to discover local attitudes and perceptions about a particular resource. This may be about the land use, water conservation measures, seasons, weather conditions, rainfall pattern or rainfall distribution, intensity and efficiency. These are assessed to determine the extent they affect and influence the way of life within the community. This helps to provide a better understanding of constraints and opportunities for possible development interventions. A graph is usually drawn in a matrix format displaying these constraints and opportunities.

PRA LIMITATIONS AND CHALLENGES

There are limitations in PRA and it is not the answer to all rural development problems and these limitations must be acknowledged and caution should be exercised so as to avoid unrealistic expectations and disappointments.

- **(FORMALISM)-** the urge to standardize in-order to produce quality by keeping to strict manuals can do more harm than help. Practitioners must take risks, experiment, improvise and be creative.
- **(FADDISM)** PRA could easily be discredited by misuse, i.e. calling every development intervention with local community a "PRA" especially when it is one that excludes them from active involvement in decision-making and planning.
- **(RUSHING)-**the rationale behind PRA is to make time to find the poorest and move at their own pace, to learn from them, and to empower them. Poorly conceived and rapid interventions, lack of commitment from officials to followup, compound errors and mean that the poor are, once again, neither seen, listened to, nor learnt from(Chambers, 1997).

Since PRA seeks to assist local people to plan, implement, monitor and evaluate their own action plans, in theory PRA should be used only during the implementation of a project. Since PRA aims at people taking action themselves it is most suited for the community level.

PRA presents a major step forward from RRA. Local people do the analysis and plan for the future. Their own values, needs and priorities are the point of departure. They themselves develop criteria to classify aspects of their life. This not only leads to a better understanding of the situation (for both the in-

and the outsiders) and therefore increases the chance for realistic plans, it also generates a much higher commitment of the people to the planned activities(Scrimshaw, 1992).

The many different perspectives on daily reality and the visualisation offer good opportunities to go beyond the most obvious and dominant points of view in the community. The only warning here should be that too much attention to group discussions/ -activities might enable some groups to dominate the discussion(Cornwall, 2008).

The methodology is open to modification; everybody can develop new tools and new ways of organising things. This makes PRA applicable in a very wide range of situations. Indeed, it has been used in both rural and urban areas, both in developing countries and industrial countries, in agriculture, in health care and in social programmes. PRA can also be used to collect data; local people are able to generate and/or collect reliable data which they themselves analyze and use for planning (Mukherjee, 1992).

five key principles that form the basis of any PRA activity:

1. **PARTICIPATION :**

PRA relies heavily on participation by the communities, as the method is designed to enable local people to be involved, not only as sources of information, but as partners with the PRA team in gathering and analyzing the information.

2. **FLEXIBILITY :**

The combination of techniques that is appropriate in a particular development context will be determined by such variables as the size and skill mix of the PRA team, the time and resources available, and the topic and location of the work(Dunn, 1991).

3. **TEAMWORK :**

Generally, a PRA is best conducted by a local team (speaking the local languages) with a few outsiders present, a significant representation of women, and a mix of sector specialists and social scientists, according to the topic.

4. **OPTIMAL IGNORANCE :**

To be efficient in terms of both time and money, PRA work intends to gather just enough information to make the necessary recommendations and decisions.

5. **SYSTEMATIC :**

As PRA-generated data is seldom conducive to statistical analysis (given its largely qualitative nature and relatively small sample size), alternative ways have been developed to ensure the validity and reliability of the findings. These include sampling based on approximate stratification of the community by geographic location or relative wealth, and cross-checking, that is using a number of techniques to investigate views on a single topic (including through a final community meeting to discuss the findings and correct inconsistencies).

Steps in participatory planning

PRA has steps of planning:

1. Defining the objective of PRA

2. Site selection and clearance form local administrative officials. Fro programmed implantation (or) problem solving purpose. For site selection, use-ranking methods with local people and outsiders; then select the sites for intervention(Ekins, 1992).

3. Preliminary visit

- Survey team visit

- Extended discussion with local leaders

- Agreement to do a PRA

- Sharing responsibilities with the people

4. Data collection

- Local people and survey team collect information

- The data includes:

- Spatial data

- Time related information

- Data on institutions and social structures

- Technical information

5. Data analysis

- PRA team spends days organizing information

- Make large charts and tables of trends, maps transects etc

- Compile a list of all the problems mentioned

- Summarized the problems

6. Ranking problems

- Present to the community data collected in a large meeting

- Include line department staff DA s etc

- Rank the problems by discussion and voting

7. Formulate and rank opportunities

- From discussion groups on the solutions of the problems

- Obtain advise from the technical officers on:

- Feasibility

- Sustainability

- Productivity

- Equity of the solutions

- Rank opportunities

- Set an action plans

8. Adoption of action plans

- Look for technical information to develop a comprehensive plan

- Specific expert join PRA team

- Line ministry departments take part in the implementation

9. Implementation

- All partners in development contributes to activities as:
- Manpower allocation
- Materials needed
- Time needed
- Funds required(Pretty, 1993)

PRA are good for:
• Providing basic information in situations where little in known
• Identifying and assessing problems
• Appraising, designing, implementing, monitoring, and evaluation programs and projects
• Getting a better picture of needs and organizations' ability to meet them
• Developing and transferring appropriate technologies
• Appraising emergencies
• Planning projects that are more relevant, restructuring administrations, assisting in decision-making and policy formation
• Generating hypotheses, ruling out inappropriate ones
• Providing guidelines for survey designs and assessing the applicability of their results to other places.
• Fleshing – out complementing, interpreting, or giving depth and context to information obtained through other methods.

7.5 PRA is not very useful for:
Working in situations in which the problem is not usefully addressed at the local or group level, for example, in situations where large-scale structural reorganization is necessary (but even then, local views may help to shape the change).

PREPARATIONS BEFORE THE PRA:
Proper preparations determine the success of PRA for it involves learning-by-doing and depends on team contributions. In addition to selecting the site where PRA is to be conducted and collecting secondary information regarding the specific sites and their neighborhoods, it is necessary to:
• Establish a PRA Team;
• Establish a Kushet PRA Committee;
• Conduct Preliminary Visits to the Community;
• Developing PRA Schedule.

1. The PRA Team:
The PRA Team consists five faculty members of the faculty of business and economics.
Note that other member(s) already involved in development activities in or near the specified areas shall be included if found necessary, for in PRA, the Team is expected to have the necessary technical know how in different areas (agriculture, health,

education, infrastructure, credit, marketing, culture, etc.). It also needs to have a fair gender composition. Although every team member should be familiar with all aspects of the PRA, each team member is also designated for specific tasks which are described as follows(NCAER, 1993):

a. PRA team leader: One of the PRA Team members will be designated as a leader in each of the four PRAs. That is one team leader will be assigned for each of the four villages. The team leader will be selected in such a way that four members will alternatively serve as team leaders for each of the four PRAs. The role of the team leader is to(Scoones, 1993):
• Play the leading role in the formation of the village PRA committee;
• Ensure that all preparatory work has been done;
• Make sure that the objectives of each session are achieved;
• Coordinate preparation of the PRA report;
• Resolve any problems which may arise;
• Assign facilitators and note-takers for each session;
• Organize the reports from the note-taker/s into a coherent whole;
• Work as the principal editor of that particular PRA report.

Importantly, the PRA team leader is also responsible for ensuring that all technical areas are appropriately covered. Though not intended to do so, many PRA exercises may reflect the technical bias of the facilitators or note-takers as opposed to community needs and interests. This should be avoided at all costs, and the PRA team leader should ensure that.

b. Facilitator: For each PRA session, one individual should be designated as the lead facilitator (note that the team leader may also serve as a facilitator in some of the sessions). As a key objective of the PRA is to promote active community participation, the role of the facilitator is very important and includes:

Before the Session:
• Knowing the contents of their session very well in order that they rarely have to look at the manual for guidance
• Ensuring that the site is well prepared – that there are enough places to sit, that there is not too much noise close by, that the area is well shaded, etc.
• Ensuring that the seating arrangement is good – and that participants can be seated in a circle so that they can see the facilitator, other participants, as well as any flipchart or blackboard which may be

used. Important: if participants are not properly seated, have everyone get up and rearrange the meeting place. During the Session

- Ensuring that all participants understand and contribute to the discussions.
  1. If one participant is talking too much, thank him/her for his/her comments and ask another opinion;
  2. If some participants are not contributing at all, ask them directly what they think;
  3. Do not let only one person or a small group of participants dominate the discussions;
  4. Pay special attention to women and the poor who may not feel comfortable contributing.
- Ensuring that team members share their ideas only after the community members have provided their own, and that the team members avoid influencing the community's decisions.
- Managing the time available for the session to ensure that all objectives are achieved.
- At the end of the session, thank participants for their contributions and explain to them the next activity(Drummond, 1992).

c. Note taker: Because much information is generated throughout the PRA, the task of taking notes is very important to the program's success. One person shall be assigned as a note-taker for each session. The role of the note taker includes(Uphoff, 1992):

- Sitting among participants and take notes (it may preferably be done in such a way that the participants are not so aware that someone is taking notes);
- Noting all main discussion points, and paying special attention to the comments of participants concerns:
  1. What they feel are problems;
  2. What they believe are the causes of these problems;
  3. Possible solutions, and especially how the community has solved these problems in the past;
  4. Special beliefs, customs and religious practices.
- Asking participants to repeat comments if they are not well understood;
- Assisting the facilitator by reminding if some important things are left out or not properly taken care of;Copying information presented on big paper into a notebook;

- Reviewing the notes with the facilitator to make sure that they are complete and correct;
- Copy the notes to a laptop at the end of each day's work.

d. Technical Resource Persons: Specific team members should be designated to serve as resource persons for key technical areas. If appropriate technical persons are not available with the team, the support of government bureaus or NGOs will be sought(Swift, 1991).

These individuals may serve as facilitators for sessions related to their technical area, or may simply assist the PRA team, the PRA committee or other participants in identifying community problems, causes and possible solutions. Note that even though Technical Resource Persons may have much expertise, they should share their ideas only after community members have discussed their own, and avoid influencing the community's decisions. In addition to focus group discussions, technical persons could be used during transect walk(Appleyard, 1998).

2. Preliminary Visits to the Community:

After selecting the specific areas where PRA is to be conducted, the PRA Team (all members need preferably attend) needs to conduct a visit to meet members (local leaders), development workers in the area, government workers, health workers, teachers, and religious leaders with the following duties:

- Introducing the PRA approach to local administrators and community leaders and explaining the objectives of the PRA;
- Explaining the contents and schedule of the PRA program;
- Requesting that a Village PRA Committee be established;
- Deciding on the dates for the PRA;
- Making necessary logistical arrangements, including:
  1. Identifying sites to conduct large and small group meetings;
  2. Discussing lodging arrangements for the PRA Team (if the PRA team decides to stay in the area during the PRA work).

3. The Kushet PRA Committee (KPC):

Because the success of a PRA depends on strong community participation, a KPC (also called Village PRA committee – VPC) is established. Among others, the KPC is used as an important means to win the trust of the community. The following describe its major characteristics:

1. Composition of the KPC: To ensure the committee is well-suited for its tasks, it should be composed of:

- Six members with an equal number of men and women;
- One of the six members need to be a member of leadership to facilitate the success of the PRA;
- One of the female members need to be selected from the women's association;
- One member of the committee should come from the church (orthodox) leadership;
- Most appropriately the other members (apart from the representative from the or administration and the representative from the women's association) should be elected by the community during a meeting of the community members. If found difficult, another mechanism may be thought;
- Representatives of different areas of the village; different economic groups; and different religious groups in the case of more than one religion;
- Members who have the time to not only help in preparing the community for PRA, but also to help during the PRA, as well as after PRA with the follow-up activities.

2. Roles and Responsibilities of the KPC: The major tasks of the committee include:

- Explaining to other community members the objectives, schedule and importance of the PRA;
- Arranging the place where meetings take place;
- Encouraging all community members to actively participate throughout the PRA;
- If possible, note-taking and similar tasks;
- Meeting with the PRA Team at the end of each day to discuss issues, team findings, problems, etc.;
- Assist participants in the different sessions of the PRA;
- Assisting in PRA follow up activities.

4. Developing the PRA Schedule:

About two weeks before the PRA, the PRA Team should meet to review activities already undertaken and develop the actual schedule for the PRA. Important activities include:

- Fixing the dates for the different PRA sessions in consultation with the PRA committee;

- Indicate the particular sessions and their specific dates including the results expected of each session;
- Specify the roles of each member (facilitator, note taker, etc.)
- Material preparation;
- Logistics (transport, lodging, food preparation);
- Indicate the specific date when the preliminary report should be produced;
- Others.

The PRA team leader is responsible for developing the schedule and makes follow up.

5. Activities upon arrival in the Community:

As final preparatory activities, and once the PRA Team has arrived in the community and has settled lodging and meal arrangements, they should undertake to:

- Meet with the KPC and or leaders to review
    1. The purpose of the PRA;
    2. The PRA Schedule;
    3. The role of the PRA Committee and village leaders;
    4. Preparatory activities the village has undertaken; and
    5. Other important issues (e.g. PRA Team lodging, if applicable)
- Visit PRA Meeting Sites: With the PRA Committee, the Team should visit sites designated by the village for general meetings and small discussion and working groups(Hahn, 1991).
- Plan Transect Walk: The PRA Team should take some time to walk through and around the village – both to familiarize them with the village and to allow villagers to familiarize themselves with PRA Team members. During this time, the PRA Team should also decide what areas they will walk through when they conduct the Transect Walk exercise.
- Review of the Schedule and Roles: Finally the PRA Team should meet to review all of the above, and make any final preparations and review the roles of Facilitators and Note Takers.

CONCLUSION:

The main objectives of the current PRA are:
1. empowerment of rural communities by assisting them to systematically utilize their local knowledge to identify problems and strengths, develop skills of analysis, and design appropriate mechanisms for intervention by themselves and/or by development agents;

2. advancement of understanding by academicians/researchers of local knowledge and acknowledgement of the capacity of communities to gather data, conduct analysis, and identify as well as prioritize problems and solutions;

3. utilization of the research questions/problems identified during the PRAs for further investigation;

4. documenting and presenting the outcomes of the PRAs to development agents (governmental and non-governmental) and other stakeholders so that they could undertake interventions in line with the findings.

PRA consists of a series of participatory exercises which help community members better assess their history, resources, and overall situation as concerns agriculture, health, marketing, credit, coping mechanisms, education, and other important areas. During the conduct of the PRAs, rural communities in the selected villages will gather information on the resources they already possess; organize their knowledge; share experience among themselves; learn from each other; identify and prioritize local development needs; and develop action plans which respond to these needs.

The many different perspectives on daily reality and the visualisation offer good opportunities to go beyond the most obvious and dominant points of view in the community. The only warning here should be that too much attention to group discussions/ -activities might enable some groups to dominate the discussion. The methodology is open to modification; everybody can develop new tools and new ways of organising things. This makes PRA applicable in a very wide range of situations. Indeed, it has been used in both rural and urban areas, both in developing countries and industrial countries, in agriculture, in health care and in social programmes. PRA can also be used to collect data; local people are able to generate and/or collect reliable data which they themselves analyze and use for planning.

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Effect of a Specific Combination of Mannan-Oligosaccharides and β-Glucans Extracted from Yeast Cell Wall on the Health Status and Growth Performance of Ochratoxicated Broiler Chickens

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Abstract: The effect of a specific combination of Mannan-oligosaccharides (MOS) and β-glucans extracted form the cell wall of a specific strain of *Saccharomyces cerevisiae* (AGRIMOS®) was investigated on zootechnical performance, ochratoxicosis and immune dysfunction caused by ochratoxin in broiler chickens. Three hundred and sixty, one day-old chickens were randomly allocated in a 2x2 factorial design for 5 weeks: supplementation of 2kg/ton of MOS (presence or absence) and feed contamination (presence or absence) with 50 µg/kg of ochratoxin A (OTA) for the first 3 weeks of life was done. Obtained results revealed that OTA did affect bird's growth one week after the contamination, although the final weight gain after 5 weeks was not different from the control. The use of AGRIMOS® stimulated the overall daily gain compared to the OTA group. Feed intake and feed conversion were not affected by the dietary treatments. Cumulative mortality was similar between treatments and performance indexes significantly improved with AGRIMOS® for the OTA challenged regimes. AGRIMOS® supplementation reduced macroscopic and microscopic lesion scores associated with ochratoxicosis. Also, it corrected the depression in phagocytosis induced by ochratoxin intoxication and it had strong immunomodulation as it stimulated the immune response to vaccination. It could be concluded that administration of a specific combination of Mannan-oligosaccharides and β-glucans extracted form yeast cell wall (AGRIMOS®) to chickens improved zootechnical parameters had a potent immunomodulatory effect, evoked immune response and enhanced vaccination effectiveness. It helps not only in controlling chicken ochratoxicosis but also can play a positive role in treating chicken immune dysfunction.
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Keywords: Mannan-oligosaccharides**,** AGRIMOS®**,** β-glucan**,** Ochratoxins, Chickens.

1. Introduction

All the antibiotic growth promoters have been banned in the European Union (EU) since the year (2006). Eventually, many other countries will have to follow these measures to export to the EU. The challenge will be to fight microbial imbalances in young animals. Feed formulators need efficient alternatives to use as commodities. Gastrointestinal tract (GIT) of poultry harbors microflora, which is formed immediately after the bird is hatched and is an important barrier against colonization of potentially pathogenic microorganisms. The bird's microflora is potentially depleted for a period of time at hatching and following any medication with an anti-microbial product. Consequently; natural alternatives concepts based on natural ingredients for non-antibiotic growth promotion and antibacterial action are highly commendable.

Mycotoxins are often found as natural contaminants in grains (Walker 2002). Ochratoxin A

(OTA) is the major component of a group of secondary metabolites produced by several fungi such as *Aspergillus ochraceus* or *Penicillium verrucosum*. There have been several investigations on the mode of action of this toxin, suggesting inhibition of protein synthesis, formation of DNA adducts, and provocation of DNA single-strand breaks as a result of oxidative stress (Anke *et al*. 2003). Some of the major deleterious effects of ochratoxin in poultry include depressed growth rate, nephrotoxicity, reproductive failure and immunosuppression (Marquardt and Frohlish, 1992). The immunosuppressive effect of ochratoxins in chicken immune system will lead to immune dysfunction that can lead to exacerbation of diseases (Gill and Cross, 2002 and Wang *et al*. 2009).

The term immunosuppression is defined as a state of temporary or permanent dysfunction of the immune response, which insult to the immune system leading to increased susceptibility to diseases

(Domhs and Saif 1983). It is undoubtedly true that factors contributing to immune-suppression will lead to immunodeficiency. The latter is a hazard-anticipating causative agent of serious economic impacts in poultry industry allover the world. Recognition and scientific identification of factors encountered in immune deficiency have lead to increased perusal investigation in the counterattacking modulators to accomplish immune-stimulation. Eventually, it has already known that many diseases/disorders, that have immunomodulated components, can be modified by administration of biological compounds that activate key pathways in the immune system (Podleski 1988 and Xue *et al*. 2010). They strengthen the defense and immune mechanisms of the body and currently usable for stimulating the non-specific immune responsiveness in both the human and veterinary medical practice (Emmerich *et al*. 1988, and Awaad *et al*. 1999, 2000 a, b).

This study was conducted in an attempt to investigate the possible effect of a specific combination of Mannan-oligosaccharides (MOS) and β-glucans (AGRIMOS®) extracted from the yeast cell wall of *Saccharomyces cerevisiae* on productive performance, ochratoxicosis and immune dysfunction caused by ochratoxin in broiler chickens.

2. Material and Methods
Experimental design:

Three hundred and sixty, one day-old Arbor Acres plus broiler chickens were used in this study. The birds were allotted into 4 equal groups (I-IV) of 90 birds assigned to 3 replicates of 30 each. Those of groups I and III were fed on ration containing ochratoxin for the first 3 weeks of age (OTA and OTA+AGRIMOS® groups, respectively), while those of groups II and IV were fed on plain ration *ad libitum* (control and AGRIMOS® treated groups, respectively). Chickens of groups III and IV were fed on a standard feed containing a specific combination of Mannan-oligosaccharides and β-glucans extracted form the cell wall of a specific strain of *Saccharomyces cerevisiae* (AGRIMOS®, Lallemand SAS) supplemented at 2 kg/ ton of feed. AGRIMOS® is obtained by a specific production process consisting in the extraction of the cell wall from a unique primary grown yeast *Saccharomyces cerevisiae* (process guaranteeing consistent purity and high level of Mannans and ß-glucans). Birds of group II were kept as blank control. Chickens of all groups were vaccinated via intra-ocular route and subcutaneous route, respectively with Hitchner B1+H120 vaccine and avian influenza vaccine (Inactivated H5N2 vaccine from Intervet International, B.V. BOXMEER-Holland) at 7th and

10th day of age, respectively. La Sota vaccine (Pfizer) and 228E IBDV vaccine (freeze-dried live vaccine Noblis Gumboro 228E strain from Intervet International, B.V. BOXMEER-Holland) were given at 14 days of age via intra-ocular route.

At 35 days of age, 10 chickens from each group were challenged with velogenic viscerotropic Newcastle disease virus (VVNDv) at a dose of $10^{6.8}$ EID_{50} / ml / bird by intramuscular injection and kept under close observation for clinical signs and mortality for 2 weeks. At the end of the observation period, dead as well as sacrificed birds (at 49 days) were subjected to post-mortem examination for lesion scoring of Newcastle disease virus (NDv) (Amer 1980).

Diets:

Chicks were fed *ad libitum* commercial starter diet (23% CP and 3000 k cal ME/kg diet) during the first two weeks of age, commercial grower diet (22% CP and 3150 kcal ME/kg diet) from 2 to 4 weeks of age, and then commercial finisher diet (19% CP and 3200 kcal ME/kg diet). The diets compositions are indicated in table (1). Birds had free access to water. Semduramicin was added at a concentration of 25 p.p.m as a coccidiostat.

Ochratoxicated feed preparation:

Ochratoxin-A (OTA) contaminated raw materials were prepared according to Sansing *et al*. (1973) by artificial contamination of sterile yellow corn with *Aspergillus ochraceus*. Artificially OTA-contaminated yellow corn was then thoroughly mixed with broiler ration to provide a final concentration of 50 µg/kg of feed. The mycotoxin concentration was determined by immune affinity chromatography according to Hansen (1990) and Truckess *et al*. (1991).

Measured parameters:

Chicken zootechnical performances were determined according to North and bell (1990) for Body Weight (g), Body Weight Gain (g), Feed Consumption (g/day/bird), Feed Conversion (g feed/g live body weight), and Performances Indexes defined as follows:

- Point Spread= (Live body weight in pounds) - (Feed conversion) X 100.
- Performance Index = Live body weight (Kg) / Feed conversion X 100.
- European Performance Efficiency Factor (EPEP): A / B x 10000

Where:

A= Average live weight (kg) X Livability.

B= Marketing age (days) X Feed conversion.

Birds were individually weighed.

Immunoassays:

The possible effect of AGRIMOS[®] on the cell mediated immunity was investigated using phagocytic activity of macrophages, lysozyme and Nitric oxide activities (Muller *et al*. 1995) on blood samples taken at 3 and 5 weeks of age on 5 birds randomly chosen per group. The possible effect of AGRIMOS[®] on the humoral immunity was assessed through haemaglutination inhibition (HI) test for determining antibody titers against ND (Amer 1980). Blood samples were collected from 10 randomly chosen birds at weekly intervals (0-7 weeks of age) from all groups, centrifuged for 10 minutes at 1,000 x g and serum samples were subjected to HI test.

As the primary lymphoid organ, Bursa of Fabricius is of particular interest to look at for cell mediated immune parameters, this organ was withdrawn from 5 birds / replicate at weeks 3 and 5 of age, sacrificed and the bursa / body weight ratio was calculated according to Sharma *et al*. (1989) as follows: Organ weight (g) x 1000 / Total body weight (g), expressed as the arithmetic mean for each group of birds.

Histopathology assay:

Liver, kidneys, Bursa of Fabricius, spleen and thymus glands were collected from the sacrificed 5 chickens per replicate at 3 and 5 weeks of age and fixed in 10% buffered formalin. Paraffin-embedded sections were routinely prepared and stained with Hematoxylin and Eosin (Bancroft *et al*. 1996), and scored for histopathological lesions according to the method described by Rosales *et al*. (1989).

Statistical analyses:

Two-way analysis of variance was used using SAS software general liner models procedure (SAS Institute 1999). The main factors were ochratoxin contamination and yeast cell wall supplementation. Mean values were assessed for significance using Duncan's multiple range test with significance set at P<0.05.

3. Results
Zootechnical performance:

As shown in table (2), no significant (P<0.05) difference was recorded between the control and the AGRIMOS[®] treated group at any time for all the zootechnical parameters concerned. However OTA contamination had a significant (P<0.05) deleterious effect on growth from week 2 and the body gain slowed down until the end of the OTA challenge. At the end of the experiment; control and OTA compromised chickens had not statistically different body weights even if the OTA group exhibits the lowest final body weight. Finally, no

significant (P<0.05) difference was detected between the control and the OTA group for any of the other zootechnical parameters. Interestingly, OTA+ AGRIMOS[®] treated chicken did not exhibit the same trend compared to control than the OTA group according to body weight and body weight gain. Indeed AGRIMOS[®] supplementation compensated the growth delay due to OTA contamination. Chickens from the OTA+ AGRIMOS[®] group maintained similar body weight and body weight gain than the control and the AGRIMOS[®] groups. Furthermore, body weight was found to be significantly (P<0.05) higher in the OTA+ AGRIMOS[®] group compared to the OTA group two weeks after the first feeding to the end of the trial, while body weight gain was also higher but only during the second and the third week of the trial. Point Spread and Performance Index were also significantly (P<0.05) higher in the OTA+ AGRIMOS[®] group versus OTA.

Finally, the cumulative mortality rate of AGRIMOS[®] group recorded the lowest value, but without significant (P<0.05) differences as compared to other groups. Only Control group recorded the highest mortality at week four of the study.

Immune status assessment:

Cell mediated immunity parameters are reported in table (3). No significant (P<0.05) difference was measured between the two examined dates (3rd and 5th weeks of age) for phagocytic percentage and phagocytic index in group II (control), whereas lyzozyme and Nitric oxide increased significantly (P<0.05) from the third to the fifth week of rearing. However this phenomenon was not observed in group IV (AGRIMOS[®]) as cell mediated immune parameters were already high after 3 weeks of rearing. Indeed, Nitric oxide, as well as phagocytic indexes were found significantly (P<0.05) higher in group IV (AGRIMOS[®]) compared to group II (control) at 3 weeks of age. These differences remained significant (P<0.05) at 5 weeks of age for the macrophage activity indexes. No effect of OTA contamination has been reported in this study on cell mediated immune parameters when compared to control (group II), even if phagoctytic index and phagocytic % tended to be systematically affected by ochratoxicosis at both sampling times. Indeed, lower values for these parameters were systematically recorded for group I (OTA); while almost no difference between group III (OTA+ AGRIMOS[®]) and group II (control) was detected whatever the parameter concerned even if a higher lyzozyme activity was measured in group III after 3 weeks of rearing. Concerning Nitric oxide activity, at 3 weeks

of age, group I (OTA) produced the lowest figure with significant (P<0.05) differences compared to groups III (OTA+ AGRIMOS®) and IV (AGRIMOS®). On the other hand, the AGRIMOS® group produced the highest amount of Nitric oxide at week three. At five weeks of age, no difference was recorded between groups.

As regards humoral immunity, the haemaglutination inhibition (HI) test (Figure 1) indicated that the AGRIMOS® treatment in group III corrected the depression in humoral immunity induced by OTA. As a matter of fact, after one week of feeding the OTA contaminated diet, the chicken that did not receive AGRIMOS® showed a significant (P<0.05) decrease in HI, while this drop was attenuated in the group III (OTA+ AGRIMOS®). The same phenomenon was recorded during week 4, and lasted over the fifth week of rearing.

Bursa of Fabricus live weight and Bursa / Body weight index were not affected by the treatments after 3 weeks of rearing. However, at 5 weeks of age, chickens from group I (OTA) showed significant (P<0.05) lower Bursa weight and Ratio index compared to groups II (control) and IV (AGRIMOS®), while group III (OTA+ AGRIMOS®) showed intermediate results (Figure 2).

Histopathological changes:

In ochratoxicated (immunocompromised) chickens, the main organs such as liver and kidneys were differently affected according to the AGRIMOS® supplementation or not.

Concerning liver; OTA group, showed vacuolar degeneration of hepatocytes, hyperplasia of epithelial lining bile duct associated with appearance of newly formed bile ductuoles and fibroplasia in the portal triad. Some examined sections revealed chronic cholangitis manifested by fibrous connective tissue proliferation and massive inflammatory cells infiltration (mainly heterophils, macrophages and lymphocytes) in the wall of bile duct (Photo 1). Focal area of hepatic necrosis replaced by mononuclear leucocytes (Photo 2) was observed in all examined sections. Regarding liver of chickens of AGRIMOS® treated group (IV) showed no histopathological changes except vacuolar degeneration of centrolobular hepatocytes (Photo 3). Chickens of group III (OTA+AGRIMOS®) showed improvement in the histopathological picture as the examined sections revealed few vacuolar degeneration of hepatocytes, slight thickening in the wall of bile duct associated with leucocytic cells infiltration mainly heterophils (Photo 4) together with small focal area of hepatic necrosis replaced by leucocytic cells.

At the kidneys level, chickens of group I (OTA) revealed vacuolar degeneration of epithelial

lining of the renal tubules, congestion of intertubular blood vessels and capillaries, subcapsular hemorrhage and interstitial hemorrhage (Photo 5) accompanied with multiple focal areas of tubular necrosis which completely replaced by massive leucocytes (Photo 6). Those of group IV (AGRIMOS®) revealed no histopathological changes. Chickens of group III (OTA+AGRIMOS®) showed congestion of intertubular blood vessels and capillaries, peritubular leucocytic cells infiltration (Photo 7) together with focal area of tubular necrosis replaced by leucocytic inflammatory cells infiltration. As regards the major immune organs, Bursa of Fabricius of chickens of group I (OTA) showed vacuolation of lymphoid follicles (Photo 8), lymphocytic necrosis and depletion especially in the medulla of lymphoid follicles as well as hyperplasia and vacuolation of follicle associated epithelium. While in groups III and IV examined sections revealed no histopathological alterations (Photo 9). Concerning spleen; OTA group revealed focal splenic hemorrhage, lymphocytic necrosis and depletion, hyperplasia of reticular cells associated with atrophy of the lymphoid follicles (Photo 10). While in groups III and IV examined sections revealed no histopathological alterations (Photo 11). Thymus glands of group I (OTA) showed focal thymic hemorrhage (Photo 12) associated with lymphocytic depletion in the medulla. While in groups III and IV examined sections revealed no histopathological alterations (Photo 13).

All examined sections from group II (blank control) revealed no histopathological changes.

Concerning the macroscopic lesion scoring after the challenge with velogenic viscerotropic Newcastle disease virus (VVNDv) conducted at day 35 on ten birds per treatment, the results indicated a significant (P<0.05) higher lesion score in the OTA group (group I) (2.6) while no significant (P<0.05) difference was observed among the three other groups (0.9, 0.7 and 0.8; for groups II, III and IV, respectively) (Figure 3).

4. Discussion

Our results indicate that OTA contamination has a negative effect on growth parameters of broiler chicken. Interestingly we showed that a contamination of the feed with OTA at 50 µg / kg induced a negative effect that can be detected after two weeks of feeding. The reduction in growth parameters due to OTA in the present study is consistent with the previous reports of Raju and Deveyowda (2000), Kumar et al. (2003), Verma et al. (2004) and Elaroussi et al. (2006). Huff et al. (1974) reported that OTA impaired protein metabolism system. Hsieh (1987) showed that OTA

inhibits the activity of phenylalanine t-RNA synthetase, an enzyme involved in the initial step of protein synthesis. Marquardt and Frohlich (1992) explained that OTA affects DNA, RNA and protein synthesis as well as carbohydrate metabolism. Elaroussi *et al*. (2006) showed that the reduction in growth parameters, feed conversion and feed consumption of OTA treated broiler chickens resulted from the decrease in serum T4 and increase in serum T3 hormones concentration. They also found that body weight and T4 concentrations in the control group were significantly higher than in the OTA-treated group. Wentworth and Ringer (1986) reported that T3 and T4 had a significant effect on growth of broiler chickens. However, it is important to emphasize that our results indicated that at the level of contamination used in the present study, the induced delay in growth can be catch up one week after feeding back an uncontaminated diet. It can be then concluded that the contamination level applied in this study was possibly too low to induce important and irreversible damages on chicken performances.

Interestingly, the AGRIMOS® supplementation had compensated the growth delay induced by OTA at week two and three, and resulted in a higher final body weight compared to the OTA group (I). Previous researches demonstrated that MOS and β-glucans could enhance broiler performance especially under unhygienic conditions (Tzianabos 2000, Huff *et al*. 2006, Tokic *et al*. 2007 and Yang *et al*. 2007). The enhancement of these agents can be explained in part by the improvement of intestinal function or gut health through the increase of villi height, uniformity and integrity (Huff *et al*. 2006). Surprisingly, at the end of the trial the OTA+AGRIMOS® group (III) showed a significant higher final body weight compared to the other treatments. This result is somewhat difficult to explain at this stage, but it can be assumed that the low contamination level in OTA and the associated effects on immune modulation has been responsible for an increase in the animal health status providing a benefit over the uncontaminated group.

Furthermore, no significant differences were observed throughout the experimental period between groups for both feed consumption and feed conversion. Opposite results were reported by Kubena *et al*. (1989), Raina *et al*. (1991) and Elaroussi *et al*. (2006). They mentioned that OTA contamination reduced feed consumption and deteriorated the feed conversion ratio. Indeed; the difference between the present and previous results might be due to the dose of OTA, then the age of the birds and the period of administration used in our study. Finally, the cumulative mortality rate of the

AGRIMOS® group (IV) recorded the lowest value, but without significant differences as compared to other groups. During the experimental weeks, no significant differences were recorded between groups, except after four week of age when, control group recorded the highest mortality rate.

The OTA group had the worst economic parameters: both Point spread and Performance index of OTA group (I) were significantly lower than OTA+AGRIMOS® group (III), while control (II) and AGRIMOS® group (IV) had almost similar value without any significant differences. However, no significant differences were found between groups in European performance index. It appears from these experiments that the use of AGRIMOS® in the feed of broiler chickens can significantly improve chicken zootechnical performance.

Boorman *et al*. (1984) already reported the immunotoxic effects induced by ochratoxin on cell mediated immunity with respect to both structural and functional alterations: decreased splenic cell count, reduce thymus size and increase bone marrow depression. Ochratoxin had also been shown to interfere with natural killer cell activity (Luster *et al*. 1987). Our results confirmed the immunotoxic effect of OTA on macrophage activity. This result agrees with Muller *et al*. (1995) who stated that ochratoxin has a non selective suppressive effect on various immune and defense reaction. Dhuley (1997) recorded significant decrease of chemotactic activity of murine macrophages, marked reduction in production of interleukin-1 (IL1) and tumor necrosis factor (TNF) alpha in mice intoxicated with ochratoxin. Singh *et al*. (1990) recorded significant reduction in cell mediated immune response as well as depression in phagocytic activity of splenic macrophages in broiler chickens fed with diet intoxicated with OTA.

Our results also indicated that group IV (AGRIMOS®) birds revealed significantly higher phagocyte percentage and phagocytic index over all the other groups at both 3 and 5 weeks of age; while OTA group (I) had the inferior values but without significant differences than OTA+AGRIMOS® (III) and control (II) groups. Additionally, group III chickens did not show any significant change as compared with control group (II). This may traduce that AGRIMOS® corrected the depression in phagocytosis induced by ochratoxin. Williams *et al*. (1999) suggested that the protective effect of β-glucans might be due to the antioxidant capacity as well as the inhibition of early activation of tissue muscular nuclear factor-KB (NF.KB) and NF-IL6. Mowat (1987) and Stokes *et al*. (1987) reported that β-glucans are known to possess antitumor, antioxidant, and antimicrobial activities by enhancing

the host immune functions. Chae *et al*. (2006) stated that β-glucans are beneficial for growth performance in broilers and increased CD8 cell. Vetvicka *et al*. (2007) recorded significant increase in phagocytosis of peripheral blood cells and antibody production induced by oral administration of β-glucans in balb/C mice. Macrophages are known to play an important role in resistance to infection. They are part of the non-specific first line of defense because of their ability to engulf and degrade invading microorganisms (Sharma and Tizard, 1984). Enhancement of phagocytic function is even expected to be applicable for therapy of microbial infection and cancer (Popov *et al*. 1999). Tizard (1996) and Stafford *et al*. (2002) reported that macrophages perform a variety of functions other than phagocytosis; they act as secretor cells, produce Nitric oxide that kill intracellular microorganisms, secrete many different proteins such as lysosomal enzymes and cytokines that play a key role in regulating immunity. With respect to lyzozyme activity, control group (II) at 3 weeks of age had the lowest values when compared to OTA+AGRIMOS® group (III). At 5 weeks of age, no differences were found between all groups. Lysozymes are proteins of low molecular weight found in polymorphonuclear cells and synthesized also in mononuclear cells. They are present in most tissue fluid except cerebrospinal fluid, sweet and urine. Lysozymes are considered as a number of innate humoral factors that elaborated from the body and showed domestic increase in their concentration (Weir 1983). Similar results were obtained by Karkowski *et al*. (1999) who recorded non significant differences in serum lysozme activity of pregnant mares injected with β-glucans.

It has been shown that ochratoxin exposure affects humoral immunity by reducing antibody production which results in significant decrease in haemagglutination inhibition (HI) antibody titers (Creppy *et al*. 1983, Stoev *et al*. 2000, Verma *et al*. 2004, Elaroussi *et al*. 2006 and Xue *et al*. 2010). In the present investigation, AGRIMOS® enhanced the humoral immunity in both immunocompromized and non-immunocompromized chickens. Karakowski *et al*. (1999) recorded significant increase in gamma globulin fraction, level of IgG in pregnant mares injected with β-glucans. Shashidhara and Devegowda (2003) in studying the effect of dietary MOS on broiler breeder production traits and immunity reported that the antibody responses against infectious bursal disease virus (IBDV) were significantly higher in MOS treated group and that the maternal antibody titers in progeny were also influenced by MOS supplementation.

The influence of OTA on relative Bursa weight was observed only at the fifth week of age. At this age OTA group had significantly lower relative Bursa weight than control. This result is concordant with previous studies looking at this indicator (Campbell *et al*. 1983, Chang *et al*. 1981, Huff and Doery, 1981 and Wang *et al*. 2009). For instance, Hatab (2003) reported that OTA contamination resulted in a significant reduction in relative Bursal weight. However, in this case, the reduction was observed as early as one week of age.

We also showed that AGRIMOS® lowered the macroscopic lesion score after VVNDv challenge and limited the histopathological lesions attributed to ochratoxicosis. Hanif *et al*. (2008) fed OTA and yeast (*Trichosporon mycotoxinivorans)* based toxin deactivator for broilers and found lowered lesion scores by 4. These results strengthen that AGRIMOS® is able to implement the innate-cell mediated immunity as well as the humoral immune response in both OTA intoxicated and non intoxicated chickens. Zou *et al*. (2006) studied the effect of MOS on growth performance and immunity of broilers and reported that Mannan oligosaccharides are natural oligosaccharides, composed of various mannose chains that block pathogens colonization within the digestive tract of the animal, while β-glucans are essential components of yeast cell walls that are particularly important to support the non-specific immune system of the animal. They concluded that MOS limits pathogenic bacterial development within the gastro intestinal tract, favors beneficial intestinal flora without being used as a substrate for pathogens, and stimulates the immune response. MOS and β-glucans have been shown to improve immune response and to block bacterial adhesion (especially enteric pathogens) to gut lining (Volman *et al*. 2008).

In conclusion; administration of a specific combination of Mannan-oligosaccharides and β-glucans extracted form yeast cell wall (AGRIMOS®) to chickens improved zootechnical parameters and had a potent immunomodulatory effect in the form of evoking immune response and enhancing vaccination effectiveness. Also, it helps not only in controlling chicken ochratoxicosis but also can play a positive role in treating chicken immune dysfunction.

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Table 1. Composition of the broilers 3-phase diets (g/kg as fed) and their calculated chemical composition (on as fed basis)

Ingredients	Starter	Grower	Finisher
Yellow corn	524.5	544.2	628.5
Soybean meal 44%	332.4	299.1	221.1
Corn gluten meal 60%	70	70	66.5
Oil	30	43.8	40
Di-calcium phosphate	18	18	18
Lime stone	13	13	13
D.L. Methionine	2.2	2.1	2.3
Lysine hydrochloride	2.9	2.8	3.6
Sodium chloride	4	4	4
Premix[1]	3	3	3
Calculated analysis			
Crude protein %	230	220	190
Metabolizable energy (kcal/kg)	3000	3150	3200

[1]Each gram of mineral mixture contained: vitamin A (trans-retinyl acetate), 9,000 IU; vitamin D3 (cholecalciferol), 2,600 IU; vitamin E (dl-α-tocopheryl acetate), 16 mg; vitamin B1, 1.6 mg; vitamin B2, 6.5 mg; vitamin B6, 2.2 mg; vitamin B12 (cyanocobalamin), 0.015 mg; vitamin K3, 2.5mg; choline (choline chloride), 300 mg; nicotinic acid, 30 mg; pantothenic acid (d-calcium pantothenate), 10 mg; folic acid, 0.6 mg; d-biotin, 0.07 mg; manganese (MnO), 70 mg; zinc (ZnO), 60 mg; iron (FeSO4 H_2O), 40 mg; copper ($CuSO_4$ $5H_2O$), 7 mg; iodine [$Ca(IO_3)_2$], 0.7 mg; selenium (Na_2SeO_3), 0.3 mg.

Table 2. Average body weight, body weight gain, feed intake, feed conversion, mortality and performance indexes (two-way ANOVA)

	I OTA	II Control	III OTA+AGRIMO	IV AGRIMOS	SEM	P value
Body weight (g)						
on d 1	40.4	39.8	40.7	39.6		
wk 1	149.7[b]	153.2[a]	152.1[a]	156.9[a]		
wk 2	360.0[b]	404.1[a]	405.4[a]	414.7[a]		
wk 3	746.2[b]	813.0[a]	838.4[a]	823.6[a]		
wk 4	1454.2[b]	1507.3[ab]	1542.3[a]	1549.4[a]		
wk 5	2022.7[c]	2061.2[bc]	2157.2[a]	2120.4[ab]		
Body gain (g)						
wk 0-1	109.3	113.2	111.4	117.3		
wk 1-2	210.7b	250.9[a]	253.4a	258.3a		
wk 2-3	381.0[b]	408.9[ab]	427.1[a]	409.3[ab]		
wk 3-4	691.9	660.8	688.5	710.8		
wk 4-5	578.4	554.0	586.2	571.0		
wk 1-5	1982.3[c]	2021.5[bc]	2116.9[a]	2080.7[ab]		
Daily feed intake (g/head)						
d 1-35	157.1	145.5	148.3	157.1		
FCR						
d 1-35	1.577	1.477	1.480	1.537		
Mortality (%)						
wk 1	0.0	0.0	3.03	0.0		
wk 2	0.0	3.03	3.03	0.0		
wk 3	0.0	6.07	0.0	6.07		
wk 4	6.07b	0.0a	0.0a	0.0a		
wk 5	3.03	0.0	0.0	3.03		
wk 1-5	9.1	9.1	6.07	9.1		
Point spread (%)	288.2[b]	306.5[ab]	329.2[a]	314.2[ab]		
Performance Index	283.5[b]	308.3[ab]	322.8[a]	305.0[ab]		
EPEF	317.0	342.9	359.9	351.7		

FCR: Feed conversion rate; EPEF: European Performance Efficiency Factor
Values in the same row with different superscripts a,b,c were significantly ($P < 0.05$) different. Number of samples per group = 90

Table 3. Macrophage activity, serum lysozyme activity and Nitric oxide content at 3 and 5 weeks of age.

	Age	I OTA	II Control	III OTA+AGRIMOS	IV AGRIMOS	SEM	P value
Phagocytic %	3 wk	58.33[b]	61.25[b]	61.00[b]	65.50[a]		
	5wk	59.00[b]	60.50[b]	63.75[b]	71.00[a]		
Phagocytic index	3 wk	0.080[b]	0.123[b]	0.133[b]	0.253[a]		
	5wk	0.100[b]	0.140[b]	0.160[b]	0.258[a]		
Lysozyme (µg/ml)	3 wk	9.85[ab]	2.73[b]	17.00[a]	9.85[ab]		
	5wk	9.85[a]3	6.28[a]	9.85[a]	7.53[a]		
Nitric oxide (µg/ml)	3 wk	10.75[c]	13.25[bc]	17.75[ab]	19.50[a]		
	5wk	17.50[a]	21.25[a]	24.50[a]	17.50[a]		

Values in the same row with different superscripts a,b,c were significantly (P<0.05) different. Number of samples per group = 10.
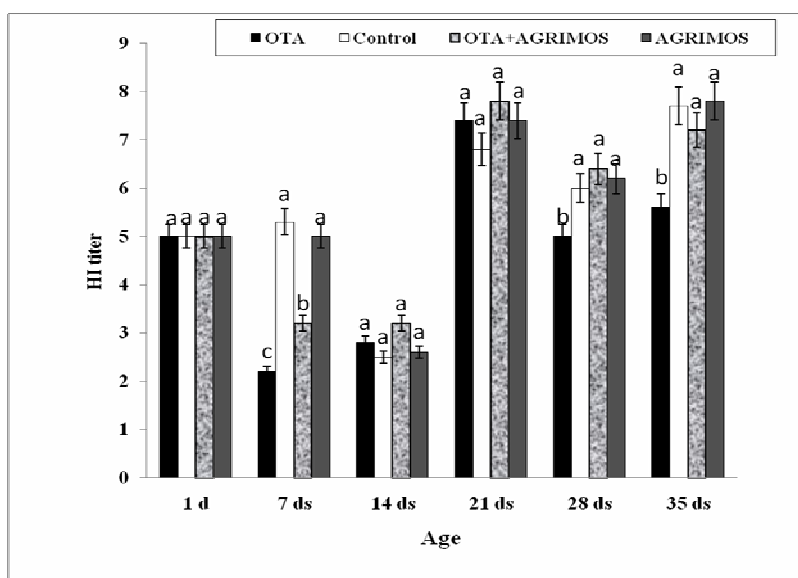
Figure 1. Haemaglutination inhibition (HI) against Newcastle disease virus (NDv) during the first 35 days of chickens' life.
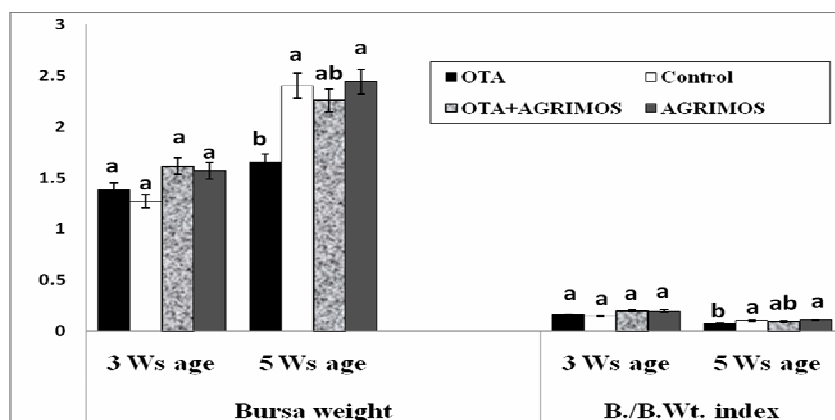
Figure 2.   Bursa weight and Bursa/Body weight indexes of ochratoxicated and non-ochratoxicated, AGRIMOS® treated and untreated chickens versus blank control chicken groups.
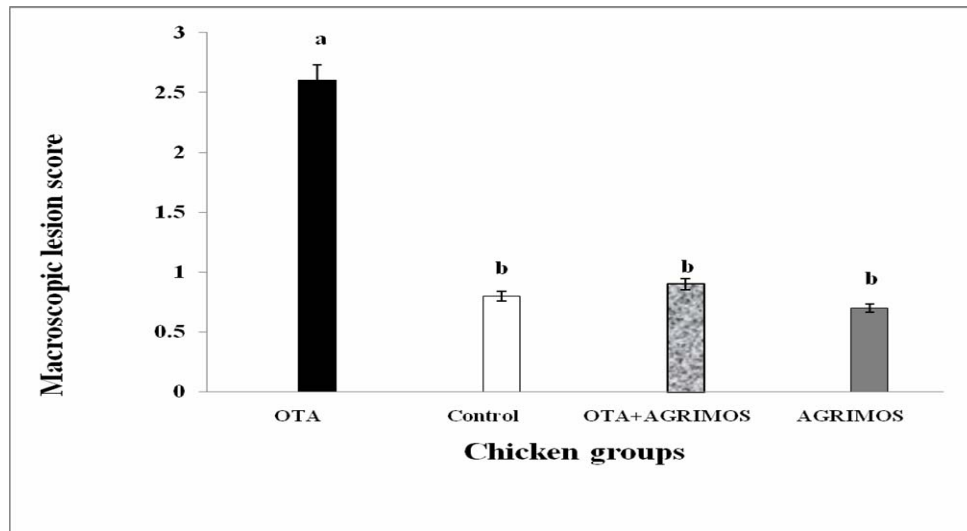
Figure 3. Results of macroscopic lesion scores of velogenic viscerotropic Newcastle disease virus (VVNDv) challange of ochratoxicated and non-ochratoxicated AGRIMOS® treated and untreated chickens versus blank chicken group.
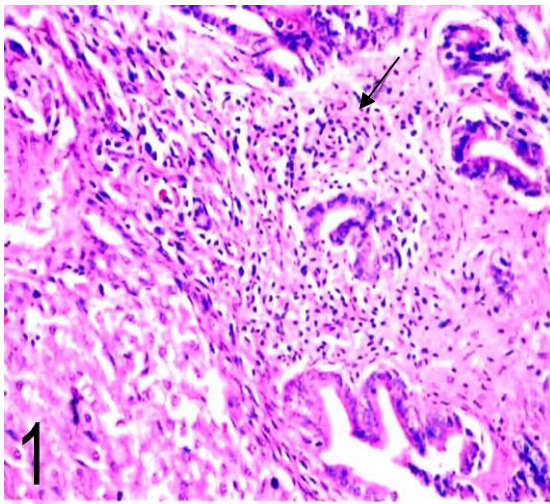
Photo 1: Liver (gr.I) showing chronic cholangitis. Notice the fibrous connective tissue proliferation and massive inflammatory cells infiltration in the wall of bile duct (arrow) (H&E x200)	Photo 2: Liver (gr.I) showing focal hepatic necrosis replaced by mononuclear leucocytes (arrow) (H&E x200)
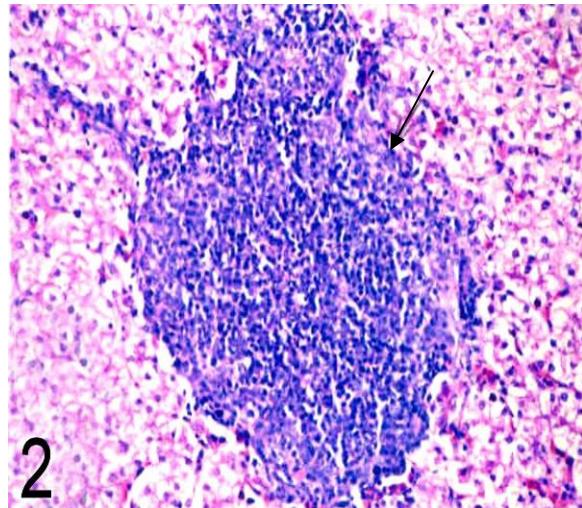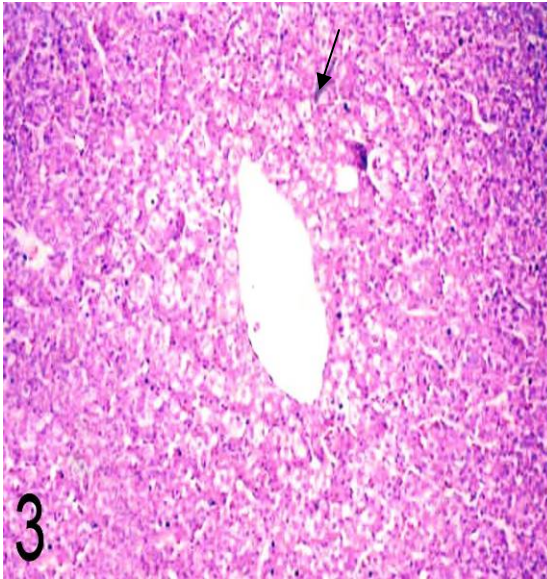
Photo 3: Liver (gr.IV) showing vacular degeneration of centrolobular hepatocytes (arrow) (H&E x200)
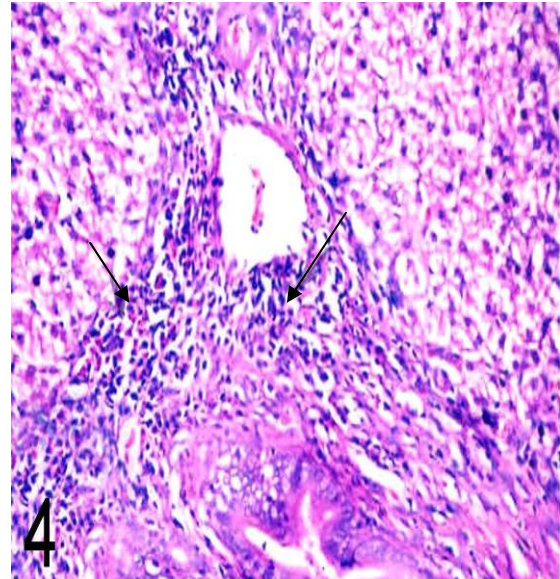
Photo 4: Liver (gr.III) showing vacular degeneration of hepatocytes, slight thickening in the wall of bile ducts associated with leucocytic cells infiltration (arrow) (H&E x200)
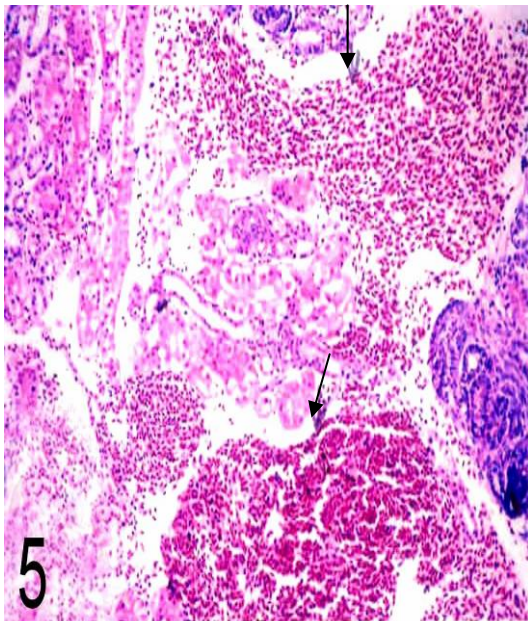
Photo 5: Kidney (gr.I) showing massive interstitial haemorrhage (arrow) (H&E x100)
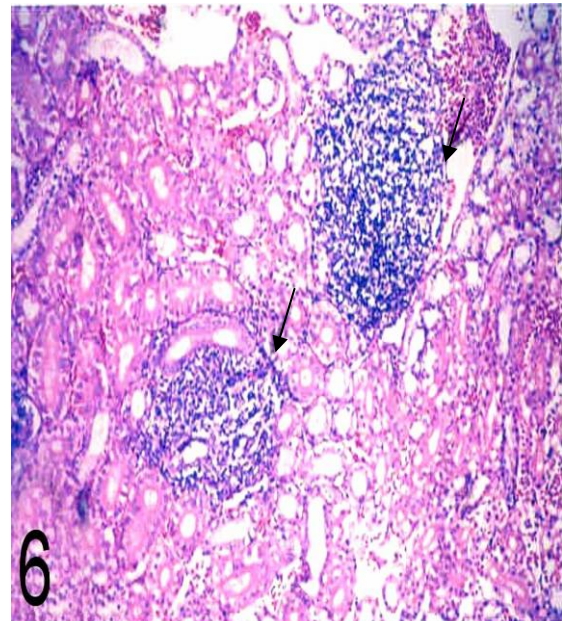
Photo 6: Kidney (gr.I) showing multiple focal areas of necrosis completely replaced by massive leucocytes (arrow) (H&E x100)
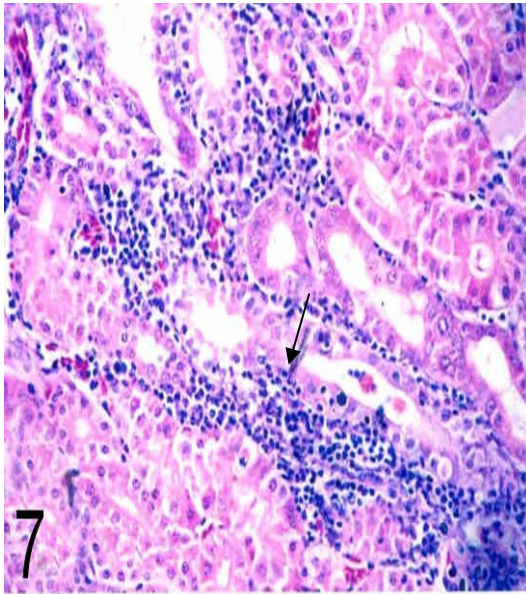
Photo 7: Kidney (gr. III) showing peritubular leucocytic cells infiltration (arrow) (H & E x200)
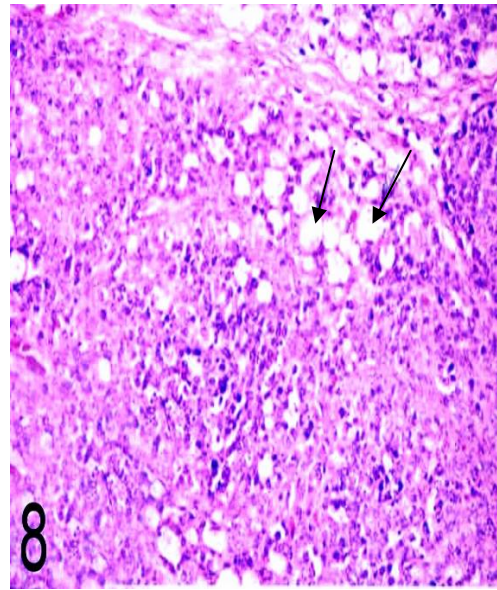
Photo 8: Bursa of Fabricius (gr. I) showing vaculations of lymphoid follicles (arrow) (H & E x200)
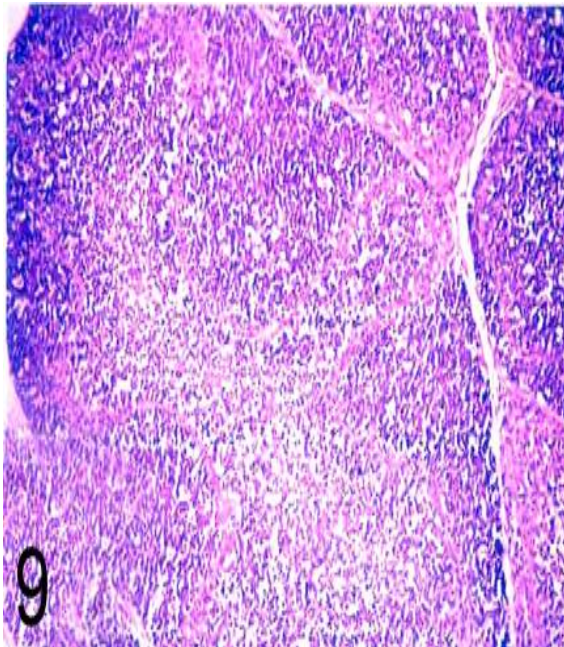
Photo 9: Bursa of Fabricius (gr. III & IV) showing no histopathological changes (H & E x100)
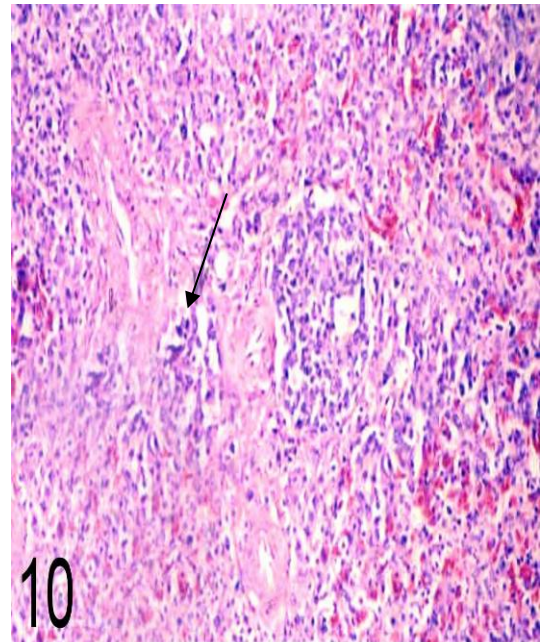
Photo 10: Spleen (gr. I) showing atrophy of lymphoid follicles (arrow) (H & E x200)
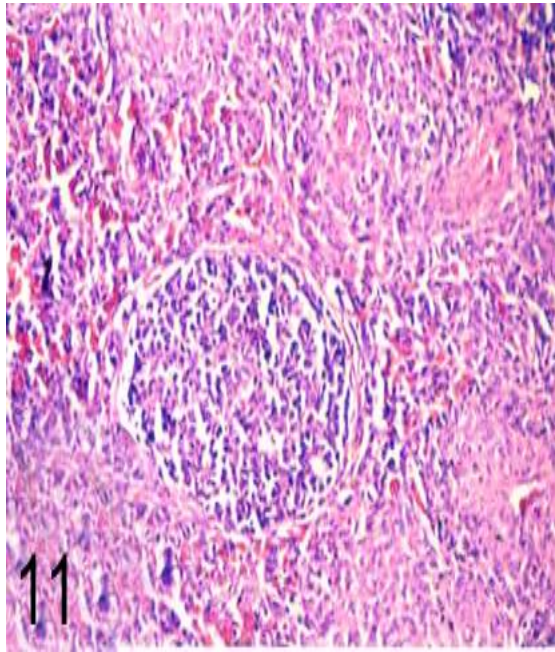
Photo 11: Spleen (gr. III & IV) showing no histopathological changes (H & E x200)
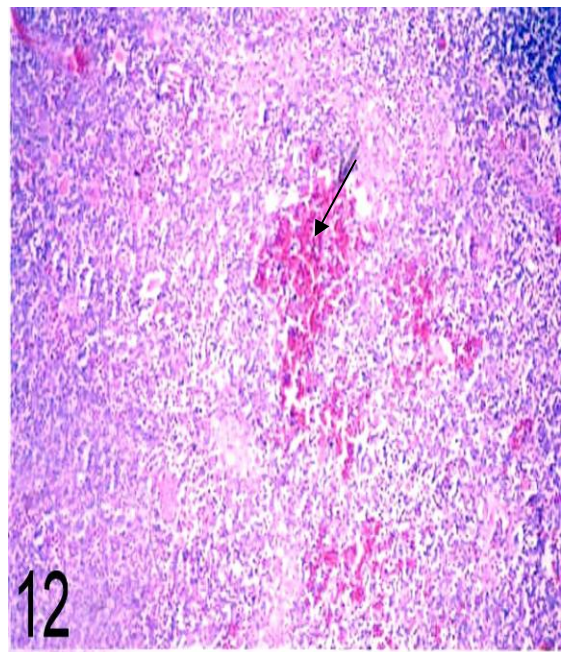
Photo 12: Thymus gland (gr. I) showing focal thymic haemorrhage (arrow) (H & E x100)
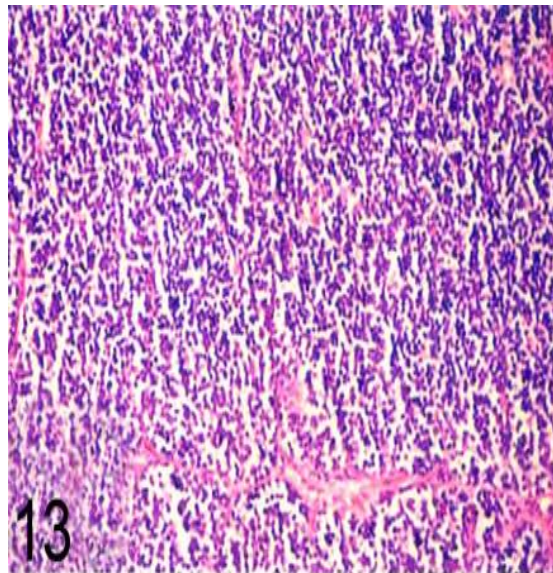
Photo 13: Thymus gland (gr. III & IV) showing no histopathological alterations (H & E x00)

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Pathological Studies on Experimental Systemic Candidiasis induced by Candida albicans Isolated from Different Animals in Immunosuppressed Mice

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Abstract: Fifty male mice (6 weeks old) were used to evaluate the severity of the pathological lesions induced by *Candida albicans* isolated from different animals (goats, sheep, cattle and buffaloes). The mice were immune suppressed by subcutaneous injection of 0.5 mg cortisone/kg B wt for 5 successive days before the beginning of the experiment and extended to the first 5 days after *Candida albicans* inoculation. These mice were randomly assigned to five groups (n=10). These groups intravenously (via tail vein) inoculated with 0.5 ml suspension of candida albicans 1 x 10^6 *blastospores* isolated from goats (gp 1), sheep (gp 2), cattle, (gp3) or buffaloes (gp 4), besides the gp (5) which inoculated with phosphate buffer solution (PBS) as a control group. The clinical signs, mortalities and the gross lesions were recorded before different specimens from lungs, heart, liver, kidneys, spleen and brain collected and were routinely processed for histopathological examination. Multiple granulomas were detected replacing the pulmonary tissue, pleura, myocardium, hepatic and renal parenchyma of gps (1 and 2). Such granulomas were represented by central basophilic structureless mass containing blastospores, pseudohyphae, hyphae and oval yeast cells, 3-8 μm in diameter, surrounded by a thick zone of mononuclears mostly of macrophages and lymphocytes besides few polymorphnuclear cells. Fibrinonecrotic pseudomembranes and multifocal suppurative areas were observed in the pleura and pericardium. Meanwhile, the gps (3 and 4) showed minimal lesions and poor fungal growth besides lowering in mortalities from 70-80%(gps 1 and 2) to 30-40% (gps 3 and 4). Finally, it could be concluded that the *Candida albicans*, isolated from goats and sheep, induced severe multiple lesions than that isolated from cattle and buffaloes.

1. Introduction

Candidiasis is a disease caused by a dimorphic Candida sp. which is part of the normal flora found in the upper respiratory, gastrointestinal and female genital tract of the human body (Brawner and Cutler 1989 and Kumamoto and Vinces 2005). Most cases of Candida infection result from *Candida albicans*, which is an opportunistic infection as it does not induce disease in immunocompetent individuals but can only do so in those with impaired host immune defenses (De Repentigny et al 1992 and Jarvis and Martone 1992).

Nowadays, the incidence of invasive fungal infection has been increasing, mostly due to advances in medicine that may produce immunocompromised individuals (Nakayama et al 2010). Candida species are implicated in cases of superficial and disseminated candidiasis due to the administration of broad-spectrum antibiotics, corticosteroids and immunosuppressive drugs (Fraser et al 1992 and Ozcan et al 2006). Diabetes mellitus, viral infections, and urinary and venous catheters were also reported as risk factors (Hamir et al 2000 and Pressler et al 2003) and the patients are often resistant to

conventional antifungal therapy and may cause high morbidity and mortality rates (Morgan 2005 and Spellberg et al 2006) . *Candida* pathogen was accounted for approximately 50 to 60% of all *Candida* blood culture isolates (Wisplinghoff et al 2004) and by far the most common species causing infections in humans (Vazquez and Sobel 2002) and can invade and damage a wide range of host tissues during systemic infections that often results in death, even in patients treated with antifungal agents (Roilides et al 2003).

Omuta et al (2007) reported that the systemic candidiasis tends to cause lesions in the lungs, kidneys, heart, spleen and brain. *C. albicans* infection was assessed by evidence of lesions and by presence of hyphae on the affected organs (O'Grady and Reade 1993). Multiple pyogranulomatous lesions with blastospores, pseudohyphae, and true hyphae of *Candida albicans* were observed in various organs in dogs with systemic candidiasis (Tunca et al 2006 and Matsuda et al 2009). Hosogi et al (2008) observed apoptosis of alveolar epithelial cells in mice that given an intravenous injection of Candida albicans inducing acute lung injury. Ashman (1998) reported

that the mouse model of acute infection with *C. albicans* is a valuable experimental model for studying microbial pathogenesis, as it includes many of the clinical features of the human condition.

The objective of this work was to evaluate the severity of the pathological lesions of *Candida albicans* isolated from different animals species (goats, sheep, cattle and buffaloes).

2. Material and Methods
Animals:

Fifty male mice (6 weeks old and weighed 20 to 25 gm) were obtained from the Unit of Laboratory Animal, Faculty of veterinary medicine, Zagazig University, Egypt. Mice were placed in polycarbonate cages with stainless-steel wire tops and maintained at 24 to 26°C with 55 to 75% humidity and a 12-h light/dark cycle, and fed a commercial rodent diet and given water ad libitum. The mice received humane care. They were immune suppressed by subcutaneous injection of 0.5 mg cortisone/kg B wt• for 5 successive days before the beginning of the experiment and extended to the first 5 days after *Candida albicans* inoculation (Kamai et al 2001).

Source and Culture of Fungal Strains

Samples obtained from the tongues with lesions of goats, sheep, cattle and buffaloes (table,1) were plated directly in Sabouraud dextrose agar (Difco Laboratories, Detroit, MI, USA) added with chloramphenicol (0.1 mg/ml), and incubated at 37°C for 48 h (Cruickshank et al 1975 and Freire-Garabal et al 1999). The isolated organisms were identified as *C. albicans* by the germ tube test, chlamydospore production as described by Schaar et al (1974) and

the API 20 C AUX kit "biomérieux, Marcy-L'Etoile, France" (Nowotny 1979 and Sandvén 1990).

Table (1): Source and number of total and positive sample

Animal Source	No. of sample	No. of positive sample	%
Goats	28	16	57.14
Sheep	25	14	56
Cattle	23	13	56.52
Buffaloes	26	11	42.31
Total	102	54	52.94

Inoculum preparation

Candida albicans isolated from the different animals were subcultured on Sabouraud dextrose agar and incubated at 37°C for 3 days. Loopful from the growth was suspended in phosphate buffer solution (PBS) and compared with MacFarlane number 3 for turbidity containing 1×10^6 cfu/ml (Hoyer et al 1999).

Mice inoculation

On day 6 after cortisone injection, these mice were randomly assigned to five groups (n=10). These groups intravenously (via tail vein) inoculated with 0.5 ml suspension of 1×10^6 Candida albicans *blastospores* isolated from goats (gp 1), sheep (gp 2), cattle, (gp3) or buffaloes (gp 4), besides the gp (5) which inoculated with phosphate buffer solution (PBS) as a control group (table, 2).

Table (2):Mice groups, number of mice, treatment and number of dead and sacrificed mice.

Groups	No. of mice	Treatment $1x10^6$ blastospores of	No. of dead mice within 8-14 day post inoculation	No. of sacrificed mice on 15th day post inoculation
1	10	C. albicans from goats	7	3
2	10	C. albicans from sheep	8	2
3	10	C. albicans from cattle	4	6
4	10	C. albicans from buffaloes	3	7
5	10	Control (PBS injection)	0	10
Total	50		22	28

• Fortcortin contain 8 mg/2 ml dexamethasone, Merck-Germany

Pathological Examination

The clinical signs and mortality rate were evaluated. At the end of the experiment, all remaining mice were sacrificed for complete necropsy and all macroscopic abnormalities were recorded in *C. albicans*-infected mice in each group. Specimens from the lungs, heart, liver, kidneys, spleen and brain were collected and fixed in 10% neutral buffered formalin solution. Five micron thick paraffin sections were prepared and stained by hematoxylin and eosin (HE) and periodic acid-Schiff (PAS) for histopathological examinations (Bancroft and Stevens 1996).

3. Results

Clinical Signs and Mortality rates:

Dyspnea, anorexia and distended abdomen besides emaciation and ruffled hair coat were the most common clinical signs of the *C. albicans* particularly that isolated from goats and sheep. The mortalities were high in gps 1 and 2 (70% and 80%), respectively and they were low in mice of gps 3 and 4 (40% and 30%), respectively.

Pathological Findings:

Macroscopically, the mice infected with *C. albicans* isolated from goats (gp 1) or from sheep gp (2) showed yellowish-white mottled lungs with numerous red spots 1-3 mm in diameter. Multifocal pale yellow foci on the liver (Fig 1), the epicardium and the adjacent myocardium were noticed.

Microscopically, multiple granulomas were detected replacing the pulmonary tissue (Fig 2), pleura (Fig 3), myocardium (Fig 4), hepatic (Fig 5) and renal (Fig 6) parenchyma. Such granulomas were represented by central basophilic structureless mass containing blastospores, pseudohyphae, hyphae and oval yeast cells, 3-8 μm in diameter, surrounded by a thick zone of mononuclears mostly of macrophages and lymphocytes besides few polymorphnuclear cells (Fig 7). The fungal elements were stained pink by PAS reaction (Fig 8).

Fibrinonecrotic pseudomembranes and multifocal suppurative areas were observed in the pleura (Fig 9) and pericardium (Figs 10 and 11). Basophilic suppurative emboli in the perialveolar (Fig 12) and peritubular (Fig 13) capillaries were detected in the lungs and kidneys; respectively besides myomalacia cordis in the myocardium (Fig 14). Interstitial aggregations of mononuclears were seen in the kidneys (Fig 15), liver and heart. Periportal hepatocytes showed coagulative necrosis,

represented by pyknosis and karyorrhexis (Fig 16), and the cytoplasm of some necrotic hepatocytes showed refractile eosinophilic granules (Fig 17). Congestion, hemorrhage and degenerative changes (Fig 18) besides cellular casts were visualized in the affected kidneys (Fig 19). Sometimes, the granulomas revealed numerous neutrophils with minimal fibroblastic proliferation, particularly in the lungs and kidneys (Fig 20). Zenker's necrosis and edema with widely separated cardiac muscle fibers were noticed in the heart of most infected cases.

Focal and mild microscopic lesions were also seen in the brain and spleen. The brain showed edema in the Virchow Robin spaces and ventricles besides some degenerated neurons, satellitosis and neuronophagia (Fig 21). Few scattered neutrophils were observed infiltrating the pia mater. Meanwhile, the spleen revealed significant depletion in the lymphocytes of white pulp and hemosiderosis in the red pulp (Fig 22).

Meanwhile, the mice infected with *C. albicans* isolated from cattle (gp 3) or from buffalo (gp 4) showed congested and edematous lungs. The liver and other organs were moderately congested. The described lesions were mostly minimal with poor fungal growth (blastospores and budding cells) in the tissue comparing with the previous groups. The lungs showed focal thickening of the interalveolar septa with septal cells proliferation and polymorphnuclear cell infiltrations. Bronchiolitis with desquamated lining epithelium, severe congested blood vessels and inflammatory cells infiltration was also seen (Fig 23).

Perivascular and alveolar edema were visualized. The heart revealed thickening of the pericardium with edema infiltrated with few neutrophils (Fig 24). Such edema was extended to the myocardium represented by widely separated cardiac muscles fibers and around the cardiac blood vessels (Fig 25). The liver showed congestion of the hepatic blood vessels and sinusoids (Fig 26) with few round cells infiltrating the portal areas. Small areas of coagulative necrosis were rarely encountered. The kidneys showed congestion of the renal blood vessels and hemorrhage among the renal tubules (Figs 27 and 28). Some renal tubular epithelia showed hydropic degeneration and coagulative necrosis. Focal interstitial aggregations of leukocytes predominantly neutrophils were seen.
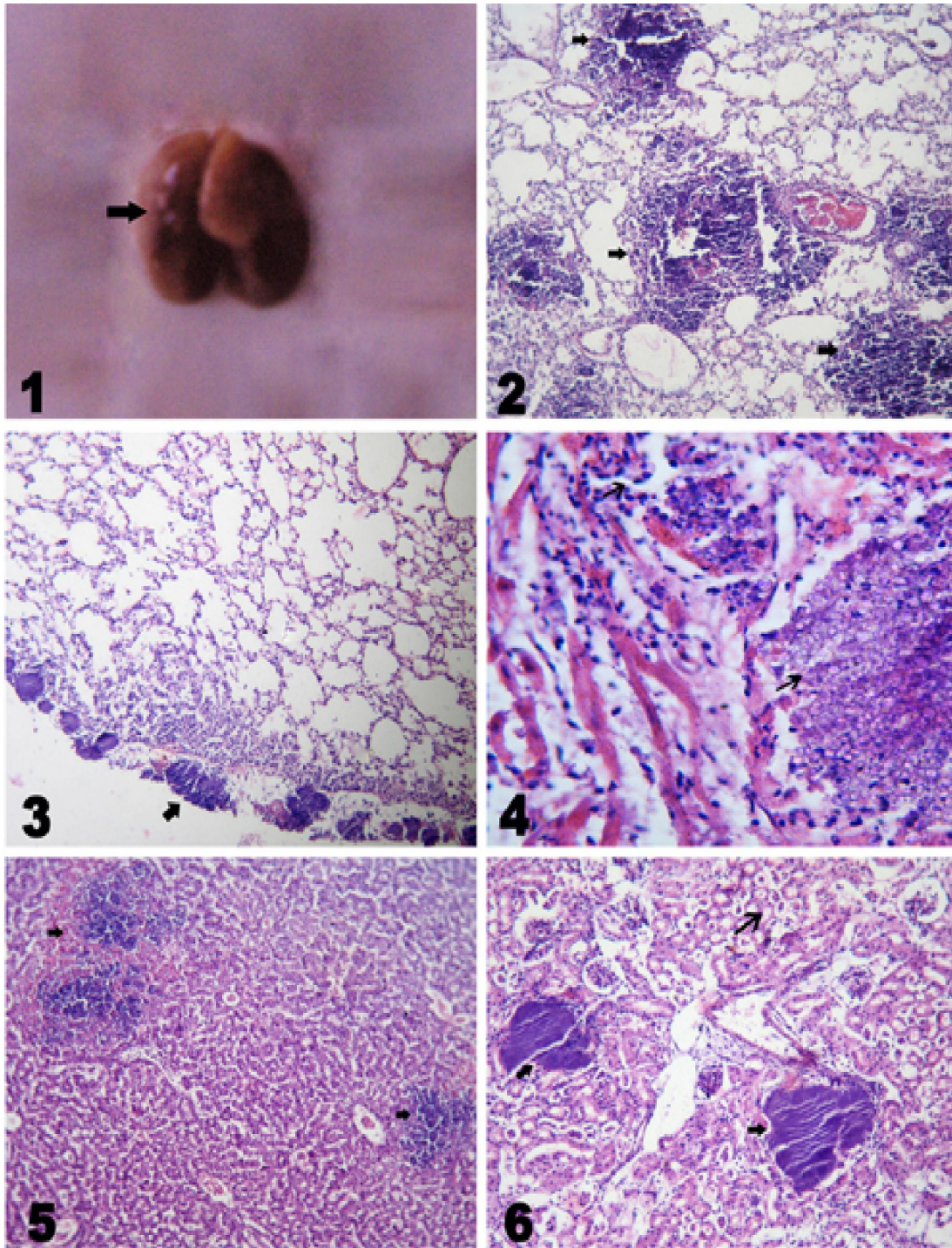
Figs (1-6): Groups (1 and 2) are similar.
Liver showing multifocal pale yellow foci **(1).**
Multiple granulomas replacing the pulmonary tissue ,HE x300 **(2)**, pleura, HE x300 **(3)**,myocardium, HE x1200 **(4)**,hepatic, HE x300 **(5)** and renal parenchyma, HE x300 **(6).**
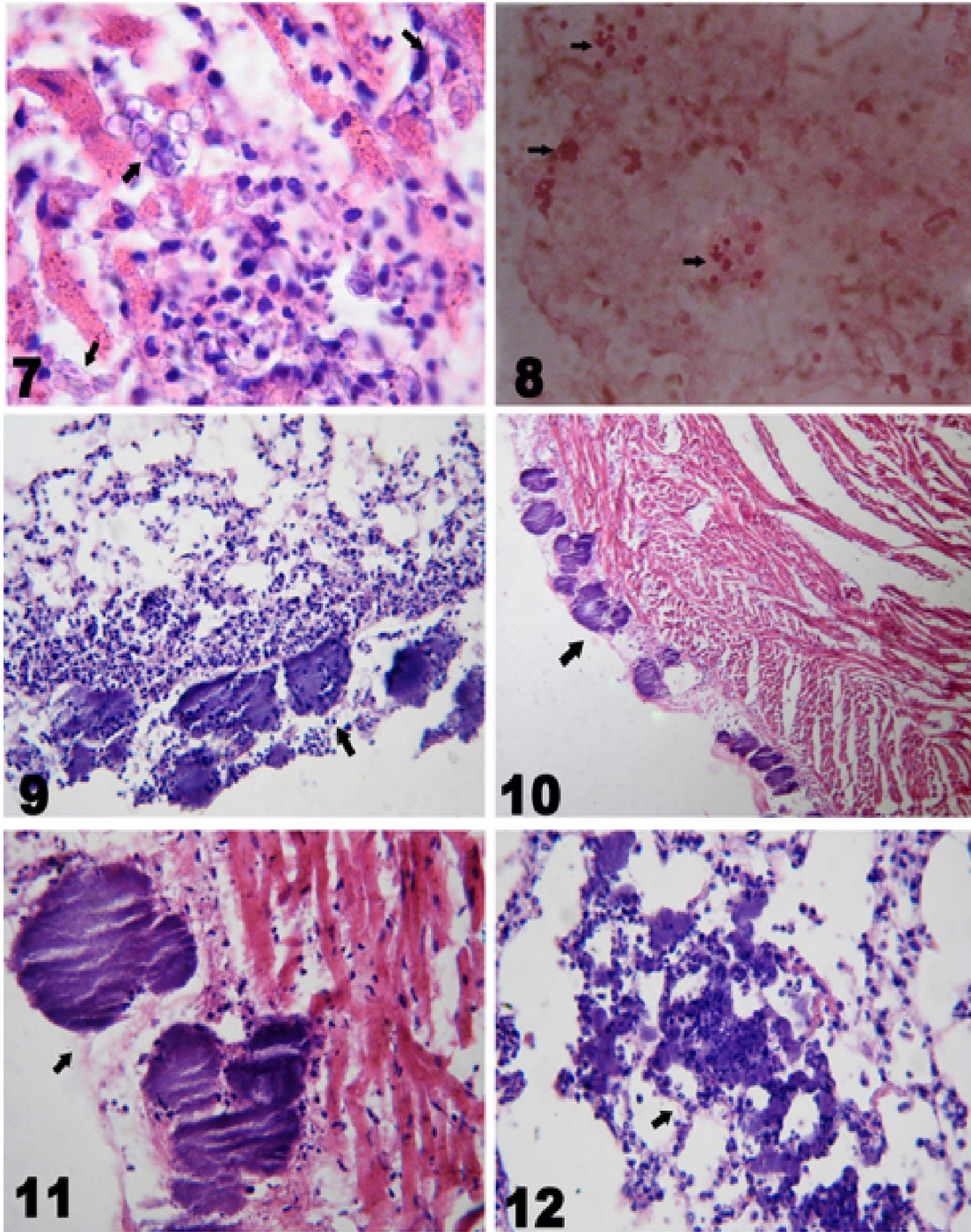
Figs (7-12): Groups (1 and 2) are similar.
Heart showing blastospores, pseudohyphae and hyphae among the fragmented cardiomyocytes,HEx3000 **(7).** The blastospores were stained pink by PAS stain x3000 **(8).**
Fibrinonecrotic pseudomembranes and multifocal suppurative areas were observed in the pleura,HEx1200 **(9)** and pericardium, HEx300 **(10)** and HEx1200 **(11).** Lung showing basophilic suppurative emboli in the perialveolar capillaries, HEx1200 **(12).**
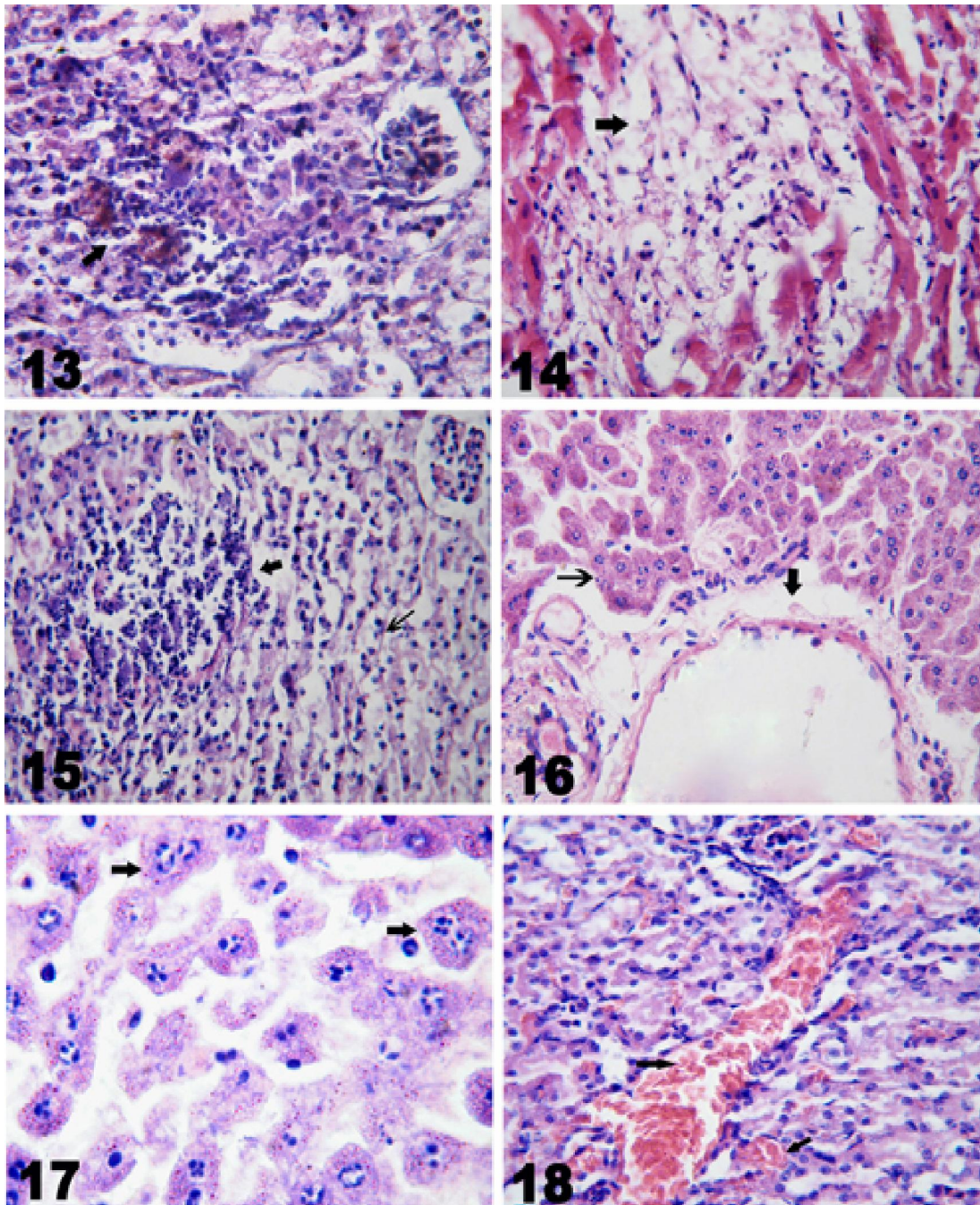
Figs (13-18): Groups (1 and 2) are similar.
Kidney showing basophilic suppurative emboli in the peritubular capillaries,HEx1200 **(13).** Heart showing myomalacia cordis in the myocardium, HEx1200 **(14)**. Kidney showing interstitial aggregations of mononuclears , HEx1200 **(15).** Liver showing coagulative necrosis of periportal hepatocytes, represented by pyknosis and karyorrhexis, HEx1200 **(16)**, and the cytoplasm of some necrotic hepatocytes showed refractile eosinophilic granules, HEx3000 **(17).** Kidney showing congestion, hemorrhage and degenerative changes, HEx1200 **(18).**
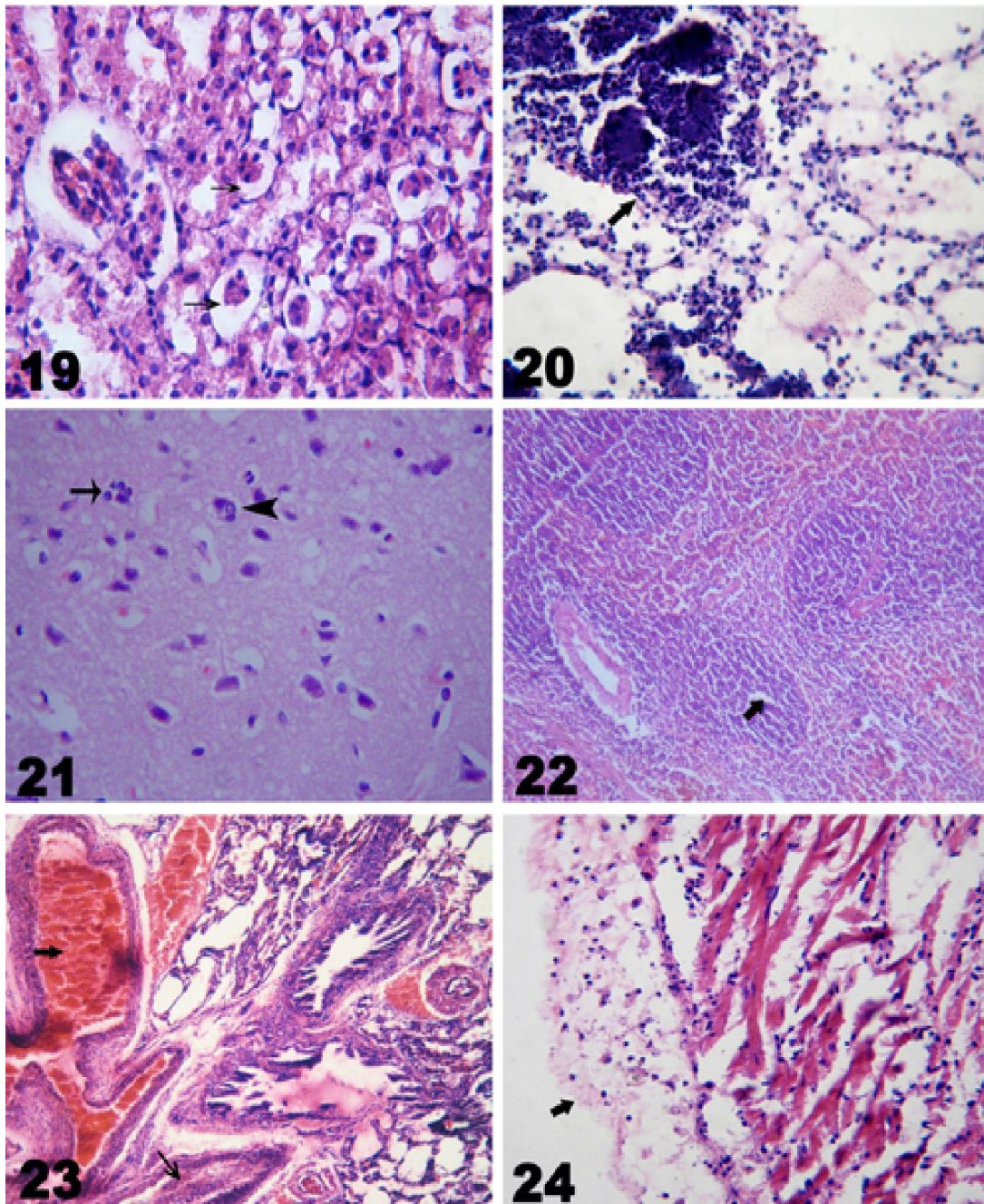
Figs (19-22): Groups (1 and 2) are similar.

Kidney showing cellular casts inside the lumens of some renal tubules , HEx1200 **(19).** Lung showing granuloma with numerous neutrophils with minimal fibroblastic proliferation, HEx1200 **(20).** Brain showing some degenerated neurons, satellitosis and neuronophagia, HEx1200 **(21).** Spleen showing depletion in the lymphocytes of white pulp , HEx300 **(22).**

Figs (23&24): Groups (3 and 4) are similar

Lung showing bronchiolitis with desquamated lining epithelium, severe congested blood vessels and inflammatory cells infiltration , HEx300 **(23).** Heart showing thickening of the pericardium with edema infiltrated with few neutrophils, Hex1200 **(24).**
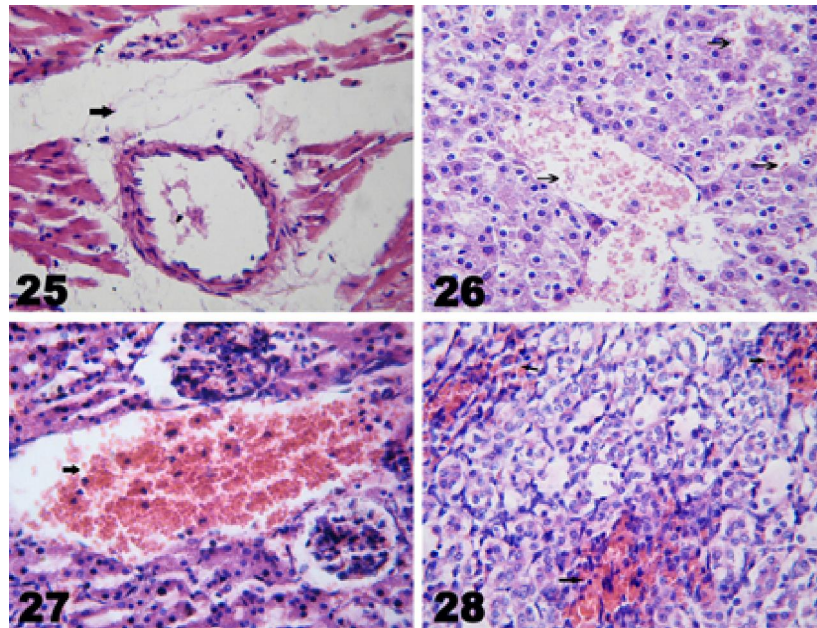
Figs (25-28): **Groups (3 and 4)** are similar

Heart showing edema represented by widely separated cardiac muscles fibers and around the cardiac blood vessels, HEx1200 **(25).** Liver showing congestion of the hepatic blood vessels and sinusoids, HEx1200 **(26).** The kidneys showed congestion of the renal blood vessels **(27)** and hemorrhage among the renal tubules, HEx1200 **(28).**

4. Discussions

It is evident that the *C. albicans* isolated from sheep and goats were the most pathogenic among the *C. albicans* isolated from the different animals (cattle and buffaloes) used in this study. They induced high mortalities among the examined mice of gps (1 and 2). Such high mortality (70 and 80%) may be due to the presence of large numbers of blastospores in the examined organs. Blastospores were more rapidly and consistently fatal to mice than the hyphae (Evans 1981). Allendoerfer et al (1993) found that a high-dose intravenous injection of *C. albicans* was associated with increased levels of TNF- and an increased mortality in mice. Scoring of candidiasis and its associated lesions in the lungs, heart, liver, kidneys, spleen and brain could explicate the pathogenesis of *C. albicans* in mice and its spread through the blood (Clancy et al 2000).

C. albicans isolated from sheep and goats induced multiple granulomas replacing the pulmonary tissue, pleura, myocardium, hepatic and renal parenchyma. Such granulomas were represented by central basophilic structurless mass containing blastospores, pseudohyphae, hyphae and yeast cells; surrounded by a thick zone of mononuclears mostly of macrophages and lymphocytes besides few polymorphnuclear cells. Fibrinonecrotic pseudomembranes and multifocal suppurative areas were observed in the pleura and pericardium. Basophilic suppurative emboli in the

perialveolar and peritubular capillaries were detected in the lungs and kidneys; respectively besides myomalacia cordis in the myocardium. Focal areas of coagulation necrosis, represented by pyknosis and karyorrhexis, and interstitial aggregations of mononuclears were seen in the kidneys, liver and heart. Congestion, hemorrhage and degenerative changes besides cellular casts were visualized in the affected kidneys. Zenker's necrosis and edema with widely separated cardiac muscle fibers were noticed in the heart of most infected cases. The brain showed edema in the Virchow Robin spaces and ventricles besides some degenerated neurons, satellitosis and neuronophagia. Meanwhile, the spleen revealed significant depletion in the lymphocytes of white pulp. Moreover, the significant correlation between the severities of lesions was accompanied by the *C. albicans* isolates, where it became mild and caused the least damage and, quite notably, formed fewer blastospores and budding cells in tissue of gps (3 and 4) and with low mortalities (30 and 40%).

The previous results are in agreement with the experimental studies conducted by Vose et al (2001), Feman et al (2002) and Tunca and Haz roglu (2004).They observed that systemic candidiasis tend to cause lesions and candida was observed in the lungs, kidneys, heart, liver spleen, myocardium, pericardium and brain. Tunca et al (2006) also observed multiple pyogranulomatous lesions besides blastospores, pseudohyphae, and true hyphae of

Candida albicans in various organs in male dogs with systemic candidiasis. The current study revealed that *C. albicans* isolated from sheep and goats induced suppurative pneumonia infiltrated by neutrophils, lymphocytes and some macrophages. Blastospores were also seen in the centers of the lesions. This result is disagreement with Trudeau and Saranac (1990) who found that the pulmonary tissue was quantitatively very resistant to *C. albicans* infection because of the ability of resistant pulmonary alveolar macrophage to rapid phagocytozed and kill yeast. Moreover, the presence of neutrophils in the pyogranulomatous lesions, could help the macrophages in the phagocytic process and play an important role in the prevention of fungal growth and the invasion of tissues. Defects in neutrophil number and function have been consistently implicated in the pathogenesis of disseminated candidiasis (Fradin et al 2005). Subsequently, neutropenia has been consistently implicated as a risk factor for the development of disseminated candidiasis (Hope et al 2002, Sallah et al 2001 and Uzun et al 2001). The exact mechanisms of pathogenesis of systemic candidiasis remain incompletely understood and no specific virulence factor is dominant (Calderone and Fonzi 2001). Rather, pathogenesis depends upon the coordinated expression of multiple genes in a manner that facilitates proliferation, invasion and tissue damage within the given in vivo milieu (Mahan et al 2000, Staib et al 2000 a and b and Fradin et al 2003). A number of *C. albicans* genes are likely to play roles in the pathogenesis of candidal disease at diverse tissue sites (Mahan et al 2000, Staib et al 2000 a and b and Fu et al 2002), however, other genes are likely to make distinct temporal-spatial contributions to virulence (Muhlschlege and Fonzi 1997 and De Bernardis et al 1998). Past studies have shown that *C. albicans* produces farnesol in vitro (Hornby et al 2001) and that increased production of farnesol in vivo is accompanied by increased virulence of *C. albicans* (Navarathna et al 2005). Hornby et al (2001) proposed that the farnesol excreted during infection would alter the membrane fluidity of host cells, allowing *C. albicans* to penetrate host tissues and thus indirectly acting as a virulence factor.

Moreover, the greater severity of the lesions induced by the *C. albicans* isolated from sheep and goats relative to the other isolates may be due to the adhesive capacity of the organism (Calderone and Braun 1991). Chaffin et al (1998) , Fukazawa and Kagaya (1997) and Hostetter (1994) reported that the *C. albicans* adherence to host tissue has identified several adhesion proteins in the organism. Genes of the *C. albicans* ALS (agglutinin-like sequence) family encode proteins with features of cell surface adhesion glycoproteins (Hoyer et al 1998). Moreover, Shimizu et al (1995) reported that the *C. albicans* was the only species which could produce four enzymes (hyalurinidase, chondroituin sulphatase, proteinase and phospholipase) simultaneously from a single strain and the *C. albicans* strains which failed to produce one or more of the four enzymes seemed to be less virulent.

Finally, it could be concluded that the *Candida albicans*, isolated from goats and sheep, induced severe multiple lesions than that isolated from cattle and buffaloes.

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Selective Antimicrobial properties of Leaf extract of *Samanea Saman* against *Candida albicans*, *Staphylococcus aureus* and *Escherichia coli* using several microbial methods

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ABSTRACT: Antibacterial and antifungal activities of *Samanea Saman* were investigated against pathogenic microorganisms: S.aureus (gram+ve), E.coli (gram-ve) and *C.albicans* using the Stokes disc diffusion, Well diffusion, streak plate methods and a dilution method. The solvent type extracts were obtained by three extractions each with hexane, CH_2Cl_2, EtOAc and CH_3CH_2OH respectively. Solvents were removed in *vacuo* to yield viscous oils and paste which were made up to a concentration of 0.035g in 0.01L(10 mL) of the respective solvents. These were tested in varying volumes of 0.2-0.6ml/plate (i.e. concentrations of 0.03-0.18 mg/10 mL agar). Solvents were used as control whereas ampicillin and nystatin were used as references for bacteria and fungal species respectively. The solvents had no effect on the microorganisms whereas ampicillin and nystatin inhibited microbial growth. Saman Samanea showed selective antimicrobial inhibitory activity, with activity most prominent for the CH_3CH_2OH and CH_2Cl_2 extracts and negligible with the hexane. Its the first time in our study that the CH_2Cl_2 extracts is found to be more potent antimicrobially than the EtOAc extract. This study suggests that the CH_3CH_2OH and CH_2Cl_2 extracts of *Saman Samanea* can be used as herbal medicines in the control of *E.coli* and *S.aureus* and *C.albicans* induced diseases, following clinical trials.
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Key words: Antimicrobial; *Samanea Saman; S.aureus; E.Coli*; *C. albicans*, Stokes Disc diffusion; Well diffusion; Streak plate; Dilution Method; Selective; herbal medicines.

1.   Introduction:

This paper focuses on the antimicrobial (antibacterial and antifungal) properties of leaf extract of *Samanea Saman*, commonly called "rain tree or "monkey pod" from the coastal plain of the Guyana flora and its possible use as an herbal cream/herbal medicine. Antimicrobial properties were investigated against *S. aureus* (SA)(gram+ve), *E.coli* (EC) (gram-ve) and *C.albicans* (CA*)* strains using the Stokes disc diffusion assay, Well diffusion, Streak plate and a dilution method.

There is an urgent need to revolutionalsed research in herbal medicine and isolated drug discovery, considering the presence of incurable diseases such as HIV AIDS and the threat of new emerging disease such as SARS, bird flu etc. Plants extracts and fractionated plant extracts have been a good source of herbal medicine and natural products/ phytochemicals over the years (Kendal et. al, 1994 to Jagessar, R.C, 1998). Guyana has a rich biodiversified flora whose crude extracts, both organic and aqueous are currently been screened for their antimicrobial activity [(Jagessar, R.C et. al, 1994 to Jagessar, R.C.et.al, 2008) in addition to their role as global CO_2 sinks (in the context of global

warming) and national Low carbon development strategy (Jagdeo, B, 2009). Also, the specified plants parts fractionated or screened for natural products whose antimicrobial activity can also be investigated and compared with the crude extracts. Following this, clinical trials of crude extracts or fractionated natural products can lead to the formulation of an herbal plant cream or herbal medicine. A few herbal medicine shops are established in Guyana and the "bush" medicine man is still an important figure in Guyana's culture. Plants are known to synthesize antimicrobial natural products whose structure usually correlates with biological activity. However, there are a large number of plants whose antimicrobial activity need urgent investigations. Besides used as an herbal cream, following clinical trials, crude plant extracts can be chromatographed, leading to the isolation and purification of new and known bioactive natural products/phytochemicals, whose medicinal activity can also be investigated. For example, alkannin (1), shikonin (2) and their derivatives (3-8) isolated from the extract of *Arnebia euchroma* have been found to be the active principles against methicillin resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant *enterococci* (VRE). The derivatives (3)-(9)

showed stronger anti-MRSA activity (minimum inhibitory concentrations (MICs) that ranged from 1.56 to 3.13 ug/ml than alkannin or shikonin (MIC = 6.25 ug/ml). Anti-MRSA activity of derivatives was bacterial with minimum bactericidal concentration (MBC/MIC) < 2. Bactericidal activity against MRSA was achieved within 2h. Derivatives (3)-(9) were also active against vancomycin-resistant *Enterococcus faecium* (F935) and vancomycin resistant *Enterococcus faecalis* with MICs values similar to those with MRSA (Shen, C. C, 2002).
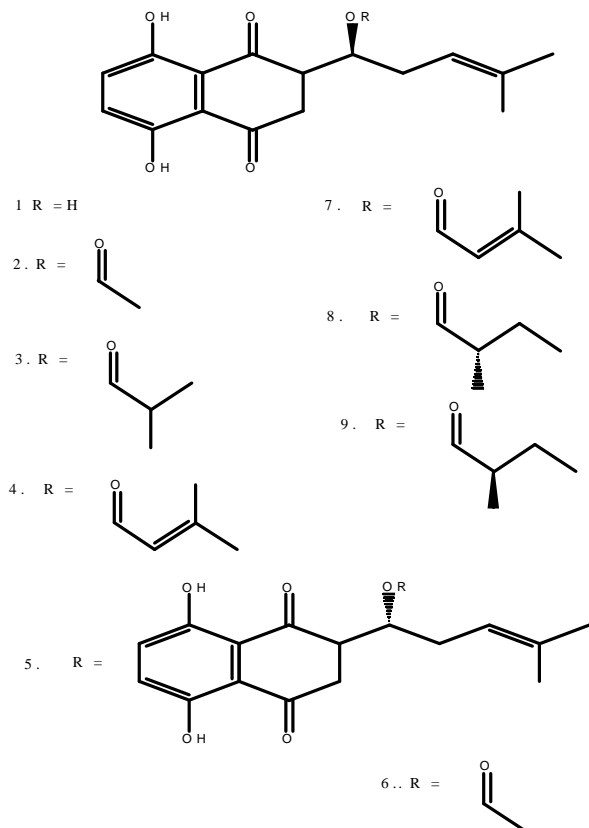
Fig. 1.0. Structure of Antibacterial Naphthazarins from *Arnebia euchrom*

Many synthetic drugs owe their discovery and potency as a result of a mimic of structures from natural products isolated from plants rather than totally to the creativity and imagination of contemporary organic chemists (Scalbert, A, 1991, Hadlington, 2006, Shen, C.C, 2002, Hepeng, 2008 to Macor, 2008). As part of an ongoing project to investigate extracts and chromatographic fractions from plants of the Guyana's flora (Jagessar, R. C. 2007 to Jagessar, R .C, 2008) for their antimicrobial activity, in correlation with folklore practices, we report here, the antimicrobial properties of *Samanea Saman* .

In Guyana's traditional medicine, there is no documentation of *Saman Samanea* as a medicinal plant, however, in neighbouring Venuzuela, the roots are used against stomach cancer and in the West Indies, the seeds are used for sore throat ((**http://www.tropilab.com/raintree.html,** En.wikipedia.org/wiki/*samanea_saman.*

Samanea Saman, the "rain tree", "monkey pod" also "cow tamarind", is a tall tropical canopied tree with a large symmetrical crown up to 240 ft broad with a beautiful shade (Scientific Classification of *Samanea   Saman* (**http://www.tropilab.com/raintree.html,** En.wikipedia.org/wiki/*samanea_saman*). It is native to the mainland neotropics, from Mexico south to Peru, Brazil and Guyana but has been widely introduced to the Pacific islands, including Hawaii where it is categorized as an invasive species. The tree can reach a height of 25 meters and a diameter of 40 meters. Leaves are alternate bipinate, while the flowers are small pinkish green. It has flat oblong seedpods containing oblong brown seeds. Several flowering version are available e.g. with reddish pink and creamish golden colored flowers. Its usually found on the coastal land of Guyana. The classification of the plant is given in Table 1.0:

Table 1.0 Scientific Classification of *Samanea Saman* (**http://www.tropilab.com/raintree.html,** En.wikipedia.org/wiki/*samanea_saman*.

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Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Fabales
Family	Fabaceae
Sub Family	*Mimosoideae*
Genus	*Samanea*
Species	S. *Saman*

Pathogenic microorganism investigated were *Escherica coli (EC), Staphylococcus aureus* (*SA) and C. albicans (CA). Escherica. coli* can cause several intestinal and extra intestinal infections such as urinary tract infections, meningitis, peritonitis, mastitis, septicemia and gram-negative pneumonia (http://en.wikipedia.org/wiki/Escheria_coli.).
Staphylococcus aureus, the yellow type can cause furuncles (boils), carbuncles (a collection of furuncles)(http://en.wikipedia.org/wiki/Escheria_coli.). In infants, *Staphylococcus aureus* can cause a severe disease Staphylococcal scalded skin syndrome (SSSS). Staphylococcal *endocarditis* (infection of the heart valves) and pneumonia may be fatal. *Staphylococcus aureus* can cause food poisoning

(http://en.wikipedia.org/wiki/*Staphylococcus_aureus*). *Candida Albicans* is a diploid fungus (a form of yeast) and is a casual agent of opportunistic oral and genital infections in humans (http://en.wikipedia.org/wiki/Candida_albicans). It is responsible for the infectious disease, candidiasis, thrush etc (Chenn. P, Murray, J, 1997).

2.   Materials and Methods

The leaves of the above plant was collected from the University of Guyana and the plant identified at the Biodiversity centre of the University of Guyana. A voucher specimen of the plant is deposited at the herbarium. The detached plant leaves were subjected to aerial drying for three weeks, removed and placed in separate conical flasks. They were then extracted with the required solvents of varying polarity.

Using selective solvent extraction, the ground leaves were first extracted thrice in hexane over a period of five days (Jagessar, R.C, 2007 to Jagessar, R.C, 2008). .Water was removed from the solvent extract by stirring over anhydrous Na_2SO_4 and extract was filtered. Solvents were removed in *vacuo* using a rotor vapor. The extracts was placed in sample vials and allow to evaporate. Further drying was done in a dessicator to remove residual solvents. Extracts were stored in capped vials and were weighed. The above procedure was repeated with the same leaves but with different solvents of increasing polarity: CH_2Cl_2, EtOAc, and then CH_3CH_2OH. At the end of drying process, plant extract was either viscous oils, solid or paste.

Approximately 0.035g of dried crude extract of *Samanea Saman* was weighed and transferred to a 10 ml volumetric flask. The respective solvent was then added to make up the 10 ml solution i.e 0.035g/0.01L. Pathogenic micro organisms: *Staphylococcus aureus, Escherichia coli and Candida albicans* were obtained from the Georgetown Public Hospital (GPH) microbiology laboratory and were stored in a refrigerator at the Berbice campus, Johns.

Two types of agar were used, nutrient agar to make up the medium for bacteria and PDA (Potato Dextrose Agar) (Murray, 1995 and Chenn, 1997) to make up the medium for fungi. The potato *was* peeled and 100g was measured, finely chopped and boiled to a mash in distilled water.The dextrose was measured (12.5g) and placed in a 1L measuring cylinder. Agar was measured (12.5g) and added to the measuring cylinder (with the dextrose).The potato mash was

stirred and strained into the cylinder. Hot distilled water was added to make up to 500mL.The contents was continuously poured and stirred until consistency was achieved. The content was then poured into a conical flask, plugged with cotton wool, over which aluminium foil was tightly wrapped. The flask was then autoclaved at 121 ^{0}C for 24hrs. The pH range was between 6.5-7.0.

Nutrient agar was purchased from the International Pharmacy Association in Guyana. 14g of nutrient agar was suspended in 500ml of distilled water in a 1L flask, stirred, boiled to dissolve and then autoclaved for 15 minutes at 121^{0}C. The pH range was between 7.0-8.0. The plates were poured in a sterile environment and allowed to cool for 2 hours. Under aseptic conditions, micro organisms were streaked onto separate plates and the discs were applied with a forceps.They were labeled and placed in an incubator at 37 ^{0}C for 24 and 48 hours for bacteria and fungi respectively.

Luria –Bertani broth (LB broth) (Murray, 1995 and   Chenn, 1997)   is a rich medium used to culture bacteria such as *E.Coli and S.aureus*. To make it, tryptone (10g), yeast extract (5g) and sodium chloride (10g) were measured and placed in a 1L cylinder. Distilled water was added to make up the 1L solution and the mixture was poured and re-poured until the contents were dissolved. The pH of the solution was adjusted to 7.4 using sodium hydroxide. 3mL each of LB broth was placed in 56 test tubes. The tubes were plugged with cotton wool foil and wrapped over each top. The tubes were placed into a beaker and autoclaved at 121 ^{0}C for 2h. These tubes were used in the dilutions experiments.

The references were antibiotic in nature. *Ampicillin* and *Nystatin*. *Ampicillin* was choosen as the reference for all bacterial species used: *E.Coli* and *S.aureus*. *Nystatin* was used as the reference for the fungus, *Candida. albicans*. The Control experiment consists of a plate of solidifying agar onto which was inoculated pure solvent with microorganism mixed in a 1:1 portion (Murray, 1995 and Chenn, 1997)

The aseptic chamber which consists of a wooden box (1m x 1m x 0.5m) with a door, was cleaned with 70% ethanol and irradiated with short wave UV light (from a lamp). These were made by culturing *C.albicans* on PDA. A sterilized 6 mm cork borer was used to cut agar discs in the plate. Stokes Disc diffusion sensitivity technique, Well Diffusion (Diffusion plate) method, Streak Plate Method and Dilution method.

Using Stokes Disc diffusion   sensitivity testing technique (Murray, 1995 and   Chenn, 1997), an inoculum containing bacterial or yeast cells was applied onto nutrient agar plates. On each plate, a reference antibiotic was also applied.The reference antibiotic disc contained 200mg antibiotic/ml. The

discs were made by cutting discs (5-6mm) from a filter paper with a perforator, placing 5 of these discs in a vial and adding 0.2mL of each extract solution. These were left to dry. Discs were also made for the controls: Ampicillin for the bacteria and nystatin for the fungus. Each disc was impregnated with the anticipated antimicrobial plant extract at appropriate concentration of 200 mg/ml using a microlitre syringe. This was then placed on a plate of sensitivity testing nutrient agar which was then incubated with the test organism:Bacteria/fungi. Incubation was done at 37ºC for 24 hr and 48 hr for the bacteria and *Candida albicans* species respectively. The antimicrobial compound diffuses from the disc into the medium. Following overnight incubation, the culture was examined for areas of no growth around the disc (zone of inhibition). The radius of the inhibition zone was measured from the edge of the disc to the edge of the zone.The end point of inhibition is where growth starts. Larger the inhibition zone diameter, greater is the antimicrobial activities. It is anticipated through the antimicrobial activity of plant extract, no area of growth will be induced around the disc. Bacteria or fungal strains sensitive to the antimicrobial are inhibited at a distance from the disc whereas resistant strains grow up to the edge of the disc. Discs applied to the plates already streaked with bacteria and the fungus.

For the Well Diffusion (Diffusion plate) method, A fungus (*Candida albicans*) was inoculated into test tube containing three ml of distilled water (medium), using a flamed loop. Drops of fungus/water culture was mixed with the warm, melted, autoclaved PDA and poured into separate plates under aseptic conditions.The plates were covered and allowed to cool. As soon as the agar was partly solidified, the plates were inverted and left for 2h. When cooled, a well was made at the centre of the plate. The well was made by using a 6 mm cork borer or puncher that was sterilized with alcohol and flame. Plant extracts dissolve in solvent at final concentration of 0.035g/0.01L was pipette into the different wells in a sterilized environment at different volumes (0.2-0.4-0.6ml) in separate plates, using a micro liter syringe. The four solvents (hexane, CH_2Cl_2, EtOAc and CH_3CH_2OH) at different volumes were used as control whereas nystatin dissolved in CH_2Cl_2 at same concentration with plant extract (0.035g/0.01L) at different volumes(0.2-0.4-0.6ml) was used as the reference. The plates were labelled, covered, inverted and placed in a fume hood (no incubator was available) for 48h

Fig. 3.0. Well diffusion for *C.albicans*, Control Experiment.

Nutrient agar was prepared as described above and 10 mL was poured into plates. Plant extracts dissolved in solvent at a final concentration of 0.035g/0.01L were pipette into three sterilized plates under asceptic conditions at different volumes (0.2-0.4-0.6 ml), using a micropipette. The plates were allowed to cool and then the bacteria were streaked onto the surface of the solidified agar/plant extract medium. A flame loop was used to inoculate the bacteria from their cultures These plates were left for 24 hours in a dessicator. The plates with inhibition were used in further experiments. A reference experiment was setup using an antibiotic (ampicillin capsule) at the same concentration as plant extracts (0.035g/0.01L) at different volumes (0.2-0.4-0.6ml). Controls were also setup using solvents: hexane, CH_2Cl_2 and EtOAc and CH_3CH_2OH at the different volumes.

This Dilution method was used to test the plant extracts for antimicrobial activities against bacteria by investigating whether there was turbity or not. Turbidity represents microbial growth, while no turbidity represents inhibition of microbes. One set of tubes containing LB (*Louria Bertinieia*) was inoculated with *Staphylococcus aureus* and the second set was inoculated with *Escherichia coli* using a loop, flame and alcohol. Under aseptic conditions, the plant extracts (dissolved in solvent at concentration 0.035g/0.01L) and showed inhibition in the streak plate were added to the one set of test tubes containing *E. Coli* and the other set, *S.aureus* with LB broth(medium) in differing volumes (0.2-0.4-0.6ml). Two sets of four tubes each were treated with the four solvents (hexane, CH_2Cl_2, EtOAc and CH_3CH_2OH). One set was inoculated with *S.aureus* and the other with *E.coli*. Cotton wool was used to plug test tubes. The tubes were observed after 24 hrs.

Retention Factor, R_f was calculated using the formula,

$$R_f = \frac{\text{Distance moved by sample}}{\rule{3cm}{0.4pt}}$$

Distance moved by solvent front.

In general, the most polar compound has the lowest R_f value

For Thin Layer Chromatography (TLC), A baseline was drawn on the TLC plate. A spot of the plant extract was placed on the baseline with use of the pipette and allowed to dry. The plate was placed in the developing jar with the solvent. When taken out of the jar, the solvent front was drawn. The plates were then held in the iodine jar for a few seconds, shaken and taken out. They were examined under the UV/Vis lamp and the spots were circled with a pencil. The plate was

further examined under UV lamp and any new spots were marked. The spots were labeled and their distances from the baseline were measured. The distance between the baseline and the solvent front was measured. The R_f values were calculated.

3. Results:

Mass of dried leaves used for *Samanea Saman species was* 8.55g respectively. These extracts were in the concentration of 0.035g in 10 ml (0.0003mg/uL) of solvent except for *Samanea Saman* with CH_3CH_2OH which was 0.5g in 25ml (0.02mg/uL). Reference compounds were: Ampicillin and nystatin in a concentration of 200mg/ml.

Table 2.0. Using the Disc diffusion method above, the Antimicrobial activity of Plant extract as shown by the inhibition zone diameter**.**

Area of inhibition. (mm^2) using *E.Coli*	Area of inhibition. (mm^2) using *S.aureus*	Area of inhibition. (mm^2) using *Candida albicans*	Plant Extracts *Samanea Saman species*	Reference compound (Ampicillin) (mm^2)	Control Experiment
< 5	< 5	< 5	Hexane extract	27	No zone of inhibition
20	27	26	CH_2Cl_2 extract	28	No zone of inhibition
<5	< 5	<5	EtOAc extract	30	No zone of inhibition
22	29	28	CH_3CH_2OH extract	33	No zone of inhibition

Table 3.0. Results of Well diffusion for plant extracts *Samanea Saman* against *C.albicans.*

Plant Extract dissolved in solvent at concentration: 0.035g/0.01L	Volume of Extract (mL)	Presence of zone of Inhibition	Diameter of Zone of Inhibition (mm^2)
Samanea Saman with Hexane	0.2	No zones of inhibition visible, scattered colonies.	<5
	0.4	" "	<5
	0.6	" "	<5
Samanea Saman with CH_2Cl_2	0.2	Zones of inhibition visible.	45
	0.4	" "	55
	0.6	" "	74
Samanea Saman with EtOAc	0.2	No Zones of inhibition visible.	<5
	0.4	" "	10
	0.6		< 5
Samanea Saman with CH_3CH_2OH	0.2		50
	0.4		61
	0.6		65
Reference(Nystatin)	0.2	Zone of Inhibition	76

	0.4	" "	**76**
	0.6	" "	**76**
Controls Hexane, CH$_2$Cl$_2$, EtOAc, CH$_3$CH$_2$OH	0.2	No zone of inhibition	**< 5**
	0.4	No zone of inhibition	**< 5**
	0.6	No zone of inhibition	**< 5**

Table 4.0. Results obtained from Streak plate method for the bacteria's *Escheria coli(EC)* and *Staphylococcus aureus(SA)* against different volumes of dissolved plant extracts at a final concentration of 0.035g/0.01 L and controls. Inhibition or no growth of microbes were represented by a positive sign (+), while the negative sign (-) represents no inhibition or growth of microbes**.**

Plant extract dissolved in solvent	Volume of dissolved plant extract used in (ml) at concentration 0.035g/0.01L	Inhibition or no growth of microbe, *Escherichia coli*	Inhibition or no growth of microbe *Staphylococus aureus*
Samanea Saman dissolved in hexane	**0.2**	-	-
	0.4	-	-
	0.6	-	-
Samanea Saman dissolved in CH$_2$Cl$_2$	**0.2**	+	-
	0.4	+	-
	0.6	+	-
Samanea Saman dissolved in Et(OAc)	**0.2**	-	-
	0.4	-	-
	0.6	-	-
Samanea Saman dissolved in CH$_3$CH$_2$OH	**0.2**	+	+
	0.4	+	+
	0.6	+	+
Reference (Ampicillin with same concentration as dissolved plant extracts(0.035g/0.01L).	**0.2**	+	+
	0.4	+	+
	0.6	+	+
Hexane	**0.2**	-	-
	0.4	-	-
	0.6	-	-
CH$_2$Cl$_2$	**0.2**	-	-
	0.4	-	-
	0.6	-	-
EtOAc	**0.2**	-	-
	0.4	-	-
	0.6	-	-
CH$_3$CH$_2$OH	**0.2**	-	-
	0.4	-	-
	0.6	-	-

For the Dilution Method , Results were recorded in terms of turbidity.

T_0 = No Turbidity = Inhibition
T_1 = Slightly Turbid = Moderately Inhibited
T_2 = Moderately Turbid = Lightly Inhibited
T_3 = Very Turbid = No Inhibition

Table 5.0 shows the degree of turbidity of dissolved *Samanea Saman* extracts at concentration of 0.035g/0.01L at different volumes against *Escheria coli* (EC) microbe.

Plant extract dissolved in solvents at concentration of 0.035g/0.01L	Volume of dissolved plant extract (ml) 0.2 ml	Volume of dissolved plant extract (ml) 0.4ml	Volume of dissolved plant extract (ml) 0.6 ml
Samanea Saman with hexane	T_3	T_3	T_3
Samanea Saman with CH_2Cl_2	T_3	T_0	T_0
Samanea Saman with EtOAc	T_3	T_3	T_3
Samanea Saman with CH_3CH_2OH	T_0	T_0	T_0

Table 6.0. Table showing the degree of turbidity of dissolved plant extract at concentration of 0.035g/0.1 L at different volumes against *Staphylococcus aureus (SA)* microbe.

Plant extract dissolved in solvents at concentration of 0.035g/0.01L	Volume of dissolved plant extract (ml) 0.2 ml	Volume of dissolved plant extract (ml) 0.4ml	Volume of dissolved plant extract (ml) 0.6 ml
Samanea Saman with hexane	T_3	T_3	T_3
Samanea Saman with CH_2Cl_2	T_0	T_0	T_0
Samanea Saman with EtOAc	T_3	T_3	T_3
Samanea Saman with CH_3CH_2OH	T_0	T_0	T_0

Table 7.0. Antimicrobial activity of different controls used in the dilution method.

	Volume of dissolved plant extract (ml) 0.2 ml	Volume of dissolved plant extract (ml) 0.4ml	Volume of dissolved plant extract (ml) 0.6 ml
Reference (Ampicillin at same concentration as dissolved plant extract 0.035g/0.01L	T_0	T_0	T_0
Control			
Hexane	T_3	T_3	T_3
CH_2Cl_2	T_3	T_3	T_3
EtOAc	T_3	T_3	T_3
CH_3CH_2OH	T_3	T_3	T_3

Table 8.0. TLC analyses for all the extracts of *Samanea Saman*.

Eluent (90:10, v/v)	Plants Extracts	Number of spots visible by UV	R_f value
CH$_2$Cl$_2$/Hexane	Hexane Extract	2	0.043 0.109
CH$_2$Cl$_2$/Hexane	CH$_2$Cl$_2$ Extract	2	0.051 0.974
EtOAc/Hexane	EtOAc Extract	3	0.032 0.081 0.35
Ethanol/Hexane,	CH$_3$CH$_2$OH Extract	3	0.081 0.251 0.512

Bar graphs are shown in Fig. 4.0 (a) and 4.0 (b)

(a)

Disc Diffusion: Zone of Inhibition for CH$_2$Cl$_2$ and Ethanol Extract vs Microorganisms
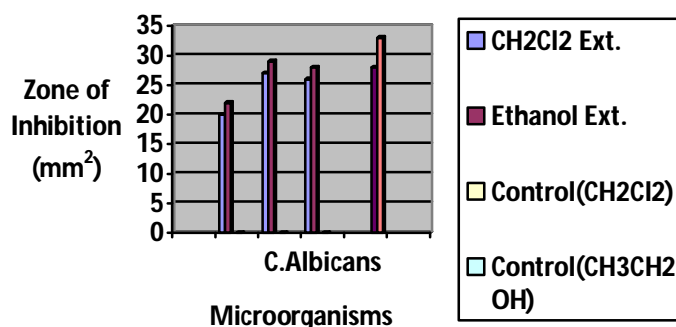
(b)

Well Diffusion: Zone of Inhibition for CH$_2$Cl$_2$ and CH$_3$CH$_2$OH extract, vs. Volume of Extract
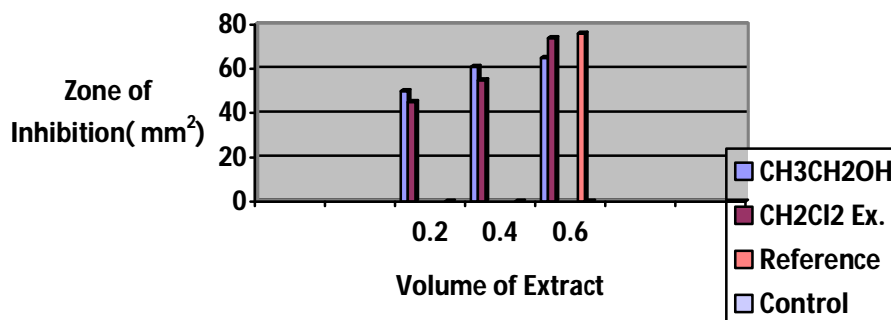
4. .**Discussion**:

All four methods: Stokes disc diffusion techniques,Well diffusion Streak plate and a dilution method were successful in determining crude hexane, CH_2Cl_2 and CH_3CH_2OH *extracts of Samanea Saman* for antimicrobial activities. Several trends are noted and these will be discussed first. Each solvent type extracts was added in increasing volume (0.2-0.4-0.6) ml to the microbial medium. Unlike previous publications (Jagessar, R.C, 2007 to Jagessar, R.C, 2008) antimicrobial potency follow the sequence: CH_3CH_2OH extract > CH_2Cl_2 extract> EtOAc extract > hexane extract, suggesting that *Samanea Saman* antimicrobial compounds are localized in the CH_3CH_2OH and CH_2Cl_2 extract. For example, with disc diffusion method, zone of inhibition of 22 mm^2, 29 mm^2 and 28 mm^2 were obtained for the CH_3CH_2OH extract against *E.Coli*, *S.aureus* and *C.albicans* in contrast to 20 mm^2, 27 mm^2 and 26 mm^2 for the CH_2Cl_2 extract. For the hexane and EtOAC extract, zone of inhibition of < 5 mm^2 were obtained against all microbes. In a comparative Well diffusion method, zone of inhibition of 65 mm^2 was obtained for the CH_3CH_2OH extract when the well was filled with 0.6 ml of extract compared with a zone of inhibition of 74 mm^2 for CH_2Cl_2 extract when the volume of the well was 0.6 ml, suggesting greater potency. This is the first example of a plant, we have studied whose CH_2Cl_2 extract is more potent that the EtOAc extract against microbes. All of our plants reported to date displayed the common antimicrobial potency trend: CH_3CH_2OH extract > EtOAc extract > CH_2Cl_2 extract > hexane extract (Jagessar, R.C, 2007 to Jagessar, R.C, 2008).. For all methods used, the control experiments which necessitate the use of pure distilled solvent alone, rather than pure plant extract induced negative result i.e no zone of inhibition or in the case of the dilution method, turbidity in test tubes containing LB (*Luria-Bertani)* broth with bacterial microbes. The reference antibiotic ampicillin for bacteria and Nystatin for fungi induced positive results as anticipated, Figure 1.0 and Figure 2.0. For example, for the disc diffusion method, ampicillin induced zone of inhibition of 33 mm^2 for the CH_3CH_2OH extract whereas Nystatin induced zone of inhibition of 76 mm^2 at a volume of 0.6 ml for the Well diffusion method. These results suggest that *Samanea Saman* antimicrobial properties are due to the plant active constituent rather than to a solvent effect.
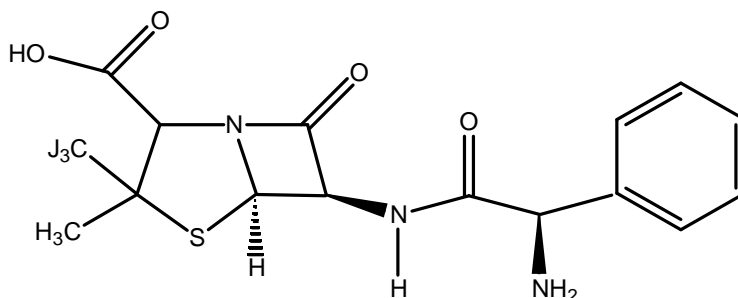
Figure. 1.0 Ampicillin
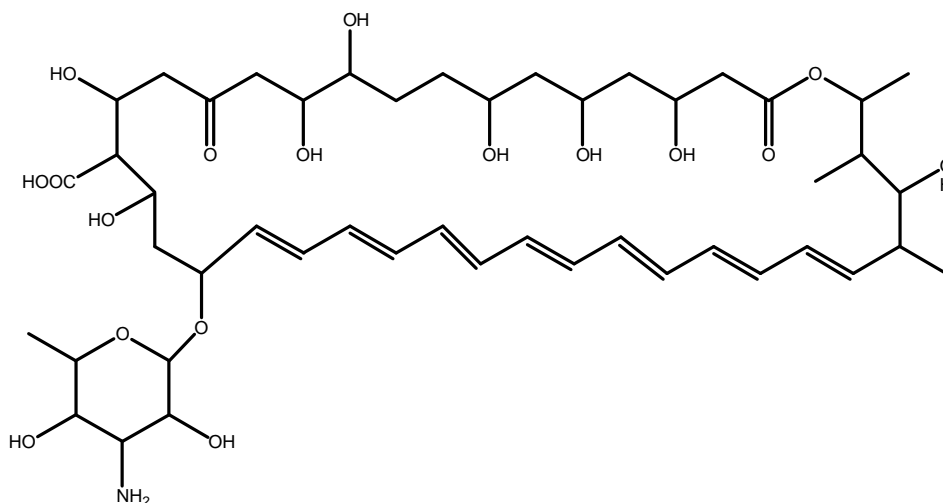
Figure. 2.0. Nystatin

Each method display interesting results and these can be further discussed. Stokes Disc diffusion indicates that the plant extract was more selective potent against *S.aureus* as compared against *E.coli* and *C.albicans*. For example, for the CH_2Cl_2 extract, zone of inhibition of 26 mm^2 and 27 mm^2 were obtained for *C.albicans and S.aureus* respectively. Of the two bacteria, *S.aureus*. showed a larger zone of inhibition as compared with *E.Coli*, inspite of the fact that *E.coli* has an outer membrane that acts as a selective barrier against antibiotics and toxins. Contrary, gram positive *S. aureus* lack the outer membrane but possess a much thicker cell wall of peptidoglycan layer which is not an effective permeability barrier. However, for the Streak plate method, *E.Coli* showed inhibition whereas *S. aureus* showed no inhibition for the CH_2Cl_2 extract. Comparatively, for the EtOAc extract, no inhibition zone was observed. For the CH_3CH_2OH extract extract, inhibition was observed against both *E.Coli* and *S.aureus*.

The Well diffusion method was used to investigate *C. albicans's* antimicrobial activity. A larger zone of inhibition was observed compared with the Stokes Disc diffusion method. This difference may be ascribed to the higher sensitivity of the Well diffusion as compared with the Disc diffusion method. For example, for the CH_3CH_2OH extract, a zone of inhibition of 65 mm^2 was observed at a volume of 0.6 ml. Compared with the Well Diffusion method, the ethanol extract induce zone of inhibition of 28 mm^2 against *Candida albicans* for the Disc diffusion method.

The Streak plate method indicated selective solvent and microbial inhibition at increasing volume of 0.2-0.6 ml. Results are tabulated as positive or negative inhibition. Hexane and EtOAc extract showed negative inhibition against *E.Coli* and *S.aureus*. However, for the CH_2Cl_2 extract at a volume of 0.2 to 0.6 ml, positive inhibition was observed for *E.Coli* whereas a negative inhibition was observed for *S.aureus*. Ethanolic extract induce positive inhibition against both *E.Coli* and *S.aureus* from 0.2 to 0.6 ml. The reference ampicillin showed positive inhibition.

The Dilution method was used to test plant extracts for antimicrobial activity against bacteria: *E.Coli* and *S.aureus*. The plates with inhibition from the Streak plate method were used in these experiments. Results were recorded in terms of turbidity. In general, no turbidity (T_0) indicates inhibition. LB (*Louria Bertinieia*) broth was used as a rich medium to foster or stimulate the growth of the bacteria. *E.Coli and S.aureus* microbes induced no inhibition (very turbid mixture, T_3) for the hexane and EtOAc extract. Complete inhibition was observed for the CH_2Cl_2 extract at a volume of 0.4-0.6ml for *E.Coli* and *S.aureus*. CH_3CH_2OH extract at a volume 0.2 to 0.6 ml induce complete inhibition, T_o. The reference

compound, ampicillin and solvent control at a volume range from 0.4-0.6 showed inhibition and non inhibition respectively. It is interesting to note that the zone of inhibition induced by the antibiotics ampicillin and nystatin was greater than each solvent type extract.

Figure 4.0 (a) represent for the Disc diffusion method, using the CH_3CH_2OH and CH_2Cl_2 extract, a comparative bar graph plot of the zone of inhibition (mm^2) vs. type of microorganism. Figure. 4.0 (b) represent for the Well Diffusion method, a bar graph plot of the zone of inhibition for CH_2Cl_2 and CH_3CH_2OH extract vs volume of extract. For the disc diffusion technique, a larger zone of inhibition was observed for the CH_3CH_2OH extract as compared with the CH_2Cl_2 extract. The largest zone of inhibition was observed for *S.aureus* in both cases. For the Well Diffusion method, as the volume of plant extract increased in the well so too is the zone of inhibition. However, the largest zone of inhibition was seen for the reference antibiotic, ampicillin (light blue). In contrasts, zero inhibition was seen for the solvent control (red).

TLC analyses in various solvent system for each solvent type extract revealed the presence of spots that range from one to two, Table 8.0. Each spot has a specific R_f value. The larger the R_f value, the lower the polarity of natural product/phytochemicals For example for *Samanea Saman* ethanol extract using the solvent system, Ethanol/hexane (90:10, v/v), three spots of R_f values 0.081, 0.251 and 0.512 were seen.

In conclusion, *Samanea Saman* has antimicrobial properties which are localized primarily in the CH_3CH_2OH and CH_2Cl_2 extract. However, antimicrobial activity is selective and solvent dependent with the CH_3CH_2OH extract, the most potent and hexane the least. In general, the order of antimicrobial activity follow the sequence: CH_3CH_2OH extract > CH_2Cl_2 extract > EtOAc extract > hexane extract. In our study, it's the first plant in which the CH_2Cl_2 extract is found to be more potent than the EtOAc extract against microbes. Thus, the CH_3CH_2OH and CH_2Cl_2 extract of *Samanea Saman* can be used as the active constituent of an antimicrobial cream. Future work such as isolation and purification of bioactive constituents should target the CH_3CH_2OH and CH_2Cl_2 extract of *Samanea Saman*.

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Diagnosis of Recurrent Pyoderma in Dogs by Traditional and Molecular Based Diagnostic Assays and Its Therapeutic Approach

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Abstract: Canine recurrent pyoderma is a common skin problem encountered in small animal practice and also resistant staphylococci may cause hazards in contact human. The main objective of the present investigation was to study the underlying etiologies of recurrent pyoderma and antimicrobial resistance guidelines of staphylococci on traditional and molecular basis. Also, the present workup was aimed to select satisfactory antimicrobial prescriptions for cases of recurrent pyoderma on empirical and bacteriological basis. A total number of 44 dogs were thoroughly examined for dermatological lesions and classified into 32 empirically treated dogs and 12 treated dogs based on bacteriological results. Pyoderma were classified into surface (13.6%), superficial (66%) and deep pyoderma (20.5%) with main clinical signs of pruritus, skin lesions (papules and pustules), marked alopecia (specially in superficial and deep pyoderma) and epidermal collarettes. The common recurrent pyoderma was German Shepherd pyoderma (38.6%) and the common pathogen was *Staphylococcus intermedius* (100%). *S. intermedius* was isolated alone in 58.3% and 41.7% in combination with *Corynebacterium spp.* and *Staphylococcus aureus* from skin of 12 examined dogs. The present study was recorded multidrug resistance exhibited by 75% of the 12 *S. intermedius* isolates. Oxacillin MIC testing revealed 6 *S. intermedius* isolates (50%) to be resistant, which included 2 strains with the mecA gene. The mec A (Methicillin resistant *Staphylococcus intermedius*, MRSI) was detected by PCR in 5 isolates (41.7%). Amoxicillin-clavulinic acid, cephalosporines and flouroquinolones were achieved magic results on empirical and antibiogram basis in treatment of idiopathic recurrent pyoderma. It was concluded that our data provided the first Egyptian guidelines in companion animals for common bacterial pathogens with antibiogram for bacterial resistance and Antimicrobial therapy with selected antibiotics and suitable period for treatment of each type of pyoderma.

1. Introduction:

Canine pyoderma is one of the most common diseases. Pyoderma literally means pus in the skin and can be caused by infectious, inflammatory and/ or neoplastic etiologies; any condition that results in the accumulation of neutrophilic exudates can be termed pyoderma. Most commonly, however, pyoderma refers to bacterial infections of the skin. Pyodermas are common in dogs and less common in cats (Leib and Monoroe, 1997; Frank *et al.*, 2003; Loeffler, 2005 and Morris, 2010). Pyoderma classified according to the depth of infection into surface, superficial and deep pyodermas. Surface pyoderma are those infections that restricted to the surface of the skin and not extend into the follicle, it does not extend deeper than the stratum corneum or into hair follicle. Superficial pyodermas include infections that involve the hair follicle but do not extend into the dermis. Deep pyodermas are infections that extend into the dermis and underlying panniculitis (Ihrke, 1996; and Leib & Monoroe, 1997). Pyoderma should be suspected if the patient has a history of pruritus, especially if the pruritus has previously responded to antimicrobial therapy. The presence of papules, pustules and epidermal collarettes should create a high index of the suspicion for pyoderma. Epidermal collarettes are circular scale- crust lesions that represent the end stage of pustule (Carlotti, 1996; Leib and Monoroe, 1997; and Scott *et al.*, 2001).

Pyoderma is caused most frequently by *Staphylococci*. Bacterial pyoderma is usually triggered by an overgrowth/ over colonization of normal resident or transient flora. Most canine cutaneous staphylococcal infections occur as secondary infections to predisposing factors such as atopic dermatitis (AD), flea allergy dermatitis, demodicosis or hypothyroidism (Gross *et al.*, 2005), immature immune system, such as in young animals or in those taking steroids precipitate to pyoderma. Animals with short coats, skin folds, or calloused skin were anatomically predisposed to pyoderma. Trauma from grooming, scratching, or rooting in dirt or garbage was also recorded to induce pyoderma.

The German shepherd dog has a deep pyoderma that may respond to treatment only partially and frequently recurs (Miller, 1991; Mason *et al.*, 1996; Saijonmaa-Koulumies et al., 1998)

Staphylococcus intermedius is regarded as the primary pathogen in deep pyoderma which may develop from superficial pyoderma (Gross et al., 2005). *S. intermedius* is regarded as a zoonotic pathogen. A common route of invasive infection in human is through dog bite wounds and several instances of life-threatening infections of human have been reported (Talan *et al.*, 2003 and Pottumarthy *et al.*, 2004)**.** Thus, S. intermedius, especially MRSI (Methicillin resistant *Staphylococcus intermedius*), represents a potential serious public health concern. *Staphylococcus intermedius* attaches to epidermal cells of healthy dogs, but shows greater adherence to epidermal cells of atopic dogs (McEwan, 2000; Simou *et al.*, 2005 and McEwan *et al.*, 2006). It is possible that AD alters the availability of cutaneous receptors for staphylococci and facilitates bacterial adherence.

Normal resident bacteria in canine skin also include coagulase negative *Staphylococci, Streptococci, Micrococcus spp,* and *Acinebacter spp.* Transient bacteria in canine skin include *Bacillus spp, Corynebacerium spp, Escherichia coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa* (Patel, 2006). Surface pyodermas included acute moist dermatitis and intertrigo. Acute moist dermatitis (hot spot or pyotraumatic dermatitis or thick coated pyoderma) was secondary to self-induced trauma from pruritus, ectoparasitism and alterations of microenvironment of the skin (Mason & Lloyd, 1990 and Asher *et al.*, 1995). The microenvironmental change was exacerbated in long or thick coated breeds and in high relative humidity or heat and is compounded by some behaviors of animals, such as swimming. It was manifested by moist, exudative areas of erythema in addition to severe pruritus. Alopecia was the result of self inflicted trauma. Intertrigo (skin fold pyoderma or frictional dermatitis) often develop in skin folds of Brachycephalic breeds, such as English and French Bull dogs and Boston terriers (facial fold pyoderma) after Lloyd & Lamport (1999). Lip-fold pyoderma occurs in Spaniel breeds, Irish Setters. Vulvar folds or animals with corkscrew tails may develop skin fold pyoderma. The Chinese Shar-Pei can develop skin fold pyoderma in almost any area of the body. Purulent exudates and classical pyoderma lesions restricted to affected areas. It is manifested by rubbing of the face, scratching, or scooting the perineal area on a carpet, depending on the area that is involved (Allaker *et al.*, 1991 and Leib & Monoroe, 1997). The treatment included surgical correction of a conformational problem in addition to pyoderma treatment.

Superficial pyodermas are include impetigo and folliculitis. Impetigo or juvenile pyoderma characterized by development of subcorneal papules and pustules. It is caused by specific immunodeficiency syndromes, inadequate nutrition, poor husbandry (e.g. crowding, inadequate housing) and stress (Valensi, 1990 and Mason & Lloyd, 1993). Staphylococci and streptococci are most frequently isolated from the lesions. Folliculitis (short coated dog pyoderma or lumpy- bumpy disease) is defined as inflammation of the hair follicle. There are three diseases in which folliculitis are a common feature: demodecosis, dermatophytosis and bacterial infections of the skin (Mason *et al.*, 1996). It is manifested by patchy or diffuse alopecia and hairs easily epilated. The large numbers of papules has led to the term lumpy- bumpy disease (Deboer *et al.*, 1990 and Leib & Monoroe, 1997). Systemic antimicrobial therapy should not less than 21-28 days (Carlotti, 1996 and De Jaham, 2003).

Deep pyodermas are include furunculosis and interdigital pyoderma. Furunculosis is defined as disruption of the hair follicle as a result of any underlying disease process. The condition may be seen in any breed predisposed to folliculitis. It is commonly occur in German Shepherd (Miller, 1991 and Chabanne *et al.*, 1995). Systemic antimicrobial therapy may take from 4 weeks to several months (Carlotti *et al.*, 2004). Furunculosis is clinically similar to folliculitis. However, as the lesions progress, draining tracts, nodules, ulcerations, and lichenification of the skin are seen. It is manifested by moderate to severe pruritis and a serosanguineous blood tinged fluid from the skin in affected areas (Wisselink, 1989; Day, 1994 and Leib & Monroe, 1997). furunculosis is often classified according to the location of lesions into canine acne (face and chin), facial pyoderma, pressure point pyoderma and interdigital pyoderma (Leib & Monoroe, 1997 and Curtis *et al.*, 1999). Pododermatitis or interdigital pyoderma is a form of folliculitis and furunculosis of the interdigital areas. Erythema, papules, pustules, ulcerations and fistulous tracts are found in the interdigital spaces on the dorsum of the foot and between the pads on the ventrum. In severe cases, the feet may become severely swollen, resulting in lamenss. The prescapular and popliteal lymph nodes are enlarged. Foot soaks in antiseptic solutions may provide relief to some patients but are not effective as active hydrotherapy (Wisselink, 1989 and Day, 1994).

Although data on the occurrence of antimicrobial resistance for many different animal species are reported through reporting systems in the

world as European Food Safety Authority, data on the occurrence of antimicrobial resistance in bacteria from companion animals are absent or scarce. The occurrence of antimicrobial resistance in companion animals may however be of significance to human health. Considering the shared environment of human and companion animals, transfer of resistant bacteria or mobile resistance determinants between companion animals and human is likely to occur and has been indicated in some studies (Guardabassi *et al.*, 2004 and VanDuijkeren *et al.*, 2004)**.** However, the extent to which this exchange occurs is essentially unknown. In Denmark, few reports on the occurrence of antimicrobial resistance among bacteria from companion animals have been published and most of these investigations have focussed on *Staphylococcus intermedius* (Wegener & Pedersen, 1992 and Pedersen & Wegener, 1995).

The use of antimicrobials in companion animals has received little attention and remains unregulated, whereas antimicrobial use in farm animals is regulated in many countries by guidelines or legal restrictions (Heuer *et al.*, 2005). Some antimicrobials that are widely used in animals belong to classes of antimicrobials that are regarded as critically important for use in human (e.g. cephalosporins and fluoroquinolones) after Sternberg, 1999, and the use of these antimicrobials in farm animals is restricted or prohibited in some countries (Heiene *et al.*, 2004). Long term therapy of deep pyoderma was performed by 3 regimens. Intermittent therapy was the first regimen used in cases of recurrence, used 2- 3 times annually. Pulse therapy was the second regimen achieved by treatment 2- 7 days and stopped for 5- 32 days. Slow dose or tapering was the third regimen applied by decreasing dose gradually which suitable for cephalosporines (Negre *et al.*, 2009)

Methicillin and oxacillin are members of a class of antibacterials known as the semi-synthetic penicillinase-resistant penicillins (SSPRP). Due to its superior stability *in-vitro*, oxacillin is now used by most microbiology laboratories as the surrogate for testing the susceptibility of bacteria to this entire class of antibiotics (Chambers, 1997). Even so, the term "methicillin resistant" (MR) has persisted in the common vernacular and in most scientific publications. The SSPRP class was developed to circumvent staphylococcal resistance to the first generation penicillins, which is mediated by bacterial production of penicillinase enzymes. Although the SSPRP class is unaffected by penicillinases, it is susceptible to an acquired penicillin-binding protein (PBP), known as PBP2a. This staphylococcal PBP is encoded by the mecA gene, which confers an intrinsic resistance to all beta-lactam antibiotics and

their derivatives (including all classes and generations of penicillins and cephalosporins) after Berger- Bachi & Rohrer (2002). Since the early 1960's, the incidence of MR has escalated within human hospital strains of *S. aureus*, and hospital-acquired MR *S. aureus* (HA-MRSA) has now become the most prevalent pathogen causing nosocomial infections of people throughout the world (Diederen & Kluytmans, 2006)**.** The broad antimicrobial resistance patterns inherent to HA-MRSA contribute significantly to the morbidity and mortality associated with human nosocomial MRSA infection. Since pets predominantly acquire the HA-MRSA strains, retrospective studies have attempted to test the hypothesis that outcomes for MRSA-infected pets are worse than for non-resistant *S. aureus* infections (Faires *et al.*, 2010).

Transmission of *S. intermedius* between animals and veterinarians may also be present in Egypt, and might be an emerging problem for public health (Tanner *et al.*, 2000). Presently, we sought to determine whether *S. intermedius* strains was present among various cases of canine pyoderma using samples obtained from different canine races and to determine the antibiotic susceptibility of *S. intermedius* isolates. Also, the target of present study was to detect underlying etiologies and antibiotics of choice for cases of recurrent pyoderma either empirically or based on antibiotic sensitivity testing.

2. Materials and Methods

A total number of 44 dogs admitted to private clinics in Giza governerate were thoroughly investigated. Age, breed and sex were recorded for each examined dog. An accurate clinical history of previous treatments, routine health care, such as internal parasite control and vaccination was collected. All investigated dogs were vaccinated and were received one tablet/ 10 Kg once of Drontal® plus (50 mg praziquantel, 150 mg Febantel, 144 mg pyrantel- Embonat, made in Germany by Bayer) as internal worm prophylaxis. Physical dermatological examinations were performed by inspection of different clinical signs. All areas of the skin should be carefully palpated and visually examined. A hand lens or other magnification was often helpful to identify lesions. The veterinarian's sense of smell may helpful to identify bad odor occurred in pyoderma. The appearance of pelage (hair coat), the ease of hair removal from follicles (epilation) and the pattern of lesions on the skin should be noted. The present study was carefully recorded findings on a special dermatologic examination form containing a silhouette of a dog for recording the location of the lesions. At each visit (every 2 weeks), They were

evaluated with lesion, pruritus and body scores (Leib & Monoroe, 1997 and Mueller & Stephan, 2007).

Skin scrapings were performed for all examined dogs (Leib & Monoroe, 1997). Fecal concentration floatation also was performed (Thiopont et al., 1986). Hypoallergenic (Elimination) dietary trial was performed in cases of adverse reaction to food (Leib & Monoroe, 1997).

The present study was classified examined dogs into:
Group (1): Thirty two empirically treated dogs:
The present group was performed on 9 German shepherd dogs, 9 griffon dogs, 5 American Staffordshire dogs, 4 Mastiff dogs, 3 Rottweiler dogs, one Siberian Husky dog and one Mongrel dog. Sex of these dogs was 20 male dogs and 12 female dogs. The age of dogs in the present group were ranged from 5 months into 12 years.

Firstly, affected areas were clipped and sheaved for application of topical medicaments. Topical treatments selected for all cases were Betadine® shampoo (povidone iodine 7.5%, Mundi pharmaceutical company) and Fucidin® ointment (fusidic acid 2%, Leo pharmaceutical company). Topical treatment applied every 12 hours and period of treatment differed according to the form of pyoderma. Choice of systemic antibacterial differed also according to the form of pyoderma. Four cases of surface pyoderma were treated by only topical treatment for 10 days- 2 weeks as described by Saijonmaa-Koulumies et al. (1998) and Loeffler et al. (2005). Twenty three cases of superficial pyoderma were treated by topical treatment for 2 weeks- 1 month. Systemic antimicrobial was chosen for superficial pyoderma was Augmentin® capsules (clavulinic acid potentiated amoxicillin 156, 312, 487, 625, 1g., GSK pharmaceutical company) which used/ 12 hours for 2 weeks- 1 month in a dose of 10 mg/ Kg and clavulinic acid 2.5 mg/ kg . Five cases of deep pyoderma were treated by topical treatment for 4 weeks- 3 months. Systemic treatment was selected for deep pyoderma was several antibacterial. The used antibacterial shifted every 2-3 weeks. The beginning antibacterial was Augmentin® capsules (clavulinic acid potentiated amoxicillin 156, 312, 487, 625, 1g., GSK pharmaceutical company) then shifted into Rocephin® vials every 24 hours in a dose of 15 mg/ Kg (ceftriaxone 500, 1000, Roche pharmaceutical company) and then ended by Ciprocin tablets® in a dose of 5 mg/ Kg every 12 hours (ciprofloxacin Hcl 250, 500, and 750, Eipico pharmaceutical company) as mentioned by Kruse et al. (1996); Holm et al. (2002); Negre et al. (2007) and Mueller & Stephan (2007).

All previous treatment was beside treatment of primary cause if detected. Cases of demodectic mange were treated by Dectomax® injectable solution every 2 weeks (10 mg doramectin/ ml, by Pfizer Egypt veterinary pharmaceutical company) in a dose of 1 ml/ 50 kg, Amitraz ® solution 0.5 ml of the solution/ L of water (emulsifiable concentrate containing 125g amitraz per liter, made in Egypt by ADWIA veterinary pharmaceutical company) and Ketrax® tablets as immunostimulant one tablet/ 5 Kg (levamisol Hcl 40 mg/ tablet, by Zenc pharmaceutical company). Cases of sarcoptic mange were treated by Dectomax injectable solution® every 2 weeks and Amitraz® solution 0.5 ml of the solution/ L of water. Flea allergic dermatitis cases were treated by Dectomax injectable solution® every 2 weeks beside treatment of allergy. Allergic skin disease cases were treated principally by avoidance of putative allergens and control of pruritis. Control of pruritis was by the use of one tabet/ 5kg prednisolone tablets® by tapering regimen every 7 days (prednisolone, 5mg/ tablet by Adco pharmaceutical company). These prescriptions were selected according to Leib & Monroe (1997).

Group (2) 12 treated dogs based on bacteriological results
The present group was performed on 8 German Shepherd dogs, 2 Rottweiler dogs and 2 Labrador dogs. Sex of these dogs was 8 male dogs and 4 female dogs. The age of dogs in the present group were ranged from 3 months into 9 years.

Microbial isolation
Cotton swab specimens (BD BBL Culture Swabs, Becton-Dickinson, Sparks, MD, U.S.A.) were collected from the canine skin lesions and inoculated into nutrient broth, then incubated at 37°C for 24 hours. a loopfull from each broth culture was inoculated onto 5% (v/v) sheep blood agar plate, manitol salt agar, nutrient agar and cetremid agar. The plates were then incubated at 37°C for 24 to 48 hours. Presumptive identification of different colony types was made morphologically following visual evaluation, then subsequently identified using procedures that included gram stains, and when appropriate, an evaluation for production of catalase and oxidase. Additional identification criteria for bacterial isolates were based on methods described in microbiology manuals (Isenberg, 1998). Primary identification of Staphylococci was made on the basis of colony morphology, Gram staining, and conventional catalase test. The staphylococcal isolates were further tested for coagulase synthesis, lack of colony pigmentation and acetoin production with additional confirmation being done using API 20-STAPH (bioMérieux; Marcy l'Etoile, France) and polymerase chain reaction (PCR) with previously

described *S. intermedius*-specific primers (Baron *et al.*, 2004).

Molecular identification of isolated S. intermedius

Extraction of bacterial DNA was performed by suspending 3–4 colonies of freshly subcultured strains to be investigated in 180 ul TE buffer (10 mM TrisHCl/l, 1 mM of ethylenediaminetetraacetic acid (EDTA)/l, pH 8 and 8 ul lysostaphin (1.8 U/ml; Sigma, Steinheim, Germany). After incubation for 1 hour at 37°C 20 ml proteinase K (Quiagen, Hilden, Germany) was added and the suspension was incubated for 2 hours at 56°C. The DNA was subsequently isolated with Dneasy Tissue-Kit (Qiagen) according to the manufacturer's recommendations. A PCR mediated amplification of *S. intermedius* was done according to Baron *et al.* (2004) using *S. intermedius* ATCC 29663 as reference strain.

Antimicrobial Susceptibility Tests

Antimicrobial susceptibilities of S. intermedius isolates to 16 different antimicrobials from 11 classes were tested by a disk diffusion test according to the Clinical and Laboratory Standards Institute (CLSI, M2-A9) guideline. The tested antibiotics and their concentrations were ampicillin (10 μg); amoxicillin-clavulanic acid (30 μg); amikacin (30 μg); chloramphenicol (30 μg); clindamycin (2 μg); ciprofloxacin (5 μg); cefotaxime (30 μg); erythromycin (15 μg); gentamicin (10 μg); penicillin (10 units); trimethoprim-sulfamethoxazole (23.75 μg, 1.25 μg); tetracycline (30 μg), and vancomycin (30 μg) (BD BBL). In addition, the

minimal inhibitory concentrations (MICs) to oxacillin (Sigma- Aldrich, St. Louis, MO, U.S.A.), were determined by a broth microdilution method according to the CLSI guideline (M2-A9). Isolates with oxacillin MIC≥4mg/l were classified as being methicillin resistant, and were further confirmed by PCR using primer sets targeting the mecA gene as MRSI.

Detection of the mecA Gene by PCR

The presence or absence of the mecA gene among the S. intermedius isolates was determined by the PCR using the mecA-specific primers as previously described by Zubeir et al., 2007. The oligonucleotide primer sequences used for identification of the resistance gene mecA were initially described by Strommenger et al. (2003). Details of the primer sequences and thermal cycler PCR programs are summarized in Table 1. The PCR reaction mixture (50 ul) contained 0.5 ul of each primer (50 pmol/ul), 25 ul 2X master mix (Finzyme) and 19 ul deionized H2O. Finally 5 ul DNA preparation was added to the PCR reaction mixture. The reaction mixtures were then subjected to thermal cycling (Gene Amp PCR System 2400, Perkin Elmer, Germany). The presence of PCR products was determined by electrophoresis of 10 ul of reaction product in an 1.5% agarose gel (Gibco BRL, Karlsruhe, Germany) with Tris-acetate electrophoresis buffer (TAE, 4.0 mmol/l Tris, 1 mmol/ l EDTA, pH 8.0) and visualized under UV light (Image Master VDS, Pharmacia Biotech, Freiburg, Germany).

Table (1). Oligonucleotide primer sequences and PCR conditions used in the present study

Primer	Program [a]	Sequence	Suspected length	References
SInuc1 SInuc2	1	CAA TGG AGA TGG CCC TTT TA AGC GTA CAC GTT CAT CTT G	125 bp	Baron *et al.* (2004)
mecA1 mecA2	2	AAA ATC GAT GGT AAA GGT TGG C AGT TCT GCA GTA CCG GAT TTG C	532 bp	Strommenger *et al.* (2003)

[a]PCR program 1: 1cycle (95 ºC, 240 s), 30 cycles- (95 º C, 30 s; 55 º C, 30 s; 72 º C 30 s), and 1cycle (72 º C, 420 s). 2. 1 cycle (94 º C, 240 s), 40cycle (94 º C, 30 s; 55 º C, 30 s; 72 º C 60 s), and 1cycle (72 º C, 300 s).

3. Results:

The clinical presentation included panorama of clinical signs which differed according to the form of pyoderma. Six cases of surface pyoderma (13.6%) manifested by erythema, scales, crusts, pruritis, weepy skin (purulent exudates) and salivary staining on rump when bitting it (Fig. (I), A and B). Twenty nine cases of superficial pyoderma suffered from massive erythema, pustules, erythema, cracks, excoriations, epidermal collarettes, pruritis, alopecia, bad odour of skin and easily epilated hairs (Fig.(I), C, D, E and F). Nine cases of deep pyoderma (20.4%) showed massive alopecia allover the body, erythema, very offensive odour of skin, small abscesses and pododermatitis (Fig. (I), G and H).

The initiating etiology of empirically treated dog was illustrated in table (2). Flea allergic dermatitis (8 cases out of 44) and Demodectic mange (8 cases out of 44), each one represented by 18.2 % as primary cause of pyoderma. Cases of flea allergic dermatitis were manifested by presence of fleas or flea dirt, intense pruritus, signs of secondary pyoderma. In demodectic mange, mostly animal kept in captivity). Cases of sarcoptic mange (3 cases out of 44) represented by 6.8% and showed signs of secondary pyoderma, crusty ears and massive itching. Allergic conditions included contact allergic dermatitis (4 cases out of 44- 9%), atopy (2 cases out of 44- 4.5%), food allergy (2 cases out of 44- 4.5%) and flea allergic dermatitis. Cases of allergic conditions were manifested by signs of secondary pyoderma and intense pruritus.

Figures (I): Clinical presentation of different forms of pyoderma

A-6.3 Ys old German Shepherd dog suffered from surface idiopathic pyoderma showing crusty lesion with purulent exudates (Hot spot or pyotraumatic pyoderma by self-inflicted trauma) in the skin of wither.; B-4.7 Ys old German Shepherd dog suffered from surface pyoderma displaying salivary staining as self induced trauma, moist exudative areas of erythema and detructed hairs in the skin of back (flea allergic dermatitis).; C-5 months old Bull Mastiff female puppy suffered from superficial pyoderma manifested by papules, pustules and crusts (Juvenile pyoderma or impetigo) in the skinof ventral abdomen.; D-2.9 Ys old Rottweiler bitch suffered from superficial pyoderma showing patchy alopecia, ulceration and purulent exudate (idiopathic short coated dog pyoderma) in the skin of hunch.

E-9.4 Ys German Shepherd dog suffered from superficial pyoderma displaying massive ulceration, papules, pustules, crusts and patchy alopecia (folliculitis after contact allergic dermatitis) in the skin of back.; F-6.5 Ys German Shepherd dog suffered from superficial pyoderma manifested by diffuse alopecia, large numbers of papules and pustules (Lumpy- bumpy disease).; G-3.7 Ys Neapolitan Mastiff dog suffered from deep pyoderma showing lichenification of skin and patchy alopecia in different body regions (furunculosis after demodicosis detected microscopiaclly).; H- 2.4 Ys old Labrador dog suffered from deep pyoderma showing erythema and ulcerations (pododermatitis or interdigital pyoderma).

The form of idiopathic pyoderma in 12 dogs treated on the basis of bacteriological investigations was 2 cases of surface pyodermas, 6 cases of superficial pyodermas and 4 cases of deep pyodermas.

Types of pyoderma in both groups (1) and (2) was arranged in order where the highest percentage was superficial pyoderma (29 out of 44 dogs- 66%), followed by deep pyoderma (8 out of 44 dogs- 20.5%) and surface pyoderma (6 out of 44 dogs- 13.6%). Idiopathic cases without underlying etiologies (17 cases) represented 38.6% of all pyoderma cases in both groups (1) and (2).

*Table (2): classification of cases according to depth and initiating cause of Group 1 (32 empirically treated dogs)

Initiating cause	Surface P.	Superficial P.	Deep P.	total
FAD	one dog	7 dogs	0 dog	8
Atopy	0 dog	One dog	One dog	2
Food allergy	0 dog	2 dogs	0 dog	2
Contact allergic dermatitis	One dog	3 dogs	0 dog	4
Demodectic mange	0 dog	5 dogs	3 dogs	8
Sarcoptic mange	0 dog	2 dogs	One dog	3
idiopathic	2 dogs	3 dogs	0 dog	5
total	4	23	5	32

*P. = pyoderma   *FAD = flea allergic dermatitis

Skin scraping revealed 8 cases of Demodex canis and 3 cases of Sarcoptes spp. While fecal examination demonstrated 8 cases of Dipylidium caninum egg nests.

Response to treatment in group (1) differed according to the form of pyoderma. All cases of surface pyoderma responded efficiently to topical treatment. The treatment period lasted 7-14 days. Cases of superficial pyoderma cured by topical treatment and Augmentin® within 13 days to 4 weeks without recurrence except 4 cases. The four cases suffered from demodectic mange (2 cases) and food allergy (2 cases). The used antibacterials in both conditions shifted every 2 weeks till complete cure into Rocephin® vials then Ciprocin® tablet. The response delayed in demodectic mange upto 6-7 weeks. While in food allergy was responded in 6 weeks by the elimination trial. Cases of deep pyoderma responded to topical treatment and systemic antimicrobials within 3 weeks- 13 weeks (except 4 cases). One case of generalized demodecosis euthanized as it was not responded to treatment. There were 3 cases of recurrence, 2 cases of demodectic mange and one case of atopic dermatitis which delayed the treatment of deep pyoderma.

Results of microbial isolation

S. intermedius, which preliminary identified based upon their properties in culture, beta-hemolysis on sheep blood agar, a positive coagulase, a negative Voges Proskauer and the results of API 20-STAPH, was isolated from 12 samples (100%) taken from the skin during the time period under investigation and was isolated alone from the skin of 7 dogs (58.3%). It was isolated in combination with Corynebacterium spp. and S. aureus from the other samples (41.7%) as shown in the table (3); the species identity confirmed by PCR yielding positive reactions with the S. intermedius nuc gene specific oligonucleotide primer.

Table (3) results of microbial isolation

Animal species	No. of samples	S. intermedius	S. aureus	Corynebacterium spp.
German Shepherd	8	8 (100%)	1 (12.5%)	4 (50%)
Rottweiler	2	2 (100%)	0 (0%)	1 (50%)
Labrador	2	2 (100%)	0 (0%)	2 (100%)

Antibiotic resistance of S. intermedius isolated strains

The result of disk diffusion test is shown in Fig. 1. Resistance to more than three antimicrobial classes represented multidrug resistance (MDR); MDR was exhibited by 9 (75%) of the 12 S. intermedius isolates. Oxacillin MIC testing revealed 6 S. intermedius isolates (50%) to be resistant, which included 2 strain with the mecA gene that did not display oxacillin resistance in the MIC test.

Identification of MRSI Using mecA Gene PCR

PCR was performed to detect the mecA gene in all 12 S. intermedius isolates. The mecA gene was detected in 5 (41.7%) isolates; of these, 3 exhibited MIC-determined oxacillin resistance.
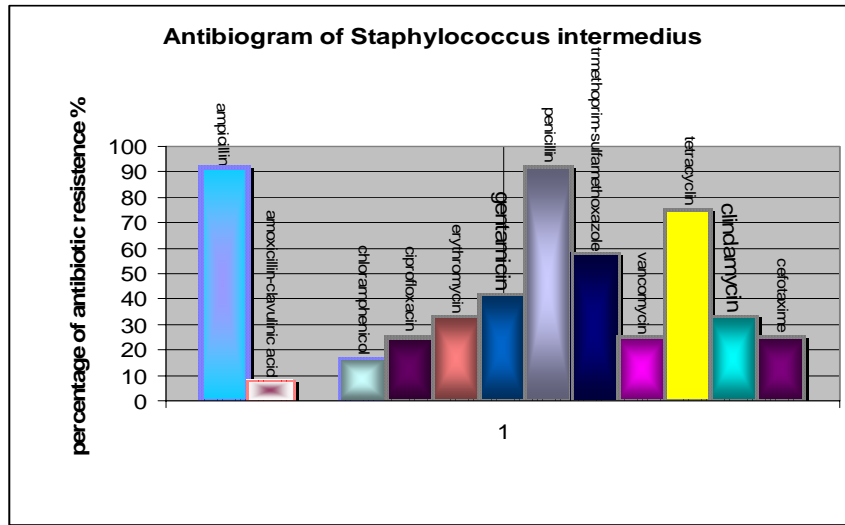
Fig. 1. Antibiogram of *Staphylococcus intermedius* isolates
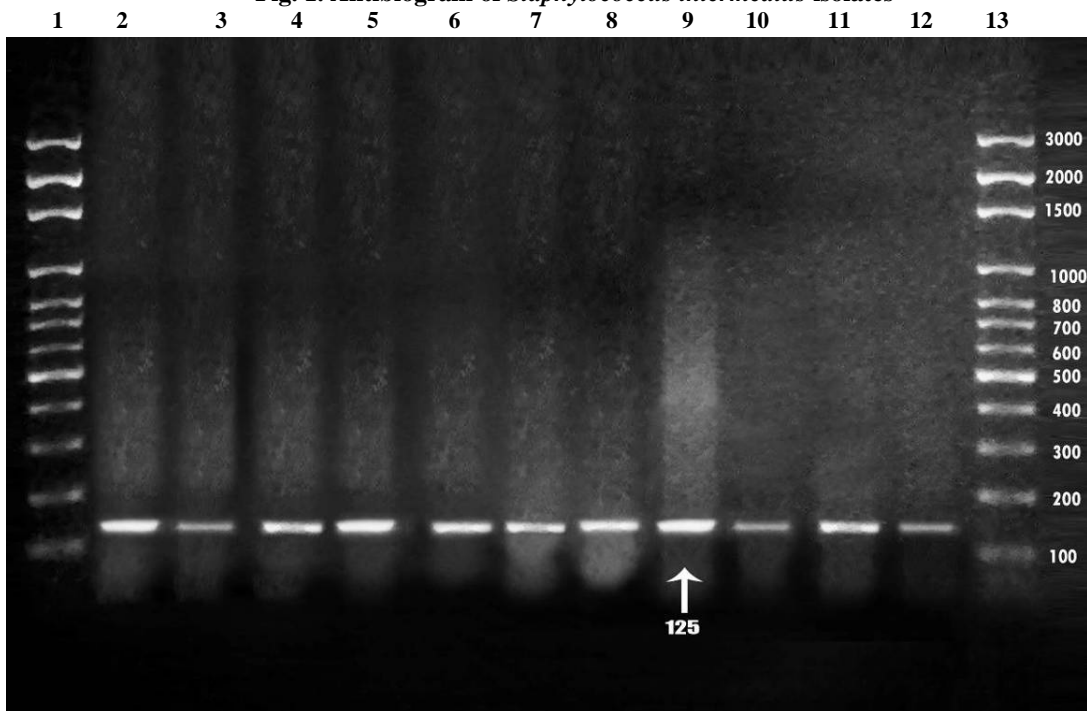
Fig. 2. PCR assay for identification of *S. intermeius*. Lanes 1&13 DNA ladder; Lanes 3-12 *S. intermedius* isolates from pyoderma cases; Lane 2 reference *S. intermedius* strain.
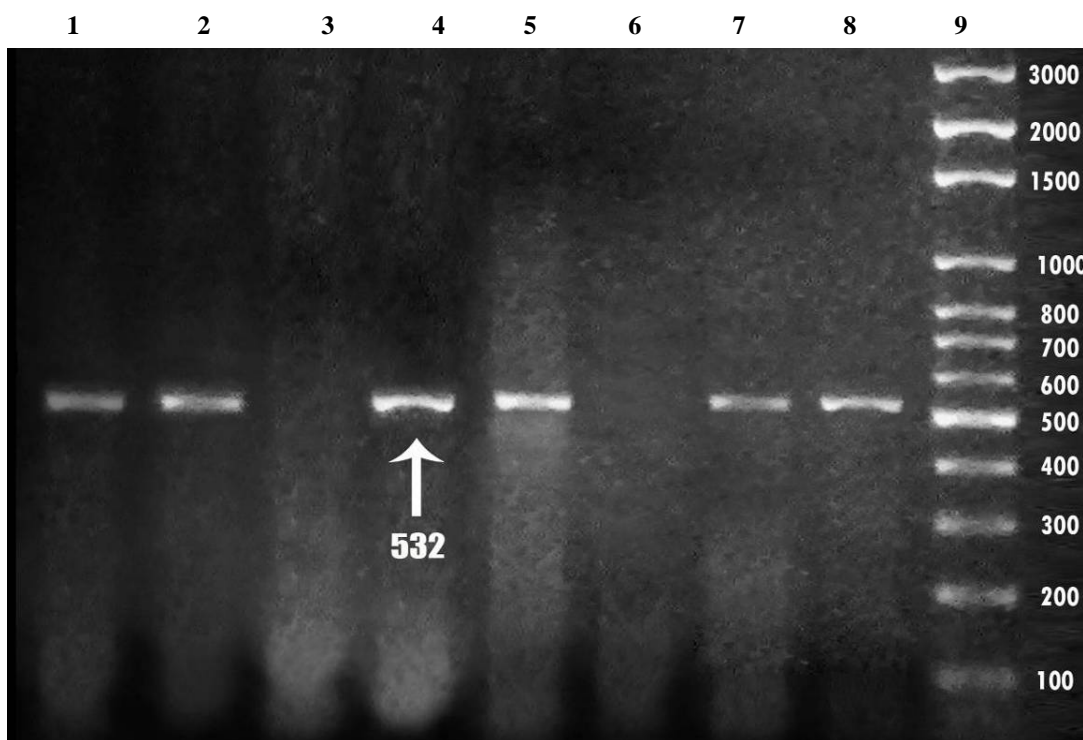
Fig. 3. PCR assay for identification of methicillin-resistant *S. intermedius*. Lanes 1, 2, 4, 5& 7 MRSI isolates; lanes 3& 6 non MRSI isolates.

Response to treatment in group (2):

Six cases were responded to amoxicillin-clavulinic acid within 2- 3 weeks. Four cases were treated effectively by ciprofloxacin within 3- 6 weeks. While 2 cases were treated to cefotraxione, where one case was responded within 3 weeks and the other case was responded within 5 weeks.

4. Discussion:

Canine pyoderma is a complex of diseases involving bacterial infection at different levels of the skin (Table 2) and requiring different approaches to therapy. These diseases are nearly always secondary and so it is important to identify underlying factors. Commonly these are allergies but endocrinopathy, immunodeficiency, ectoparasitic infestation, follicular dysplasia and breed predisposition may be involved. Diagnosis of underlying conditions may not be easy. Treatment during the diagnostic phase was designed to advance diagnosis and to avoid camouflaging diagnostic clinical signs. Antibiotic therapy is a good diagnostic strategy as it eliminates pyoderma and helps expose underlying conditions (Scott *et al.*, 2001 and Patel, 2006).

For many reasons, clients may not permit or be able to afford all diagnostic procedures deemed appropriate by the clinician. Therefore, empiric treatment is often prescribed by the veterinarian. Whenever treatment is administered or prescribed without a clear diagnosis, the treatment should be considered diagnostic procedure. Antimicrobials used to rule out or reduce bacterial dermatitis. The choice of antimicrobials was differed according to form of pyoderma (Kruse *et al.*, 1996; Holm *et al.*, 2002 and Loeffler *et al.*, 2005).

Pyoderma was long-standing problems that may predispose for development of resistance due to repeated or prolonged antimicrobial treatment (Guardabassi *et al.*, 2004 and Morris *et al.*, 2010). It is noteworthy that 81% of the total amount of antimicrobials prescribed for companion animals was the broad-spectrum compounds, cephalosporins, extended-spectrum penicillins (69% with clavulanic acid) and trimethoprim potentiated sulphonamides, which is much in contrast to human medical practice (Heuer *et al.*, 2005).

Primary disease processes should be considered whenever a case has been recurring pyoderma or pyoderma that does not respond to the appropriate therapy. A number of factors predisposed dogs to a bacterial infection of the skin. Underlying diseases such as ectoparasitism or flea allergic dermatitis (18.2%), atopy (4.5%), adverse reactions to food (4.5%), contact allergic dermatitis (4.5%),

sarcoptic mange (6.8%), resulted in secondary pyoderma caused by self inflicted trauma. Non pruritic conditions associated with bacterial infections include specific immunodeficiency syndromes as immunoglobulin A deficiency in German shepherd (38.6% in our study) as reported by Miller (1991) and demodicosis (18.2% in our study) as recorded by Leib & Monoroe (1997).

Dogs get pyoderma more readily than people due to the unique characteristics of dog skin. Dog skin has a thin stratum corneum with less lipid material and unprotected hair follicles that are increased risk for bacterial invasion and subsequent colonization and overgrowth. This may lead to superficial bacterial folliculitis. There is the potential for the transfer of bacterial resistance genes from drug resistant staphylococci of dogs to human pathogenic staphylococci (Divriese et al., 2005 and Takashi et al., 2007).

German Shepherd dog pyoderma is a deep pyoderma that has a familial tendency in the breed. The mode of inheritance is hypothesized to be autosomal recessive. The etiology is unknown, but an immunologic defect is suggested (Divriese et al., 2005; Stegemann et al., 2006 and Takashi et al., 2007)

Staphylococcus intermedius is the primary pathogen of canine skin. It is a coagulase-positive, B-lactamase-producing staphylococcus (Bannoehr, 2007). The cell wall of this bacterium contains the substance protein A, which has many biological effects, including activation of complement, induction of immediate and delayed hypersensitivity reactions, lymphocyte stimulation and inhibition of phagocytosis (Cox et al., 1986; Miller, 1991 and Day, 1994). Staphylococci also produce a variety of enzymes that may participate in the pathological processes (Leib & Monoroe, 1997 and Scott, 2001). Although coagulase-positive Staphylococci are not considered part of the resident microflora of canine skin, they are consistently present on normal canine hair (Allaker, 1991 and McEwan et al., 2006). Adherence of bacteria to the epithelial cells is required for bacterial colonization, environmental (temperature and humidity), host (underlying diseases) and organisms characteristics affect adherence.

Six cases of surface pyoderma was represented by 13.6%. Acute moist dermatitis was attributed to bacterial proliferation on the skin and subsequent release of bacterial toxins and enzymes resulted in inflammation and pruritus. As the animal traumatizes the skin in response to the pruritus, The infection became more severe and a vicious circle of pyoderma and pruritus was established (Leib & Monoroe, 1997 and Loeffler et al., 2008).

Twenty nine cases of superficial pyoderma represented by 66%. Folliculitis was occurred as follicle growth cycle shifted to the telogen phase after inflammation occurs in and around the follicle. Hairs were the easily epilated, resulting in diffuse or patchy alopecia (Leib & Monoroe, 1997 and King et al., 2006).

Seven cases of deep pyoderma (Furunculosis- 20.5%) was the end stage of untreated cases of folliculitis. When furunculosis occured, the infectious agent responsible for folliculitis and the keratinized structures of the follicle (e.g., hair) were released into the surrounding dermis. The result was a deep infection of the skin and secondary foreign body reaction in the dermis. Draining tracts developed if the follicle was destroyed (Leib & Monoroe, 1997). High percentage of superficial and deep pyoderma in our work-up was attributed to carelessness of companion animal owners about the dose and duration of antimicrobials. When the pets achieved clinical remission, the owners stopped the treatment. So, the bacterial resistance and multi-drug resistance were the end result. Response to treatment in our study by tapering method using amoxicillin-clavulinic acid, cefotraxione and ciprofloxacin was within 3-13 weeks except 3 cases of demodecosis and one case of atopy. Also results of Mueller & Stephan (2007) indicated that pradofloxacin as a member of flouroquinolones was an efficacious therapy comparable to amoxicillin- clavulinic acid for deep bacterial pyoderma in dogs. While Negre et al. (2009) recorded that deep folliculitis or furunculosis was treated within 4-6 weeks and in some cases of scarred granulomatous lesions was cured within 3-6 months.

Pododermatitis was associated with foreign material (e.g. plant awns), bacterial folliculitis, trauma, atopy, adverse reaction to food, demodecosis and contact allergic dermatitis (Leib & Monoroe, 1997). The bacterial component of the pododermatitis may be a primary factor or an opportunistic infection (King et al., 2006 and Bannoehr et al., 2007).

With discharging lesions, antimicrobial washes and soaks were useful to remove pus and debris, and were accelerated the recovery. Clipping was helpful, enables the extent of lesions to be demonstrated and can be useful in persuading clients to comply with treatment. Prolonged systemic antibiotic treatment with bactericidal antibiotic was necessary and was continued for at least two weeks beyond clinical cure. Where lesions were in areas with poor blood supply or large granulomatous lesions, fluoroquinolones, which penetrate well, are particularly useful. Plant et al. (1992); Rosenkrantz (2006) and Loeffler et al. (2008) also reported that cleaning of the skin and topical therapy in some cases

2-3 times a week was very effective in preventing recurrent pyoderma and bacterial overgrowth and yeast as well as irritants and allergens. In addition to moisturizing the skin resulted in decreasing of pruritus. For MRS, daily therapy was needed and generally was best with shampoos but sprays and rinses may also be effective.

Despite the reported escalation of methicillin resistance (MR) in staphylococci of veterinary origin, the majority of staphylococcal strains residing on dogs and cats continued to be susceptible to most classes of antibiotics, including the beta-lactams. Therefore, empirical therapy of first-time skin and soft tissue infections with "pet friendly" drugs such as amoxicillin-clavulanic acid, cephalosporins, and clindamycin continued to constitute acceptable practice (Abraham et al., 2007; Griffeth et al., 2008 and Morris, 2010). The absence of resistance to amoxicillin with clavulanic acid in this study is encouraging, albeit surprising, since this drug combination for many years has been one of the most often prescribed antimicrobials for dogs, in particular for skin infections, due to the very frequent resistance to penicillin (Heiene et al., 2004).

To our knowledge, this study was the first published analysis of the antibiograms of S. intermedius isolated from dogs in Egypt. 12 S. intermedius isolates (8 German Shepherd, 2 Rottweiler, and 2 Labrador) were isolated in a percentage of 100%, Which was a higher isolation rate than that reported in a previous study (Morris et al., 2006 and Youn et al., 2010). The reasons for the high rate of S. intermedius isolation from the skin may be due to the samples were collected from wound area and S. intermedius is a temporary bacterium on the skin and hair coat of dogs (Hartmann et al., 2005). The high antibiotic resistance of S. intermedius isolates against penicillin and ampicillin (Fig. 1) might be due to the frequent use of penicillin G and ampicillin in small animal hospitals in the past few years in Egypt. The results of the disk diffusion test of S. intermedius isolates from the present study were different from previous 2 studies (Boerlin et al., 2001 and Loeffler et al., 2007) while they were agree with that obtained by another previous study (Youn et al., 2010). All four studies showed high resistant rates against penicillin, whereas the S. intermedius isolates for example displayed 100% resistance against amoxicillin-clavulanic acid (Loeffler et al., 2007) which is markedly higher compared with resistance rates of 8.3% determined presently and the rate of 2.74% and 1.37% determined in the other studies (Boerlin et al., 2001 and Youn et al., 2010, repectively). These differences might be due to the variation in the sampling source (wound area versus wound-free

area), diverse selective pressure exhibited by the use of different antibiotics in different regions (Germany, Switzerland, Korea and Egypt), and sampling sources. Presently, oxacillin MIC results indicated a resistance rate of 50%, which is markedly different from the rate of 0% reported by five other studies. The occurrence of S. intermedius resistant to all antimicrobials commonly used for systemic therapy in small animal medicine is alarming. This is of special concern, since S. intermedius has so far not presented as a therapeutic problem in pets in Egypt and resistance to several antimicrobial classes appears rare (Ganiere et al., 2005; Lloyd et al., 1996 and Manian, 2003). In addition, a total of 5 S. intermedius isolates (41.7%) possessed the mecA gene and thus the abbreviation MRSI is appropriate in analogy to MRSA.

In conclusion, the emergence of MRSI in Egypt as a cause of canine skin infection is alarming and this highlights the importance for vigilance by hospital staff, who may serve as carriers for the pathogen. To prevent transmission and avoid the outbreak of disease caused by S. intermedius and MRSI, prudent use of antibiotics and strict infection control practices in animal hospitals should be enforced. In addition, continuous monitoring and molecular epidemiological studies should be followed. As the animals spend the main time with their owner and in their homes, continuous sampling of these two groups will give us more information about the spread and antibiograms of S. intermedius. The prudent strategic use of antimicrobials, reserving systemic application for deeper and more complicated infections, may limit the spread of multiresistant staphylococci in patients with skin disease. With antimicrobial resistance as one of the most pressing public health problems for the future, the veterinary profession needs to be proactive in monitoring and controlling antimicrobial resistance and use in small animals at the local, national and international level. Advice on the zoonotic potential of staphylococci is important when dealing with multidrug resistant isolates and for immunocompromised owners or those undergoing surgery and this should only be assessed by medical doctors together with their full medical history.

5. Conclusion:

Canine recurrent pyoderma is a group of various skin diseases and an accurate diagnosis is mandatory. In the cases of broadly drug-resistant pyoderma, intensive topical therapy is often beneficial as either the sole treatment for localized pyoderma, or as an adjunct to systemic treatment of more generalized disease. Antibiotics must be prescribed carefully at an adequate dose for an

appropriate duration. This investigation provided data on occurrence of antimicrobial resistance in important pathogenic bacteria from dogs as the first record in companion animals in Egypt, which may be useful for the small animal practitioner. Resistance was low to the compounds that were most often used, but unfortunately, these compounds were broad-spectrum. Data on resistance and usage may form a background for the establishment of a set of recommendations for prudent use of antimicrobials for companion animals.

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Morphological and Anatomical Evaluation of a new five Stone Fruit Rootstocks

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ABSTRACT: Comparative study was recorded for the five stone fruit rootstocks: GF677 – Tetra pdm 5450 – Saint Julian- Myroblan 29c and Nemaguard throughout 2007 and 2008 seasons. This investigation included: leaf shape and dimensions; Vegetative and floral bud patterns;Stomata shape and dimensions; vegetative and floral buds patterns ;stomata shape and dimensions; tree canopy and growth habit; reproductive under Egyptian condition; fruit set percentage and date; chlorophyll percentage; root distribution (Number, length and weight of different root diameters through the soil profile), as well as, cross section of the stem dimensions (epidermis, cortex, pholeom, xylem and pith).
Data showed a great variation of the studied characters, so a clear key was made to identify these rootstocks.
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Key words: rootstocks, stem dimensions, peach, peach-almond hybrid, plum, seed germinations,

1.INTRODUCTION

Rootstocks (the below – ground portions of fruit trees) play a major role in modern orchards. Recently, the importance of the rootstock, which has an essential value for fruit yield is noticed. The rootstock together with the grafted cultivar, influence the vegetative and generative mass and profitability of fruit production (Racsko *et al*., 2004). Moreover, choosing the proper stock is one of the major factors that influence the growth and production of stone fruit tree (Dozier *et al.* 1984).

On the other hand, the most important agricultural traits and the tree as a biotic unit : such as vigor, blossom initiation, fruit set, fruit size and fruit flavour, etc. ; may be, substantially, influenced by the rootstock (Tubbs, 1974 and Dozier *et al*., 1984). Moreover, the rootstock determines the ecological fitness of the tree. Their effects can be recognized in the health status of critical tree phonological stages, tree kilter and tree sensitivity to pests and diseases (Holb, 2000 and 2002). Also in the efficiency of pest and disease management programs and fruit yield (Holb *et al*., 2003 *a* and *b*). Rootstocks with good ecological fitness are in caressingly important in environmentally, friendly fruit production (Racsko *et al*., 2004).

Also, Antonio *et al*., (2008) studied the influence of eight rootstocks on fruit quality of (pioneer) Japanese plum and stated that, rootstock effect was variable because of the strong interaction due to (rootstock x year). Apricot, peach and Japanese plums are the important stone fruit crops grown in Egypt. In addition, the total stone fruit area made up in the old lands represents 16% with production of

86.933 tons / year. Meanwhile, 84% of this crop in the new reclaimed soils with production of 389.916 tons / year (Anonymous, 2005).

Five stone fruit rootstocks namely GF677, Saint Julian, Myroblan 29c, Tetra pdm 5450 plum and Nemaguard were study.

GF677 (Almond X Peach): originated near lot-Et-Garonne in south western France is a natural hybrid of peach x almond discovered in 1938 by Silored and Soaty introduced in 1965. Very vigorous, it makes tree 10% to 20% larger than trees on peach rootstocks. It is a clone rootstock difficult to propagate by conventional methods. Tolerant of high lime concentration in soils sensitive to root asphyxia (Cummins, 1991).

Kamali *et al* (2001 b) reported that, GF677 (peach-almond hybrid) is one of the most suitable rootstock used in calcareous soils to overcome lime induced chlorosis, but susceptible to root –Knot nematodes.

Saint Julian (plum): is Semi –dwarf rootstock for areas with fluctuating spring temperatures due to inconsistent spring weather conditions preferred over Eitatin in north coastal mountains and Oregon.

Tetra pdm 5450 (plum) is suitable for all kinds of soil even heavy soils in which peach generally suffers from water logging Nicotra and Moser (1997). {Reighard (2000)} added that Tetra is resistant to root – knot nematode, but moderately resistant to *Meloidogyne Javanica*,

Concerning the studied rootstocks Saint Julian and Tetra considered dwarf and semi-dwarf rootstocks respectively .

Jackson (1986) summarized, dwarfing rootstocks reduce vegetative vigor and tend to cause more flower –bud formation in younger trees. This being termed (precocity) two ways in which they could reduce vigor. Firstly, cross sections through the trunk of dwarfing rootstocks reveal a higher proportion of bark relative to wood than in vigorous rootstocks. This may alter the pattern of translocation in such a way that vegetative growth is reduced. The second possibility is that hormones produced by the roots affect the vegetative growth of stems, thus; dwarfing rootstocks may produce fewer growth promoters and or more inhibitors. Erez (1976) demonstrated that dwarf tree walls are desirable because of the very high cost of hand harvest of large trees.

Myroblan 29c seedlings (plum): used as the principle rootstock for Japanese (*prunus salicina*) and European (*prunus domestica*) plums in Ontario. There is significant variability in size and performance among trees grown on Myroblan 29c seedling rootstocks. It is native to south Eastern Europe and south western Asia, and widely adaptable to different soil types and moisture condition. It is not tolerate to extremely heavy soils. Myroblan29c seedling is a vigor rootstock in North America. The main advantage of Myroblan seedling is that it provides better tree anchorage than other plum rootstocks. Its principal disadvantage is the variability in tree performance in the orchard due to difference among seedlings.

Nemaguard (Peach rootstock): is originated in Fort Valley, Georgia, by the U.S. Dept. Agr. Hort. Field Laboratory and introduced in 1961, A selected seedling from seeds obtained in 1949 from a commercial importer as *Prunus davidiana* Tree and fruit characters resemble peach (*P.Persica*) yet it may be of hybrid origin, Tests of seed germination compatibility with various peach nematode resistance and top growth of scions have been made,

Percentage of seed germinations is high, peach buds compatible; showing very species satisfactory growth, resistance to root knot nematode species equally to any other stock tested at Belts ville and fort valley tests. (Brooks and Almo, 1972).

The use of trichomes and stomata morphology in taxonomy have specific role in the classification of genera with species and in analyzing interspcific hybrids {Fahn 1974 ; Metcalfe and chalk 1979}.

This investigation was carried out to detect quantitavely the morphological characters, vegetative growth, flowering parameters of five new stone fruit rootstocks introduced from Italy. Also, Root distribution cross histological studies was

carried out under tip shoots of one year old. Stomata imprison to clear the range of different aspects between them, in addition a key has been constructed for each examined stock separately and Anatomy study of stem was also carried out.

2.MATERIALS AND METHODS

present study was conducted throughout 2007 and 2008 seasons to evaluate performance and adaptability some new introduced deciduous fruit rootstocks under Egyptian conditions.

These rootstocks namely GF677 (peach x Almond), Tetra pdm5450 (Plum), Saint Julian (Plum), Myrobolan29C (plum), and Nemaguard (peach) were introduced from Italy in 2004 Season. Rootstocks seedlings planted 5m apart in Kafer Ashma nursery in Monofia governorate on a sandy loam soil and flood irrigation system. Each rootstock was representing in three replicates each was 3 rootstocks. The following determinations were measured.

2.1.Morphological study:

Five , one – year – old shoots were tagged on each cardinal point on each tree of each rootstocks. These shoots were leaf un-pruned to determine morphological study which included:

2.1.A) Rootstocks characteristics:
* Stem parameters: Stem shape – stem surface (Cylindrical - glabrous Shape).
* Leaf characteristic: system of leaves on shoot – leaf apex shape (obtuse or Tapering) – Leaf base shape (obtuse or tapering) – Tear base shape – leaf shape (oval or sereat) – margin crenate – Upper and lower surface (Coarse or smooth) - stipulate leaf- Flower study: Number of petiole – colour of petiole – number of calyx.

2.1.B) Bud break:
* Bud style: Compound or single bud – flower or vegetative bud.
* Beginning of bud break.
* Stomata system: Laracytic or Anisocytic – Shape of guard cells (Kidney or elliptical) - Average of stomata number- length and width of stomata.

2.2.Vegetative growth:
- Growth shape: Growth habit (erect, semi erect, spread).
- Enlarge of tree canopy: It was measured by meter.
- Tree height: It was measured by meter.
- Average number of main branches.
- Diameter of main stem: It was measured at 10cm above soil surface.
- Trunk diameter at shoot spot:
- Average number of shoot per branch.

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- Average length of shoot.
- Average number of internodes per branch.
- Average length of internodes.
- Average number of leaves per branch.
- Length and width of leaf.
- Leaf area: It was measured at the end of the growing season (August). Samples were taken from the fourth to the sixth leaves from the top of the selected shoots (three leaves per shoot X Five shoots) to measure their area in October by using LI – COR – Portabe area meter model LI -3000. Area was expressed as Cm^2.
- Chlorophyll percentage in leaf: At the end of the growing season, percentage of chlorophyll content was recorded using a spd 502 chlorophyll meter (Minolta corporation, Ramsey, N.J., USA) as chlorophyll readings (Vadava 1986).
- Stomata Imprison: Number of stomata per square millimetre in the lower Surface of the leaf was counted using the micrometer slide method and the light microscope (Williams *et al.* 1965). In addition, the same method was used to determine the length and width of the stomata for leaves were recorded.
- Dates of the beginning of vegetative growth.

2.3.Flowering parameters:

Only two of the five rootstocks flowered because of the high chilling requirements of the other three rootstocks. Date of the beginning of flowering , average number of flowers on shoot , period length of flowering ,the beginning of fruit set and fruit set percentage were determined.

2.4. Roots distribution study:

It was make two hole from each opposite side. One of them 50cm and the second 100cm distance from trunk with soil depth 30-60 cm. At each depth, it was taken samples to study number, length and diameter of root.

2.5. Anatomy study of stem:

Apical samples were taken and put in FAA (Killing and fixation solution) to study stem anatomy. After that, it used wax, Microtom cutting and dyeing. Cross section was discussed to separate between them. Diameter determined to textile in cross section were tabulated and discussed.

Data were statistically analyzed according to the method by L.S.D according to {Sendecor and Cochrar (1980)} in each season were used for comparison between means of each rootstock.

3.RESULTS AND DISCATION
3.1.Morphological study
3.1.A) Rootstocks characteristics:

All the studied rootstocks have cylindrical stem shape with smooth surface are shown in table (1). At the same time all of them had Irregular alternate leaf arrangement with stipulate ordinary leaf base and both of Nemaguard and GF677 had sereat leaves shape while Tetra pdm 5450, Saint Julian and Myroblan 29c had ovate leaf shape. Also, the studied rootstocks had crenate leaf margin. All the rootstocks had obtuse leaf apex.

The studied rootstocks had smooth Upper surface, on the other hand both of Tetra and Saint Julian had coarse on the lower surface while Nemaguard, GF677 and Myroblam had smooth lower surface. The leaves of the studied rootstocks had stipulate leaf and at the same time both of Tetra and Saint Julian had simple auxiliary bud along the stem ,while Nemaguard , GF677 and Myroblan 29c had compound buds each of them contain three buds one of them was flower bud.

From another point of view, Tetra pdm5450 had the appearance of unsatisfied chilling requirement that reads to irregular bud break at spring with vegetative bud break at the upper part of the stem only while the rest of its stem was without leaves. Stomata system was paracytic for all the studied rootstocks except for Nemaguard rootstocks it was anisocytic. All guard cells had Kidney shape, except Nemaguard had elliptical guard cell shape. Only both of Nemaguard and GF677 rootstocks were reproductive under Egyptian condition where the flowers had 47 stamens for both of them.

GF677 rootstock had 5 villot petals. Nemaguard has 3-10 light pink minute petals in the inner whorles and 5 petals in the outer whorles. Both Nemaguard and GF677 rootstocks had five sepals in their flowers. At the same time, Nemaguard trees had hypogenous flowers while GF677 had normal flowers, (Fahn, 1974; Metealfe and chak 1979)

The use of trichomes and stomata morphology in taxonomy was well known and important in the classification of genera with species and in analyzing inter-specific hybrids.

However, Attala (1993) stated that, leaf apex is acute in all cases except apricot which is obtuse. The base is acute in almond, hastate in peach, cordate in local apricot and rounded in myro B. The margin is always serrate except in apricot where it is dentate. Both flordaguard and Florda $^9/_3$ have a distinct purplish reddish colour. The highest shape index was that of Nemaguard. Blade and the lowest of local apricot which has the highest petiole index value. Longest stomata

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were those of Okinawa and Bitter almond while the narrowest were of Okinawa and Nemaguard. Moreover, Gorgi *et al*. (2005) working with peach rootstocks outlined that GF677 promoted the highest vegetative development. Also, Zielinski (1955) Showed that peach leaves are alternate and simple, deciduous or persistent mostly serrate and sometimes ovate.

3.1.B) **Bud break**:

Vegetative bud break was influenced by both winter chilling and G.D.H. in spring. However the results in

Table (2) stated that, bud break of Nemaguard rootstock was at 17-19March. Followed by both GF677 (21-24 March)and Myroblan 29c (24- 25March). While ,Saint Julian and Tetra rootstocks bud break were at 29-31 March on the other hand, GF677 rootstock flower bud break was at 1-5 of March and Nemagrad flower bud break was at 17-19 March in the two studied seasons, meanwhile Tetra pdm 5450, Saint Julian and Myroblan 29c rootstocks did not flower under local conditions .

Table (1): list of trees characters of different rootstocks under study.

rootstocks different characteristic	GF677	Tetra Pdm 5450	Saint Julian	Myroblan 29c	Nemaguard
Stem characteristic: Cylindrical shape (+)	+	+	+	+	+
Glabrous surface (+)	+	+	+	+	+
Leaf characteristic: * Arrangement: Irregular Alternate (+)	+	+	+	+	+
* base : stipulate ordinary (+)	+	+	+	+	+
* leaf shape: ovate (+) sereat (-)	-	+	+	+	-
* Leaf margin crenate (+)	+	+	+	+	+
* Apex: obtuse (+) tapring (-)	-	+	+	-	-
* base of blade Symmetrical (+)	+	+	+	+	+
Upper surface – Smooth (+)	+	+	+	+	+
Lower surface coarse (+) Smooth (-)	-	+	+	-	-
Stipulate leaf (+)	-	+	+	-	-
Buds compound (+) Vegetative (-)	+ -	-	-	+ -	+
Stomata System: Paralytic (+) Anisocytic (-)	+	+	+	+	-

Shape of guard cells Kidney (+) elliptical (-)	+	+	+	+	-
Flowers: (1)Num. of stamens (+)	47 stames	----	----	----	47stamens -3-10minute petals in the inner whorles.
(2)Num. of petiole	5 petals	----	----	----	-5petals in the outer whorles
(3)Color of petiole	villot	----	----	----	Light Pink -
(4)Num of calyse	5 sepals	----	----	----	5 sepals hypogenous flowers)-

   Attala (1993) summarized Variation in time and indices of bud burst for different stocks, Myroblan 29c is consistently delayed than other stocks. Bitter almond has the highest number of burst and Myroblan 29c has the lowest. While Myroblan29c seedling is a vigorous rootstock in North America.

   Rato *et al* (2008) working with different plum rootstocks grown reported that, GF677 10-2 promoted the largest fruit set and higher calcium fruit level and the highest firmness pulf values.

   However, the beginning of fruit set was at 17-19 March with GF677 while was 7-9 April with Nemaguard rootstocks throughout 2007 and 2008 Seasons. So, Flowering period of GF677 was shorter (15-16 days) than Nemaguard rootstock (19-23days). Generally, Nemaguard rootstock showed a High fruit set percentage (40-50%) comparing with GF677 (20-22%). Number of flowers/ branch were high with GF677 (14-25) Than with Nemaguard (3.5- 5.5) in the two studied seasons.

Table (2): Dates of vegetative and flowering bud break–flowering period-fruit set and No. of flowers per branch during 2007-2008 seasons.

Years	Rootstocks	Beginning of vegetative growth	Beginning of flowering	Ave No. . of flowers for branch	Flowering period	Beginning of fruit set	fruit set %
2007	GF677	24 Mar.	5 Mar.	25	15 days	19 Mar.	20
	Tetra pdm 5450	31 Mar.	-	-	-	-	-
	Saint Julian	30 Mar.	-	-	-	-	-
	Myroblan 29c	24 Mar.	-	-	-	-	-
	Nemaguard	19 Mar.	19 Mar.	5.5	19 days	7 Abr.	40
2008	GF677	21 Mar.	1 Mar.	14	16 days	17 Mar.	22
	Tetra pdm 5450	30 Mar.	-	-	-	-	-
	Saint Julian	29 Mar.	-	-	-	-	-
	Myroblan 29c	25 Mar.	-	-	-	-	-
	Nemaguard	17 Mar.	17 Mar.	3.5	23 days	9 Abr.	50

3.1.C) Stomata system:

Data in table (3) Indicated that, GF677 developed the highest significant number of stomata per unit area (12.8) followed by Saint Julian (11.3), Nemaguard (10.7) Tetra pdm 5450 (8.7). While, Myroblan 29c have the lowest significant stomata (6.7) concerning leaf stomata characteristic Tetra rootstock induced the highest significant stomata length.

Fergoni and Roversi (1968) stated that average number of stomata per unit of leaf surface did not differ appreciably in 10 peach varieties. While, Meidner and Mansfield (1968) stated that the number of stomata per unit area varies not only between specied but also, within any one species owing to the influence of environmental factors during growth.

Attala (1993) found that the highest number of stomata in the lower surface of leaves was found in Nemagard and the lowest in florda and sweet almond. Also, the longest stomata were in Okinawa and Bitter almond while the shortest ones were in sweet almond.

Concerning the length of stomata, Moore and Petersen (1968) found no difference in length of stomata in 10 peach cvs. (0.145 µ) and length/ width (L/W) ratio (2.07) but the least stomata width (0.07 µ). Myroblan 29c rootstock also has longer stomata (0.12 µ) and L/W ratio (1.54) but less width (0.078µ).

In the same view GF677 rootstock has middle length (0.111 µ) and L/w ratio (1.14) but the largest diameter (0.097µ).

The present results shed light on number, dimensions and the shape of leaf stomata, as well as, they agreed with Dejong and Ryugo (1985) who stated that, the stomata have an active mechanism for controlling their opening to permit just enough carbon dioxide into the leaf to allow photosynthesis to continue, thus higher number of stomata and big size (concerning the length and width) were promoted to increase the CO_2 entering the leaf.

Table (3): Number, length, width and length/width ratio of stomata per leaf for the different rootstocks under study.

Rootstocks / stomata system	Ave. num of stomata	Ave length of stomata (µ)	Ave diameter of stomata (µ)	Length / width ratio of stomata (µ)
GF677	12.8 A	0.111 B	0.097 A	1.14D
Tetra pdm 5450	8.7 D	0.145 A	0.070 C D	2.07A
Saint Julian	11.3 B	0.106 B	0.117 D	0.91E
Myroblan 29c	6.7 E	0.120 B	0.078 BC	1.54B
Nemaguard	10.7 C	0.111 B	0.086 B	1.29C

Means within each column followed by the same letter (S) are not significantly different at P = 0.05

3.2.Vegetative growth:
 - Enlarge of tree canopy – Tree height –
Number of main branches – growth shape –
trunk diameter:

Concerning table No. (4) only GF677 rootstock had the largest large canopy while Myroblan 29c and Nemaguard had medium canopy. On the other hand, both Tetra pdm 5450 and saint Julian rootstocks had the smallest Canopy.

Concerning rootstock height, GF677 rootstock induced the highest trees followed by Myroblan 29c, Tetra and Nemaguard, while Saint Julian recorded the shortest rootstock height.

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Number of the main branches was recorded for the five studied rootstocks; results revealed that the highest number was induced by GF677, Myroblan 29c and Nemaguard. On the contrary, Tetra pdm 5450 and Saint Julian showed the lowest number of main branches.

There are two types of branching habit erect and spread. Both of Tetra and Saint Julian rootstocks have erected growth habit while both GF677 and Myroblan 29c have semi – erect. On the other hand Nemaguard rootstock has spread branching Habit.

Concerning, trunk diameter GF677 developed the highest significant trunk diameter followed by Myroblan 29c and Nemaguard while the lowest significant trunk diameter developed by both Tetra and Saint Julian rootstocks. At the same time both GF677 and Myroblan 29c induced the highest diameter of breast height followed by Nemaguard. While, Tetra pdm 5450 and Saint Julian produced the lowest ones.

Concerning the tree canopy, GF677(hybrid of peach x almond) was a natural, very vigorous it made the tree 10% to 20% larger than trees on peach rootstock (Cummins 1991). Also Elfviny and Tahrani, (1980) demonstrated that there is a significant variability in size and performance among trees grown on Myroblan 29c seedling. Myroblan 29c is a very vigorous rootstock, because of its genetic uniformity. Trees on Myroblan 29c were uniform in size and performance in the orchard. Meanwhile, Giorgi *et. al.* (2005) stated that GF677 rootstock promoted the highest vegetative development for Suncrest peach CV. followed by Julior, Ishtara then Barrier rootstocks while were similar for their effect on plant yield. On the other hand, Attala. (1993) said that stock diameter could not be taken as a criterion for differentiation between stone fruit stocks, while Nemaguard had the highest tendency of feathering.

On the other hand, Pavline et al. (2007) stated that Romea and Catherina peaches grafted on GF677 produced the largest fruits (mean 189 gm).

-Number of shoots, shoot length and number of internodes per brunch:

Data in Table (5) showed that Number of shoots per branch revealed no significant differences among rootstocks during the first season but in the second season, GF677 rootstocks had the highest number of initiated shoots per branch, the least number was exhibited by other rootstocks.

Average shoot length per branch showed that the GF677 developed the highest significant shoot length followed by Nemaguard; the lowest length was recorded to Tetra, Saint Julian and Meroblane29c at the first season.
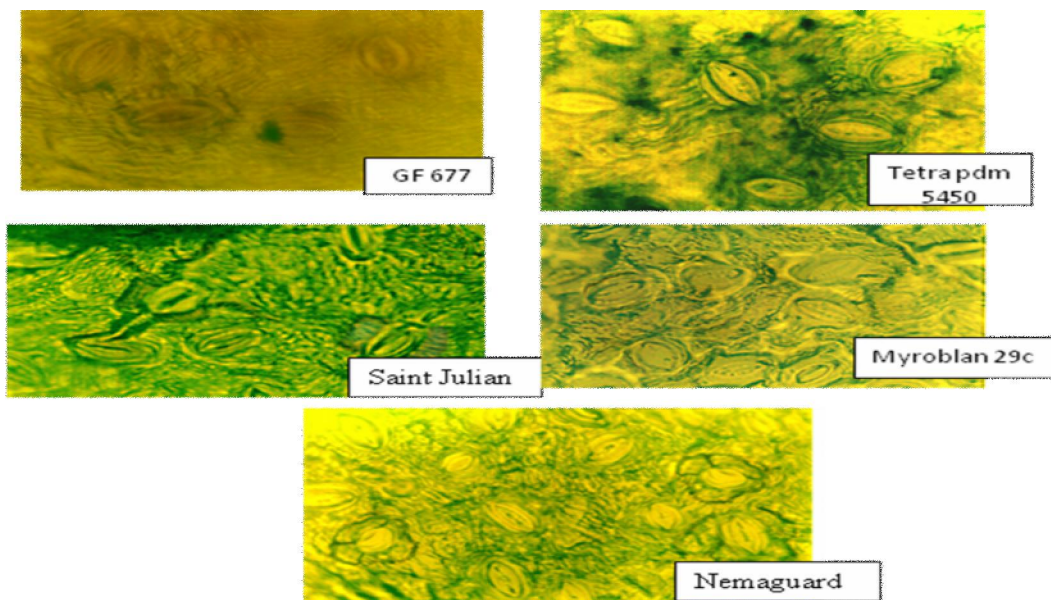
Fig. (1):- Stomata frequency per (μ) in different stone fruit rootstocks under study.

Table (4): Evaluation of the vegetative characters of the different rootstocks under study

Rootstocks	Trees canopy	Height	Ave. No. of Main branches	Growth habit	Ave. diameter trunk (cm)	Ave. diameter breast height (dbh)
GF677	7.5 m large	4.5 m	3.67 A	Semi -	38.00 A	43.0 A
Tetra pdm 5450	2.20 m. small	1.60 m	2.90 B	Erect	8.67 C	8.17 C
Saint Julian	1.2 m small	1.00 m	2.80 B	Erect	8.83 C	8.66 C
Myroblan 29c	4.54 m. medium	2.60 m	3.67 A	Semi - erect	33.00 A	37.0 A
Nemaguard	4.5 m	1.60 m	3.00 A	spread	16.33 B	17.83 B

Means within each column followed by the same letter
(S) are not significantly different at P=0.05

   At the same time GF677 rootstock induced the highest significant shoot length in the second season followed by Myroblan 29c, Nemaguard, Tetra and Saint Julian rootstocks.
    Concerning average length of internodes GF677 rootstock induced the highest significant length of shoot internodes followed by Myroblan 29c and Nemaguard. The least numbers were exhibited by Tetra, Saint Julian rootstocks in the first season. While in the second season GF677, Myroblan 29c and Nemaguard recorded the highest significant length of internodes followed by Saint Julian since Tetra induced the shortest internodes.
    On the other hand, Average number of internodes per branch didn`t affect significantly along the different studied rootstocks in the first season, while Myroblan 29c rootstock developed the highest significant number of internodes per branch in the second season, Nemaguard recorded the least number of internodes.
    Attala (1993) mentioned that local apricot stock has the highest number of internodes on side shoots while Myroblan 29c has the lowest.
    She found that the longest internodes was recorded to Nemaguard stock but Myroblan 29c had the shortest one.
    Rato *et al* (2008) demonstrated that GF 8 rootstock promoted the highest vegetative development comparing to GF 10-2 who promoted the highest fruit set.
    Erez (1979) reported that rootstocks for dwarfing sweet cherries were desirable because of the very high cost of hand harvest of large trees.
   At the same time ,Westwood (1993) outlined that dwarf stocks for *Prunus domesttica* plums showed promise as semi dwarfs stocks but they attain about $^{3}/_{2}$ the size of trees on peach or about $^{1}/_{2}$ size of trees on Mariana 4001 a very vigorous stock .

-Leaf measurement and chlorophyll percentage:
    Table (6)showed that mean initiated leaves per branch Nemaguard rootstock occupied the highest significant mean followed by Myroblan 29c, Saint Julian , Tetra pdm5450. Whereas GF677 rootstock occupied the lowest significant leaves per branch in the first season. The second season showed that Nemaguard produced the highest Number of leaves per branch followed by Nemaguard rootstock occupied the highest significant mean followed by myroblan29c initiated leaves followed by Meroblan29c and Tetra pdm5450 recorded the lowest . Meanwhile, both GF677and Nemagrad introduced the highest significant leaf length followed by Myroblan 29c since Tetra pdm5450 recorded the lowest significant leaf length in the two studied seasons.
    At the same time both Saint Julian and Myroblan 29c occupied the highest leaf width followed by Tetra pdm5450, GF677 whereas, Nemaguard produced the lowest significant leaf width in the first season. The second season, Myroblan 29c and Saint Julian developed the highest leaf diameter followed by Tetra pdm5450, Nemaguard and GF677.
    Regarding leaf area of different rootstocks GF677occupied the highest significant leaf area in the two seasons (40 and 44 cm ^{2}) followed by Nemaguard, Myroblan 29c, Tetra and Saint Julian Concerning leaf chlorophyll percentage both Myroblan 29c and Saint Julian rootstocks introduced the highest significant leaf chlorophyll percentage followed by Tetra pdm5450 and GF677, since Nemaguard produced the lowest significant leaf chlorophyll percentage in the first season. Also, Saint Julian and Tetra pdm5450 indicated the highest chlorophyll percentage followed by Myroblan 29c and GF677 since Nemaguard indicated the lowest significant chlorophyll percentage.
    Attala (1993) revealed that the highest number of leaves is developed on Okinawa peach, local apricot and sweet almond during the season. Leaf measurements indicate a significant elongation of peach leaves than the other stocks

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while local apricot had the widest leaves. Also, Turrel (1961) determined the total leaf area and crown surface area for Valencia orange tree of various ages.

Similarly, Hamouda (1971) recorded the leaf length width and area in six mandarin varieties. He found that the width and the area varied among the varieties.

Table (5): Average No. ,length of shoot and internodes per branch for the different rootstocks 2007-2008 seasons.

Years	Rootstocks	Ave. No. of shoot per branch	Shoot length (cm)	Ave. No. of internodes /brand	Ave. length of internodes (cm)
2007	GF677	3.67 A	68.33 A	26.33 A	2.833 A
	Tetra pdm	2.67 A	31.0 C	30.34 A	0.64 C
	Saint Julian	3.0 A	30.33 C	40.0 A	0.80 C
	Myroblan 29c	5.0 A	31.00C	37.0 A	2.0 B
	Nemaguard	3.67 A	53.0 B	26.35 A	1.67 B
2008	GF677	2.66 A	51.67 A	34 B	2.00 A
	Tetra pdm 5450	1.00 B	35.33 C	34 B	0.23 C
	Saint Julian	1.33 B	33.67 C	33.3 B	1.17 B
	Myroblan 29c	1.00 B	48.00 AB	45.3 A	2.00 A
	Nemaguard	1.33 B	38.67 BC	30 C	1.80 A

Means within each column followed by the same letter
 (S) are not significantly different at P=0.05

Table (6): Leaf measurement and chlorophyll present age for the different   rootstocks during 2007-2008 seasons

Years	Rootstocks	Ave. No. of leaves per branch	leaf Length	Leaf width	Leaf area (cm^2)	chlorophyll % in leaf
2007	GF677	27.0 C	10 A	2.67 B	40.0 A	33.9 BC
	Tetra pdm 5450	29.3 BC	4.2 D	2.82 B	11.0 C	38.9 AB
	Saint Julian	36.7 ABC	5.4 C	3.63 A	10.75 C	44.8 A
	Myroblan 29c	40.0 AB	6.6 BC	3.77 A	18.49 BC	45.8 A
	Nemaguard	45.0 A	9.67 A	2.13 C	25.69 B	30.3 C
2008	GF677	31.0 C	9.73 A	2.7 B	44 A	34.3 B
	Tetra pdm 5450	27.3 D	3.88 D	2.8 B	12.33 C	38.1 A
	Saint Julian	33.3 C	4.98 C	3.7 A	11.33 C	40.9 A
	Myroblan 29c	39.0 AB	5.30 BC	3.9 A	19.67 BC	36 AB
	Nemaguard	41.7 A	8.58 B	2.77 B	27 B	29.2 C

Means within each column followed by the same letter (S) are not significantly different at P=0.05

3.3. **Roots distribution study:**
-Root length:

Tables (7 and 8) showed that, GF677 and Myroblan 29c rootstocks significantly produced longer roots at 50 (111.2 and 71.6 cm) and 100 cm (54.0 and 40.3cm) from the tree trunk. On the other hand, Tetra pdm 5450 and Saint Julian rootstocks significantly produced shorter roots at 50 (36.3 and 35.6cm) and 100cm (31.6 and 23.3cm) from stem. However, Nemaguard rootstock produced long roots at 50cm but short roots at 100cm (24.0cm) from stem.

Meanwhile, the studied rootstocks produced longer roots (80.13cm) at 50cm from the tree trunk within 0-30cm than within 30-60cm soil depth (45.92cm). While, at 100cm from stem the rootstock roots were Longer within

30 - 60cm Soil Profile than within 0-30cm .Generally, The studied roots were markedly longer at 50cm than at 100cm from the tree trunk.

Moreover, GF677, Tetra pdm 5450, Saint Julian, Myroblan 29c and Nemaguard rootstocks produced longer fine rootstock roots{< 2mm}(101.0 and 65.14cm) than either medium roots{2-6mm} (57.81 and 26.44cm) or thick roots (>6mm) (30.27 and 12.37cm). It was also noticeable that roots were greatly longer at 50cm than at 100cm from the tree trunk.

Table (7): Length of roots (cm) at 50 cm from the stem of rootstocks under study as affected by soil depth and root diameter.

(A) Rootstocks		Length of roots (cm)			
	(B) Soil depth (cm)	Root diameter (C)(mm)			Ave. (A x B)
		< 2	2-6	>6	
GF677	0 - 30	204.5 B	235.7 A	43.67 KL	161.3 A
	30 - 60	72.4 GF7	34.5 l	76.33.GF	61.08 DE
Ave. (A X C)		13.8.4 A	135 A	60 DE	Ave. (A) 111.2 A
Tetra pdm 5450	0 - 30	106.7 E	8.167 MN	15.37 M	43.4 F
	30 – 60	79.83 G	1.0 N	7.73 MN	29.19 G
Ave. (A X C)		93.25 C	4.08 IJ	11.55 HI	Ave. (A) 36.29 D
Saint Julian	0 - 30	7.73 MN	66.33 HI	104.3 EF	56.89 E
	30 - 60	1.0 N	7.73 MN	66.33 HI	14.28 H
Ave. (A X C)		49.5 F	57.25 DEF	1.0 J	Ave. (A) 35.58 D
Myroblan 29c	0 - 30	104.3 EF	1.0 N	32.67 L	73.73 B
	30 - 60	10.17MN	1.0 N	153.6 C	69.57 BC
Ave. (A X C)		114 B	37.63 G	63.15 D	Ave. (A) 71.65 B
Nemaguard	0 - 30	1.0N	52.33 JK	18.17 M	65.33 CD
	30 - 60	93.67 F	57.67 IJ	15.17 M	55.5 E
Ave. (A X C)		109.6 B	55 EF	16.67 H	Ave. (A) 60.42 C
Ave. (B X C)	0 - 30	131.3 A	28.97 E	35.52 D	Ave. B 80.13 A
	30 - 60	80.10 B	70.67 C	31.58 DE	Ave. B 45.92 B
Ave. (C)		101 A	57.81 B	30.27 C	-

Means within each column followed by the same letter
(S) are not significantly different at P=0.05

Table (8): length of roots (cm) at 100 cm from the stem of rootstocks under study as affected by soil depth and root diameter.

(A) Rootstocks		Length of roots (cm)			
	(B) Soil depth (cm	Root diameter (C)(mm)			Ave. (A x B)
		< 2	2-6	>6	
GF677	0 - 30	75.33 C	63.17 DE	55.67 G	64.72 A
	30 - 60	60.67 DEFG	43.5 H	25.67 IJ	43.28 D
Ave. (A X C)		68.0 C	53.3 D	40.67 E	Ave.(A) 54.0 A
Tetra pdm 5450	0 - 30	28.73 I	2.4 lM	1.0 M	10.38 H
	30 – 60	133.4 A	20.4 jk	4.8 LM	52.88 C
Ave. (A X C)		81.1 B	11.4 G	2.4 H	Ave. (A) 31.63 C
Saint Julian	0 - 30	26.17 IJ	30.67 I	1.0 M	18.94 G
	30 - 60	62.17 DEF	20.67 JK	1.0 M	27.61 F
Ave. (A X C)		44.17 E	25.67 F	1 H	Ave. (A) 23.28 D
Myroblan 29c	0 - 30	112.3 l	56.53 FG	6.70 l	58.51 B
	30 - 60	64.2 D	2.33 lM	1.0 M	22.18 G
Ave. (A X C)		88.25 A	29.43 F	3.35 H	Ave. (A) 40.34 B
Nemaguard	0 - 30	30.33 I	6.67 l	1.0 M	12.33 H

30 - 60	58.07EFG	18.07 K	30.83 i	35.66 E
Ave. (A X C)	44.2 E	12.37 G	15.42 G	Ave. (A) 23.99 D
Ave. (B X C)	0 - 30	54.57 B	12.47 E	20.99 D	Ave. B 32.98 B
	30 - 60	31.89 C	75.71 A	12.26 E	Ave. B 36.32 A
Ave. (C)	65.14 A	26.44 B	12.37 C	-

Means within each column followed by the same letter
(S) are not significantly different at P=0.05

Generally, GF677 rootstock produced the longest roots within 0-30cm in the soil profile (161.3cm). Especially fine roots (138.4cm) and medium (135.0cm). However, fine roots were always longer (131.3cm) within 0-30cm in the soil at 50 cm from the tree trunk, while medium roots were longer (75.7cm) at 100cm from the stem within 30-60cm in the soil. So GF677 rootstock within 0-30cm in the soil with fine and medium roots was the best interaction under study. Generally the previous data showed that, Myroblan plum rootstock had the lightest root system ascomparing to Sweet and Bitter almond, Okinawa, Nemaguard, Flordaguard, Flord 9/3 and local apricot (Attala, 1993).

Table (9): Numbers of roots at 50 cm from the stem of rootstocks under study as affected by soil depth and root diameter.

(A) Rootstocks	Number of roots			
	(B) Soil depth (cm)	Root diameter (C)(mm)		Ave. (A x B)
		< 2	2-6	>6	
GF677	0 - 30	21.33 A	23.33 A	4.0 IJK	16.22A
	30 - 60	16.0 B	8.0 FGH	13.33 BCD	12.44 B
Ave. (A X C)		18.67 A	15.67 B	8.67 C	Ave.(A) 14
Tetra pdm 5450	0 - 30	21.0 A	0.661 KL	2.33 JKL	8.0 D
	30 – 60	12.33CDE	1.0 L	1.33 KL	4.556 EF
Ave. (A X C)		16.67 AB	0.33 FG	1.83 FG	Ave.(A) 6.
Saint Julian	0 - 30	7.33 FGHI	8.33 FGH	1.0 L	5.22 E
	30 - 60	7.0 GHI	2.67 KL	1.0 L	3.0 F
Ave. (A X C)		7.17 CD	5.17 DE	1.0 G	Ave. (A) 7
Myroblan 29c	0 - 30	22.67 A	1.0 L	10.67 DEF	11.1 BC
	30 - 60	12.67 BCDE	12.33CDE	5.667 HIJ	10.22 C
Ave. (A X C)		17.67 AB	6.17 CD	8.17 C	Ave. (A) 1
Nemaguard	0 - 30	15.0 BC	5.667 HIJ	2.67JKL	7.78 D
	30 - 60	16.0 B	9.33 EFG	3.0 JKL	9.44
Ave. (A X C)		15.5 B	7.5 CD	2.83 EF	Ave. (A) 8
Ave. (B X C)	0 - 30	17.47 A	3.93 D	6.33 C	Ave. (B) 9.
	30 - 60	7.6 C	12.8 B	4.667 D	Ave. (B) 7.
Ave. (C)		15.0 BA	6.97 B	4.30 C	-

Weight of roots:

   Our results in Tables (11 and 12) significantly appeared that, GF677 rootstock produced the most heavy roots at 50 and 100cm from the tree trunk followed by Myroblan 29c at 50cm.Different studied rootstocks significantly produced heavier roots at 0-30cm from the soil profile (15.62G) than 30-60cm (6.74G) at 50cm from the stem while at 100cm the roots were similar (5.11 and 5.14G). Furthermore, the thick roots (>6mm) were significantly heavier (20.3 and 12.56G) than both medium (4.18 and 2.07G) and fine roots (1.05 and 0.7Means within each column followed by the same letter
(S) are not significantly different at P=0.05

The interaction between rootstock and soil depth revelled that, GF677 followed by Myroblan 29c rootstocks produced the heaviest roots specially within 0-30cm in the soil profile (55.19 and 12.11g respectively) at 50cm from the tree trunk while at 100cm from the stem GF677 rootstock was the superior (20.74 and 18.20G) within 0-30 and 30-60cm in the soil respectively.

If we consider the reaction between the studied rootstock and root diameter, we can note much thick roots (>6mm) with GF677 rootstock (91.93 and 52.75G) at 50 and 100cm from the tree trunk respectively. Moreover, Myroblan 29c and Nemaguard rootstocks produced much more thick roots (>6mm) at 50 from the stem (32.40 and 11.84G respectively) than the other interactions. Meanwhile, the studied rootstocks significantly produced thick roots (>6mm) much more medium roots (2-6mm) and also than fine roots (<2mm) at both 50 and 100cm from the tree trunk.

However, this phenomenon was much obvious within 0-30cm in the soil profile (39.42, 6.50 and 1.17G) than within 30-60cm (17.40, 2.26 and 0.95g) respectively at 50cm from the tree trunk. Moreover, roots within 0-30 and 30-60cm at 100cm from the tree stem had not clear trend. Finally, the reaction between the three studied factors (The rootstock, the soil depth and the root diameter) significantly showed that, GF677 rootstock produced the heaviest roots with the skeletal roots (>6mm) within 0-30cm from the tree trunk both at 50cm (145.7) and at 100cm (57.2g).

Table (10): Number of roots at 100 cm from the stem of rootstocks under study as affected by soil depth and root diameter.

(A) Rootstocks	(B) Soil depth (cm)	Numbers of roots			
		Root diameter (C)(mm)			Ave. (A x B)
		< 2	2-6	>6	
GF677	0 - 30	11.0 C	8.0 DEF	7.33EFG	8.778 A
	30 - 60	11.0 C	9.33 CDE	7.33 EFG	9.22 A
Ave. (A X C)		11 B	8.667 C	7.33 CD	Ave.(A) 9.00 A
Tetra pdm 5450	0 - 30	6.00 FGH	1.33 KLM	1.0 M	2.44 EF
	30 – 60	24.33 A	2.00 JKLM	1.33 KLM	9.22 A
Ave. (A X C)		15.17 A	1.667 EFG	0.667 FG	Ave. (A) 5.833 B
Saint Julian	0 - 30	4.004H IJK	3.667 HIJKL	1.0 M	2.556 EF
	30 - 60	10.67 CD	2.33 IJKLM	1.0 M	4.333HIJ
Ave. (A X C)		7.333 CD	3.00 E	1.0 m	Ave. (A) 3.444c
Myroblan 29c	0 - 30	16.0 B	6.0 FGH	1.0 LM	7.667 AB
	30 - 60	11.0 C	1.33 KLM	1.0 m	4.111 DE
Ave. (A X C)		13.5 A	3.667 E	0.500 FG	Ave. (A) 5.889 B
Nemaguard	0 - 30	4.0 HILM	1.33 KLM	1.0 M	1.778 F
	30-60	8.667 CDEF	4.33 HIJ	5.00 GHI	6.00GH
Ave. (A X C)		6.33 D	2.833 E	2.50 EF	Ave. (A) 3.889 C
Ave. (B X C)	0 - 30	8.2 B	1.667 E	3.867CD	Ave. B 4.644 B
	30 - 60	4.067 C	13.13 A	2.733 DE	Ave. B 6.578 A
Ave. (C)		10.67 A	3.96 B	2.20 C	-

Means within each column followed by the same letter (S) are not significantly different at P=0.05

Table (11): weight of roots at 50 cm from the stem of rootstocks under study as affected by soil depth and root diameter.

(A) Rootstocks	(B) Soil depth (cm)	Weight of roots			
		Root diameter (mm)			Ave. (A x B)
		< 2	2-6	>6	
GF677	0 – 30	2.497 IJK	17.4 D	145.7 A	55.19 A
	30 - 60	1.793 IJK	2.46 IJK	38.2 B	14.15 B

		2.145 E	9.93 C	91.93 A	Ave. (a) 34.67 A
Ave. (A X C)					
Tetra pdm 5450	0 – 30	0.593 JK	0.153 K	6.35 GH	2.37 EFG
	30 – 60	1.583 IJK	1.0 K	4.313 HIJ	1.966 FG
Ave. (A X C)		1.09 E	0.0767 E	5.33 D	Ave. (A) 2.166 D
Saint Julian	0 – 30	0.57 JK	12.63 EF	0.1 K	4.401 DE
	30 - 60	0.12 K	0.96 JK	1.0 K	0.36 G
Ave. (A X C)		0.34 E	6.797 D	1.0 E	Ave. (A) 2.38 D
Myroblan 29c	0 – 30	0.7 JK	1.0K	35.63 B	12.11 BC
	30 - 60	0.573 JK	5.067 HI	29.17 C	11.6 C
Ave. (A X C)		0.642 E	2.53 E	32.4 B	Ave. (A) 11.86 B
Nemaguard	0 – 30	1.457 IJK	1.297 IJK	9.34 FG	4.033 DEF
	30 - 60	0.673 JK	1.8 IJK	14.34 De	5.606 D
Ave. (A X C)		1.07 E	1.548 E	11.84 C	Ave. (A) 4.819 C
Ave. (B X C)	0 – 30	1.17 D	6.50 C	39.42 A	Ave. B 15.70 A
	30 - 60	0.95 D	2.26 D	17.40 B	Ave. B 6.837 B
Ave. (C)		1.057 C	4.38 B	28.41 A	-

Means within each column followed by the same letter (S) are not significantly different at P=0.05

Table (12): Weight of root at 100 cm from the stem of rootstocks under study as   affected by soil depth and root diameter.

(A) Rootstocks	Weight of roots			
	(B) Soil depth (cm)	Root diameter (C) (mm)		Ave. (A x B)
		< 2	2-6	>6	
GF677	0 – 30	0.517 E	4.5 D	57.2 A	20.74 A
	30 - 60	1.5 D	4.8 D	48.3 B	18.20 A
Ave. (A X C)		1.01E	4.65C	52.75A	Ave(A)19.47A
Tetra pdm 5450	0 – 30	0.153 E	1.15 E	1.0 E	0.77C
	30 – 60	0.617 E	1.03E	0.51E	0.72C
Ave. (A X C)		0.39E	1.09E	0.76E	Ave(A)0.74D
Saint Julian	0 - 30	0.186 E	4.67 E	1.0 D	1.95C
	30 - 60	0.32 E	0.606 E	1.0 E	0.64C
Ave. (A X C)		0.52E	2.64D	1.0E	1.30 C
Myroblan 29c	0 - 30	1.067 E	2.3 de	1.13E	1.5C
	30 - 60	0.447 E	0.93D	1.0E	0.79C
Ave. (A X C)		0.76	1.62E	1.07DE	1,15D
Nemaguard	0 - 30	0.267 E	0.443 E	1.0E	0.57C
	30 - 60	2.33 DE	0.317E	13.4 C	5.35B
Ave. (A X C)		1.30E	0.38E	7.2B	Ave. A 2.96B
Ave. (B X C)	0 - 30	0.44D	2.61B	12.27A	Ave B 5.11A
	30 - 60	1.04C	1.54BC	12.84A	Ave B 5.14 A
Ave. (C)		0.74C	2.07B	12.56A	-

Means within each column followed by the same letter (S) are not significantly different at P=0.05

From the previous results it could be concluded that number of roots, Root length and weight of roots of the rootstocks: GF677, Tetra pdm 5450. Saint Julian, Myroblan 29c and Nemaguard. The study included root system perforated at 0-30 and 30-60cm soil depth. The root system divided to <2, 2-6 and >6mm root thick.

The percent results showed that, GF677 and Myroblan 29c rootstocks significantly produced more, longer and heavier roots than the other stocks.

Root length and weight at 50cm from the tree trunk significantly were more at 0-30cm than at 30-60cm within the soil profile. On the other hand, the rest results were about the same.

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Concerning the root diameter, number of roots and root length were markedly better with < 2 than 2-6 and than > 6mm roots. While, root weight at 50 and 100cm from the tree trunk was heavier with > 6 then 2-6 then < 2mm. The interaction between rootstock and soil depth showed the superiority of GF677 and Myroblan 29c at both studied soil depths (0-30 and 30-60cm). However, the root weight at 100cm from the tree trunk failed to show this trend when it appeared the superiority of GF677 rootstock only.

Meanwhile, the interaction between rootstocks and root diameter appeared an obvious descent of both number and length of roots parallel to increase of root diameter from < 2 to 2-6 and to > 6mm. However, weight of roots at 50and 100cm from the stem showed the adverse trend.

Nevertheless of studied rootstocks, fine roots (< 2mm) were more in number and length within 0-30cm in the soil profile while the weight of thick roots (> 6mm) was more in 0-30cm soil layer. Within deeper layer (30-60cm), medium roots (2-6mm) were more and longer but thick roots (> 6mm) were heavier.

The interaction between rootstock, soil depth and root diameter showed different habits. Myroblan 29c plum rootstock proliferate with more number and longer roots within 0-30cm in the soil profile specially with fine roots (2m). Also GF677 produced more, longer and heavier roots within 0-30cm especially with medium (2-6m) and thick (>6m) roots. Generally, the previous reports showed that, Myroblan plum rootstock had the lightest root system in comparing to sweet and Bitter almond, Okinawa, Nemaguard, Floridaguard and local apricot (Attalh, 1993).

Table (13): studies of Cross sections of sub apex of one year old shoots of different deciduous rootstocks .

Thickness(μ)	A Epidermis	B Cortex	C Phloem	D xylem	D Pith
GF677	0.12 A	0.051 C	0.051 A	1.14	2.88
Tetra pdm 5450	0.09 B	0.63 B	0.54 A	1.41	3.27
Saint Julian	0.09 B	0.64 B	0.33 C	3.12	4.15
Myroblan 29c	0.051 D	2.042 D	0.03 D	1.82	3.6
Nemaguard	0.06 C	0.84 A	0.39 B	0.57	4.14

Means within each column followed by the same letter (S) are not significantly different at P=0.05

Also, Westwood (1993) stated that, in a coarse sand low in nutrients few roots went deeper than 0.9m, with ¾ of the roots in the top 30cm. However, root distribution was affected by soil conditions, as well as ,genetics

Glenn and Miller (1995) outlined that, the root length density of peach roots was greatest in the 0-30cm depth and was promoted by irrigation and was reduced by root pruning in the 0-90cm root Zone.

3Anatomy study of stem :

Data in (Table13) showed the average thickness of stem section (epidermis , cortex , pholeom , xylem and pith)in different rootstocks .

GF677 rootstock recorded the highest significant epidermis thickness 0.12μ (Table 13) followed by Tetra, Saint Julian and Nemaguard , since Myroblan 29c stock had the lowest epidermis thickness at the sub apes shoot (0.051 μ).

Moreover Nemaguard developed the highest significant shoot Cortex thickness 0.84 M followed by Saint Julian, Tetra pdm 5450 and GF677 rootstocks since Myroblan 29c introduced the lowest Cortex thickness (0.049 μ). Meanwhile, Tetra pdm 5450 rootstock introduces the highest significant cortex thickness (54 μ) followed by GF677.

Moreover Saint Julian produced the highest xylem Thickness (3.12 μ) followed by Myroblan , Tetra pdm 5450, GF677 since Nemaguard produced the lowest (0.57 μ).

Concerning, pith thickness both Saint Julian (4.14 μ) and Nemaguard (4.14 μ) occupied the highest significant pith thickness followed by Myroblane 29c Tetra pdm 5450 and GF677 (2.88 μ) stocks.

However, Jackson (1986) showed that, cross sections through the trunk of dwarfing rootstocks reveal a higher proportion of bark relative to wood than in vigorous rootstocks. This might alter the pattern of translocation in such a way that vegetative growth is reduced.

Guirguis *et al.* (1994) said that, the perineum covers the outer layer of the stem and was composed of 1-2 phylum layers. Such layer was thick in *prunes Davidiana* and Nemaguard rootstocks as compared to Okinawa. Inward to the phloem layers was the cortex with 8-10 layers which varying in cell size and intercellular spaces.

3.4.Cross structure of the stem under the apex bud was investigated:

- **Cross section of GF677 stem rootstock:**

The epidermis: Consisted of one layer of parenchyma cells covered with cuticle on the outer walls.

Cortex Consisted of parenchyma cells with 10-11 layers, thickness of about (0.06- 0.054μ) it contains cells with star crystal (calcium oxalate

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crystals).While the cells of the endodermis contained tannins.

The phloem: Primary phloem, sieve cells and parenchyma cells were crushed and compacted as a result of the secondary phloem (prevent cells, sieve tubes and companion cells

Xylem: The secondary xylem consisted of xylem vessels, fibers of secondary xylem. The fibers cells were smaller than those of xylem vessels. At the end there were rays of primary xylem while these new cells are green parenchyma cells.

Pith: Consists of parenchyma cells contained star crystal (calcium oxalate crystals)
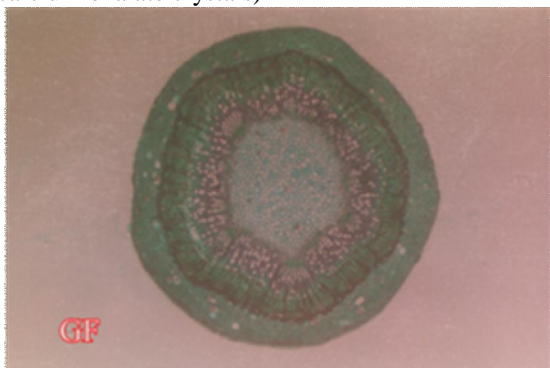
Fig. (2): Cross section of GF677 stem rootstock

- **Cross section of Tetra pdm 5450 stem rootstock:**

The epidermis: consists of one layer of small cells covered with cuticle.

Cortex: consists of both parenchyma and collenchymas cells. Tannins spread in cortex, endodermis, between phloem and parenchyma rays between phloem vessels , pith and there was no crystals in cortex layer. Cambium layer were between phloem and xylem consisted of 8 layers of cells which had more thickness in comparison with both GF677 and Nemaguard rootstocks. The secondary growth in Phloem and xylem were higher than those of GF677 and Nemaguard rootstocks.

Pith: parenchyma cells contained star crystals the pith was differ in shape because the growth protoxcylem penetrate the pith . Myroblan 29c showed a nearly circular shape. While GF677 stem cross section had pentagon pith. On the other hand, the pith of Tetra was unique shape which was in between the two mentioned shapes. Moreover, the longiest diameter shapes. Moreover, the pith of Nemaguard had the longest diameter while Tetra Pdm5450 had the narrowest one.

The vascular tissues: As the stem grows in length, the secondary tissues form the vascular cambium. The secondary phloem developed toward the outside of the stem by the vascular cambium. Guirguis *et al* (1994) stated that in all the studied stocks, pith differ in shape and diameter. The general shape of the pith differs from round to pentagon. Nemaguard, Myroblan 29c showed nearly circular shaped pith. Sweet and Bitter almond and Okinawa showed a pentagon one. However, Local apricot showed a unique shape which was in between the two mentioned shapes.

Xylem:The widest xylem tissues was that of Flordguard followed by Sweet almond. However, the narrowest was that of bitter almond

Bark and phloem, Okinawa and Myroblan 29c had the thinnest tissues however, bitter almond had the thinnest tissues .Cross sections would be very useful in distinguishing various stocks in the field specially after leaf shedding.
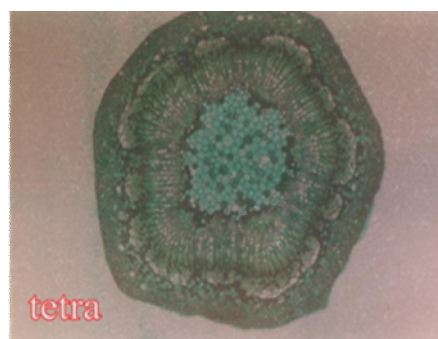
Fig. (3): Cross section of Tetra pdm 5450 rootstock.

- **Cross section of Saint Julian stem rootstock:**

The epidermis: consisted of two layers of small parenchyma cells coated with cuticle layer.

Cortex: consisted of collenchymas and parenchyma cells width intervals between the large cells. Tannins spread in the waves between the phloem and xylem till the pith.

The amount of secondary xylem was too large in comparison with those of GF677 and Nemaguard Primary xylem consists of 2 layers of growth, dark cells around the pith.

The pith: consisted of parenchyma cells with stars crystals and tannins while the crystals were rarely. Primary phloem consisted of 1to 2 dark layers of large cells. Secondary phloem 6 layers of small parenchyma cells became smaller towards inside.
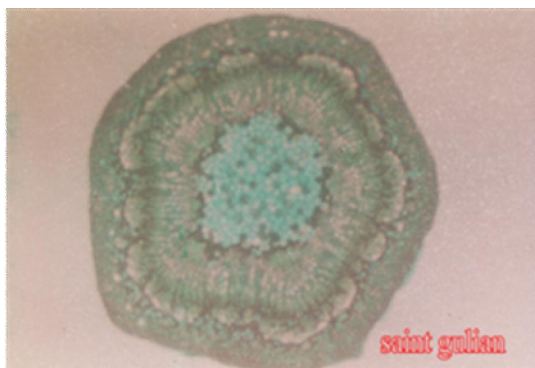
Fig. (4): Cross section of Saint Julian rootstock.

- **Cross section of Myroblane29 c stem**

The epidermis: Consisted of one layer of parenchyma cells covered with cuticle.

The cortex: Consisted of parenchyma and collenchymas cells tannins were rarely in endodermis cells but the rest of tissues were free of tannins. The secondary growth in phloem and xylem were more than those of Nemaguard but less than GF677. It was evident the high thickness of the secondary phloem compared with other section.

Pith: Consisted of parenchyma cells contain stars crystals with low percentage than the other rootstocks.

Fig. (5): Cross section of Myroblane 29c stem

- **Cross section of Nemaguard stem rootstock:**

The cortex: consists of 15:16 layers of parenchyma cells, it contained many stars – crystals. There were no tannins in the endodermis cells but the parenchyma rays which were between the phloem groups contained tannins.

The cambium: consists of 4 layers of meristmatic cells.

Phloem and xylem: the amount of secondary phloem and secondary xylem and fibers cells of xylem were less than those of GF677.

The pith: pith radical diameter was larger than that of GF677. Due to decrease of secondary growth we could record number of vascular vessel.

pith: All the studied stocks had solid pith which differed in shape and diameter. The pith of Nemaguard and Myroblan 29c showed a nearly circular shape .While GF677 stem cross section had pentagon pith. On the other hand, the pith of Tetra was unique shape which was in between the two mentioned shapes .Moreover; the pith of Nemaguard had the longest diameter while Tetra had the narrowest pith diameter.

The vascular tissues: As the stem grew in length, the secondary tissues form the vascular cambium. The secondary phloem developed toward the outside of the stem by the vascular cambium and the secondary xylem forms in – worldly. However, the diameter of vascular tissues differed with the diameter of secondary tissues, where Tetra rootstock cross section has the largest secondary tissues (the vascular cambium was clearer than in any other studied stock) followed by Myroblan 29c and GF677. While Nemaguard had the least secondary tissues (cells were still in juvenile stage). On the other hand Saint Julian stock had the largest secondary xylem tissues.

The cortex: Stem cortex lies just beneath the epidermis and encircles the inner core of the vascular tissue. Calcium oxalate crystals distribute through the cortex in Nemaguard stem tissues while distribute through the pith in Tetra, Saint Julian and Myroblan 29c. However these crystals distribute through both pith and cortex in GF677 rootstock. Also, tannins concentrated through most tissues in Tetra rootstock, while were as traces in Myroblan 29c but were not seen in the other stocks.

The epidermis: The epidermis comprises of one layer of parenchyma cells which covered with cuticle in all studied rootstocks.
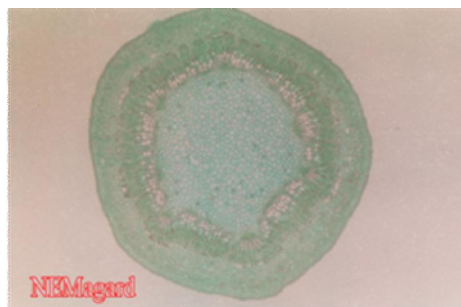
Fig. (6): Cross section of Nemaguard rootstock

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Conclusion For the Diagnosis of the examined new rootstocks

1) GF677

-Sereat leaves shape – tapering apex margin – smooth leaf lower surface – compound auxiliary bud – paracytic stomata – guard cells had kidney shape – reproductive Under Egyptian condition - vegetative bud break at (21-24) March – flower bud break at 1-5 March - Fruit set at 17-19 march % fruit set(20-22%) No. flowers / branch(14-25)- No. stomata 12.8 – dimensions stomata $(0.111/0.097\mu)$– tree canopy 7.5 m – tree height 4.5m –No. main branches 3.67 – growth habit semi erect – trunk diameter 38.0cm – the diameter of breast height 43.0 – No. shoots/ branch(2.66 -3.67) – shoot length(51.7 – 68.3cm) – No. internodes / branch (26.3 – 34.0)– internodes length (2.0 -2.8cm) – No. leaves /branch (27-31)– leaf area (40-44 cm^2)- % chlorophyll (33.2 -34.3) thickness of stem section (epidermis 0.12 μ – cortex 0.051 μ – phloem 0.051 μ - xylem 1.14 μ - pith 2.88 μ) .

2) Tetra pdm 5450:

Ovate leaves shape – obtuse leaf apex – coarse leaf lower surface – simple auxillary bud – paracytic stomata – guard cells had Kidney shape – non-productive under Egyptian condition – vegetative bud break at(29-31)March – No. stomata 8.7 – stomata dimensions (0.145/0.07 μ) – tree canopy 2.2m – tree height 1.6m – No. main branches 2.9 – growth habit erect – trunk diameter 8.67cm – the diameter of breast height 8.17 – No. shoots/branch (1.0-2.67) – shoot length(31.0-35.3 cm) – No. internodes / branch (30.3 – 34)– internodes length (0.23 – 0.64cm) – No. leaves / branch (27.3 – 29.3) – leaf area (11.0 – 12.3 cm^2) - %chlorophyll 38.1 – 38.9 – thickness of stem section(epidermis 0.09 μ – cortex 0.6 μ – pholeom 0.54 μ – xylem 1.41 μ – pith 3.27 μ).

3) Saint Julian:

Ovate leaves shape – obtuse leaf apex – coarse leaf lower surface simple auxiliary bud – paracytic stomata – guard cells have kidney shape – non productive under Egyptian condition – vegetative bud break at 29-31 March – No. stomata Egyptian condition – vegetative bud break at 29-31 March – No. stomata 11.3 – stomata dimensions(0.106/0.117 μ) – tree canopy 1.2 m- tree height 1.0m –No. main branches 2.8 – growth habit erect – trunk diameter 8.83cm – the diameter of breast height 8.66 – No. shoots/ branch(1.3-3.0)– shoot length (30.3 -33.7cm) –No. internodes / branch(33.3-40.0)– internodes length(0.80 -1.17cm) –No. leaves / branch (33.3-36.7)- leaf area (10.8 -11.3cm^2)-% chlorophyll (40.9-44.8) – thickness of stem section (epidermis

0.09 μ - cortex 0.64 μ – phloem 0.33 μ – xylem 3.12 μ – pith 4.15 μ).

4) Myroblan 29c:

Ovate leaves shape – tapering apex margin – smooth leaf lower surface – compound auxiliary bud – paracytic stomata – guard cells have kidney shape – non-productive under Egyptian condition vegetative bud break at 24-25 March – No. stomata 6.7 – stomata dimensions (0.12/0.078 μ) – tree canopy 4.5m – tree height 2.6m – No. main branches 3.67 – growth habit semi erect – trunk diameter 33.0cm – the diameter of breast height 37.0 – No. shoots/ branch(1.0 -5.0)– shoot length(31.0 – 48.0cm) – No. internodes/ branch (37.0 – 45.3) – internodes length 2.0cm – No. leaves /branch(39-40) – leaf area (18.5 – 19.7 cm^2)- % chlorophyll (36 -45) – thickness of stem section(epidermis 0.051 μ – cortex 0.042 μ – phloem 0.03 μ – xylem 1.82 μ – pith 3.6 μ).

5) Nemaguard:

Sereat leaves shape – tapering apex margin – smooth leaf lower surface – compound auxiliary bud – anisocytic stomata – guard cells have elliptical shape – reproductive under Egyptian condition – vegetative bud break at 17-19 March – flower bud break at 17-19 March – fruit set at 7-9 April - % fruit set 40-50% - No. flower /branch (3.5-5.5) – No. of stomata 10.7 – stomata dimensions (0.111/ 0.086 μ) – tree canopy 4.5m – tree height 1.6m – No. main branches 3.0 – growth habit spread – trunk diameter 16.33cm – the diameter of breast height 17.83 –No. shoots/branch(1.33 – 3.67)– shoot length (38.7 – 53.0cm) – No. internodes / branch(26.4 – 30.0)internodes length(1.67- 1.8cm). No. leaves/branch(41.7 – 45.0)- leaf area (25.7 -270cm^2)- % chlorophyll (29.2 – 30.3) – thickness of stem section(epidermis 0.06mcortex 0.84 μ – pholeom 0.39 μ – xylem 0.57 m – pith 4.14 μ).

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The study of Signal Propagation in Electromagnetic –Measurement While Drilling (EM-MWD) telemetry systems

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Abstract: Electromagnetic measurement while drilling (EM-MWD) telemetry can provide real time-large amount of data to the drilling crew and this is the reason for its rapid development in the recent years. For effective and efficient design and utilization of the EM-MWD tool, one needs to understand the behavior of the electromagnetic signal as it propagates along the drill string as well as through the formation. Based on electromagnetic theory, this paper examines the behavior of the signal such as attenuation, propagation velocity with varying operating frequency and earth resistivity.

1. Introduction

High depth drilling, increased activity offshore and rapidly escalating costs have focused attention on all potential methods of drilling safer and cheaper. Real time data delivery from the bottom of the borehole (at the drill bit) to the surface offers the greatest potential for achieving these needs. Oil company management and engineers place a lot of emphasis on well control and directional information (McDonald, 1978). It is therefore, important to understand how the data can be transferred from bottom to surface. A number of systems have been used namely hardwired telemetry, Acoustic telemetry, mud pulse telemetry, and electromagnetic telemetry. Although mud pulse telemetry is a well developed and commercially available, it has some limitations such as the high demand for drilling fluid rendering it useless in underbalanced drilling which is common in air and foam drilling. EM-MWD also offers higher data rates for good resistant earth cases, and hence more varieties of quantities can be measured simultaneously (Xia and Chen, 1993).

Electromagnetic telemetry transmits data through low-frequency electromagnetic waves which propagate through the subsurface formations from the drill string and are received by surface antennas. The successful implementation of electromagnetic telemetry requires understanding of the formation types and associated resistivities. And also knowing the behavior of the signals as it moves through the formation. The signal propagation has been studied by several authors (Jose and Flavio, 2002; Poh, David and Andrew, 2005; Xia and Chen, 1993). In this paper, the signal propagation through the drill string and formation of an electromagnetic telemetry is studied based on the Electromagnetic field theory (Bhag and Huseyin, 1998) and analysed using Matlab codes (James, 2001).

2. Methodology

2.1 Working principle of EM-MWD

The signal source emits the signal which propagates through the formation to the receiving antenna from which the surface equipments are connected as shown in figure1 above. The surface equipment decodes the data and puts in a form which is easily understandable by the driller. The driller can also send commands to the downhole assembly through the surface equipment.
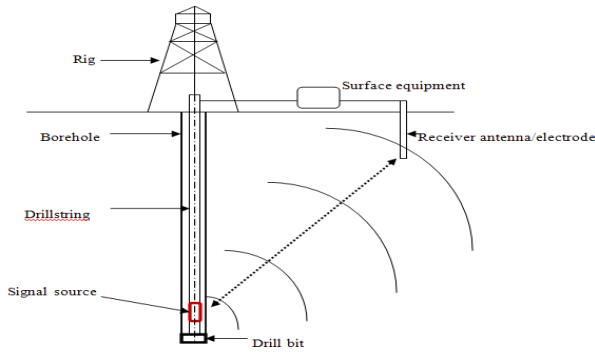
Figure 1 Schematic diagram of showing the Principle of operation of the electromagnetic MWD system. An emitting antenna in the drill string transmits the data to the surface electrodes.

2.2 Description of the configuration and formulation of solution

The geometry of the problem is illustrated in Fig.2. To specify the position of the source in the configuration, we employ the coordinates (r, θ, φ) as a spherical coordinate system with origin O (Ivo, 1996; John et al..2007; Wu et al..2009). The source is a vertical infinitesimal electric dipole antenna J=żIdzδ(z) immersed in a dielectric medium of infinite extent and excited by an impulsive current. The electric dipole antenna, located at the origin O and oriented vertically in the z-direction, is short in length, "dz", carrying a current "I".
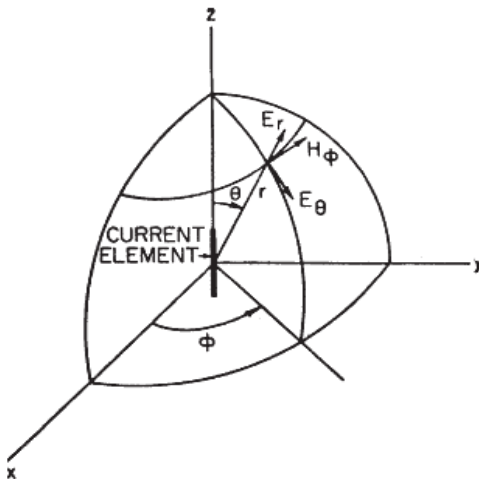
Figure 2: showing the coordinate system for an electric dipole

Maxwell's equations lead to the following formulations:

$$\nabla^2 E + \omega^2 \mu \varepsilon E = \frac{1}{\varepsilon}\nabla\sigma - j\omega\mu J \qquad (1)$$

$$\nabla^2 H + \omega^2 \mu \varepsilon H = -\nabla \times J \qquad (2)$$

$$k^2 = \omega^2 \mu \varepsilon \qquad (3)$$

Where: E=Electric field intensity (v/m); H=electric field intensity (v/m); J=electric field intensity (v/m^2); B=Magnetic flux density (wb/m^2); σ=conductivity (Ω/m); ε= permittivity F/m); and μ= permeability (H/m)

Considering the infinitesimal electric dipole antenna J=żIdzδ(z), as illustrated in figure 2 above, the electric and magnetic field components obtained from equation (1) or (2) are given by

$$E_r = \frac{k^2 Idz\, e^{-j\omega t}}{2\pi\omega\varepsilon}\left[\frac{1}{(kr)^2} - \frac{j}{(kr)^3}\right]e^{-jkr}cos\theta$$

$$E_\theta = \frac{jk^2 Idz\, e^{j\omega t}}{4\pi\omega\varepsilon}\left[\frac{1}{kr} - \frac{j}{(kr)^2} - \frac{1}{(kr)^3}\right]e^{-jkr}sin\theta$$

$$H_\varphi = \frac{jk^2 Idz\, e^{-j\omega t}}{4\pi}\left[\frac{1}{kr} - \frac{j}{(kr)^2}\right]e^{jkr}sin\theta$$

3. Results and Discussions

3.1 Case I: Near field (r<<)

When the distance (r) between the observer and the centre of the electric dipole is too short, only values of high-order terms are of significant effect, hence low-order terms can be ignored yielding the following set of equations:

$$E_r = \frac{jIdz}{2\pi\omega\varepsilon r^3}e^{-j\omega t}cos\theta$$

$$E_\theta = \frac{-jIdz\, e^{j\omega t}}{4\pi\omega\varepsilon r^3}e^{j\omega t}sin\theta$$

$$H_\varphi = \frac{Idz}{4\pi r^2}e^{-j\omega t}sin\theta$$

It can be observed from the above set of equations for the near field that when the electric field is at its maximum, the magnetic field is zero and vice versa, and the average energy-flux density vector is zero. This means that there is no radiation in the near field.

3.2 Case II: Far field (r>>)

When the distance (r) between the observer and the centre of the electric dipole is too large, only values of low-order terms are of significant effect, hence high-order terms can be ignored yielding the following set of equations:

$$E_r = 0$$

$$E_\theta = \frac{-jIdz\omega\mu}{4\pi\varepsilon r}e^{-j(\omega t - kr)}sin\theta$$

$$H_\varphi = \frac{-jIdz\omega\sqrt{\mu\varepsilon}e^{-j\omega t}}{4\pi r}e^{-j(\omega t - kr)}sin\theta$$

3.3 Attenuation

The earth being a loss dielectric, k the propagation constant is generally a complex number and can be expressed as:

$$k = \alpha + j\beta$$

Where

$$\alpha = \omega\sqrt{\frac{\mu\varepsilon}{2}}\left[\sqrt{1 + \left(\frac{\sigma}{\omega\varepsilon}\right)^2} + 1\right]^{1/2}$$

$$\beta = \omega\sqrt{\frac{\mu\varepsilon}{2}}\left[\sqrt{1 + \left(\frac{\sigma}{\omega\varepsilon}\right)^2} - 1\right]^{1/2}$$

Considering $\frac{\sigma}{\omega\varepsilon} \gg 1, \alpha = \sqrt{\frac{\omega\mu\sigma}{2}}$, which leads to the relationship between attenuation, frequency and resistivity as below:

$$\alpha = \sqrt{\frac{\omega\mu}{2\rho}} = \sqrt{\frac{\pi f\mu}{\rho}}$$

A plot of attenuation against frequency (using Matlab) is shown below:
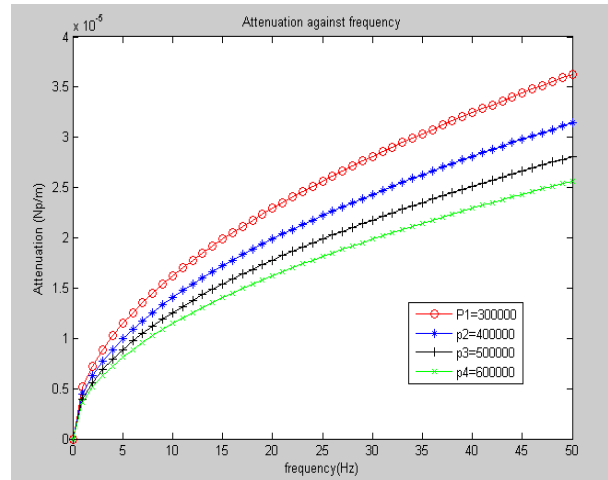
3(a)
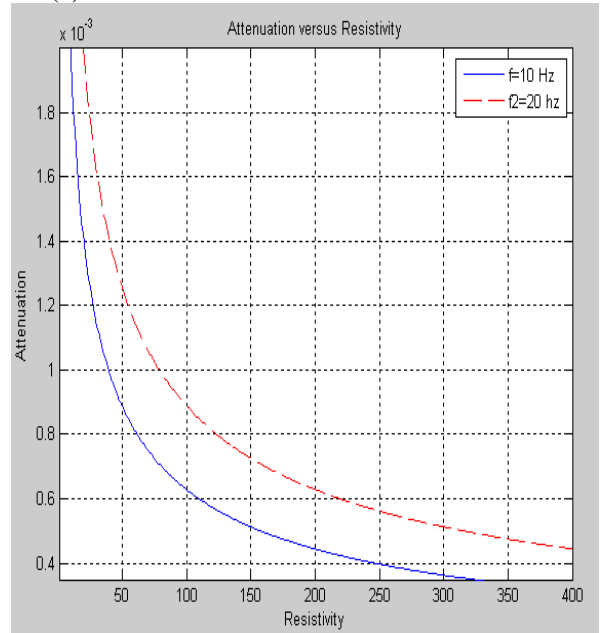
3(b)

Figure 3(a) Shows attenuation against frequency with resistivity's p1=300,000Ω.m, p2=400,000Ω.m, p3=500,000Ω.m and p4=600,000Ω.m; and 3(b) shows attenuation against resistivity at frequencies of 10Hz and 20Hz.

The signal attenuates as it propagates through the media (formation or drill pipe) and this attenuation with increasing frequency but reduces with increasing resistivity of the medium (fig.3).

3.4 Velocity of propagation (u_p)

The velocity of propagation (also referred to as the wave propagation speed) is defined as the speed at which an electromagnetic signal passes through a medium [4], and it is expressed as:

$$u_p = \sqrt{\frac{2\omega\rho_m}{\mu_m}}$$

A plot of the velocity of propagation against frequency through media of different resistivity is shown below:
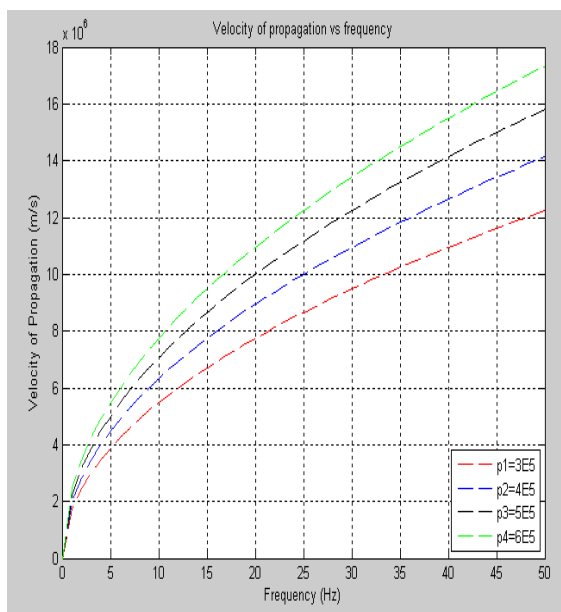
Figure 4 Shows velocity of propagation against frequency with changing resistivity

It is observed from figure (4) that the velocity of propagation increases with both increasing frequency and resistivity. This implies that velocity of propagation reduces with increasing conductivity of the medium through which the signal is passing. The increase in propagation velocity means increased data rate transfer from the bottom to the surface and vice versa.

4. Conclusion

In the process of electromagnetic wave propagation, the signal will be lost gradually with increasing frequency and reducing formation resistivity, indicating that a high frequency cannot be used. However, data transfer rate increases with both increasing frequency and resistivity. In this study frequencies above 20Hz may lead to a higher attenuation where as those below 5Hz will limit the data rate transfer.

The above conclusion has a strong influence during the design of an EM-MWD tool as one must strike a balance between frequency and the data rate transfer as they greatly affect the performance of the tool.

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Repair Maintenance of Diesel Engine Cylinder Head

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Abstract: This paper presents many trials to repair a diesel engine cylinder head made of pearlitic grey cast iron, which was used in a truck. The cylinder head was repaired due to the existence of cracks at the junction between the valve seat and the heater plug seat. Shielded metal arc welding (SMAW) process using different electrodes was applied. The increase in preheating temperature resulted in a formation of a continuous carbide layer in the partial fusion zone and a decrease in martensite phase formed at the heat affected zone. However, the decrease in preheat temperature resulted in an increase of martensite at the heat affected zone and a decrease in the carbide layer at the partial fusion zone. Most of the SMAW electrodes resulted in the formation of regions with high hardness values which imply that the repair welding of the cylinder head using these electrodes is inefficient. Application of the powder flame spray method in repair welding of the cylinder head resulted in partial fusion zone and heat affected zone with hardness values comparable to that of base metal. Preheating in furnace to 500 °C then immediately putting the specimen in the furnace at the same temperature for 1 hour after applying powder flame spray gave excellent hardness results for the heat affected zone (HAZ) and partial fusion zone (PFZ).
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Keyword: Failure of cylinder head, Pearlitic gray iron, SMAW, Flame spray method, Heat affected zone, Partial fusion zone, Hardness distribution, Microstructure.

1. Introduction:

In an internal combustion engine, the cylinder head sits above the cylinders consists of a platform containing part of the combustion chamber and the location of the valves and heater plugs. In Diesel engines, the cylinder heads are usually made of grey cast iron. The cylinder head is a crucial part of all combustion engines, and its cracking can result in catastrophic damage to the engine. The most common cause of cylinder head cracking is overheating. When a vehicle overheats, it puts stress on all of its metal components, including the cylinder head, which is often at the center of the heat. Xu and Yu studied the failure on a diesel engine cylinder head made of grey cast iron. The crack was initiated from the interior wall and propagated toward the exterior surface of the cylinder head. Their results showed that longer graphite flake and more amount of ferrite are the general metallurgical characteristics of the failed cylinder head, compared with the specified, which may lead to the lower whole hardness of the material. They added that in the crack origin zone, a network of grain boundary connected flake graphite was found. The appearance of this network grain boundary flake graphite decreases the strength of this zone and initiates the crack [1].

Cast irons are among the most difficult metals to weld because they contain high carbon content, and a wide range of microstructures. The part of the base metal that reaches a temperature high enough to cause metallurgical changes, but not high enough to cause melting is the HAZ. In this zone, the matrix is transformed to hard martensitic structure upon rapid cooling which cause brittleness. Brittleness associated with martensite which can be reduced by maintaining a high preheat during welding, followed by slow cooling, or a post weld heat treatment [2-7]. The weld metal and fusion zones are other potential problem areas, when molten cast iron is cooled rapidly; the carbon is not rejected from the melt as graphite, but forms hard brittle iron carbide which is susceptible to cracking and difficult to machine [4-7].

Thermally sprayed nickel-based alloy coatings are used in a variety of applications, e.g. as bond coats for thermal barrier coatings (TBCs) on turbine components, as restorative layer for machine parts, as bond coats in internal combustion engine cylinders, for corrosion protection of boiler tubes and in other numerous applications requiring wear, high temperature and corrosion resistant surfaces [8–13]. Repair maintenance of pearlitic gray-cast iron cylinder head using thermal spray technique should be systematically studied in order to give better insight into the capability of this technique.

In this study shielded metal arc welding (SMAW) with different electrode types were used to repair a pearlitic gray cast iron and the results were compared with that obtained by using thermally sprayed nickel-based alloy powder.

2. Experimental work
2.1 Material

2.1.1 Cylinder head
In an internal combustion engine, the cylinder head sits above the cylinder and consists of a platform containing part of the combustion chamber and the location of the valves and heater plugs. The most common cause of cylinder head cracking is overheating. The cracks were detected using magnetic particle test. One of the cracks detected by fluorescent magnetic particle test is shown in Fig. 1.

Fig. 1 Crack as detected by fluorescent magnetic particle test

Chemical analysis of cylinder head material is carried out using optical emission spectrometer. The composition of the cylinder head material is shown in Table 1.

Table 1 Chemical composition of cylinder head, mass%

Element	C	Si	Mn	P	S	Cr	Mo	Ni	Fe
mass %	3.76	1.94	0.669	0.0321	0.0218	0.165	0.37	0.283	balance

2.1.2 Repair welding materials
Different types of Shielded Metal Arc Welding (SMAW) electrodes are applied in this work. Table 2 shows the different electrodes used and the chemical composition of their weld metals.

Table 2 Chemical composition of weld metal for different electrodes, mass%

Electrode \ Element	C	Mn	Si	P	S	Cu	Ti	Al	Ni	Fe
JIS DFC Fe	0.15	0.8	1	0.03	0.04	_	_	_	_	Balance
AWS A5.11 E Ni –Cu-7	0.04	3	0.7	0.01	0.001	29	0.7	0.3	Balance	1
AWS A5.15 E Ni-Fe-CI	0.5	_	_	0.2	0.001	_	_	_	53	Balance

Also, a thermal spray powder is applied for the repair welding process; its chemical composition is shown in Table 3. The average particle size of the powder is -53 to +20 μm; the hardness of the powder is in the range 190-260 HV.

Table 3 Chemical composition of flame spray powder, mass%

Element	C	Fe	B	Si	Ni
mass%	0.03	0.5	1.4	2.4	Balance

2.2 Repair Welding Procedure

2.2.1 Test specimens

To examine the best technique for repair of the pearlitic grey cast iron cylinder head, test specimens with a groove angle of 90º and a groove depth of 15 mm was prepared from the cylinder head.

2.2.2 Welding conditions
Three different electrodes were applied in SMAW process. The welding conditions are shown in Table 4.

Peening is applied after each pass to remove residual stresses and oxide layers formed. After welding, all specimens were allowed to cool slowly in sand.

2.2.3 Repair welding using thermal spray powder

Powder flame spraying process was also applied in repair welding of cylinder head. Preheating temperatures of 500°C is applied before thermal spray application followed by cleaning of the groove faces by grinding and brushing.

Thermal spraying process was applied using two different post weld heating cycles. In the first one, the specimen after thermal spray was left to cool to room temperature in sand. In the other, the specimen was held for 1 hour at 500°C in a Muffle furnace then furnace cooled to room temperature.

Table 4. Welding conditions for different SMAW electrodes

Electrodes	Voltage, V	Current, A	Electrode size, mm	Preheating Temperature, °C	Polarity
AWS A5.15 E NiFe-CI	24	100	3.2	100 and 400	DCEP
JIS Z3252 DFC Fe	24	100	3.2	100	DCEP
AWS A5.11 E NiCu-7	24	100	3.2	100	DCEP

2.3 Microstructure Observation

Microscopic examination is conducted at the cross section specimens. The specimens were cutout using fine cutter with cooling. The cross section was then ground through grit Silicon papers (180 to 1000). Final polishing was performed using 0.5 µm alumina paste, then cleaned and dried. The polished specimens are etched by 2% Nital solution to reveal the cast iron structure. Etching solution of 50 % Nitric acid, 50% Acetic acid are used to reveal the weld microstructures of AWS E NiFe-CI and AWS E NiCu-7 welds. Microstructure of base metal and welded specimens were observed using optical microscope.

2.4 Micro-hardness Measurements

Micro-hardness was conducted using Shimadzu Vicker micro-hardness testing device with a load of 9.807 N and 15 sec loading time.

3. Results and Discussion
3.1 Cylinder head Properties

The microstructure of the base metal is shown in Fig. 2. The microstructure shows gray cast iron that contains flakes of graphite in a pearlitic matrix. The average value of the base metal hardness is 215 HV.
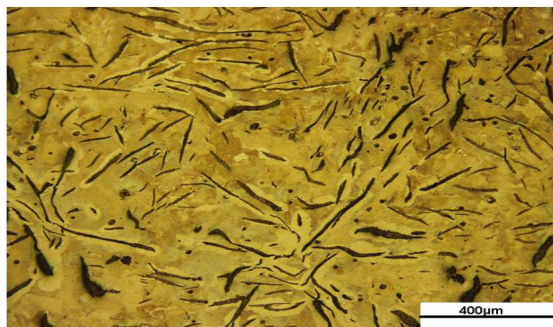
Fig.2 Microstructure of base metal cylinder head

The weldability of cast iron is very low. Thus, optimizing the welding conditions such as preheating and PWHT can improve the properties of the weldment. Three arc welding electrodes were selected to repair the cylinder head using different conditions. The results will be discussed based on the microstructures of weldments and their hardness distribution along weld metal, partial fusion zone, and heat affected zone.

For the sake of comparison, Powder flame spraying process was also applied in repair welding of cylinder head.

3.2 Repair of Cylinder head Using Carbon Steel Electrode

Cast iron was welded under welding conditions mentioned in Table 4 using JIS Z3252 covered electrode - DFCFe with preheating temperature of 100°C.

3.2.1 Microstructure

Microstructures of the cross section after welding using carbon steel electrode are shown in Figs. 3-5. Figure 3 shows the microstructure of weld metal, partial fusion zone, and heat affected zone. The weld metal near the fusion zone is in a dendritic form. The partial fusion zone contains some carbides which are not continuous as shown in Fig.3. The heat affected zone shows the existence of some martensite as shown in Fig. 4. Weld metal shows the existence of carbides and martensite as shown in Fig.5.

3.2.2 Micro-hardness distribution

Figure 6 shows micro-hardness distribution of weld metal, PFZ and HAZ. The micro-hardness value of the carbides in the PFZ is very high compared with that of the base metal.
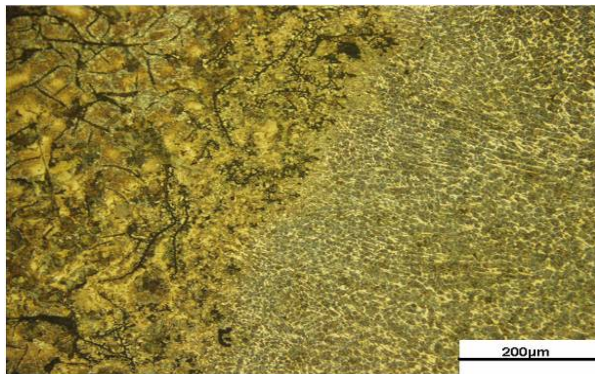
Fig. 3 Microstructure of weld metal, PFZ and HAZ of the specimen welded using carbon steel electrode
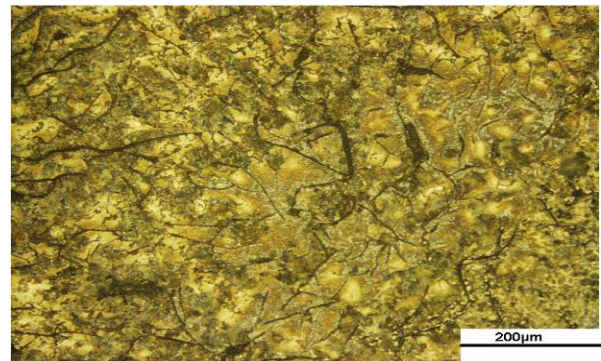
Fig. 4 Microstructure of the HAZ of the specimen welded using carbon steel electrode.
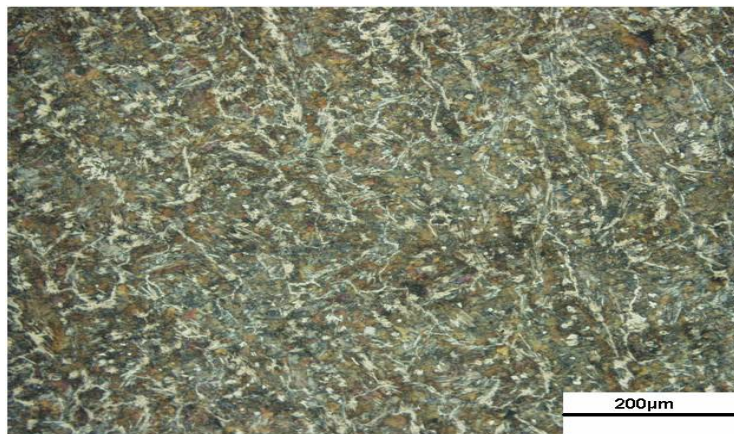
Fig. 5 Microstructure of the weld metal of the specimen welded using carbon steel electrode.
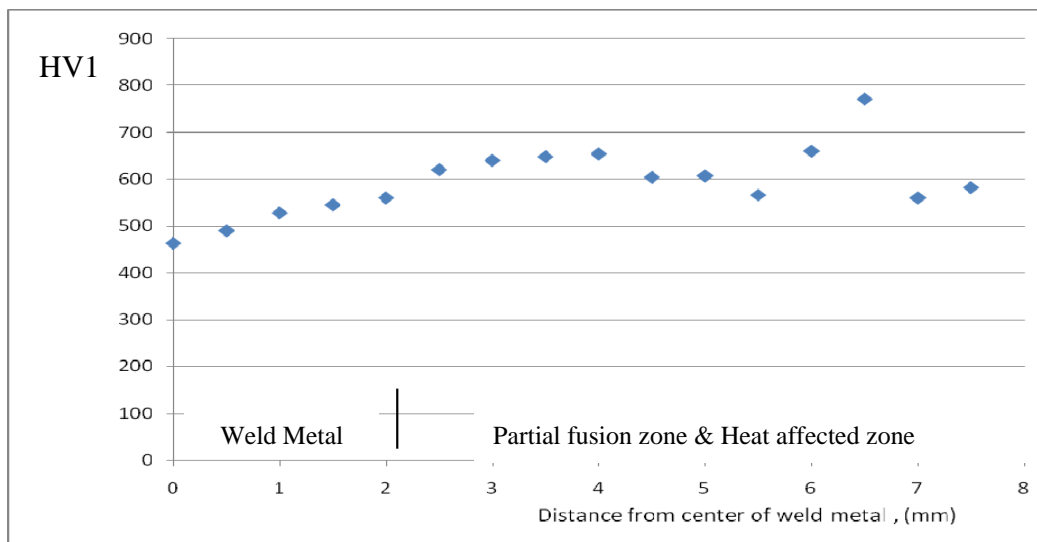
Fig. 6 Micro-hardness distribution using carbon steel electrode

3.3 Repair of Cylinder Head Using Nickel-Copper Electrode

Cast iron was welded under welding conditions mentioned in Table 4 using AWS A5.11 – E Ni Cu-7 electrode. Its chemical composition is shown in Table 2 and the preheat temperature was 100°C.

3.3.1 Microstructure

Microstructures of the cross section after welding are shown in Figs. 7-9. PFZ shows the existence of carbides as shown in Fig. 7. Microstructure of weld metal shows the dendritic structure of austenitic structure of Copper-Nickel alloy as shown in Fig. 8. The HAZ microstructure shows the existence of some martensite and a network of grain boundary connected flake graphite as shown in Fig.9.
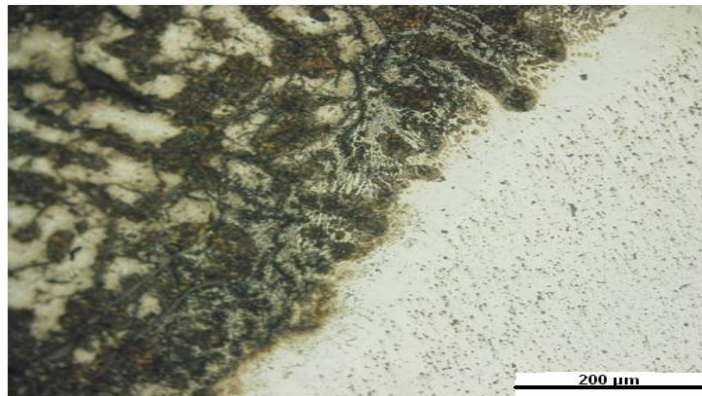
Fig. 7 Microstructure of weld metal, PFZ and HAZ using Nickel - Copper alloy electrode
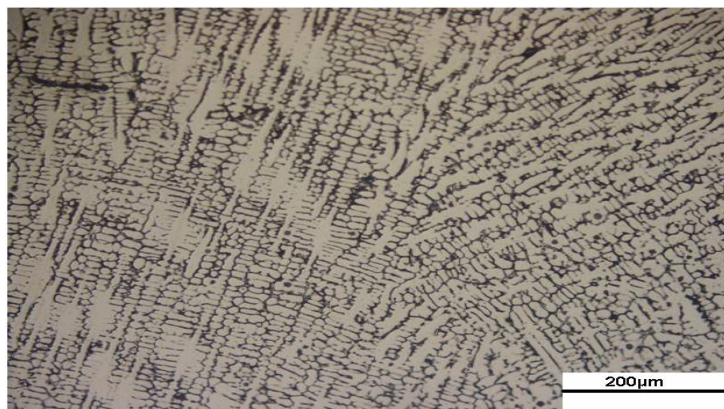
Fig. 8 Microstructure of weld metal using Nickel- Copper alloy electrode
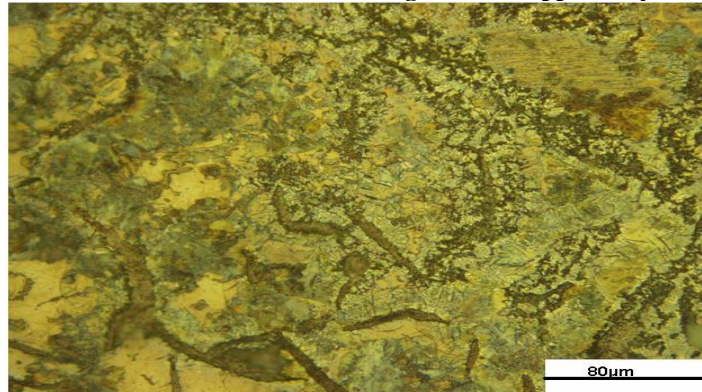
Fig. 9 Microstructure of HAZ using Nickel-Copper alloy electrod

3.3.2 Micro-hardness distribution

Figure 10 shows micro-hardness distribution of weld metal, PFZ and HAZ using Ni-Cu electrode. The micro-hardness value of the carbides in the PFZ is as high as 754 HV which is very high compared with that of base metal (215 HV) which acts as an area susceptible to crack initiation and propagation at the fusion line.
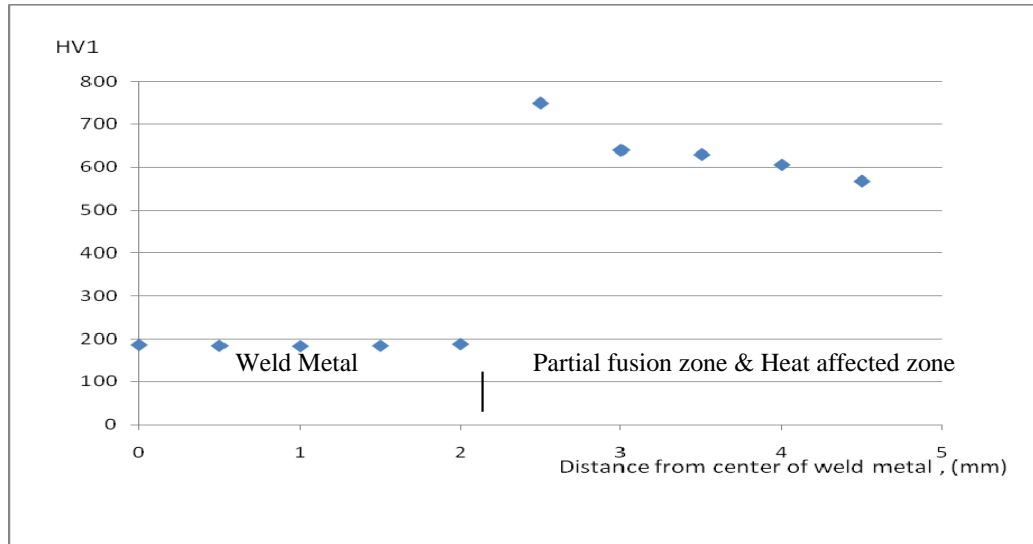
Fig. 10 Micro-hardness distribution for Nickel-Copper electrode

3.4 Repair of Cylinder Head Using AWS A5.15-E NiFe-CI
3.4.1 Using preheat temperature of 100°C

Cast iron was welded under welding conditions mentioned in Table 4 using AWS A5.15 E Ni-Fe-CI electrode. This electrode was used with a preheat temperature of 100°C.

3.4.1.1 Microstructure

Microstructures of the cross section after welding are shown in Figs. 11-13. The microstructure of the PFZ shows a few amounts of carbides which is discontinuous as shown in Fig. 11. However, Fig. 12 shows the existence of martensite at the HAZ. Figure 13 shows the dendritic structure of iron nickel alloy.
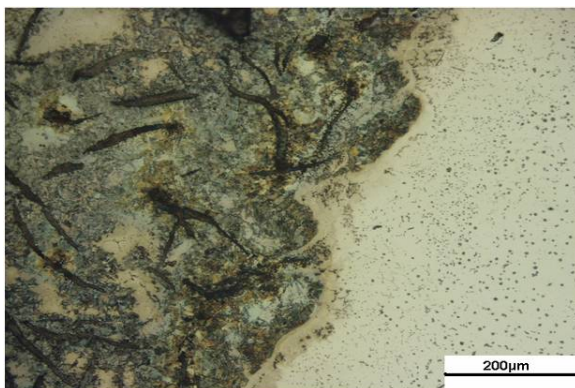
Fig. 12 Microstructure of HAZ of the specimen welded using Iron Nickel cast iron electrode; preheat temperature of 100°C.
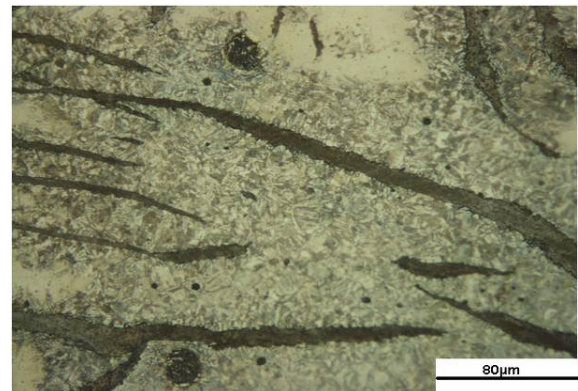
Fig. 11 Microstructure of PFZ of the specimen welded using Iron Nickel cast iron electrode; preheat temperature of 100°C.
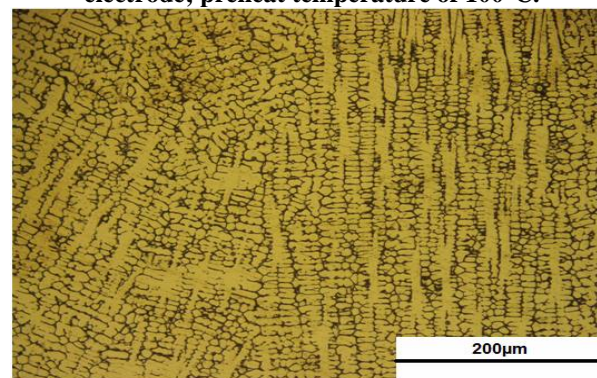
Fig. 13 Microstructure of weld metal of the specimen welded using Iron Nickel cast iron electrode; preheat temperature of 100°C.

3.4.1.2 Micro-hardness distribution

Figure 14 shows the micro-hardness distribution of the weld metal, PFZ and HAZ of the specimen welded using ENiFe-CI electrode. Martensite formed in heat affected zone caused an increase in its hardness.
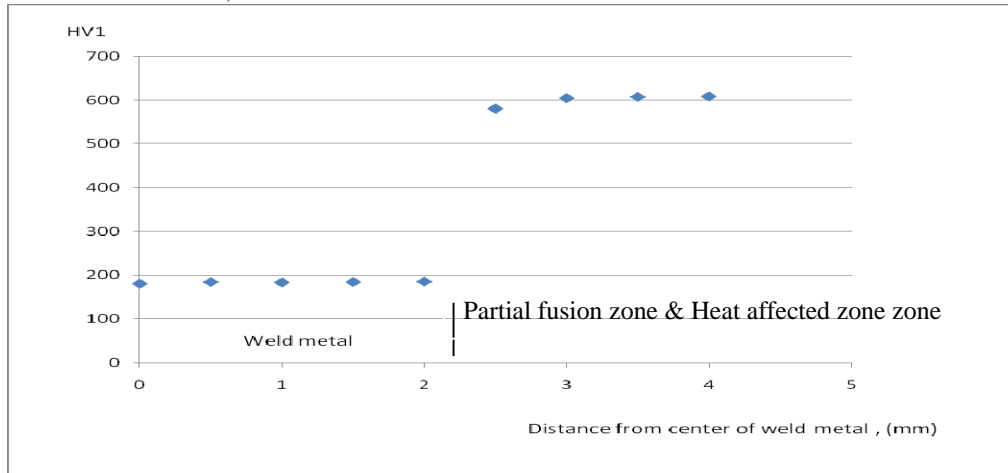
Fig. 14 Micro-hardness distribution using Ni-Fe-CI electrode and preheat temperature of 100°C.

3.4.2 Using preheat temperature of 400°C

Cast iron was welded under welding conditions mentioned in Table 4 using AWS A5.15 E NiFe-CI. This Electrode was used by applying a preheat temperature of 400°C.

3.4.2.1 Microstructure

Microstructures of the cross section after welding are shown in Figs 15 and 16. Figure 15 shows weld metal, PFZ and HAZ. There are continuous carbides in the PFZ. HAZ shows less martensite compared with the specimen preheated to 100°C as shown in Fig. 16.

Weld metal shows the dendritic structure of NiFe-CI alloy as shown in Fig. 17. Austenite grains of nickel alloy with carbides appear in Fig. 17.
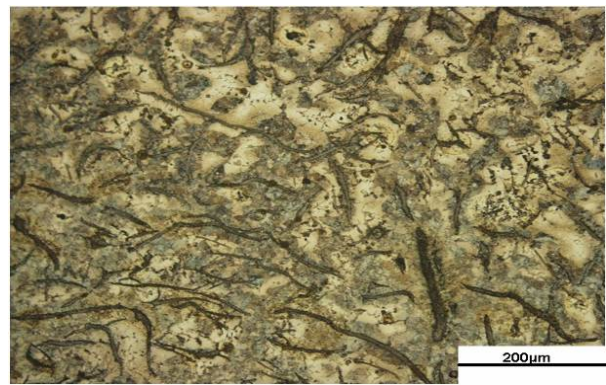
Fig. 16 Microstructure of HAZ of the specimen welded using Nickel Iron Cast Iron Electrode.
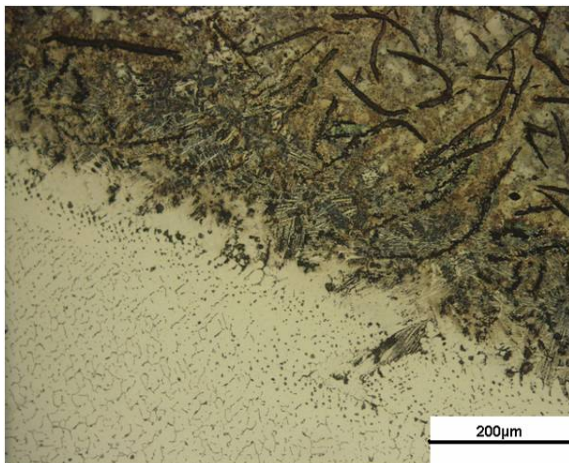
Fig. 15 Microstructure of PFZ of the specimen welded using Nickel Iron Cast Iron Electrode
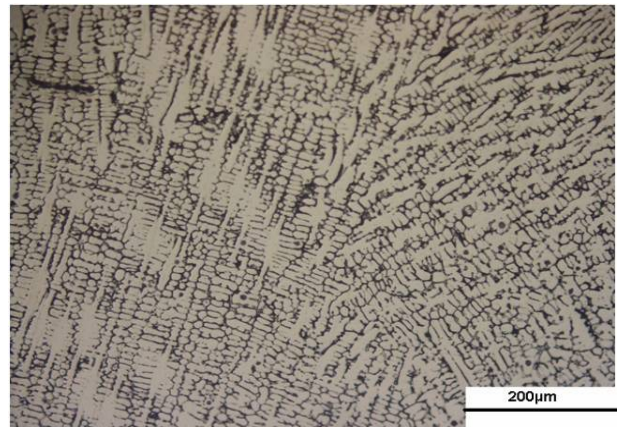
Fig. 17 Microstructure of weld metal using Iron-Nickel Iron Cast Iron Electrode.

3.4.2.2 Micro-hardness distribution

Figure 18 shows micro-hardness distribution of the specimen repaired using ENiFe-CI electrode after preheating at 400 °C.

The micro-hardness values of the partial fusion zone are much higher than weld and base metal due to presence of carbides however the heat affected zone shows lower values of hardness due to low martensite area fraction.
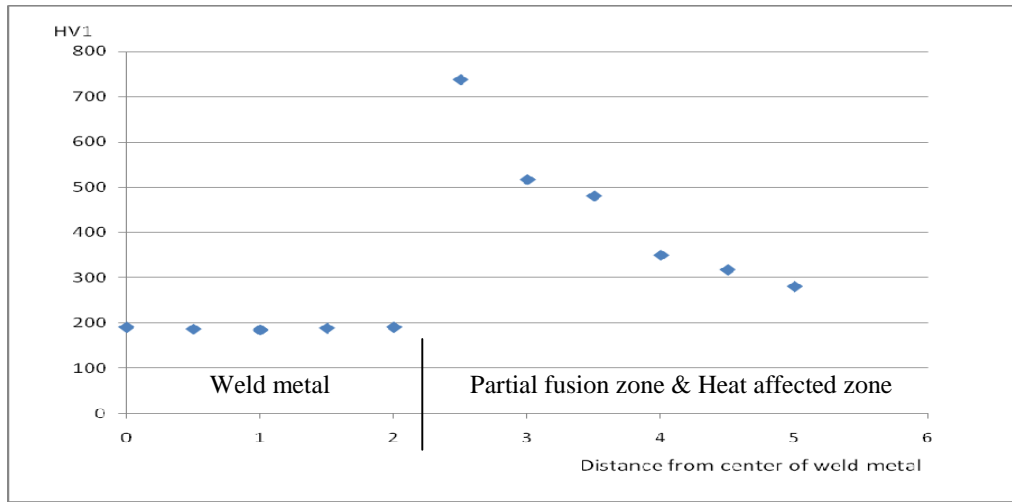
Fig. 18 Micro-hardness distribution for the specimen welded using Iron Nickel Cast Iron Electrode with preheat temperature 400°C

3.4.3 SMAW processes evaluation

The previous results using different electrodes indicated that increasing preheat temperature resulted in the formation of continuous carbides at the partial fusion zone with less martensite at heat affected zone. However low preheating temperature resulted in formation of fewer carbides at partial fusion zone and formation of large area fraction of martensite in the heat affected zone. Thus a low preheating temperature is preferred in welding of cast irons, due to the fewer amounts of carbides formed. However, it is impossible to achieve a solidification cooling rate sufficiently low to completely avoid carbide formation [15,16]. It is also possible to use low heat input as possible to minimize the formation of the PFZ, i.e., to create a steep temperature gradient which will reduce the thickness of the PFZ. In this study, the heat input was the same for all electrodes used (1.44 kJ/mm) and the Ni-Fe electrode showed the smallest PFZ (0.2mm) with a preheat temperature of 100°C. However, increasing the preheat temperature to 400°C results in larger PFZ (0.8mm) for the same electrode which is in agreement with the results obtained by Askeland and Birer obtained for tempered nodular cast iron weldments [14].

3.5 Repair Welding of Cylinder Head Using Thermal Spray Powder

Ni-base powder thermal spray is applied using oxy-acetylene flame to weld the test specimen. The powder chemical composition was shown in Table 3.

4.5.1 Specimen A

The specimen was subjected to uniform preheating at 500°C then buried in sand to slowly cool after thermal spraying.

4.5.1.1 Microstructure

Microstructures of the cross section after welding are shown in Figs. 19- 21.
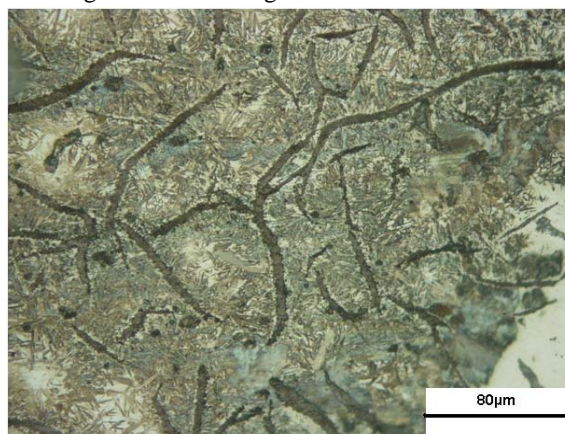
Fig. 19 Microstructure of heat affected zone of specimen A
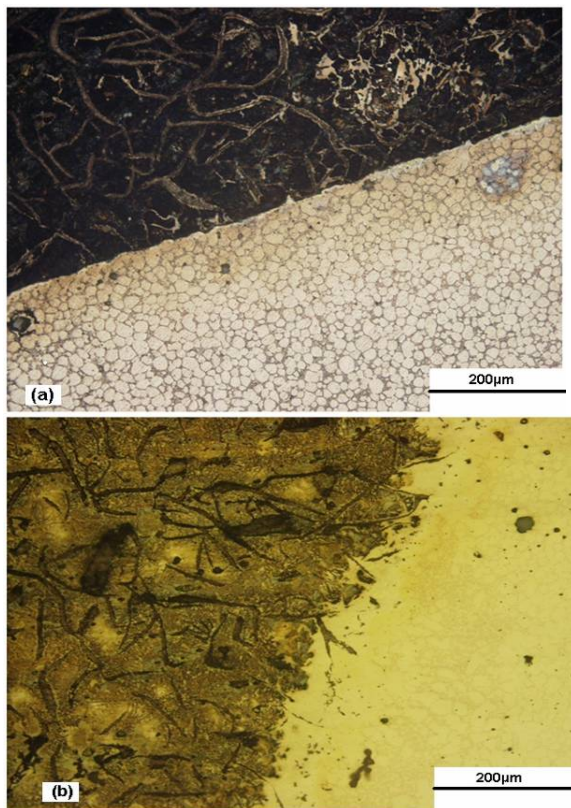
Fig. 20 Microstructure of weld metal, PFZ, and HAZ for specimen A, (a) Etching of weld metal only, (b) Etching of base metal
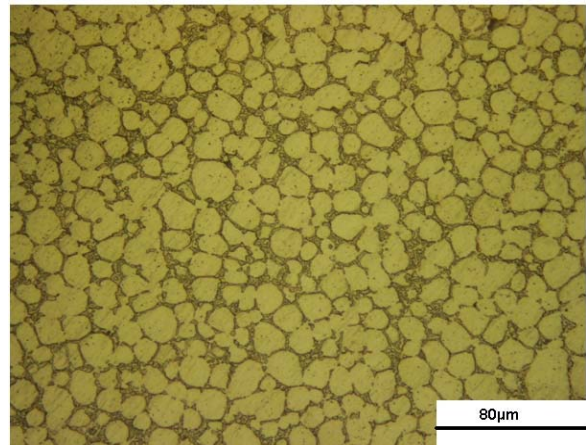
Fig. 21 Microstructure of Weld metal for specimen A

There is a doubt of the existence of partial fusion zone as shown in Fig. 20. However, HAZ shows the existence of martensite as shown in Fig. 20. Microstructures of weld metal are shown in Fig. 21 which shows the gamma phase of Nickel and the eutectic structure at grain boundaries.

3.5.1.2 Micro-hardness Distribution

Figure 22 shows micro-hardness distribution through the weld metal, PFZ and HAZ for the thermal sprayed specimen A. The presence of martensite in HAZ raised its hardness values to be in the range of 500-600 HV. This value is also higher than that of base metal.
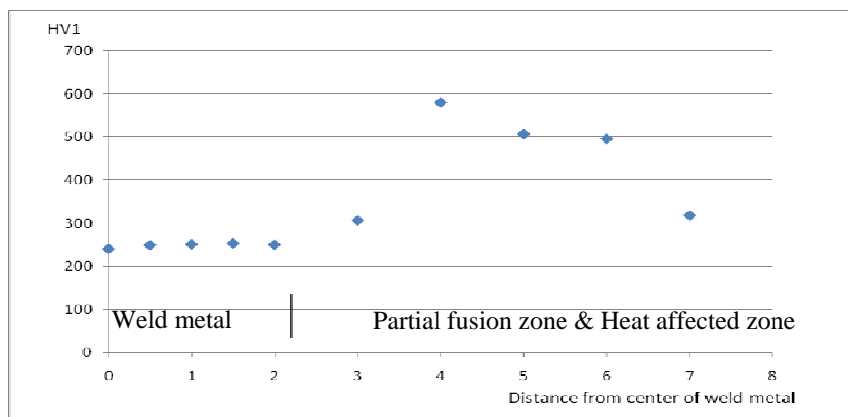
Fig. 22 Micro-hardness distribution for specimen A

3.5.2 Specimen B

The specimen was subjected to uniform preheating of 500°C; then thermally sprayed and immediately kept at 500°C for 1 hour and allowed to cool slowly to room temperature in the furnace.

3.5.2.1 Microstructure

Microstructures of weld metal, PFZ and heat affected zone are shown in Figs 23 and 24. There is a doubt of the existence of PFZ (No carbide formation)
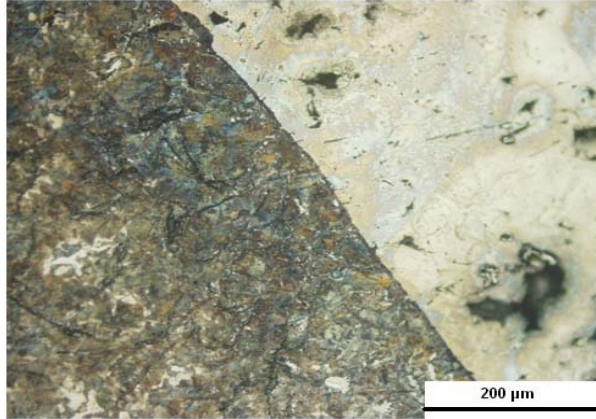
Fig. 23 Microstructure of weld metal, PFZ, and HAZ of specimen B
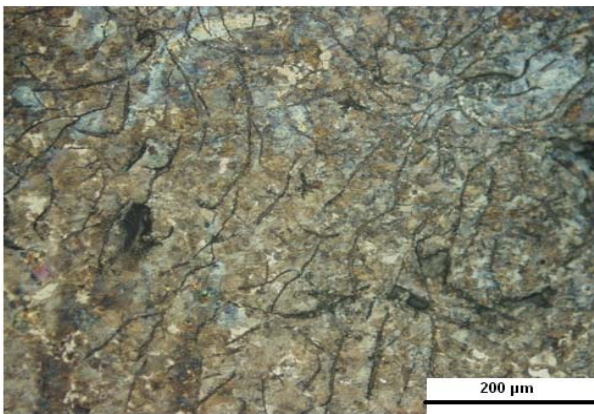
Fig. 24 Microstructure of HAZ of specimen B

and the heat affected zone is a pearlitic matrix. This achieves the two optimum conditions which are: 1. Carbides free PFZ and 2. Least martensite in the HAZ.

3.5.2.2 Micro-hardness distribution

The micro-hardness distribution of specimen B is shown in Fig. 25. The micro-hardness values of the HAZ and PFZ are close to the values of the base metal hardness. It is well known that the crack initiation and propagation in cast iron welds are due to the difference in mechanical properties between the base metal and weld metal. Using the technique followed with specimen B, such difference could be eliminated to a large extent and an average hardness of 300 HV was obtained for both PFZ and HAZ which is comparable to the hardness of the base metal (215 HV).

Some researchers [17] studied the restoration by welding of ductile cast iron and they concluded that trial and error procedure development may be necessary to get lower thickness of the martensite zone and discontinuous carbide network in addition to a pearlitic HAZ. Using thermal spray technique, the trial and error procedure which was applied in welding of cast irons may be eliminated and better welding properties were obtained.

Using of this technique, the cylinder head was repaired successfully and it is working since two years with 12 hours working time per day, which imply the reliability of this technique. Furthermore, it saves the cost of purchasing new cylinder heads for this large number of trucks.
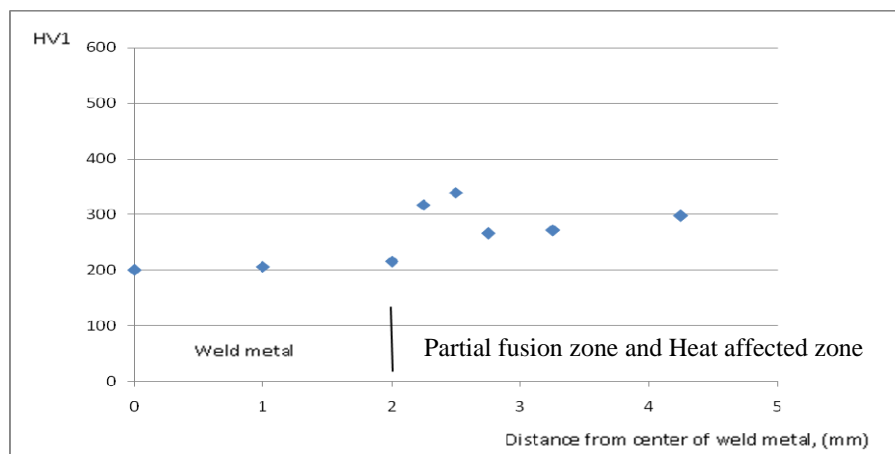
Fig. 25 Micro-hardness distribution of specimen B

4. Conclusions:

Repair welding of cylinder head was carried out primarily using shielded metal arc welding (SMAW) process with the following types of electrodes: AWS A5.15 ENiFe-CI, JIS DFC Fe (carbon steel electrode) and AWS A5.11 E Ni-Cu-7.

Welding using SMAW with AWS ENiFe-CI electrode was carried out after preheating to 100^0C and 400 ^{0}C as well. High preheating temperature caused formation of continuous carbide layer in the partial fusion zone and formation of few amount of martensite in the heat affected zone. Low Preheat temperature of 100^0C caused formation of a fewer amount of carbides in partial fusion zone, however higher amount of martensite was formed in the heat affected zone.

Hardness distribution of the specimens welded by SMAW using the three electrodes indicated that hardness values at partial fusion zone and heat affected zone were very high when compared with base metal hardness but the Ni-Fe electrode showed the lowest hardness values compared with the other two electrodes.

The other alternative was using powder flame spray process with preheating temperature of 500°C. Thermal spraying process was applied twice, at the first time the specimen was cooled in sand, and the other specimen was hold for 1 hour at 500 °C and slowly cooled in a muffle furnace at. The two specimens did not show formation of carbides, but the specimen cooled in sand showed formation of martensite in the heat affected zone. The other specimen that was cooled in the furnace showed a microstructure that is free of martensite and carbides, hardness distribution for this specimen showed comparable hardness values for partial fusion zone and heat affected zone with that of the base metal which imply the success of this technique to be applied in repair of pearlitic gray cast iron.

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Improving gladiolus growth, flower keeping quality by using some vitamins application

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Abstract: Response of growth, flowering quality and active chemical constituents of gladiolus plants by using some vitamins such as, thiamin, ascorbic acid and their combination during two seasons were studied .Plant which received the combined treatments of both vitamins recorded the highest growth, flowers quality and cormelets induction. Thiamine treatments had the lowest effect on photosynthetic pigments, while 200 ppm, thiamin+ 200ppm ascorbic acid, improved growth, delayed flowering opening of vase life , stimulated accumulation of carbohydrate and increased photosynthetic pigments and macronutrients status. Photosynthetic pigments and macronutrients. [Bedour, A. Abo Leila and Rawia, A. Eid. **Improving gladiolus growth , flower keeping quality by using some vitamins application.** Journal of American Science 2011;7(3):169-174]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: gladiolus plant flower quality, vase life and chemical constituents

1. Introduction

Gladiolus flowers is considered a main exportable ornamental plants in Egypt, and the flower can be available the year around, the foreign markets demand Egyptian gladiolus with higher quality. Ascorbic acid (vitamins C) is a product of D-glucose metabolism in higher plants which affect on plant growth and development, and play a role in electron transport system (**El-Kobisy et al., (1**). Also, **Smirnoff et al., (2)** proposed a biosynthetic pathway and identified novel some enzymes. They also reported that ascorbate is synthesized from L-galactose via GDP-mannose and GDP- L galactose. Ascorbic acid also has been associated with several types of biological activities in plants such as in enzyme co factors, antioxidant, and as a donor / acceptor in electron transport at the plasma membrane or in the chloroplast (**Conklin (3)**. A high level of endogenous ascorbate is essential effectively to maintain the antioxidant system that protects plants from oxidative damage **Cheruth (4)**. Further, **Farahat et al., (5)** on *Cupressus sempervirn* L. reported that foliar application of ascorbic acid caused pronounced increases in vegetative growth and chemical constituents as well as essential oil percent.

Thiamine (vitamin B1) is a necessary ingredient for the biosynthesis of co- enzyme. Thiamine pyrophosphates , so it plays an important role in carbohydrate metabolism in plant. It synthesis in leaves and it transported to roots where it controls growth. Thiamine is an important factor for the translocation reactions of the pentose phosphate cycle which provides pentose phosphate for nucleotide synthesis and for the reduction of NADP required for various synthetic.

Youssef and Talaat (6) found that pronounced increases in vegetative growth and chemical constituents of rosemary plants by foliar application of thiamine were observed.

The objective of the present study was to investigate the effect of ascorbic acid and thiamine on improving the flower characters and chemical constituents of gladiolus plant.

2. Materials and methods

A two pot experiment were carried out at the greenhouse of National Research Centre, Dokki, Egypt, during the two successive growing seasons of 2007/ 2008 and 2008/2009 to investigate the effect of ascorbic acid and thiamine on improving growth, flower characters and chemical constituents of gladiolus plant .The effect of gladiolus corms were kindly supplied by ornamental plants research, Ministry of Agriculture, Giza, Egypt. Ninform corms where sown on the first week of December in two seasons using plastic pots (30 cm diameter) that were filled with loamy sand soil, physical and chemical properties are illustrated in Table (1) which determined according to **Jackson (7).** Gladiolus corms were irrigated regularly with tap water to reach 80 % of water holding capacity of the soil by weighting the pots daily and the needed amount of water was added. All the normal culture practices of growing gladiolus corms were applied as usual manner. The experiment including 8 treatments in addition to the control which were two concentrations of thiamine (Th)and ascorbic acid (ASC)100 and 200 ppm applied each separately and/or in combination at concentrations of(100Th+100 ASC) ,(100 Th 100 + 200ASC), (200Th + **100** ASC)and (200 Th + 200 Th) , to the respectively in addition untreated plants.

Table (1): Some physical and chemical properties of the used soils.

Properties		Value	Nutrient content		Value
Clay	%	13.1	P	mg/100 g soil	13.4
Silt	%	24.1	K		59.2
Sand	%	62.8	Mg		17.4
Texture		Loamy sand	Fe	ppm	5.8
EC dSm^{-1}		1.98	Mn		8.3
pH		7.73	Zn		0.84
CaCO₃	%	2.15	Cu		0.96

Foliar application of thiamine and ascorbic acid were carried out two times of 30 days intervals, starting at the first week of February at both seasons. The treatments were arranged in a complete randomized block design with six replicates each replicate contained four pots and each pot contained four plants.

At the flowering stage sample was taken from representative three replicates randomly for each treatment and other three replicates for vase life, and the following parameters were determined the parameters included the following data , plant height (cm), no. of leaves per plant, as well as fresh and dry weights of leaves (g/plant), no. of florets/spike, No. of cormlets, and fresh and dry weight of cormlets (g/plant).

The flower spikes were cuts in the 7.00 am, wrapped in Kraft paper in groups inside treatments and translocated to the laboratory in cool water for 3 hours. The experiment design was a completely randomized with three replicates with five spikes per treatments.

The spikes were placed in glass bottles containing of 500 ml of water and kept into laboratory at room temperature for 15 days and the following data were recorded

Percentage of flowers wilted (on 6 days) total water uptake by the spikes and vase-life (days)

Water uptake (ml/l) by the cut spikes was estimated by subtracting the amount of water at the end of experiment from the initial volume.
Flower opening percentage after 24 h of placing the spikes in glass bottle congaing of water.

Chemical analysis

Photosynthesis pigments (chlorophyll a , chlorophyll b)and total chlorophyll (a+b) as well as carotenoids content were determined in fresh leaves as mg/g FW according to **Saric et al., (8),** Total carbohydrates were determined using colorimetric method as described by **Herbert et al., (9).** Total nitrogen was determined by the methods of **Chapman and Pratt (10)** while phosphorus

determination was carried out calorimetrically according to **King (11)**. Potassium was determined photo metrically by flame photometer method as described by **Brown and Lilland (12).**

Statistical analysis:

The recorded data (mean of the two seasons) were statistically analyzed on complete randomized design according to the procedure of **Snedcor and Cochran (13) Means were** compared by least significant differences(LSD 5% levels of probability).

3. Results
Effect of thiamine and ascorbic acid and their combination
Growth parameters:

It is evident from data in Table (2) that there are significant differences among the treatments and control in plant height, leaves number, fresh and dry weights (g/plant). The combined treatment of 200ppm thiamine +200 ascorbic acid followed by 200 thiamine + 100 ppm ascorbic acid were superior to the other treatments and control in previous mentioned parameters. The superiority of the combined treatment over the control was by about 63.41% for plant height, 53.98 % for number of leaves and 17.24 and 62.57 % for fresh and dry weights.

Table (2): Growth parameters of gladiolus plants as affected by some vitamins (mean of the two seasons).

Treatments	Plant height (cm)	Number of leaves /pl.	Leaves fresh weight	Leaves dry weight	FW /plant	DW/ plant
					(g/plant)	
Control	33.4	5.2	5.81	1.95	25.63	4.31
100 ppm Th	42.2	6.5	7.52	1.54	28.35	5.08
200 ppm Th	51.6	6.9	8.21	1.74	34.83	6.17
100 ppm ASC	47.8	8.4	10.54	2.07	36.74	6.97
200 ppm ASC	58.7	10.5	13.83	2.92	53.20	8.14
100 ppm Th+ 100 ppm ASC	49.4	7.7	9.35	1.84	56.21	9.16
100 ppm Th+ 200 ppm ASC	83.2	9.8	12.86	2.76	58.43	10.21
200 ppm Th+ 100 ppm ASC	88.0	10.7	14.58	3.62	59.09	11.34
200 ppm Th+ 200 ppm ASC	91.3	11.3	15.85	3.81	59.61	11.52
LSD (0.05)	2.12	0.23	0.43	0.11	1.12	0.21

Th=Thiamine   ASC=ascorbic acid

Florets:

Foliar application of ascorbic acid, thiamine and their combination improved florets characters of gladiolus compared with the control plants. Combined treatment of 200 ppm ascorbic acid + 200

ppm thiamine was more affective than each treatment alone and all combined treatments. However, single treatments of ascorbic acid showed intermediate effect (Table 3).

Table (3): Flower characters of gladiolus plants as affected by some vitamins (mean of the two seasons).

Treatments	No. of florets/ spike.	Spike lengt h	Diam eter of floret s	Leng th of floret s	FW of floret s/ spike	DW of floret s/ spike
			(cm)		g/plant	
Control	3.52	10.1	2.45	4.21	14.51	2.18
100 ppm Th	3.84	14.8	3.83	5.83	14.83	2.37
200 ppm Th	5.71	18.5	4.74	6.32	19.77	3.22
100 ppm ASC	6.83	17.3	5.03	6.84	20.42	3.47
200 ppm ASC	8.51	20.4	6.08	7.75	33.40	6.02
100 ppm T h+ 100 ppm ASC	10.3	17.5	6.24	8.00	35.21	7.08
100 ppm T h+ 200 ppm ASC	11.0	22.7	7.00	8.52	36.78	7.11
200 ppm T h+ 100 ppm ASC	12.2	23.8	6.90	9.00	37.42	8.10
200 ppm T h+ 200 ppm ASC	13.8	25.7	7.42	9.21	38.93	8.23
LSD at 5%	0.58	0.81	0.12	0.05	0.11	0.04

Th=Thiamine   ASC=ascorbic acid

Cormlets:

Table (4) show that application of ascorbic acid, thiamine and their combination had promotive effect on cormelets production of gladiolus compared with the control plants.

The low values were obtained by using 100 and/or 200 ppm thiamine while the combined treatment of 200 ppm thiamine + 100 ppm ascorbic acid induced the highest values for number of cormelets, fresh and dry weights (g/plant) . The increments amounted by 77.11 % for number of cormelets, 73.12 and 55.76 % for fresh and dry weights respectively over the control.

Table (4): Induction of gladiolus cormlets as affected by some vitamins (mean of the two seasons).

Treatments	No. of cormlets	Cormlets FW	Cormlets DW
		(g)	
Control	4.43	7.22	1.02
100 ppm Th	5.81	9.10	1.21
200 ppm Th	7.22	12.43	1.34
100 ppm ASC	8.42	13.63	1.95
200 ppm ASC	12.53	18.74	2.09
100 ppm T h+ 100 ppm ASC	14.22	22.11	2.00
100 ppm T h+ 200 ppm ASC	16.71	25.42	2.14
200 ppm T h+ 100 ppm ASC	18.11	26.83	2.29
200 ppm T h+ 200 ppm ASC	15.27	25.11	2.10
LSD (0.05)	0.56	0.31	0.04

Th=Thiamine   ASC=ascorbic acid

Photosynthetic pigments:

Results in Table (5) indicated that spraying ascorbic acid, thiamine and/or their combination induced gladiolus leaves with intensive Chl a, Chl b and carotenoids compared with control plants. Single treatments of thiamine gave the lowest favorable effect on photosynthetic pigments Chl a, Chl b and carotenoids, while the combined treatment of 200 ppm thiamine + 100 ppm and/or 200 ppm ascorbic acid gave the greatest promoting effect.

Table (5): Photosynthetic pigments and carotenoids content as affected by some vitamins treatments(mean of the two seasons).

Treatments	Chl (a)	Chl (b)	a+b	Carotenoids
		mg/g/fw		
Control	0.513	0.216	0.729	0.483
100 ppm Th	0.587	0.312	0.899	0.593
200 ppm Th	0.583	0.311	0.894	0.611
100 ppm ASC	0.634	0.345	0.979	0.573
200 ppm ASC	0.687	0.367	1.054	0.613
100 ppm T h+ 100 ppm ASC	0.693	0.389	1.082	0.634
100 ppm T h+ 200 ppm ASC	0.745	0.422	1.167	0.678
200 ppm T h+ 100 ppm ASC	0.814	0.432	1.246	0.719
200 ppm T h+ 200 ppm ASC	0.810	0.430	1.240	0.713
LSD.5%	0.002	0.001	0.005	0.040

Th=Thiamine   ASC=ascorbic acid

Macronutrients:

Table (6) indicated that spraying gladiolus plants with ascorbic acid, thiamine and /or their combination, gradually stimulated the concentration of N, P and K in gladiolus leaves, consequently single application of 200 ppm ascorbic acid increased N, P and K by about 74.16, 61.44 and 56.22 % over the control plants, respectively. While when 200 ppm was combined with 100 and /or 200 ppm thiamine , the highest P and K concentration were observed. However, it also clear that the combination between ascorbic acid and thiamine were more effective than each single treatment on N, P and K concentration.

Total Carbohydrates:

Data in Table (6) reveal also that vitamins treatments are favourable for increasing carbohydrate % whereas 200 ppm ascorbic acid + 200 ppm thiamine gave the greatest favourable effect, the increment over the control reached 68.18 %.

Table (6): Macronutrient status and total carbohydrates content of gladiolus plant as affected of some vitamins (mean of the two seasons).

Treatments	N	P	K	Total carbohydrates (%)
		mg/g DW		
Control	1.321	0.080	12.34	13.45
100 ppm Th	2.211	0.141	15.21	29.69
200 ppm Th	3.341	0.146	18.32	21.23
100 ppm ASC	4.423	0.152	18.79	30.45

200 ppm ASC	5.112	0.288	20.41	32.34
100 ppm T h+ 100 ppm ASC	5.642	0.219	22.32	31.23
100 ppm T h+ 200 ppm ASC	6.812	0.238	25.43	35.26
200 ppm T h+ 100 ppm ASC	7.234	0.346	28.22	32.84
200 ppm T h+ 200 ppm ASC	7.200	0.368	28.71	35.84
LSD 5%	0.230	0.004	0.82	1.28

Th=Thiamine   ASC=ascorbic acid

Vase life:

Table(7) show that spraying gladiolus plants with vitamins induced significant difference in flowers opened percentage, flowers wilted ,water uptake and vase life compared with untreated plants ,the combined treatments of 200ppm thiamine +100 and/or 200ppm ascorbic acid was the most effective treatments for delaying flowers opening% decreasing the wilting flowers and it has also increasing effect on vase life(day).Maximum water uptake by flowers were recorded by 200 ppm thiamine+100 ppm ascorbic acid treatment.

Table (7): Keeping quality of gladiolus cut flowers as affected by some vitamins (mean of the two seasons).

Treatments	Flowers opened%	Flowers wilted%	Water uptake(ml)	Vase life (day)
Control	88.34	9.21	4.60	11.41
100 ppm Th	82.53	88.32	5.30	11.00
200 ppm Th	78.41	75.43	6.22	19.21
100 ppm ASC	71.25	86.24	6.40	19.82
200 ppm ASC	60.43	72.32	7.00	21.21
100 ppm T h+ 100 ppm ASC	57.48	66.43	7.30	20.41
100 ppm T h+ 200 ppm ASC	50.23	64.25	8.10	23.11
200 ppm T h+ 100 ppm ASC	48.41	55.61	9.30	25.64
200 ppm T h+ 200 ppm ASC	45.08	55.72	9.5	25.22
LSD (0.05)	0.230	0.004	0.82	1.28

Th=Thiamine   ASC=ascorbic acid

4.Discussion

As expected some vitamins such as ascorbic acid and thiamine used in this experiment led to increase growth parameters of gladiolus plants with regard to plant height (cm), number of leaves, fresh and dry weights(g/plant). The present observation are fully corporate the finding of **El-Fawakhry and El-Tayeb (14)** on chrysanthemum, **Youssef et al (15)** on datura plants, **Mona and Iman (16)** on rose geranium and **Rawia et al**., **(17)** on *Jasminum grandiflorum*, **Nahed et al., (18)** on songonium, **Tarraf et al., (19)** on lemonegrass, **farahate et al**., **(5)** on *Cypressus sempervirent*.The regulatory effect of thiamine ,on meristem plant growth and development indirectly

through enhancing the endogenous level of various growth factors as cytokinines and gibberellins **Youssef and Talaat (6).** However, thiamine synthesized in the leaves and transported to root to control growth **Kawasaki (20).**

Regarding ascorbic acid, **Price (21)** reported that it is the most abundant antioxidant which protect plant cell and currently considered to be regulators on plant growth owing to its effect on cell division and differentiation. Our results also shown that thiamine was less effective and has little influence than ascorbic acid in this respect. This could be evident from the work of **Sakr et al., (22)** on canola and **Abdel Aziz et al**., **(23)** on gladiolus. The increment is growth parameters due to ascorbic acid are largely due to stabilize member structures **Blockhina (24),** modulating membrane fluidity in a similar manner to cholesterol and also membrane permeability to small ions and molecules (**Foyer,25**)., implicated in the regulation of cell division by influencing progression from BI to S phase of cell cycle (**Smirnoff (26).**

Cormelet and flowers characters tended to increase in the presence of ascorbic acid and/or thiamine comparing with the case of complete absence of vitamin (control). Ascorbic acid was rather than thiamine tended to be more effective and showed the most beneficial effect, and the positive response appeared to be raised with rise in the concentration.   The present observations are in agreement with the finding of **Sakr et al., (27)** on wheat. In this respect thiamine is an important factor for the translocation reaction of the pentose phosphate cycle, which provides pentose phosphate for nucleotide synthesis and for the role of NADP required for various synthetic pathway **Kawasaki, (20),** the author also added that, thiamine is a necessary for biosynthesis of Co-enzyme thiamine pro-phosphate, so it plays important role in carbohydrate metabolism which reflected on the increase in cormelets weights and numbers. The stimulatory effect of thiamine on flower characters was demonstrated by El-**Fawakhry and El-Tayeb (14)** on chrysanthemum flowers and **Rawia et al**., **(17)** on Jasmine, **Abdel Aziz (23)** on gladiolus . Similar results were obtained by **El-Quesni et al., (28)** on *Hibiscus rose sinesis* L. Ascorbic acid has been associated with several types of biological activities in plants, such as enzyme Co-factor, as antioxidant and as a donor/or acceptor in electron transport at plasma membrane on the chloroplast.   Such increments in cormelets number in our result s may be due to increase in photosynthetic pigments which reflect on carbohydrate content and cormelets weight **Conktin, (29).**

Our results demonstrated that, single application of either ascorbic acid and/or thiamine led

to increments in carbohydrates %, photosynthetic pigments and mineral ions contents N, P and K in gladiolus leaves. Apparently these observations may be due to that thiamine is a necessary for the biosynthesis of Co-enzyme thiamine pyrophosphate which plays a role in carbohydrate metabolism (**Kawasaki, 20).** The promotive effect of thiamine on total carbohydrates content may be due to their important role in the biosynthesis of chlorophyll molecule which in turn affected chlorophyll content **Youssef and Talaat,(18).**

Such observation were reported by **Hassanein (30)** on *Foeniculum vulgarire* L. and **Abo Dahab (31)** on *phelodedorn erubescene*, plants, they concluded that thiamine increase photosynthetic pigments in plants. The superiority of ascorbic acid in increasing growth parameters was accompanied by high promotive effect on the previous chemical constituents. Such finding show an analogy with those obtained by **Sakr et al., (22)** on Canola plants. In this connection ascorbic acid occurs in chloroplasts, vacuoles, mitochondria and cell wall (**Andrson et al**., **32, Ravtenkranz et al., 33).** The concentration in chloroplast can be high and probably related to its control photosynthesis **Foyer, (34).** Ascorbate acts as antioxidant (**Asoda, 35).** Co-enzyme co-factor **Davies et al.,(36).** Electrons donor for photosynthetic and mitochondrial electron transport **Asard et al**., **(37). Ascorbate** is produced of D-glucose metabolism which affects some nutritional cycle activity in higher plants **El-Kobisy et al., (1).**

The present data indicating further that supplemental addition of both thiamine + ascorbic acid greatly improved growth and chemical constituents of gladiolus growth, cormelets and flower induction with high quality and long vase life. Such positive increase could be explained by the fact that vitamins considered as a bio-regulators compounds which in little concentration exerted profound influence upon plant growth and production and may be due to the synergetic effect between ascorbic acid and thiamine in increasing plant growth and metabolism. This result hold true with finding of **Rawia et al., (17)** on *jasminum grandflorum*. Our results showed that the combined treatments between both vitamins ascorbic acid or thiamine sprayed to gladiolus plants induced flowers with high quality and long vase life. This observation are harmony with the finding of **Muhammad et al.,(38)** may be due to the pro moti*v*e effect of vitamins on most chemical constituents of plants.

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Productivity Changes of Hoteling Industry in Iran
(Case study: Kadoos Hotel of Guilan)

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ABSTRACT - Productivity is the standard by which human power in using resources to achieve their desired goal is determined. In recent years this standard has seen an up most attention in calculating technologies in industrial and service productivity industries. Value added methodology is a comprehensive way of measuring producing of all producing factors in a particular unit. In this case study we have interested recent changes in the hoteling Industry in Iran, along with our investigation we have selected kadoos hotel. A 5 stars hotel located in Giulan of Iran as our case study we have analyzed the productivity of this hotel in the period of 5 years 2005 – 2009. Results have shown that the foreign rate of occupation of hotel rooms – has increased in this hotel. This in turn has resulted in an increase in hotel revenue. But still we have not seen any creativity and change in other parts of the hotel such as food and beverage. There has not been an optimal use in new capitals and equipments in this hotel. To see more improvement In the hoteling industry in Iran, we have to have new knowledge in the field of quality management and connections to the world net of hoteling.

Introduction and Subject Definition

Productivity is the standard by which human power in using resources for the achievement of his desired goal, can be determined. Various techniques and methodologies for the better use of Machinery and human resources were introduced after Feredriks Taylors systematic approach in management In the early twentieth century. All of the Introduced techniques emphasized on the manufacturing and economical efficiency in the producing organizations.

Productivity standards are of most general standard used to better identify and evaluate the function of managing of a country in using resources. In the recent decades these standards sectors. To measure productivity for the service sectors following elements are to be considered (check & Hian, 2000):
1. Efficiency or optimal use by the resources.
2. Effectively or the conception of a good work which in the service sectors depends on three factors:
   - service quality
   - Flexibility
   - creativity and innovation

If possible, in the measurement of the productivity in the service sectors has to be diaspectual. First having supervision on the financial and economical and strategical indexes through out organization; such as value added per personnel and or the return of the capital and the ability to compete in the labor cost and then next aspect involves internal standards directly related to all, practically doing the work.

This paper, first is going to have a quick glance at the situation of hotel industry in Iran, and will discuss changes in the industry's policies, its revenue indexes and finally its place in the economy of Iran.

In the second part it will investigate the methodology in the form of value added for the calculation of productivity based on accounting concepts. This will enables one to measure the productivity and quality.

In the last part productivity indexes of kadood Hotel of Guilan for the late 20th century using value added methodology will be analyzed. In our final part, having in mind the internal and external factors, we will see the changes in the productivity of this hotel in Iran (Financial reports of Kadoos Hotel, 2009).

The situation of Hotel industry in Iran

Tourism industry has seen ups and downs and changes through out the last 3 decades of the 20th century. For this industry the last yare of 70s and 80s decades has been the time of stagnation. During these years Iran faced yet decreasing number in tourists arrival. We can mention in the year 1978 Iran was the host of 500 thousands tourist but this number decreased to 70 thousands in the next ten years. Improvement were due date the first plan of government in 1988 and with the start of a good political relationship with tourist generating countries, so Iran faced an outstanding growth in this industry (Ministry of Islamic

guidance, 2006).

In 2005 the number of tourists visiting Iran reached up to 1.3 of a million and as predictions showed by the end of the year 2009 we will see an increase in this number by the rate of 24%. So the expectant number will be 4000/000. This fast grown rate in recent years, has had strengthening effect on the place of Iran in the word of tourism (Ministry of Islamic guidance, 2008).

According to the worldly zone identification done for tourism activities.

All Asian countries except Middle East countries can be categorized into 2 groups.
  First: countries in East Asia, and pacific ocean
  second: countries in the south of Asia.

The second group is consisted of India, Iran, Nepal, Pakistan and some other countries. The number of tourist arrived in these countries (group 2) in the year 2005 was about 4477 thousand %51 of which (about 2288 thousand) was for India (having the first place) and about %10.2 of which (about 456 thousand) was for Iran (having the second place). The forecasts show an improvement in Irans place in future. (WTO, 2008).

During 2000 there has not been an appropriate growth in the number of hotels comparing to the growth of arrival tourists.

In 2007 the number of rooms summed up to 17147, this number increased to 25th thou sand in the year 2008. In other words in average there was a %7 increase in the number per year. New rooms, generally, belonged to 3 and 4 stars hotels. During these years there was not even a single 5 stars hotel built (Ministry of Islamic guidance, 2008).

Increase in the number of tourists with no appropriate hotel building to meet the need, all resulted in the over crowding of the Iranian hotels in the cities with attraction for tourists, these included Yazd, Isfahan, Shiraz. It is such that agencies face difficulty in high season In these cities.

New conditions can result in an increase related to the rate of occupation and concurrently it can enhance the rate of return.

Case study on the productivity in Kadoos hotel of Guilan

Kadoos Hotel a large hotel in Iran. It is a 5 star hotel, having 540 rooms, situated in Rasht, Guilan Tourism city of this hotel has 2 towers, Eastern tower and western tower. The eastern tower was renovated last years. In this process the most recent facility and equipments in the hoteling industry have been used.

To investigate the changes we will make use of the value added model which has most practical in

the Japanese corporation.

Calculating productivity using value added methodology

In this study we acquired a methodology from productivity center in Japan. This methodology is a mixture of social accounting methodology and company accounting.

From economics point of view in any national accounts, value added can be calculated in 2 different ways.

Since our case is in a company level, we adapt the following formula.

value added = Net revenue - [(raw material expenses + depreciation + other expenses) + (stock value at the beginning of the year)-(stock value at the end of the year) ± (value added adjustment)].

In analyzing value added, the relationships between indexes and their effect on technological factors; management, revenue, sale, and labor expenses are indicated.

In general, in this study we have acquired following indexes for our value added analysis of Kadoos Hotel:
  -value added productivity indexes
  -value added component
  -cost indexes
  -capital profiting indexes

To calculate productivity we have general accounting definitions. All number were calculated according to the rate of market for the price of dollar.

Since the number of indexes used is many in number, we have tried to use lesser indexes for the simplicity of conclusion.

Analysis on the value added productivity

The analysis of value added productivity is related to 4 factors:
  a - Technology and Management
  b - Revenue
  c - Capital
  d - Labor cost

a. Technology and Management

The equipment ratio indexes per personnel has increased from 626 in the year 2005 to 2910 in the year 2000 in other word, for each personnel there has been 4.6 times more of capital at hand. In other hand, equipment productivity shows a decrease by %3.63 during the same period. These 2 indexes show an increase in the investment put in the hotel for the purpose of resource equip ting and it also shows an inappropriate use of them. So hotels value added has no increased appropriately with its income.

b. Revenue

Hotel revenues has seen an increase by 2.5 times during the mentioned period. Increase in sale has mostly belonged to the increase in the price of the rooms. other services provided by hotel has not show an appropriate growth. one of the features related to all large hotels in Iran in the stagnation of services such as food, beverage, entertainment and sport, it shows that the market for mentioned services in not doing well.

The ratio of revenue to expenses has a decrease of %34 (%92-%59) from years 2005 to 2009 which itself show a well growth and better profiting of hotel the year 2009 in comparison to the year 2005.

During the studied the ratio of value added remained unchanged, (the ratio of value added to the revenue), showing appropriate growth in value added and revenue.

c. Labor cost

During the mentioned period value added productivity has changed from$ 4776 in 2005 to $1161 in 2009 and we from $6560 in 2005 to $15789 in 2009 in the ratio of our income to the number of personnel (Table 3). This fact shows a stability in the number of labor and also appropriate changes in salary. This fact lies according to the value of the countries currency and also the increase of revenue and the profit of hotel. The most important factor contributing to the better revenue goes to the increase of foreign rate of occupation and hotels prices being in dollar.

d. Capital

productivity indexes show that the part taken by profit in our value added during this period has known an increase. But for reason of severe increase in the capital, capital productivity of value added has seen a decrease In year 2009 to year 2005.

Discussion and Conclusion

Total analysis by value added component productivity indexes in Kadoos Hotel shows that during our period of study, this hotel has had a good growth in revenue and value added. Labor productivity, too, has seen an increase but food and beverage revenue has not seen a good growth in this times. Hotel capital for the reason of the renovation and reconstruction and also the use of good equipments, has tasted an outstanding increase but hotel management has been unable to appropriately use the new capitals. So capital productivity indexes have decreased.

Hotel productivity is effected by internal and external factors. external factors included:

Economical conditions, law related to tourism industry, labor, foreign investment, taxes and existence of other competitors.

Internal factors include (Taleghani, 2007):

Labor management, and quality management.

In external factors for hotel industry, there exist 2 threatening factors.

1. The absence of world large hoteling companies in Iran.

2. Little knowledge in the field of quality management and appropriate servicing in the hoteling industry.

Cooperation and also common investment obviously we should think of young generation and the use of young trained labor.

Mentioned factors resulted in the decrease for the qualities of services presented by hotels in Iran and has let little competitors such as hotel apartments, little restaurant, pilgrim houses compete well with hotels.

Not appropriate use of the present capital has resulted a high price for hotels for their servicing. This in fact has led to the decrease in the domestic rate of occupation. So customers would prefer hotels, competitor (Taleghani, 2007).

Since tourism industry has a good perspective in Iran. Hotels must equip themselves with new knowledge on hoteling and improve their quality and labor managements. Moreover they have to create and present various services such as food and beverage and other activities. They can lessen the price by the increase in sale and so they can compete with their domestic competitors.

Of important need for this industry in recent decade we could name:

- connection to the system of international reservation

- signing of training contracts management

- cooperation and also common investment obviously we should think of young generation and the use of young trained labor.

Table 1.Net Revenu (per $) of Kadoos Hotel of Guilan.

	2005	2009
Rooms	1,883,43	6422951
Food & beverage	1395002	1,888,473
Telecommunication	126703	377709
Laundry	30922	45995
Sport & Recreational center	31848	43719
Total Revenue	3542816	8778849
Value added	2579460	6455710

Source: Financial reports of Kadoos Hotel, (2005-2009)[2].

Table 2. Total cost of different sectors of Kadoos Hotel.(per $)

	Rooms		Food & Beverage		Telecommunication		Laundry		Total	
year	2005	2009	2005	2009	2005	2009	2005	2009	2005	2009
Wages & salary	552375	352713	799996	1067627	24541	8184	39291	45081	1418441	1475079
Consumed material	44416	145232	4554346	685417	64604	103765	2602	7119	522260	950555
Administrational expenses	257012	704588	63457	208215	2084	62400	9216	135027	335385	1170497
Depreciation	41264	152551	10549	43317	1001	838	276	4466	55799	202086
Total	895068	1355086	1328350	2004578	92230	175187	51388	191693	2381897	3798218

Source: Financial reports of Kadoos Hotel (2005-2009)[2].

Table 3. productivity indexes.

	Indexes	**2005**	**2009**
1	Value added productivity (value added / number of employee)	4776	11611
2	Value added ratio (value added/Revenue)	0.73	0.73
3	Revenues / number of employee)	6560	15789
4	Capital intensity	1711	5160
5	Equipments / number of employee	626	2910
6	Employee ratio (wages & salaries / value added)	0.55	0.22
7	Profit Ratio	0.45	0.77
8	Wages & salaries / number of employee	2626	2653
9	Cost / Revenue	0.59	0.93
10	Administration expenses / Revenue	0.19	0.13
11	Revenue / Tangible fixed assets	10.48	5.42
12	Operating profit/Revenue	0.33	0.56
13	Productivity of equipments (value added /fixed assets)	7.62	3.99
14	Purchase ratio (1-value added Ratio)	0.27	0.27
15	Productivity of capital (value profit) added/operating	2.8	2.24

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2/10/2011

siRNA Mediated-hTERT Knockdown Impedes Proliferation of Mammalian Cancer MCF7 and HepG2 Cells

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Abstract: Telomerase is an attractive molecular target for cancer therapy because it is present in most malignant cells but is undetectable in most normal somatic cells. Human telomerase consists of two subunits, an RNA component (hTR) and a human telomerase reverse transcriptase component (hTERT). Small interfering RNA (siRNA), one kind of RNA interferences, has been demonstrated to be an effective method to inhibit target gene expression in human cells. We investigated the effects of siRNA targeting both hTERT mRNA and protein expression on the inhibition of proliferation and growth of human breast carcinoma cells (MCF-7) and liver carcinoma cells (HEPG-2). Here we used two siRNAs sequences (siRNA#1 and siRNA#2) that differentially target hTERT. Our results revealed that treatment of MCF7 and HepG2 cells with either of hTERT siRNAs resulted in significant decrease in both mRNA ($p<0.05$) and hTERT protein expression ($p<0.05$). Summary, our results clearly demonstrate that siRNA mediated knockdown of telomerase has efficiently suppressed proliferation rate of MCF7andHepG2cells. From these findings, we propose that targeting telomerase using siRNA might be a rational approach in cancer therapy.

1. Introduction:

Telomeres cap the ends of eukaryotic chromosomes protecting them from being recognized from DNA repair system which may lead to degradation, fusion, and recombination (1). Hence telomeres are important for maintaining genomic stability (2). In mammalian cells telomeres maintain their length stable generally through the action of telomerase (2). Telomerase enzyme complex have two major subunits contributing to enzymatic activity: a structural RNA component (*hTER*) and a catalytic subunit with reverse transcriptase activity (*hTERT*) (3). In most normal cells, telomerase activity is absent or very low, however, telomerase is active in most malignant tumor cells (3). Telomeres shorten with each cycle of cell division during replication due to a so called "end-replication-problem" (1). When telomeres reach a critical short length, cell arrest /death will ensue. This is happened in normal cells because of the absence of telomerase. However in cancer cells, the reactivation of telomerase is thought to stabilize telomere length, thereby compensating for the cell division-related telomere shortening and providing unlimited proliferative capacity to malignant cells (4). Consistently, inhibition of telomerase in tumor cells should disrupt telomere

length maintenance causes a proliferative crisis for malignant cells which leads to cell death. Previous studies have been developed to inhibit telomerase activity in tumor cells using antisense oligonucleotides (5-7). Although these strategies have been shown to effectively inhibit telomerase activity, further investigation of PNAs as gene therapy has been hampered by the poor intrinsic absorbability of PNA in living cells.

RNAi is a sequence-specific, post-transcriptional gene silencing mechanism that uses the introduction of small interfering RNA (siRNA), a hybrid consisting of a sense and antisense strand homologous in sequence to the silenced gene(8). siRNA, 21-nt RNA with 2-nt 3′ overhang, can mediate strong and specific suppression of gene expression. RNAi technology is currently evaluated as a potentially useful method to develop highly specific gene-silencing therapies (9,10).

The main aim of the current study is to verify whether or not the use of siRNA can target hTERT and hence inhibit the proliferation/growth rate in breast and liver cancer cell lines.

2. Material and Methods
Cell culture

Breast cancer cell line (MCF7), liver cancer cell line (HepG2) and normal melanocytes cell line (HFB4) were maintained in RPMI medium (SIGMA ALORICH#R8758) supplemented with 10% fetal bovine serum (SIGMA, USA#F2442) in the presence of penicillin (100 units/ml) and streptomycin (100 µg/ml). These cells were cultured in 5% CO_2 incubator in a humidified incubator at 37 C.
siRNA transfection

Two siRNA sequences (siRNA#1 and siRNA#2) (Table 1) and negative control siRNA (scRNA, silencer negative control siRNA, Sigma) were transfected independently into cell lines using the N-TER Nano particle siRNA Transfection System (Mirus, USA) according to manufacturer's protocol. Briefly, cells were seeded in 96-well plates at densities of 5000 cells per well and were then incubated under normal growth condition for approximately 24h. The cells were then combined with 50 nmol of either of siRNAs (siRNA#1, siRNA#2) or scRNA complexed with N-TER at a ratio of 10:1. The transfected cells were then incubated at 37 °C, 5% CO_2 for 24h, 48h and 72h. Following transfection, the cells were harvested and assayed for gene expression and viability.

Table 1: Sequences of siRNA#1 and siRNA#2 that target hTERT.

siRNA	Sequence
siRNA#1	F: 5'GUGUCUGUGCCCGGGAGAA dTdT3' R: 5'UUCUCCCGGGCACAGACAC dTdT3'
siRNA#2	F: 5'GAGCAAGUUGCAAAGCAUU dTdT3' R: 5'AAUGCUUUGCAACUUGCUC dTdT3'

Analysis of hTERT mRNA expression by semi-quantitative RT-PCR

mRNA expression of hTERT was quantified using semi-quantitative RT-PCR. Total RNA was extracted from approximately $3x10^6$ cells using TRIZOL (Invitrogen). 1 µg of total RNA was used to synthesize cDNA using M-MIV reverse transcriptase (Promega) according to manufacturer's protocol. The cDNA was added to a final volume of 20µl PCR reaction including 5 pmol of each primer (Table 2). PCR conditions were performed as follows: 25 cycles of 94°C/45 sec, 60°C/45 sec, 72°C/90 sec.

Table 2 : Sequences of hTERT and b-actin primers used for RT-PCR

primer	Sequence
hTERT	F: 5'-TCTACCGGAAGAGTGTCTGGAGCAA-3' R: 5'-GCTCCCACGACGTAGTC-CATGTTCA-3'
b-actin	F: 5'-TTCAGGTTTACTCACGTCATCC-3' R: 5'-CCAAATGCGGCATCTTCAAACCC-3'

The RT-PCR products were electrophorized using 2% agarose gel with ethidum bromide. The signals were then quantified by densitometer analysis using UNSCAN-IT software. The relative expression of hTERT was calculated according to the following formula:

$$\text{Relative hTERT mRNA expression} = \frac{(\text{hTERT signal - background signal}) / (\text{b-actin signal - background signal})_{siRNA}}{(\text{hTERT signal - background signal}) / (\text{b-actin signal - background signal})_{scRNA}} \times 100$$

Western blot analysis

About $3X10^6$ transfected cells were suspended in RIPA lysis buffer in the presence of proteinase inhibitors (Roche) for at least 30 min on ice. 40 µg of total proteins were electrophorized on 8% SDS-polyacrylamide gel and transferred onto PDVF membrane (Amersham Biosciences). After blocking the membrane in 5% non fat milk for 2h at room temperature (RT), the membrane was incubated

for 1h at RT with anti-hTERT antibody at final concentration of 1:1000 (Alpha Diagnostic). The probed membrane was then washed 3 times with TBST (0.1 % Tween-20 in TBS) and then incubated for 1h at RT with horse-raddish peroxidase (HRP)-conjugated secondary antibody (1:2000). Then the

membrane was washed 3 times in TBST buffer. The bands were then visualized by chemoluminescence using ECL-PLUS reagent (Amersham Biosciences). -actin was used as a loading control. The inhibition

rate was calculated in the same manner as RT-PCR. The relative expression of hTERT was calculated according to the following formula:

$$\text{Relative hTERT protein expression} = \frac{(\text{hTERT signal - background signal}) / (\text{b-actin signal - background signal})_{siRNA}}{(\text{hTERT signal - background signal}) / (\text{b-actin signal - background signal})_{scRNA}} \times 100$$

Estimation of cell viability by TACS MTT Cell Proliferation Assay

Cellular proliferation was assayed using a sensitive in vitro TACS MTT assay. This assay is a colorimetric assay system that measures the reduction of a tetrazolium component into an insoluble formazan product by the mitochondria of viable cells. Briefly, cells were transfected with either siRNAs (1 or 2) or scRNA. 72 h-post transfection, 0.5 mg/ml MTT was added to the cells and incubated under appropriate growth condition for 4h. The absorption as an indication for cell proliferation was then measured at 570 nm.

3. Results:
Effect of siRNA treatment on mRNA expression of hTERT

Using RT-PCR we analyzed mRNA expression of hTERT after transfection with 50 nmol of siRNA of both MCF7 and HepG2 cell lines. Here we used two different siRNAs (siRNA#1 and siRNA#2) that differently target mRNA of hTERT in two different sites. Our results revealed that hTERT mRNA expression was significantly reduced (~75 % for both siRNA#1 and siRNA#2) after 24h in MCF7 (Fig. 1 A&B). Importantly, this decrease persisted for 48h- and 72h-post transfection (~70% & 74% and ~70% & 75% for siRNA#1 and siRNA#2, respectively). Indeed, no effect on hTERT mRNA expression was reported after transfection of MCF7 cells with control siRNA (scRNA, data not shown). Essentially, similar results have been reported for HepG2 cells (Fig. 1 C&D). Compared to scRNA, about 75% and 70% hTERT down-regulation was observed after 24h for siRNA#1 and siRNA#2 respectively. 48h- and 72h- post transfection, hTERT was down-regulated to similar extent in hepG2 to that in MCF7 cells (73% & ~72% and 72% & 69% for siRNA#1 and siRNA#2 respectively). Altogether, these data suggest that targeting hTERT with either of siRNA #1 or #2 has effectively suppressed hTERT mRNA expression level in both MCF7 and HepG2 cell lines.

Effect of siRNA treatment on protein expression of hTERT

Down-regulation of mRNA does not necessarily mean protein down-regulation. In order to verify that, we analyzed hTERT protein expression after transfecting both strains with 50 nmol of either siRNA#1 or siRNA#2. As shown in Fig. 2 A&B, protein expression of hTERT in MCF7 was significantly decreased 24h-post transfection (~94% for both siRNAs compared to scRNA). 48h- and 72h-post transfection of either of siRNAs has almost completely inhibited hTERT protein expression in MCF7 cells (Fig.2 B). On the other hand, hTERT protein expression was not affected after scRNA treatment (Fig. 2A, compare lane 1 and 2). Basically, similar findings have been reported in HepG2 cells (Fig. 2 C&D). hTERT protein expression was significantly down-regulated (~95%) 24h-post siRNA#1 treatment and almost no protein was detected after 48h and 72h of siRNA#1 treatment. Of note, siRNA#2 treatment was more effective in hTERT protein down-regulation in HepG2 as almost no hTERT protein was detected 24h-, 48h- and 72h-post transfection. Collectively, our results implicated that both siRNA#1 or siRNA#2 treatment have effectively down-regulated hTERT expression.

Effect of Telomerase knock-down on proliferation

Telomerase was shown to impact cellular proliferation and regulates cell growth. In order to verify that, cellular proliferation was measured using MTT assay after hTERT knockdown. Our results demonstrated that hTERT knockdown did not affect proliferation rate of normal HFB4 melanocyte cells (Fig. 3, first 3 columns). In addition, cell proliferation was not affected after treatment of either MCF7 or HepG2 cells with scRNA (Fig. 3 columns 4 and 7). Importantly, Knocking down hTERT using siRNA#1 showed a dramatic inhibitory effect on cell proliferation in both MCF7 and HepG2 cells (Fig. 3, ~36% and ~34% respectively). Similar results have been reported for siRNA#2 (~34% and ~33% in MCF7 and HepG2 respectively). These results clearly revealed that hTERT down-regulation suppresses cell proliferation in tumor but not normal cells.
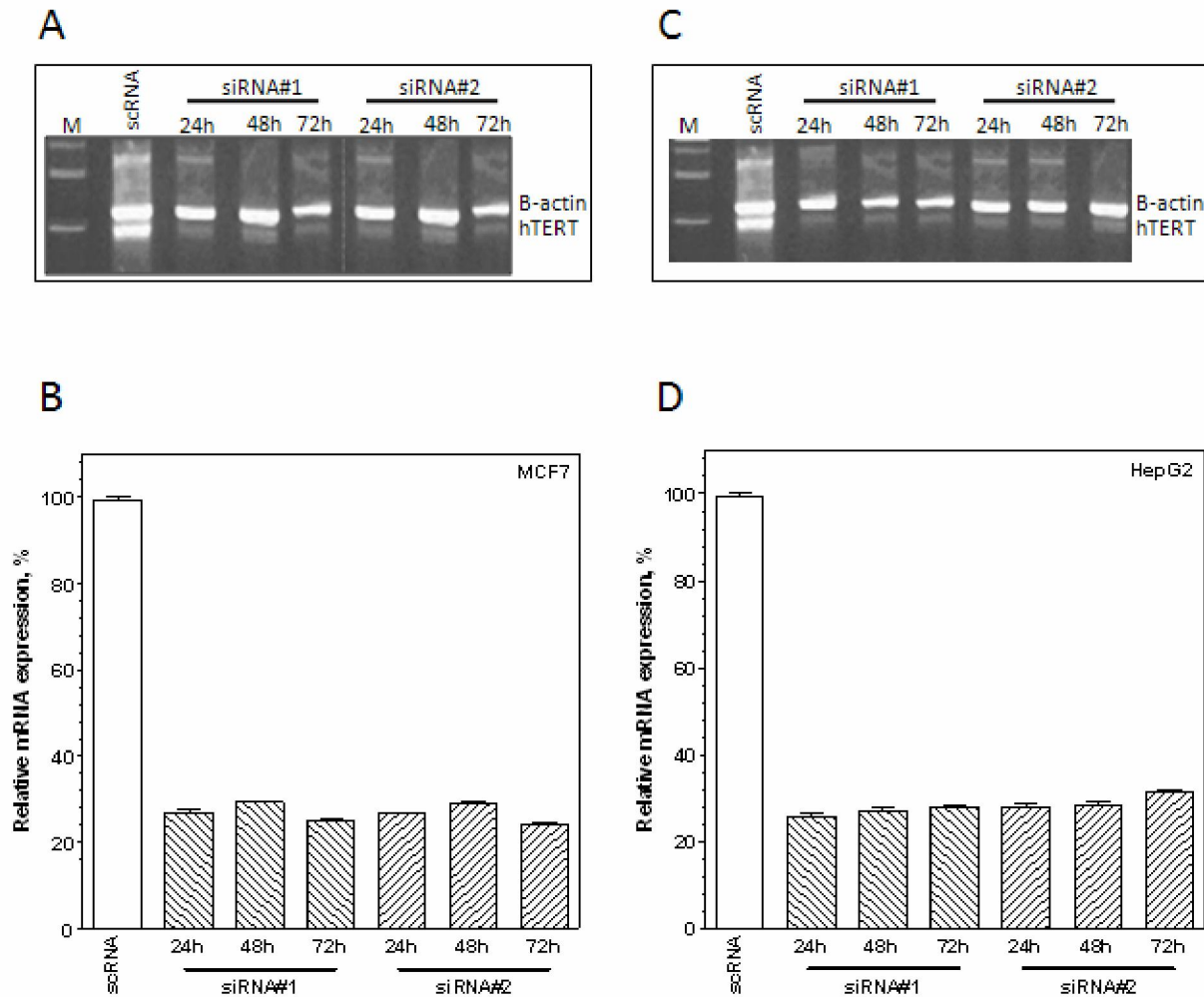
Fig.1: Down-regulation of hTERT mRNA expression in MCF7 and HepG2 cells by siRNA#1 and siRNA#2. (A) Representative examples of hTERT mRNA expression (upper bands) after transfecting MCF7 cells with 50 nmol of scRNA (lane 1), siRNA#1 (lanes 3-5) or siRNA#2 (lanes 6-8). Compared with scRNA- transfected MCF7 cells, hTERT mRNA expression was down-regulation after 24h, 48h and 72h. (B) Relative hTERT mRNA expression. Shown are mean values hTERT expressions in MCF7 of at least 3 independent RT-PCR experiments. (C) Representative examples of hTERT mRNA expression (upper bands) after transfecting HepG2 cells with 50 nmol of scRNA (lane 1), siRNA#1 (lanes 3-5) or siRNA#2 (lanes 6-8) showing hTERT downregulation after the indicated time points. (B) relative hTERT expression. Shown are mean values hTERT expressions in HepG2 of at least 3 independent experiments. M: 1kb DNA Molecular marker; sc: scRNA, #1: siRNA#1, #2: siRNA#2. B-actin was used as internal control (lower bands).
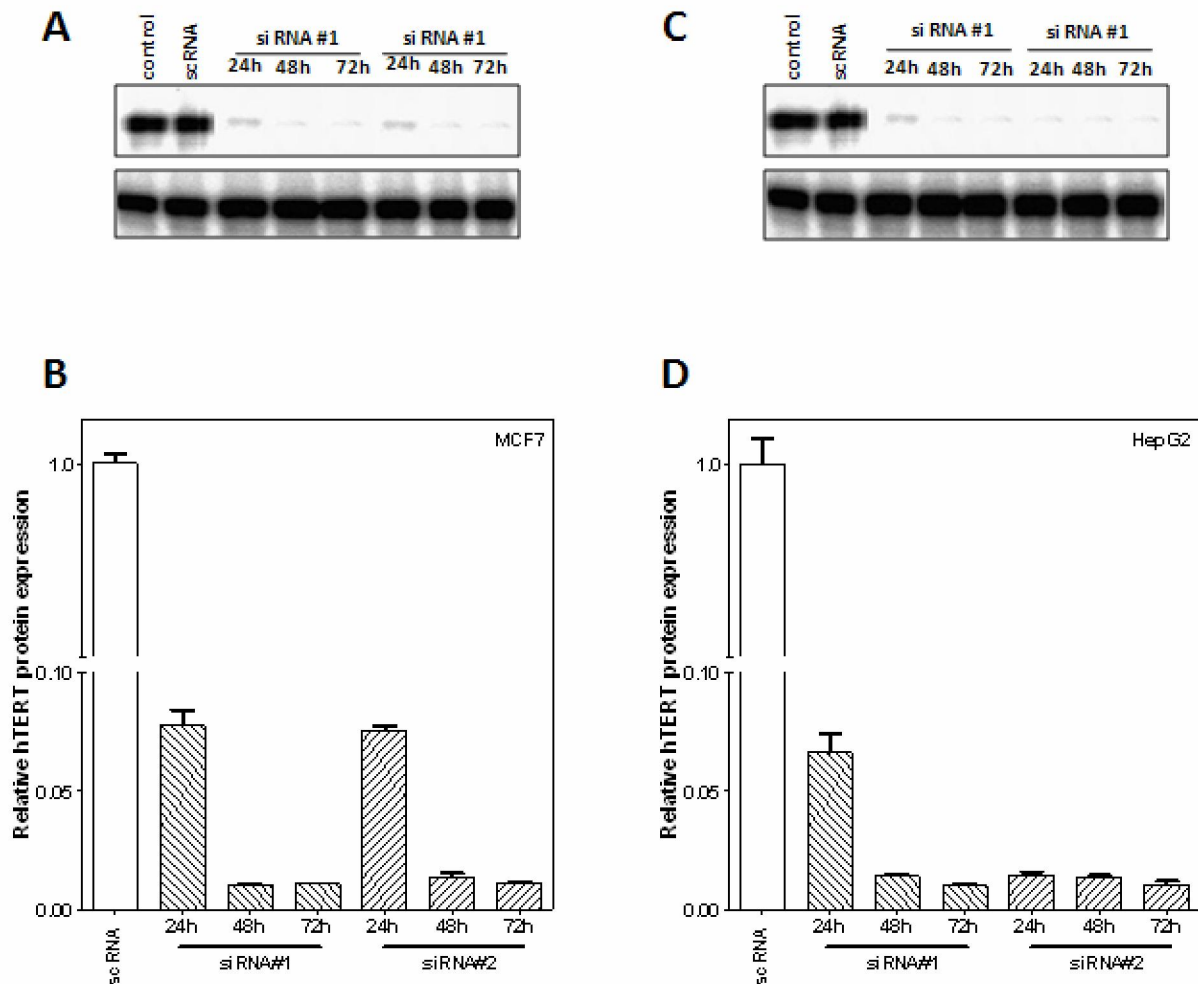
Fig.2: Down-regulation of hTERT protein expression in MCF7 and HepG2 cells by siRNA#1 and siRNA#2. (A) Representative examples of hTERT protein expression using Western blot after transfecting MCF7 cells with 50 nmol of scRNA (lane 1), siRNA#1 (lanes 3-5) or siRNA#2 (lanes 6-8). hTERT protein expression is efficiently down-regulated after treatment of MCF7 cells with either of siRNA#1 or siRNA#2. (B) Relative hTERT protein expression. Shown are mean values hTERT expressions in MCF7 of at least 3 independent blots. (C) Representative examples of hTERT protein expression after transfecting HepG2 cells with 50 nmol of scRNA (lane 1), siRNA#1 (lanes 3-5) or siRNA#2 (lanes 6-8) showing hTERT downregulation after the indicated time points. (B) Relative hTERT protein expression. Shown are mean values hTERT expressions in HepG2 of at least 3 independent experiments. control: untreated cells; sc: scRNA, #1: siRNA#1, #2: siRNA#2. b-actin was used as a loading control.
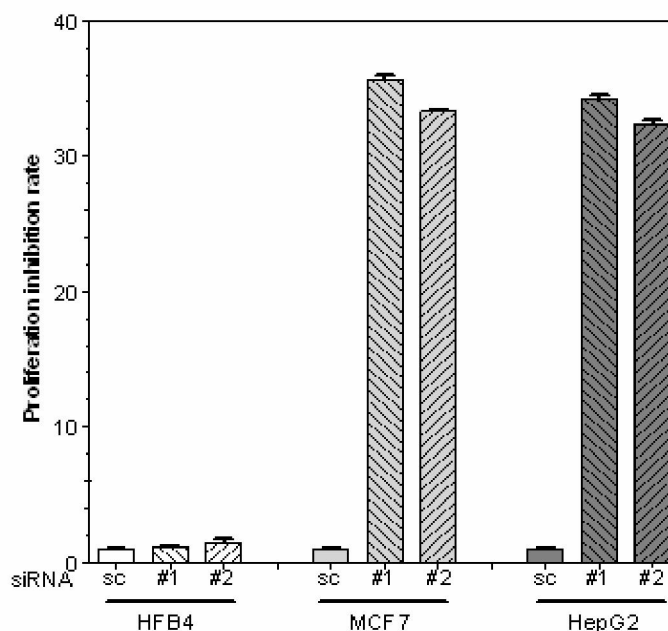
Fig.3: Inhibition of cell proliferation after hTERT knockdown by siRNA. Inhibition of cell proliferation was measured using MTT assay after transfecting HBF4, MCF7 and HepG2 cells by 50 nmol of scRNA, siRNA#1 or siRNA#2. hTERT Knock-down by either siRNA#1 or #2 showed a significant higher inhibitory (p<0.05) effect on tumor cells (MCF7 and HepG2) but not on normal HBF4 cells. Shown are mean values of at least 3 independent experiments.

4. Discussion

Telomerase maintains telomere length which in turn protects the ends of chromosomes from being detected as DNA damage by DNA repair systems. In the absence of telomerase telomeres lose nucleotides during replication which leads to shortening in the chromosomes and finally cell death (1-3,11). Telomerase activity has been reported as a valuable prognostic factor in many tumor types including neuroblastomas, bladder cancer, lung cancer and colorectal cancer (6,12-17). Giving the almost universal expression of telomerase in cancer cells but not in most normal cells, we hypothesized that targeting telomerase might be a powerful approach to cancer therapy.

Many strategies have been developed to target telomerase such as antisense nucleotides and specific inhibitors for the reverse transcriptase activity of telomerase (5,7,11,18). Although, these strategies showed a significant efficiency for inhibiting telomerase activity in cancer cells, their significant toxicity limited their clinical use. RNAi is characterized by high efficiency, high specificity and low toxicity. In the current study we used two siRNAs (siRNA#1 and siRNA#2) to target hTERT in MCF7 and HepG2 tumor cells. Our results have clearly showed that both siRNAs were efficiently down-regulated hTERT mRNA (Fig.1) and protein (Fig.2) expression in both MCF7 and HepG2 cells. In consistent with our results, several studies have demonstrated that siRNA is a powerful method to target telomerase (8,19). Down regulation of hTERT indeed impedes telomerase activity which results in preventing telomere length from being maintained. A number of genetic validation experiments indicate that telomere maintenance by the enzyme telomerase is a key event in the immortalization process and the continuous proliferation of a large proportion of human cancers (2,4,20,21). Consistently, targeting hTERT should affect cell proliferation/growth. Using MTT assay we reported in the current study that hTERT down-regulation has an efficient inhibitory

effect on cell proliferation in both MCF7 and HepG2 tumor cells (Fig.3) but not on normal cells. This indicates that telomerase plays an essential role in cell proliferation and viability control of tumor cells but properly not normal cells. Previously, it was shown that inhibition of telomerase sensitizes tumor cells to several insulting agents such as ionizing radiation (22-24). The mechanism of this sensitization is not clear. Based on our present data, we can explain this sensitization as telomerase supports proliferation. Consequently, inhibition of telomerase impedes the proliferation rate and hence leads to cell death.

Overall, this study presents a proof of principle for the use of RNA interference system to target *hTERT* as a powerful method to inhibit telomerase and hence inhibit the growth of tumor cells.

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Agronomical and Biochemical Responses of White *Lupinus albus* L. Genotypes to Contrasting Water Regimes and Inoculation Treatments

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Abstract: Two field experiments were conducted over the growing season November 15 – April 15- 2008-2009 and 2009/ 2010 at the experimental farm of Suez Canal University, Ismailia, Egypt. The purpose of this research was to study the effects of water stress and inoculation treatments on the yield, growth parameters and biochemical traits under field conditions and during two growing seasons. The experimental design for both seasons was randomized complete block in split-split plot arrangement with three replications. Where Irrigation treatments included normal (W0) and water stressed (Ws) were allocated to main-plots, two inoculation treatments: no-inoculation and inoculation with commercial inoculums were assigned to sub-plots. Five lupin genotypes including two cultivated varieties (Giza 1 and Giza 2) and three landraces (LR 1, LR 2 and LR 3) constituted the sub-sub-plots. Significant differences of irrigation, inoculation, genotype and their different interactions were detected for the most measured traits. Water stress reduced yield and growth parameters, whereas antioxidant enzyme activities were increased significantly as plants exposed to limited irrigation. Protein % was not affected by water limitation at both seasons, while 100-seeds weight was significantly affected in the first year only. There were potential beneficial effects of commercial inoculation, where it increased yield and growth parameters under water shortage condition and reduced enzyme activities. The landrace LR 1 is obviously, the best genotype in seeds yield, growth parameters over the two growing seasons and high activity of defense mechanism (activity of catalase and peroxidase enzymes) under water stress conditions and over all inoculation treatments. Thus it is considered a promising line under water limited environments.

1. Introduction

White lupin (*Lupinus albus* L.) has been cultivated in Egypt at least for four thousand years (Gladstones, 1970). It is cultivated mainly for human nutrition because of their high protein and oil contents; as a green manure contributing to improved soil structure and as ruminant feed either as green forage or as grain introduced as protein supplements in the diets of ruminants. It is originated from the Mediterranean region where drought, salinity and mineral deficiency are among the major constraints for lupines production.

In such region, water is one of the important environmental factors regulating plant growth and development (Manivannan *et al*., 2007). Drought is therefore a major threat affecting the life of plants and is responsible for limiting crop yield globally. In addition, drought has a detrimental effect on nodulation and symbiotic N_2 fixation in legumes specifically (Denison & Kiers, 2004). Therefore, the nodulation ability of the host genotype is the key feature in sustaining nitrogen fixation under stresses. Drought stress induces numerous morphological, metabolic, biochemical and physiological changes in plants. These include water status, growth, membrane integrity, pigment content, osmotic adjustment and photosynthetic activity (Zhang *et al*., 2007; Praba *et al*., 2009). It creates potential oxidative stress through accumulation of ROS, these may damage plants by oxidizing photosynthetic pigments, membrane lipids, proteins and nucleic acids (Reddy *et al*., 2004).

To eliminate ROS, all plants are endowed with detoxification mechanisms, including both enzymatic [superoxide dismuatase (SOD), ascorbate peroxidase (APX), peroxidase (POD), glutathione reductase (GR), etc.] and non-enzymatic (anthocyanins, carotenoids, ascorbic acid, etc.) antioxidants (Johnson *et al*., 2003). Kalefetog lu Macar and Ekmekçi (2009) recorded a significant increase in SOD, GR, APX and POD activities at all drought compared to control treatments in two chickpea cultivars. They concluded that the drought tolerant cultivar revealed higher antioxidant activities. In narrow-leafed lupins, Yu and Rengel (1999) found 21% increase in total SOD after 2 days of withholding water and a further increase was noted with an increase in severity of stress.

Mediterranean-type environment have shown that the number of pods, shoot length, nodule, root dry weight and seeds dry weight are dependent on developmental conditions like plant density, temperature or water stress (Lo´pez-Bellido *et al*.,

2000). Carvalho *et al.*, (2004) found that, lupines cultivars tended to accumulate crude protein and carbon compounds in seeds at the end of the water stress period (15 days after anthesis), however, Jansen (2008) recorded non significant effect of water stress on protein content when imposed at the same stage. Seeds yield and harvest index are reduced due to pod and seeds abortions (Palta *et al.*, 2007) under drought conditions, therefore the same authors (2003) suggested that seeds yield of lupines may be increased in low-rainfall by selecting cultivars with high pod retention.

Although lupines is an important legume crop worldwide, information on the effects of water stress on this species in limited. Moreover, little breeding efforts are devoted to study this species in Egypt as there are only two cultivated varieties. Therefore, the present work was designed to study the morphological and biochemical characteristics that may affect seeds yield under different water regimes including normal irrigation and artificial water stress. Therefore, the objectives of this study were to screen the lupines genotypes for yield, growth parameters, nodulation ability and enzymes activity under irrigation and inoculation treatments over two growing seasons in order to identify genotypes with superior yield and growth parameters under water stress.

2. Material and Methods
Plant material and treatments

Five lupine genotypes (Lupinus albus, ssp. termis) were used for this study, including two cultivated varieties and three landraces. The two cultivars Giza 1 and Giza 2 were obtained from Department of Legume Crops, Agricultural Research Center (ARC), Giza, Cairo. Both cultivars were evolved through individual selection from local landraces; Giza 1 is adapted for cultivation in northern region of Egypt, whereas Giza 2 is adapted for Upper Egypt region planting. The three landraces were collected from farmers' fields at Ismailia (LR1), Al-Salhia (LR2) and Almhsma (LR3) province.

Two field experiments were conducted over the growing seasons (November, 15-April, 15) 2008/2009 and 2009/2010 at the experimental farm of Suez Canal University, Ismailia. Rainfall, maximum and minimum temperatures for the two growing seasons are presented in Fig. 1.

The tested genotypes were subjected to two contrasting water regimes and two inoculation treatments. Water regimes included normal irrigation (W_0) where experiments were irrigated regularly depending on weather conditions and plant needs. Whereas water stress (Ws) treatment was irrigated when plants showed drought symptoms including

loss of leaves. Irrigation treatments were started when plants reached 40 days after planting. Soil moisture content was determined for each irrigation regime gravimetrically by weighting method (Black, 1973). Mean soil moisture content for control and Ws treatments were 2.67% and 1.72% for the first season, whereas for the second season values were 2.00% and 1.35%, respectively. Inoculation treatments consisted of un-inoculation control, and commercial Bradyrhizobium inoculum obtained from department of microbiology, ARC, Giza. For inoculation purpose, seeds were mixed with 15 % glucose solution and inoculum mixture. The control plots were sown first to prevent cross-inoculation. At sowing, 357.14 kg ha^{-1} superphosphate was broadcasting (375 kg per hectare), while 178.57 kg of potassium sulphate (125 kg per hectare) was added at flowering and seeds filling stages. No N fertilizers were added.

The experimental design for both seasons was randomized complete block in split-split plot arrangement with three replications. Main plots were two irrigation regimes, split-plots were inoculation treatments and split-split plots were the five lupine genotypes. Each plot has two rows of 3 m length with 20 cm inter-row spacing and 50 cm between rows.

Soil analysis

Soil texture of the experimental area was sandy with a pH of 8.1, 89.9% coarse sand, 5.7% fine sand, 2.7% silt and 1.7clay. Bulk density (g cm^{-3}), EC_s (dS m^{-1} in saturated soil paste) and total nitrogen (N) were: 1.59, 1.2 and 0.028%, respectively.

Growth measurements

Sixty days after planting, five plants from each plot were dug out and pink nodules were detached from the roots carefully, Roots and nodules were washed in running water and dried at 70 ℃ to estimate dry weights per plant (g). At maturity, five plants from each treatment were uprooted for recording the following data on single plant basis: number of branches, pods per plant, seed yield per plant and 100-seeds weight. Then plots were harvested by hand excluding one plant from both ends on 15 April, dried for 2–3 days for seeds yield per hectare determination.

A sub-sample of 50 g of grains was ground and the N concentration was determined using the A.O.A.C method (1990), then protein content of seeds was calculated by multiplying N% by 6.25.

Determination of activities of antioxidant enzymes

Fresh leaf samples (0.5 g) from each treatment were collected 60 days after planting and stored at - 20 ℃. Enzymes extraction was processed as described by Ni *et al.*, (2001). Briefly, extraction was done using cold phosphate buffer (0.1 M, pH 7.0) containing 1% (w/v) polyvinylpyrrolidone and

1% (v/v) triton X-100. Then samples were macerated with 1 ml of the extracting buffer. Samples were further ground with another 1 ml of the extracting buffer. An aliquot (1.5 ml) of the extract was centrifuged at 10000 g for 10 min at 4 ºC and the resulted supernatant was immediately stored at – 80 ºC for future enzyme activity assays.

The protein concentrations in the leaf extracts were determined according to the Bradford (1976) method. Catalase (CAT) activity was determined via following the initial rate of disappearance of H_2O_2 at 240 nm (Luck, 1974). Peroxidase (POD) activity was determined according to Vetter *et al*., (1958). The reaction mixture contained 200 µl sample, 1 ml of 1% o-phenylenediamine (in 95% ethyl alcohol) and 1 ml of 0.3% hydrogen peroxide (in distilled water), the reaction is allowed to proceed for 5 min at which time it is stopped by adding 2 ml of saturated sodium bisulfate. The enzyme activity was expressed as the change in absorbancy at 430 nm and expressed as O.D. units X 10^3 min^{-1} mg $protein^{-1}$.

Statistical analysis

Analysis of variance was carried out for each growing season and combined over seasons using the statistical Package MSTATC to study the main and interaction effects of the studied factors on the measured traits. Mean separation was obtained using least significant difference test at the 0.05 probability level when significant F-tests (P< 0.05) were observed.

3. Results
Yield and growth parameters

During the 2009/2010 growing season, the experiment received more rainfall compared to the first season. The maximum temperature during the pod growth and development was higher in the second season (Fig 1).

Analysis of variance for yield and growth parameters in each year demonstrated significant effects of irrigation, inoculation, genotypes and their interactions on the measured traits in both seasons (Table 1). Irrigation treatments showed significant effects on yield, nodulation and growth parameters in the first year. In the second year, only yield and nodule dry mass were significant. Significant differences among genotypes were recorded for all variables in both years, except number of branches in the first year. Inoculation did not affect 100-seeds weight in both years, branches number (in the first year) and pods number (in the second year), but showed statistically significant differences in other measurements. Significant irrigation x genotype interactions was noted for all traits except branches number, pods per plant and 100-seeds weight. Irrigation x inoculation x genotypes interactions were

significant for seeds yield, nodule mass, protein % and branches per plant in the second, whereas in the first year, only pods per plant,100-seeds weight and nodule mass per plant differed significantly.
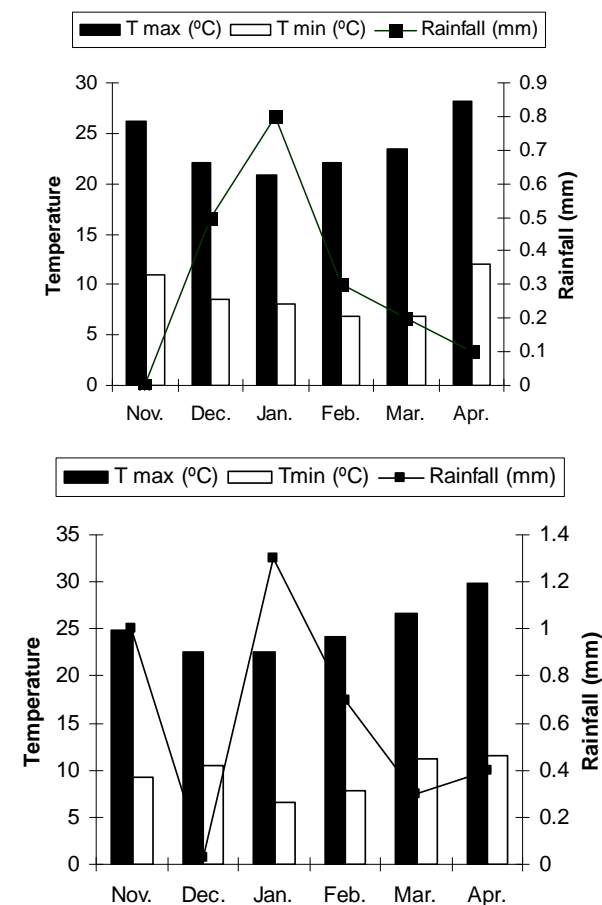
Fig.1 Monthly rainfall (mm), and maximum (T_{max}) and minimum (T_{min}) temperatures (ºC) at Ismailia, Egypt during the two growing seasons, 2008/2009 (above) and 2009/2010 (below)

Water stress resulted in a decrease in all studied traits in both years with varying values, except protein % that is showed 4.51% and 2.42 % increase at the first and second year, respectively (Table 2-9). On the other hand, commercial inoculation increased yield and growth parameters under water deficient condition in both the seasons compared to un-inoculated treatment. The highest reduction was noted for seeds yield per plant (23.93 and 24.06 %), seeds yield per hectare (23.98 and 24.00 %), pods per plant (33.97 and 13.52 %), branches per plant (11.21 and 3.92, respectively) and nodule dry weight (24.81 and 23.33 %) in the first and second year, respectively. The data for nodule dry weight demonstrates that even without Rhizobium inoculation, infection was occurred,

presumably due to a root infection by native Rhizobium strains derived from the soil or from the adjacent plot in which inoculation was practiced. There was a slight reduction in 100-seeds weight due to water stress (3.71 and 5.14 %), but inoculation caused a slow increase in this trait in both seasons (3.69 and 1.12 %). The two commercial varieties and LR 1 recorded a higher seeds yield per hectare in the second season, whereas, LR 2 and LR 3 showed lower yield. Under water stress condition Giza 2 and LR 1 out performed other genotypes in number of pods per plant and root and nodule dry weight in both seasons, protein % and seeds yield per hectare in the second season over all inoculation treatments. However, in the first season, higher seeds yield per hectare and heavier 100-seeds weight were produced by LR 1 and LR 2. The genotype LR 1 showed the lowest reduction for number of pods per plant, nodule dry weight, 100-seeds weight, number of branches per plant, increasing in protein %, and root dry weight. This favorable behavior was also confirmed by the values of the low estimated for yield per plant and hectare (10.8 and 10.76 %, respectively) during the second year.

Under normal irrigation, the same genotype produced high pods per plant, nodule dry weight at both seasons, high 100-seeds weight, seeds yield per plant at the first season only. The genotype LR 2 gave combined high number of branches (4.40), 100-seeds weight (43.86 %), protein % (35.26 %), seeds yield per plant (11.85 gm) and seeds yield per hectare (987 kg) at the first season. The commercial cultivar Giza1 with high values for all measured traits under control irrigation in the second season appears as instable genotypes when examined for the same traits under stress conditions. Poor yielding genotypes in stressed and non-stressed treatments at the second year were the landraces LR 2 and LR 3 (718.90 and 657.27; 583.40 and 492.34 kg per hectare, respectively).

Antioxidant enzyme activities

Significant differences were detected for catalase and peroxidase activities due to irrigation and genotype effects (Table 1). In contrast, peroxidase activity was not affected by nodulation, but catalase activity was statistically differed. Significant differences in the activity of both enzymes were recorded due to all types of interactions.

The activity of catalase and peroxidase enzymes was increased with water stress, where they showed 31.46 and 19.07 % increase for both, respectively (Table 10). Water stress caused increasing in catalase activity of uninoculated genotypes (49.78 %), whereas, there was slight increase incase of inoculated one (11.82 %). The same trend was observed for peroxidase activity where it recorded 20.71 and 17.48 % increase for uninoculated and inoculated treatments, respectively. Compared to controls, catalase and peroxidase activities of Giza 1, Giza 2 and LR 1 were more conspicuous as a result of water stress when compared across inoculation treatments. Over all irrigation and inoculation treatments, the same genotypes recorded the highest activities. In contrast, the LR 5 showed decrease in the activities of CAT and POD enzymes (43.62 and 1.98 %, respectively) when subjected to water deficient. There was a decrease in CAT activity in inoculated genotypes under water stress treatment except LR3, whereas POD activity decreased in Giza 2, LR 1 and LR 3 under the same environmental conditions.

4. Discussion
Yield and growth parameters

Two contrasting water treatments were used in this study, regular irrigation and water-stressed treatment which were applied when plants show wilt symptoms. In Egypt agriculture is dependent on irrigation as rainfall is very low (nearly 120 mm per year) and does not support crop productivity. The second growing season received rather more rains that the first one, whereas day temperature was higher one –two degrees in pod filling and development period. So, the first season is considered drier than the second one. Drought stress caused an observed detrimental effect on plant growth and productivity; on the other hand addition of commercial inoculums increased all the parameters under investigation compared with the uninoculated treatment. In addition, inoculation mitigated the harmful effect on yield and growth traits when plants were exposed to water stress. The effect of water stress on measured traits varied from year to year, also lupines genotypes responded to the treatment differently. It is clear that, in the first year the effect of water stress was more severe and recorded a higher decrease in pods and branches per plant; and nodule dry weight, although there was a similar decrease in seeds yield. This may be due to slight effect of water limitation on 100-seeds weight. Carvalho *et al*., (2004) attributed that the uninfluenced water stress on seeds biomass to the stems of lupines can temporary be storage sites for assimilates which are later used in seeds filling and therefore seeds weight remains unaffected.

1.

Table 1 Analysis of variance for yield, growth parameters and enzymes activities of lupine genotypes grown under irrigation and inoculation treatments in 2008/2009 and 2009/2010 seasons

SoV	df	No of branches	Pods number per plant	100-seed weight	Seeds yield (gm per plant)	Root dry weight (gram per plant)	Nodule dry weight (gram per plant)	Protein (%)	Seeds yield (kg per hectare)	Catalase activity (nmol H_2O_2 min^{-1} mg protein^{-1})	Peroxidase activity (O.D. units X10^3 min^{-1} mg protein^{-1})
						2008/2009 Season					
Irrigation	1	3.50	367.48*	34.43*	108.29*	8.12	1.61*	34.32	752028.64*		
(IR)		0.33	2.71	6.44	2.79	1.75	0.006	2.07	7997.37		
Error 1	2	0.42	14.77*	10.31	10.81*	7.98*	4.57*	132.08*	75023.30*		
Inoculation	1										
(Ino)		0.38	15.65*	5.27	2.68	1.28	0.67*	3.21	18792.79		
IR x Ino	1	0.32	52.07*	65.07*	8.69*	12.08*	2.39*	40.82*	60382.05*		
Genotype	4	0.12	23.70*	6.51	7.03*	6.41*	1.13*	39.01*	48815.18*		
(G)		0.12	0.67	8.13	1.32	1.60	0.75*	12.73*	9137.43		
IR x G	4	0.23	7.77*	33.25*	1.85	1.00	0.24*	3.79	12871.99		
Inoc. X G	4	0.25	1.86	6.01	1.45	1.13	0.003	2.02	5862.84		
IR x Inoc. X G	4										
Error 2	36										
						2009/2010 Season					
Irrigation	1	0.44	29.47	48.71	113.63*	5.18	1.79*	8.73	788833.93*	29.59*	164326.67*
(IR)		0.04	4.57	13.56	0.25	0.47	0.001	1.43	1692.75	0.11	2337.07
Error 1	2	1.07*	7.74	5.36	17.66*	4.77*	6.09*	111.87*	122609.25*	26.93*	763.27
Inoculation	1										
(Ino)		0.01	0.01	13.88	2.03*	1.08	1.08*	5.83*	14056.93*	12.29*	836.27
IR x Ino	1	0.48*	32.66*	83.44*	76.34*	6.56*	3.05*	5.59*	530389.33*	25.57*	18789.36*
Genotype	4	0.07	3.39	2.39	7.45*	2.83*	1.47*	38.42*	51827.11*	15.82*	40087.62*
(G)		0.11*	4.82	9.45	3.54*	0.58	1.04*	30.44*	24554.38*	13.85*	53712.14*
IR x G	4	0.14*	3.44	4.66	0.91*	0.33	0.32*	6.24*	6358.60*	3.27*	28081.47*
Inoc. X G	4	0.03	2.06	5.16	0.21	0.39	0.001	1.07	1449.88	0.04	742.06
IR x Inoc. X G	4										
Error 2	36										

Table 2: Effect of irrigation and inoculation treatments on branches per plant of lupine genotypes grown under field conditions in two seasons (2008-2009 and 2009-2010).

						Branches per plant								
			2008/2009				Mean			2009/2010				Mean
		Ws			W0				Ws			W0		
Genotype	0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean		0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean	
Giza1	4.25	4.12	4.18	3.90	3.58	3.74	3.96	4.10	4.50	4.30	4.10	4.10	4.10	4.20
Giza2	4.47	4.55	4.51	3.90	4.20	4.05	4.28	4.20	4.07	4.13	3.55	4.13	3.84	3.99
LR1	4.28	4.38	4.33	3.93	3.20	3.57	3.95	4.40	4.87	4.64	4.12	4.73	4.43	4.53
LR2	4.57	4.23	4.40	3.93	3.80	3.87	4.13	4.10	4.43	4.27	4.19	4.53	4.36	4.31
LR3	3.87	4.10	3.98	4.13	3.40	3.77	3.87	4.22	4.38	4.30	4.10	4.00	4.05	4.17
Mean														
Inoc	4.28	4.27		3.96	3.63			4.20	4.45		4.01	4.30		
IR		4.28			3.80				4.33			4.16		
LSD 0.05														
IR			ns							ns				
Inoc.			ns							0.095				
G			ns							0.15				
IR x Inoc			ns							ns				
IR x G			ns							ns				
Inoc x G			ns							0.21				
IR x Inoc x G			ns							0.30				

Table 3: Effect of irrigation and inoculation treatments on pods per plant of lupine genotypes grown under field conditions in two seasons (2008-2009 and 2009-2010).

							pods per plant							
	2008/2009						Mean	2009/2010						Mean
	Wo			Ws				Wo			Ws			
Genotype	0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean		0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean	
Giza1	8.64	10.08	9.36	7.51	7.82	7.67	8.52	10.87	11.80	11.33	8.73	8.80	8.77	10.05
Giza2	13.77	14.64	14.20	11.38	11.79	11.59	12.90	11.00	8.87	9.93	8.87	9.80	9.33	9.63
LR1	16.52	17.73	17.12	10.21	11.40	10.81	13.97	11.80	13.40	12.60	12.40	11.53	11.97	12.28
LR2	14.29	16.42	15.36	9.20	10.49	9.84	12.60	9.13	9.00	9.07	7.67	8.98	8.42	8.75
LR3	14.57	18.98	16.77	9.84	6.50	8.17	12.47	7.60	10.80	9.20	5.40	7.87	6.63	7.92
Mean														
Inoc	13.56	15.57		9.63	9.60			10.08	10.77		8.65	9.40		
IR	14.57			9.62				10.43			9.02			
LSD 0.05														
IR			1.83							ns				
Inoc.			0.71							ns				
G			1.12							1.18				
IR x Inoc			1.01							ns				
IR x G			1.59							ns				
Inoc x G			ns							ns				
IR x Inoc x G			2.25							ns				

Table 4: Effect of irrigation and inoculation treatments on 100-seeds weight of lupine genotypes grown under field conditions in two seasons (2008-2009 and 2009-2010).

							100-seeds weight							
	2008/2009						Mean	2009/2010						Mean
	W0			Ws				W0			Ws			
Genotype	0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean		0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean	
Giza1	39.95	36.03	37.99	36.88	39.35	38.11	38.05	38.82	35.18	37.00	35.17	35.57	35.37	36.19
Giza2	37.21	40.93	39.07	37.66	40.01	38.83	38.95	36.83	39.40	38.11	36.15	37.48	36.82	37.46
LR1	46.36	40.81	43.58	39.49	43.89	41.69	42.64	36.13	30.97	33.55	33.03	32.71	32.87	33.21
LR2	42.30	45.43	43.86	41.54	39.21	40.37	42.12	33.07	31.98	32.52	30.03	29.78	29.90	31.21
LR3	36.81	40.61	38.71	36.52	36.74	36.63	37.67	33.99	33.52	33.76	30.64	31.30	30.97	32.36
Mean														
Inoc	40.53	40.76		38.42	39.84			35.77	34.21		33.00	33.37		
IR	40.64			39.13				34.99			33.19			
LSD 0.05														
IR			2.82							ns				
Inoc.			ns							ns				
G			2.02							1.87				
IR x Inoc			ns							ns				
IR x G			ns							ns				
Inoc x G			ns							ns				
IR x Inoc x G			4.04							ns				

ns: not significant at 5% probability level.

Table 5: Effect of irrigation and inoculation treatments on root dry weight (g) weight of lupine genotypes grown under field conditions in two seasons (2008-2009 and 2009-2010).

	root dry weight (g)													
	2008/2009						Mean	2009/2010						Mean
	W0			Ws				W0			Ws			
Genotype	0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean		0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean	
Giza1	7.87	8.70	8.28	6.33	5.00	5.66	6.97	5.51	6.09	5.80	4.43	3.50	3.96	4.88
Giza2	7.26	9.21	8.24	6.66	7.27	6.96	7.60	5.08	6.45	5.77	4.66	5.09	4.87	5.32
LR1	5.74	5.81	5.78	6.54	7.10	6.82	6.30	4.02	4.70	4.36	4.54	4.97	4.76	4.56
LR2	6.59	7.09	6.84	5.13	6.09	5.61	6.22	4.61	5.25	4.93	3.59	4.26	3.92	4.43
LR3	3.83	5.59	4.71	4.42	5.80	5.11	4.91	2.68	3.58	3.13	3.10	3.97	3.53	3.33
Mean														
Inoc	6.26	7.28		5.82	6.25			4.38	5.21		4.06	4.36		
IR	6.77			6.03				4.80			4.21			

LSD 0.05		
IR	ns	ns
Inoc.	0.55	0.33
G	0.87	0.52
IR x Inoc	ns	ns
IR x G	1.24	0.73
Inoc x G	ns	ns
IR x Inoc x G	ns	ns

ns: not significant at 5% probability level.

Table 6: Effect of irrigation and inoculation treatments on nodule dry weight (g) weight of lupine genotypes grown under field conditions in two seasons (2008-2009 and 2009-2010).

	nodule dry weight (g)													
	2008/2009						Mean	2009/2010						Mean
	W0			Ws				W0			Ws			
Genotype	0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean		0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean	
Giza1	1.57	2.52	2.04	0.44	1.09	0.77	1.40	1.73	2.87	2.30	0.50	1.24	0.87	1.58
Giza2	0.89	1.24	1.06	1.27	1.06	1.16	1.11	0.98	1.41	1.20	1.55	1.21	1.38	1.29
LR1	1.14	3.07	2.10	1.14	1.83	1.48	1.79	1.26	3.50	2.38	1.30	2.09	1.69	2.03
LR2	0.75	0.84	0.80	0.64	1.06	0.85	0.82	0.83	0.96	0.89	0.73	1.21	0.79	0.93
LR3	0.39	0.89	0.64	0.67	0.82	0.74	0.69	0.43	1.01	0.72	0.76	0.93	0.85	0.78
Mean														
Inoc	0.95	1.71		0.83	1.17			1.04	1.95		0.97	1.34		
IR	1.33			1.00				1.50			1.15			

LSD 0.05		
IR	0.086	0.035
Inoc.	0.028	0.016
G	0.045	0.026
IR x Inoc	0.04	0.023
IR x G	0.064	0.037
Inoc x G	0.064	0.037
IR x Inoc x G	0.090	0.052

significant at 5% probability level.

Table 7: Effect of irrigation and inoculation treatments on protein % of lupine genotypes grown under field conditions in two seasons (2008-2009 and 2009-2010).

	protein %													
	2008/2009						Mean	2009/2010						Mean
	W0			Ws				W0			Ws			
Genotype	0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean		0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean	
Giza1	32.81	38.94	35.87	29.58	33.60	31.59	33.73	32.81	33.54	33.17	30.63	31.35	30.99	32.08
Giza2	33.25	33.51	33.38	36.31	35.70	36.00	34.69	31.72	36.46	34.09	31.72	33.54	32.63	33.36
LR1	28.88	32.04	30.46	30.10	35.88	32.99	31.72	24.06	32.81	28.43	32.45	37.88	35.17	31.72
LR2	34.30	36.23	35.26	34.30	38.59	36.44	35.85	29.17	34.64	31.90	30.26	32.45	31.35	31.63
LR3	33.08	34.12	33.60	37.28	40.95	39.11	36.36	33.18	30.26	31.72	32.81	33.18	32.99	32.36
Mean														
Inoc	32.46	34.97		33.51				30.19	33.54		31.57	33.68		
IR	33.71			35.23				31.86			32.63			
LSD 0.05														
IR			ns							ns				
Inoc.			0.74							0.54				
G			1.17							0.85				
IR x Inoc			ns							0.76				
IR x G			1.66							1.21				
Inoc x G			1.66							1.21				
IR x Inoc x G			ns							1.70				

ns: not significant at 5% probability level.

Table 8: Effect of irrigation and inoculation treatments on seeds yield per plant (g) of lupine genotypes grown under field conditions in two seasons (2008-2009 and 2009-2010).

	Seeds yield per plant (g)													
	2008/2009						Mean	2009/2010						Mean
	W0			Ws				W0			Ws			
Genotype	0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean		0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean	
Giza1	7.73	10.32	9.02	9.06	8.96	9.01	9.02	12.81	15.67	14.24	8.63	9.90	9.26	11.75
Giza2	10.26	11.91	11.08	8.38	8.19	8.28	9.68	13.55	14.61	14.08	9.78	10.71	10.25	12.16
LR1	12.91	13.06	12.99	9.61	9.07	9.34	11.16	12.50	12.51	12.50	10.74	11.57	11.15	11.83
LR2	11.76	11.93	11.85	7.70	9.33	8.51	10.18	8.31	8.94	8.63	6.88	7.12	7.00	7.81
LR3	10.19	11.98	11.09	6.79	8.10	7.45	9.27	6.53	9.24	7.89	4.82	7.00	5.91	9.60
Mean														
Inoc	10.57	11.84		8.31	8.73			10.74	12.19		8.17	9.26		
IR	11.20			8.52				11.47			8.71			
LSD 0.05														
IR			1.85							0.55				
Inoc.			0.63							0.24				
G			0.99							0.38				
IR x Inoc			ns							0.34				
IR x G			1.41							0.54				
Inoc x G			ns							0.54				
IR x Inoc x G			ns							0.76				

ns: not significant at 5% probability level.

Table 9: Effect of irrigation and inoculation treatments on seeds yield per hectare (kg) of lupine genotypes grown under field conditions in two seasons (2008-2009 and 2009-2010).

	Seeds yield per hectare (kg)													
	2008/2009						Mean	2009/2010						Mean
	W0			Ws				W0			Ws			
Genotype	0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean		0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean	
Giza1	644.09	860.09	752.09	754.82	746.25	750.54	751.31	1067.59	1305.55	1186.57	718.96	825.05	772.01	979.29
Giza2	855.08	992.42	923.75	698.17	682.77	690.47	807.11	1129.21	1217.40	1173.31	815.05	892.71	853.88	1013.59
LR1	1075.65	1088.76	1082.21	801.19	755.89	778.54	930.37	1041.67	1042.22	1041.94	895.14	964.38	929.76	985.85
LR2	979.96	994.57	987.26	641.11	777.81	709.46	848.36	692.64	745.17	718.90	573.41	593.39	583.40	651.15
LR3	849.15	998.68	923.92	566.09	685.29	620.69	772.30	544.33	770.21	657.27	401.67	583.01	492.34	574.81
Mean														
Inoc	880.79	986.91		692.27	727.60			895.09	1016.11		680.85	77.71		
IR	933.85			709.94				955.60			726.28			
LSD 0.05														
IR			99.29							45.68				
Inoc.			39.93							19.86				
G			63.14							31.40				
IR x Inoc			ns							28.09				
IR x G			89.30							44.41				
Inoc x G			ns							44.41				
IR x Inoc x G			**ns**							62.80				

ns: not significant at 5% probability level

Table 10: Effect of irrigation and inoculation treatments on catalase and peroxidase activities of lupine genotypes grown under field condition

	Catalase activity						Mean	Peroxidase activity (O.D. units X10^3 min^{-1} mg protein^{-1})						Mean
	(nmol H$_2$O$_2$ min^{-1} mg protein^{-1})													
	W0			Ws				W0			Ws			
Genotype	0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean		0 Inoc	Inoc	Mean	0 Inoc	Inoc	Mean	
Giza1	6.35	4.51	5.43	10.24	4.10	7.17	6.30	548.67	402.33	475.50	661.67	701.33	681.50	578.50
Giza2	5.53	3.12	4.32	8.97	7.63	8.30	6.31	529.00	596.00	562.50	751.00	700.00	725.50	644.00
LR1	5.14	4.23	4.68	9.17	5.48	7.32	6.00	446.33	637.00	541.67	790.33	684.00	737.17	639.42
LR2	2.90	2.98	2.94	3.97	3.50	3.73	3.34	494.33	640.67	567.50	409.67	666.67	538.17	552.83
LR3	3.42	6.31	4.86	2.53	2.94	2.74	3.80	688.67	504.00	596.33	655.00	514.00	584.50	590.42
Mean														
Inoc	4.66	4.23		6.98	4.73			541.40	556.00		653.53	653.20		
IR	4.45			5.85				548.70			653.37			
LSD 0.05														
IR			0.36							53.67				
Inoc.			0.11							ns				
G			0.17							22.26				
IR x Inoc			0.16							ns				
IR x G			0.25							31.77				
Inoc x G			0.25							31.77				
IR x Inoc x G			0.35							44.93				

ns: not significant at 5% probability level.

In this study, variation in seeds yield was observed among genotypes under water-stressed treatment at both years. The genotypes LR 1 and Giza 1 and Giza 2 maintained both high seeds yield per plant and plant under water limitation conditions at both seasons. The advantage of those genotypes was ascribed to high number of pods per plant, 100-seeds weight, root dry weight, nodule dry weight and rather low number of branches per plant. Palta *et al*., (2007) found that final pod number of the high-yielding genotypes was 85-92 % of its respective irrigated crops, whereas low –yielding genotypes recorded 48-60 %. They also reported that high pod retention genotypes resulted in high yield and consequently pod retention in lupines is an important yield-positive characteristic. The same finding was achieved by Dracup *et al*., (1998) who showed that terminal drought caused reductions in lupine through pod and seeds abortions. Although 100-seeds weight was reduced due to drought stress, the reduction was slight. Palta *et al*., (2007) found the same results as average seeds weight was unaffected by the rainfed conditions.

Nodule dry weight was decreased under water stress conditions in both years for both uninoculated and inoculated treatments. Similar results were obtained by Velagaleti and Marsh (1989) who reported a significant reduction in nodule dry mass under stress due to inadequate photosynthate supply to the roots caused by decreased plant dry mass production. Although, two genotypes Giza 2 and LR 3 showed 9.43 and 15%; 15.62 and 18.05 % increasing dry weight of nodules per plant compared to uninoculated treatment in the first and second year, respectively. However, under uninoculated treatment, all genotypes recorded some dry weight of nodule suggesting the presence of indigenous Rhizobium strains of lupin in the soil. Raza and Jørnsgård (2005) recorded 14-36 nodules per plant on lupines roots under uninoculated treatment in an experiment carried out at Ismailia governorate. The same genotypes; Giza 1, Giza 2 and LR 1 showed higher nodule and root dry mass under contrasting irrigation regimes, in addition to increasing and /or low reduction in protein %. The same conclusion was achieved by Rao *et al*., (2002) who suggest that genotypes with greater capacity for nodulation perform best under both unstressed and stressed conditions. However, the low effects of stress on Protein % may be a result of that plants were treated with water withholding from the beginning of their life. Jansen (2008) found a raise in protein % of narrow-leaf lupin due to high temperature (25 ºC). Also Carvalho *et al*., (2005) reported no effect of water deficit imposed at the beginning of seeds development (15-35 days after anthesis) on protein content of *lupinus mutabilis* and *lupinus albus*. The genotype LR 2 combined high values for seeds yield per plant, pods number per plant,

nodule and root dry mass, 100-seeds weight and protein % (rather reasonable values) under water stressed conditions and over all inoculation treatments. This suggested that LR2 could prove to be good breeding material for further breeding programs aimed at the evolution of drought tolerance in lupin.

Antioxidant enzyme activities

Plants have several physiological and biochemical strategies, such as antioxidative defense and osmotic adjustment, to prevent the damaging effect of oxidative stress, induced by drought (Tan *et al*., 2006). In the present study, the defense mechanism used by lupin genotypes was activated. This was evident from the elevated activity of catalase and peroxidase enzymes. Water limitation caused a significant increase in both enzymes in Giza 1 (32.04 and 43.32 %), Giza 2 (92.13 and 28.98 %) and LR 1 (56.41 and 36.09 %) genotypes compared to control irrigation over all inoculation treatments, which is an indication for increased production of ROS.

The genotypes LR 2 showed 26.87 % increase and 5.17 % decrease in catalase and peroxidase activity, respectively. In contrast, the genotype LR 3 recorded decreasing values in both enzymes (53.41 and 1.98 %). Our results agree partly with those of Mourato *et al*., (2009) who recorded an increase in peroxidase and non significant increase in catalase activities in *Lupinus luteus* exposing to varying Cu concentrations and suggested that, peroxidase is involved in H_2O_2 elimination in yellow lupin species while CAT is not. They also concluded that SOD and POD have the major role in the antioxidative response of the investigated lupin species. Furthermore, Macar and Ekmekçi (2009) recorded a marked elevated activity in POD, GR, SOD and APX enzymes in two chickpea genotypes in all drought treatments. This indicates that the estimated H_2O_2 scavenging enzymes probably cooperated with each other during water deficit periods. However, the activities were higher in the drought tolerant genotype. In contrast, Chatterjee and Chatterjee (2000) reported a decrease in CAT activity in cauliflower leaves subjected to micronutrients. Therefore, increasing, decreasing and unaffected activity of protective enzymes is species dependent. Interestingly, the reducing catalase (all except LR3) and peroxidase activities (all except Giza 1 and LR 2) in inoculated genotypes under water stress conditions may be attributed to the ameliorative effect of Rhizobium. Malekzadeh *et al*., (2007) concluded a potential role of Arbuscular Mycorrhiza fungus in protecting plants exposed to heavy metal stress. Zahran (1999) mentioned that, one of the adaptations of legumes to arid lands (poor in N and P) and those with low moisture availability is their infection by mycorrhizal fungi in addition to Rhizobium.

In conclusion, our results emphasize the capability of lupin genotypes to withstand drought conditions, significant differences among irrigation, genotypes, inoculations and their different interactions. Water stress resulted in yield and growth parameters reduction and increasing in antioxidative mechanisms activity. Inoculation significantly increased yield and plant growth parameters under water stress due to its ameliorative effect against water stress effect. Out of the five tested genotypes, LR1 was distinguished by its high seeds yield per plant and hectare, improved growth parameters in addition to its high catalase and peroxidase activity. This line is considered to be the most tolerant genotype compared to other tested genotypes.

Acknowledgement

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Vascular Endothelial Growth Factor (VEGF) Gene Insertion/Deletion Polymorphism and Diabetic Retinopathy in Patients with Type 2 Diabetes

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Abstract: Background: Vascular endothelial growth factor (VEGF) appears to play a central role in mediating microvascular pathology in diabetic retinopathy (DR). Aim of the study: To assess the possible association of the insertion/deletion (I/D) polymorphism of VEGF gene with diabetic retinopathy in Egyptian patients with type 2 diabetes mellitus. Subjects and Methods: This cross-sectional case-control study enrolled 87 unrelated subjects with type 2 diabetes mellitus, 43 diabetic patients without signs of retinopathy but did have type 2diabetes for more than 10 years and 44 patients with diabetic retinopathy. The control group involved 44 normal subjects without diabetes. Total genomic DNA was isolated from peripheral blood leukocytes. PCR analysis was conducted to detect the insertion/deletion gene polymorphism of the 18 bp fragment at position 2549 of the promoter region of VEGF. The frequency of D and I VEGF alleles and genotype distribution were compared in diabetics with retinopathy, diabetics without retinopathy and the control subjects. Results: There was no significant difference in genotype distribution (D/D, I/D and I/I), (p=0.43) and in (D and I) allele frequency (p=0.093) of diabetic patients with retinopathy, diabetics without retinopathy and control subjects. The distribution of the VEGF, D/D genotype was higher in patients with diabetic retinopathy compared with diabetic group without retinopathy and healthy controls (40.9% *vs*. 27.9% and 22.7% respectively), however the difference was still not statistically significant with Chi-Square= 3.637 and p value = 0.162. Despite the insignificant results, this study adjusted OR of 2.25 (95% CI, 0.672- 7.538) for D/D genotype versus I/I genotype between diabetic patients with retinopathy and controls with p value = 0.185 and the OR of 1.6 (95% CI, 0.873- 2.891) for the D allele versus I allele between diabetic patients with retinopathy and controls with p= 0.129, while the OR of the D allele versus I allele in diabetic patients without retinopathy and controls was only 1.2 and the p value was 0.539. In multivariate analysis only increased triglyceride level was the independent risk factor for diabetic retinopathy among Egyptian patients with type 2 diabetes. Conclusion: Our study suggested that I/D polymorphism in the promoter region of the VEGF gene was not significantly associated with retinopathy in Egyptian type 2 diabetic patients, however a moderate risk (i.e., OR, < 2 for D/D genotype and < 1.5 for D allele) could not be excluded. Only increased triglyceride level was the independent risk factor in the development of diabetic retinopathy detected in this study.
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Key words: Vascular endothelial growth factor (VEGF), insertion/deletion polymorphism, diabetic retinopathy, type 2 diabetes, Egyptian patients.

1. Introduction:

Many diabetic patients, especially those with poor glycemic control, develop diabetic retinopathy, which remains the major cause of blindness among diabetic adults [1]. Diabetic retinopathy is characterized by increased vascular permeability, tissue ischemia with angiogenesis [2]. Growth factors may play an important role in modifying and accelerating the tissue damage caused by hyperglycemia [3].

Vascular endothelial growth factor (VEGF) is a potent multifunctional cytokine which plays a key role in the pathogenesis of diabetic microvascular complications [4]. VEGF is produced from many cell types within the eye and it is a highly conserved homodimeric glycoprotein which promotes angiogenesis and is a potent mediator of microvascular permeability [5].

Several studies have shown that VEGF expression is increased in patients with diabetic retinopathy [6, 7, 8] and others documented that VEGF levels are markedly elevated in vitreous of the eyes of individuals with proliferative diabetic retinopathy (PDR) [9, 10]. In addition, VEGF induction of vascular permeability may contribute to the development of non-proliferative diabetic retinopathy (non-PDR) [11].

The genetic variations in the VEGF gene can influence levels of VEGF protein expression [2]. The human VEGF gene is located on chromosome 6 (6p21.3) and is highly polymorphic. Of particular interest is an insertion/deletion (I/D) polymorphism of the 18 bp fragment at–2549 position of the promoter region that has been implicated in a number of diseases, especially those with angiogenic basis [12, 13].

The aim of this study was to investigate the impact of genetic I/D polymorphism of VEGF on diabetic retinopathy in an Egyptian population with type 2 diabetes.

2. Subjects and Methods:
Subjects:

This cross-sectional case-control study enrolled 87 unrelated Egyptian diabetic patients (43 men, 44 women) with age ranged from 60 to 75 years. They were classified as having type 2 diabetes according to the current American Diabetes Association criteria for the diagnosis and classification of diabetes [14]. Patients were recruited from the Ophthalmology Clinic of the Research Institute of Ophthalmology. Control subjects were 44 healthy volunteers with no history of diabetes, or any major clinical disorders and had normal fasting blood sugar and HbA_{1C}

Methods:

Family history of DR for all patients and controls and the duration of diabetes for patients were registered. All patients underwent a complete ophthalmological examination, including best corrected visual acuity, slit-lamp examination, intraocular pressure measurement using Goldmann applanation tonometry, indirect ophthalmoscopy and biomicroscopy. Fundusg fluorescein angiography was done in needed cases using Topcon fundus camera TRC. 50 EX on image-net. Five ml of 10% sodium fluorescein was injected in the antecubital vein and photography was carried out. Retinopathy was diagnosed according to the Early Treatment Diabetic Retinopathy Study (ETDRS) criteria: the presence of microaneurysms, hemorrhages, cotton wool spots, intraretinal microvascular abnormalities, hard exudates, venous beading and new vessels [15]. Patients were classified into 2 groups: group (1): 43 diabetic patients without retinopathy, group (2): 44 diabetic patients with diabetic retinopathy (DR) and 44 healthy control group. Informed consent was obtained from participants after a clear explanation of potential risk of the study.

HbA_{1C} was measured with a cation exchange chromatography method to assess glycaemic control. The procedure is a microchromatographic methodology for the quantitation of glycosylated haemoglobin (non-diabetic reference 5.5 % - 7.7%) (GLYCO Hb Quick column procedure) [16]. Serum total cholesterol and triglycerides were measured using enzymatic methods. [17, 18].

Determination of the VEGF genotypes

Genomic DNA was extracted from peripheral blood leucocytes using QIA amp DNA mini kit (QIAGEN, Inc., Germany). The I/D polymorphism was analysed using the following primers: forward 5'-GCTGAGAGTGGGGCTGACTAGGTA-3'   and reverse 5'-GTTTCTGACCTGGCTATTTCCAGG-3'. Genomic DNA (300 ng) was amplified in a final volume of 30 µl using the following conditions: denaturation at 95°C for 6 min followed by 35 cycles at 94°C for 1 min., 57°C for 1.5 min. and 72°C for 2 min. A final extension was at 72°C for 10 min. The amplification products were separated by electrophoresis on 2.5% agarose gel stained with ethidium bromide. For the VEGF I/D polymorphism two bands were observed, 211 bp for D allele and 229 bp for I allele.

Statistical analysis:

The data were analyzed using the statistical package SPSS (version 15). They were expressed as mean ± standard deviation for quantitative variables and as number and percentage for qualitative values. Statistical differences between categorical data like gender, genotype distribution and family history of diabetic retinopathy were tested using Chi Square test. For qualitative variables, independent sample t test and ANOVA (analysis of variance) with post Hoc Bonferroni test were used for normally distributed variables as age, FBS, HbA_{1c}, total cholesterol and triglycerides**.** Associations of genotypes and alleles were assessed as OR and 95% confidence intervals (CI). Differences by univariate methods ($\chi 2$ test, unpaired Student t test) were analyzed together in a logistic regression analysis to test for significant risk factors for diabetic retinopathy. Values less than or equal to 0.05 were considered statistically significant.

3. Results:

Table (1) presents the clinical and biochemical variables of the studied diabetic subjects and controls. There was no statistically significant difference in age and gender between the control group, group (1) and group (2). Also, there was no significant difference between group (1) and group (2) in the duration of the disease. However there was a statistically significant increase of family history of retinopathy in group (2) compared to group (1) and

a statistically significant increase of fasting blood sugar, HbA$_{1c}$, total cholesterol and triglycerides was noted in patients compared to controls.

Table (2) showed that there was no statistically significant difference in genotype distribution and allele frequencies of the I/D polymorphism between the three groups in general (p-values were 0.43 and 0.093 respectively), in spite of increased the frequency of D allele in patients with diabetic retinopathy compared to both diabetic patients and controls (61.4% vs. 54.65% and 50% respectively). Further analysis showed that the distribution of the VEGF, D/D genotype was higher in patients with diabetic retinopathy compared with diabetic group without retinopathy and healthy controls, (40.9% *vs*. 27.9% and 22.7% respectively) however the difference was still not statistically significant with Chi-Square= 3.637 and

p value = 0.162 (table 3). The OR of D/D genotype versus I/I genotypes between diabetic patients with retinopathy and controls was 2.25 (95% CI 0.672-7.538) with p value =0.185 (table 4) and the OR for the D allele versus I allele between diabetic patients with retinopathy and controls was 1.6 (95% CI 0.873-2.891) with p= 0.129 (table 5), while (table 6) showed that the OR for the D allele versus I allele in diabetic patients without retinopathy and controls was only 1.2 and the p value was 0.539 (95% CI 0.64-2.29). Logistic regression analysis which included significant variables (family history of DR, fasting blood sugar, HbA1c% total cholesterol and triglycerides) plus age, gender (F vs. M) and duration of diabetes showed that only triglycerides was an independent risk factor for diabetic retinopathy in patients with type 2 diabetes with p value =0 .047 and 95% CI, 1.0- 1.033.

Table (1): Clinical and biochemical characteristics of studied subjects

	Controls (n=44)	Group 1 (n=43)	Group 2 (n=44)	p value
Gender Male **n (%)** Female **n (%)**	20(45.5) 24(54.5)	22(51) 21(49)	21(48) 23(52)	0.866
Age (years)	69.05 (±5.23)	70.63 (±3.02)	70.50 (±4.11)	0.155
Duration of disease (years)	-	11.93 (±2.58)	11.82 (±2.16)	0.826
Family history of diabetic retinopathy, n (%)	-	18(41.9%)	28(63.6%)	0.042*
FBS (mg/dl)	102.23 (±9.52)	239.35 (±45.98)	242.68 (±46.35)	<0.001*
HbA$_{1c}$ (%)	6.17 (±1.0)	9.63 (±1.87)	10.23 (±1.37)	<0.001*
Total cholesterol (mg/dl)	176.66 (±19.31)	192.60 (±34.92)	199.16 (±47.80)	0.012*
Triglycerides(mg/dl)	169.05 (±24.68)	172.60 (±33.05)	188.57 (±33.63)	0.008*

Table (2): Genotype and allele distribution of VEGF gene I/D polymorphism in type 2 diabetic patients and controls

	Controls (n=44)	Group 1 (n=43)	Group 2 (n=44)	p value
Gene polymorphism, n (%) **II** **ID** **DD**	10(22.7%) 24(54.6%) 10(22.7%)	8(18.6%) 23(53.5%) 12(27.9%)	8(18.2%) 18(40.9%) 18(40.9%)	0.43
Allelic frequency, n (%) **I** **D**	44 (50%) 44 (50%)	39(45.34%) 47(54.65%)	34 (38.6%) 54(61.4%)	0.093

Table (3): The distribution of the VEGF, DD versus ID and II genotypes among patients and controls

Genotype	DM& Retinopathy	DM	Control	Total	X^2	p value
DD Count (%)	18 (40.9)	12 (27.9)	10(22.7)	40(30.5)	3.637	0.162
ID&II Count (%)	26 (59.1)	31 (72.1)	34(77.3)	91(69.5)		
Total Count (%)	44 (100)	43 (100)	44(100)	131(100)		

DM= diabetes mellitus

Table (4): DD versus   II genotypes in diabetic patients with retinopathy and controls

Genotype	DM& Retinopathy	Control	Total	p-value	OR	95% Confidence Interval
DD Count (%)	18 (69.2)	10 (50)	28(60.9)	0.185	2.25	0.672- 7.538
II Count (%)	8 (30.8)	10 (50)	18(39.1)			
Total Count (%)	26 (100)	20 (100)	46 (100)			

Table (5): Allele distribution of VEGF gene I/D polymorphism in patients with diabetic retinopathy and controls

Alleles	DM& Retinopathy	controls	Total	P value	OR	95% Confidence Interval
D No (%)	54 (61.4)	44 (50)	98 (55.7)	0.129	1.6	0.873- 2.891
I No (%)	34 (38.6)	44 (50)	78 (44.3)			
Total	88 (100)	88 (100)	176 (100)			

Table (6): Allele distribution of VEGF gene I/D polymorphism in patients with diabetes without retinopathy and controls

Alleles	DM	controls	Total	P value	OR	95% Confidence Interval
D No (%)	47(54.7)	44 (50)	91(52.3)	0.539	1.2	CI= 0.64- 2.29
I No (%)	39 (45.3)	44 (50)	83(47.7)			
Total	86 (100)	88 (100)	174 (100)			
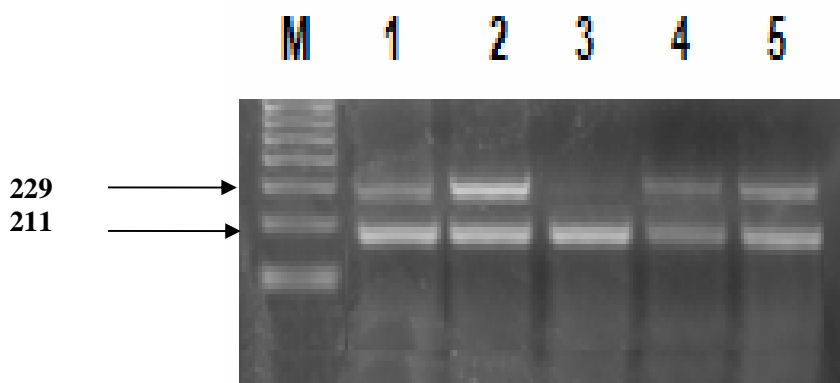
Figure (1): PCR amplification products of (I/D) VEGF gene polymorphism for patients with DR
Lane M: PCR marker 100 bp
Lanes 1, 2, 4 and 5 showed both 211 bp band of D allele and 229 bp band of I allele and lane3 showed only band of D allele.
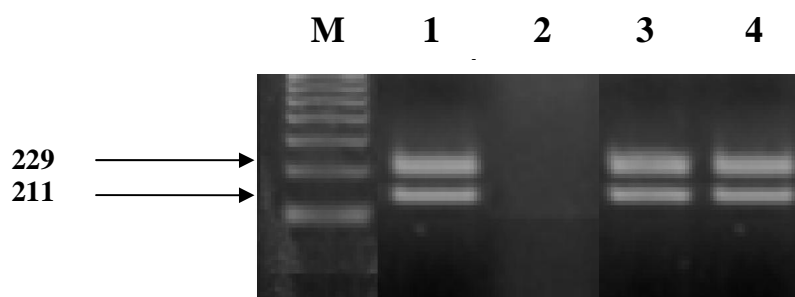
Figure (2): PCR amplification products of I/D - VEGF gene polymorphism of diabetic patients without retinopathy
Lane M: PCR marker 100 bp
Lanes: 1, 3 and 4 showed both 211bp band of D allele and 229bp band of I allele.

4. Discussion:

Diabetic retinopathy is characterized by increased vascular permeability, tissue ischemia and neovascularisation [19]. During hypoxia, hypoxia-inducible factors bind to the hypoxia-response elements and induce the expression of VEGF, which leads to the stimulation of angiogenesis and increases the permeability of the microvasculature [20]. Many cell types within the eye produce VEGF which is markedly elevated in the vitreous and aqueous fluids of patients with diabetic retinopathy [21].

Different polymorphisms in VEGF gene like I/D polymorphism in the promoter region, +405 G/C and -634 C/G polymorphisms in the 5'-untranslated region, have been studied in different ethnic groups [2, 11 and 13]. In this study we focused on the insertion/deletion (I/D) polymorphism of the 18 bp fragment at –2549 position of the promoter region.

This region has been shown to be highly polymorphic and in addition, most of the hypoxia-responsive elements are present in this region. To our knowledge, the present study is the first attempt to examine the possible association between this polymorphism and diabetic retinopathy among Egyptian patients.

In spite of increased the frequency of the D/D genotype of the VEGF gene I/D polymorphism in patients with diabetic retinopathy than diabetic patients without retinopathy and controls (40.9% vs. 27.9% and 22.7%), it did not show significant difference in genotype distribution (D/D, I/D and I/I) (p = 0.43). However we found more than a two fold increased risk of DR associated with D/D genotype and more than one and half fold increased risk with D allele in patients with DR than controls. On the other hand, there was an inconsiderable increased risk

associated with D allele in diabetic patients than controls (OR =1.2).These results suggest that the D allele has a detectable role in the development of diabetic retinopathy, however it may probably need interaction with other genetic or environmental factors or with increasing sample size in future studies we may predict a more powerful significant effect of the D allele on the development of diabetic retinopathy.

The association of the D allele at –2549 in the promoter region of the VEGF gene with the susceptibility to diabetic retinopathy can be explained in part by the enhanced level of transcription compared with the I allele. This would likely result in elevated levels of VEGF in these patients compared with the subjects carrying the I allele. Buraczynska et al. (2007), suggested that the I/D polymorphism in the promoter region of the VEGF gene is associated with retinopathy but not nephropathy in type 2 diabetes patients [13]; however Yang and his colleagues (2003), found that the D/D genotype was significantly increased in patients with diabetic nephropathy (a diabetic microvascular complication) compared to those with no complications (40.2% vs. 22.7%, respectively) [22].

Our study showed a statistically significant increase of family history of diabetic retinopathy in patients with DR than diabetic patients without retinopathy. A previous study of two hundred and twelve Egyptian families having one patient or more with diabetes mellitus showed that family history is an important risk factor for the development of DR among Egyptian patients [23]. Glycosylated hemoglobin (HbA_{1c}) was also significantly higher in patients than controls (P<0.001). HbA_{1c} value >8.0% was significantly related with sight-threatening diabetic retinopathy in a screening programme in India.The Receiver Operating Characteristic (ROC) analysis showed that the cut-off value of 8.0 had 75.6% sensitivity and 58.2% specificity [24]. Nordwall et al. (2009) documented that good glycemic control remains crucial in prevention of late diabetic complications [25].

This study demonstrated also that serum triglycerides and total cholesterol were significantly elevated in patients with DR. This agreed with The Early Treatment Diabetic Retinopathy Study (ETDRS) that suggested that lipid lowering may decrease the risk of hard exudates formation and associated vision loss in patients with diabetic retinopathy. Preservation of vision may be an additional motivating factor for lowering serum lipid levels in persons with diabetic retinopathy and elevated serum lipid levels [26]. El Haddad and Saad (1998), also documented that lowering of blood

lipids may be effective in lowering the incidence of retinopathy in controlled Omani diabetic patients [27]. On the other hand, Miljanovic et al. (2004) had failed to confirm this relationship [28]. By applying the multivariate logistic regression analysis we found that only increased level of triglycerides was the independent risk factor for diabetic retinopathy.

In conclusion, our study suggested that I/D polymorphism in the promoter region of the VEGF gene was not significantly associated with retinopathy in Egyptian type 2 diabetic patients, however moderate risk (i.e., OR, >2 for D/D genotype and > 1.5 for D allele) could not be excluded. Only increased triglyceride level was the independent risk factor in development of diabetic retinopathy detected in this study by logistic regression analysis. We recommend further investigations with increased sample size to predict a more significant effect of the D allele of VEGF gene on the development of diabetic retinopathy.

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Influence of Field Size, Depth, Nominal Dose Rate and Stem Length on Ion Recombination Correction Factor in Therapeutic Photon Beam

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Abstract: The use of ionization chamber in linear accelerator radiotherapy photon dosimetry requires various corrections to the measured charges, one of these being the ion recombination correction factor (k_s). As stated by the IAEA (2000) TRS-398 dosimetry protocol, k_s was characterized for the available thimble ionization chamber PTW 30006 using two pulsed megavoltage photon beams 6 and 10 MV. The dependence of the k_s values against the changing of field size, water depth, nominal dose rate and stem length was studied. For photon energy 10 MV, k_s shows an increase with the field size and for photon energy 6 MV, k_s values decrease from field size 4x4 cm^2 to 10x10 cm^2 and increase at field sizes larger than 10x10 cm^2. Also, k_s values are inversely proportional with the water depth and directly proportional with the nominal dose rate and stem length, for both photon energies. It is also recommended to determine the absorbed dose at lower (*p.r.f*) pulse repetition frequency or nominal dose rate. If the dose is determined at the highest (*p.r.f*), a correction must be introduced in the assessment of the dose related to the ion recombination correction factor k_s difference at different *p.r.fs*. These measurements help to correct k_s values at different dosimetry conditions and minimize the errors in the assessment of the radiotherapeutic dose calculations.
[A. I. Abd El-Hafez, Hany A. Shousha, M. S. Zaghloul and M. A. AbouZeid. **Influence of Field Size, Depth, Nominal Dose Rate and Stem Length on Ion Recombination Correction Factor in Therapeutic Photon Beam.** Journal of American Science 2011;7(3):206-213]. (ISSN: 1545-1003). http://www.americanscience.org.

1. Introduction:

In most radiotherapy clinics the dose delivered to a given point in the medium is determined by measuring the amount of charge (Q) produced in a small cavity located at that point in the medium [1, 2]. The cavity is usually an ionization chamber filled with air at ambient temperature and pressure.

The dose delivered to the medium can be calculated from the total charge, or saturation charge, produced in the air cavity according to the Spencer-Attix cavity theory, [3]. The response of a given ionization chamber depends not only on the radiation dose, dose rate and chamber polarity [4] but also on the voltage applied between the measuring and collecting electrodes of the chamber [5, 6, 7]. The charges produced in the chamber by radiation may differ from the charges that are actually collected. These discrepancies (charge losses or excess charges) occur as a result of constraints imposed by the physics of ion transport in the chamber sensitive volume and the chamber electrical design. Charge losses in the chamber are caused by ion recombination; excess charges by charge multiplication and electrical breakdown. Both the charge recombination and charge multiplication are influenced by the potential applied to the ionization chamber. A plot of chamber response, i.e., current (I) or charge (Q) against the applied voltage (V) for a constant dose rate or dose, respectively, is called saturation curve, first rising linearly with voltage at low voltages, then reaching a saturation at high voltages and eventually breaking down at even higher voltages [8, 9]. The ratio Q(V) /Q$_{sat}$ or I(V) / I$_{sat}$, where (Q$_{sat}$) and (I$_{sat}$) are the saturation values of Q and I, respectively, is referred to as the collection efficiency (*f*) of the ionization chamber at the applied voltage (V), (1).

In saturation, all charges produced by radiation are collected and produce directly the (Q$_{sat}$) and (I$_{sat}$) for use in dosimetry protocols. When the chamber is used below saturation, some of the charges produced by radiation actually recombine and lead to loss of the dosimetric signal. This charge loss occurs through three different mechanisms:

(1) Initial recombination represents the recombination that occurs between ions produced within the track of a single ionizing particle and is thus independent of dose rate.

For initial recombination, (1/Q) was shown to vary linearly with (1/V), [10, 11].

(2) General recombination, in contrast to initial recombination, applies to ions produced in different ion tracks, which meet and recombine, and thus depends on dose rate. For general recombination, (1/Q) in the near saturation region ($f > 0.7$) was found to vary linearly with (1/V^2) in continuous beams, [10, 12, 13]. In electronegative gases, such as air, it was shown by some authors [10, 11, 14, 15], that in the near saturation region, initial recombination is negligible in comparison with general recombination.

(3) Ionic diffusion loss: charges diffuse against the electric field. For thermal diffusion of ions against the applied chamber potential it was found that (1/Q) also follows a linear relationship with (1/V) in the near saturation region.

For studies of ionic recombination losses, ionizing radiation beams are placed into three categories:

(i) *Continuous radiation* (e.g., cobalt beams and orthovoltage x rays).

(ii) *pulsed beams* (e.g., non-scanned linac x ray beams and electrons).

(iii) *Scanned pulsed beams* (e.g., scanned linac beams).

On the light of the two voltages technique which suggested by Boag and Current [16], describing the ion recombination correction for a small cylindrical ionization chamber exposed to pulsed radiation, [17], proposed an approximation for the ion recombination (P$_{ion}$) or (k_s) for constructed in the form of quadratic equation and working both pulsed and pulsed swept radiation. This quadratic equation is

$$(\mathbf{P_{ion}}) \text{ or } (\boldsymbol{k_s}) = \qquad\qquad (1)$$

$$\mathbf{a_o} + \mathbf{a_1}\left(\frac{M_N}{M_L}\right) + \mathbf{a_2}\left(\frac{M_N}{M_L}\right)^2$$

Where,

(M_N) is the chamber signal determined at the normal operating voltage (V_N).

(M_L) is the chamber signal determined at a lower voltage (V_L).

(a), (a$_1$) and (a$_2$) are constants obtained from standard polynomial fitting program, (18). This equation is adopted by the [19] TRS-398 dosimetry protocol.

2. Material and Methods

The ion recombination correction factor k_s was measured for two pulsed photon radiation beams 6 and 10 MV produced by medical linear accelerator Philips SLi15 which is manufactured in England. A Farmer-type, 0.6 cm^3 vented water proof ionization chamber of model PTW30006, manufactured in Germany, was used in our experiment. The chamber is of rugged construction, since the wall material is graphite with a protective acrylic cover and the collecting electrode is made of aluminum. The readings were taken using an electrometer (Victoreen model 530) , manufactured in U.S.A., connected to the ionization chamber where the normal operating bias voltage V_N adjusted to be at the possible maximum voltage, V_N = +382.5 volts, with positive polarity. The lower operating bias voltage was chosen to be one third of V_N, V_L = +127.5 volts, as recommended by the TRS-398 dosimetry protocol [19].

The amount of irradiation necessary to provide stable readings after decreasing the bias was measured for the chamber by saturating the chamber with 500 monitor units and then reversing the bias. Readings were immediately taken at 50 monitor unit increments until a stable reading was obtained, [20].

A water phantom of model Med-Tec, manufactured in U.S.A., was used in the water measurements and it is constructed from (9.525mm)-thick clear acrylic material of volume 38 x 38 x 38 cm^3 and provided with horizontal scale and manual depth dose apparatus.

For both photon energies 6 and 10 MV, the measurements were performed under the reference conditions where the gantry angle = 0^0, the distance between the radiation focus and water surface FSD = 100 cm, the field size 10x10 cm^2 and the reference water depth = 10 cm, as recommended by the [19] TRS-398 dosimetry protocol, fig. (1).

The variation of k_s was determined against the field size changing at FSD=100 cm where the ionization chamber was located at constant reference water depth equal to 10 cm. Also, the variation of k_s was measured against the water depth changing where the field size kept constant and equal to 10x10 cm^2 at FSD = 100 cm. k_s was determined due to the variation between three available nominal dose rate 100, 300 and 600 M.U./ min.

As a study of the stem effect on k_s, the measurements were done in the air where the distance between the radiation focus and the chamber central axis (FCD) = 100 cm with two build up caps suitable for each photon energy 6 and 10 MV and the field size was equal to 10x35 cm^2 where X-side = 35 cm and Y-side = 10 cm. The zero position is considered when the center of ionization chamber sensitive volume is located at the field center. In the case of the

ionization chamber longitudinal central axis was adjusted to be parallel to the X-side, where the stem length was changing and moving away from the field center, the k_s was calculated as:

$$(k_s)^{Par.} = a + a_1 ((M_N)^{Par.}/(M_L)^{Par.}) + a_2 ((M_N)^{Par.}(M_L)^{Par.})^2. \quad (2)$$

In the other case, when the chamber longitudinal central axis was oriented in a direction perpendicular to X- side, the stem length was constant and k_s was calculated as:

$$(k_s)^{Per.} = a_o + a_1 ((M_N)^{Per.}/(M_L)^{Per.}) + a_2 ((M_N)^{Per.}/(M_L)^{Per.})^2. \quad (3)$$

Where
$(M_N)^{Par}, (M_N)^{Per}$ is the electrometer reading (in charge mode) at normal operating voltage $V_N = +382.5$ volt when the ion chamber longitudinal central axis is parallel and perpendicular to the X-side of the field respectively.
$(M_L)^{Par}, (M_L)^{Per}$ is the electrometer reading (in charge mode) at lower voltage $V_L = +127.5$ volt when the ion chamber longitudinal central axis is parallel and perpendicular to the X-side of the field respectively.

3. Results and Discussion:
Figure (2), representing the drawn data of k_s for both photon energies 6 and 10 MV as (Y) axis versus one side of the square field size as (X) axis and the data was fitted using second polynomial fitting method.

For photon energy 10MV, the value of k_s is increasing with increasing the field size. For photon energy 6MV the value of k_s is decreasing from field size 4x4 cm^2 to 10x10 cm^2, but the value of k_s is increasing when the field size increasing beyond 10x10 cm^2. As the field size is increasing, more portions of the ionization chamber stem and the cable is included in the field and that will affect the collected charges; where some of the ionized charges will be lost in the ionization chamber body and the ion recombination correction factor (k_s) needs to increase to compensate this charges loss. Also, one can observe that the values of k_s for photon energy 10MV is larger than the values of k_s for photon energy 6MV all over the field size increasing range, except at the smallest measured field size 4x4 cm^2, where the values of k_s for both photon energies become very close to each other. That is because at small field sizes the radiation field does not exceed the boundary of the ion collective sensitive volume, so no extra effects can appear from the ion chamber body. So, it is concluded that to increase the accuracy of absorbed dose determination at field sizes differ than 10x10 cm^2, it is recommended to calculate the ion recombination correction factor k_s for each field size and for all available photon energies.

The two curves in figure (3) describe the behavior of k_s for both photon energies 6 and 10 MV, as (Y) axis against water depth as (X) axis. It is obvious from the figure that the values of k_s are decreasing with increasing the water depth for both photon energies 6 and 10 MV. That is because at the small depths near the build up region, the photon beam interacts with the ionization chamber measuring electrode causing a loss of electrons from the measuring electrode which is not fully compensated by arrival electrons from the upper layers of the phantom. Also, it is notable that the values of k_s for photon energy 10MV are higher than the values of k_s for photon energy 6MV all over the increasing range of the water depth. But at the higher water depths, the values of k_s for both photon energies become very close to each other, that is because the dose rate is decreasing with increasing of water depth, which affects the ion recombination of the ionization chamber.

Table (I), shows the variation of k_s against machine nominal dose rate or machine pulse repetition frequency (*p.r.f*) changing with field size = 10x10 cm^2 and water depth = 10 cm at FSD = 100 cm, with constant water temperature and air pressure for each individual measurement at each photon energies 6 and 10 MV.

In the table (I), the values of k_s for both photon energies 6 and 10 MV are increasing with the increasing of the machine nominal dose rate or (*p.r.f*). It is obvious that the variation of k_s values in the 6MV photon beam is smaller than the k_s values for the 10MV photon beam, but at the lowest available nominal dose rate (100 M.U./min.), where the k_s values for both photon energies are close to each other. Since the value of k_s stated by equation (1) is valid if no overlapping of clouds of ions from different beam pulses occurs, [21]. So, at low (*p.r.f*), the duration time of the pulses is bigger than the collection time of the ionization chamber and that makes most of the charges produced by the ionization are well collected for both photon energies. But at higher (*p.r.f*) the pulses duration is decreased a little than the collection time of the ionization chamber, so there will be some sort of overlap between collected charges and some ions will be lost. Also, this phenomenon increases with the increasing of the energy.

Figures (4, (a) and (b)), show the curves of k_s versus the distance of the central measuring volume of the ionization chamber from the field center (distance off axis) in cm, for both photon energies 6 and 10 MV and in the two directions parallel and perpendicular to (X)-side. When the distance off axis is increasing, it means the length of the stem and cable is decreasing.

From figure (4, (a)), it can be noted that the k_s values of the photon energy 6MV are inversely proportional to the distance off axis (i.e. stem length decreases) when the chamber is directed parallel to (X) axis, and directly proportional to distance off axis (i.e. stem length is constant) in the direction perpendicular to (X) axis. The two curves of k_s values of the photon energy 10MV drawn in fig. (4, (b)), are inversely proportional to the distance off axis in a direction parallel (i.e. stem length is decreasing) and perpendicular (i.e. stem length is constant) to the (X) axis. Nevertheless, Bruggmoster et al. [7], stated that there is no influence on radiation type and energy on the recombination correction factor k_s and there is a linear relationship between k_s and the dose per pulse (DPP) up to 5 mGy/pulse. Also, they found that at dose per pulse values above 1 mGy, the method of general equations with coefficients dependent on the chamber type gives more accurate results than the Boag method. This method was already proposed by Burns and McEwen [22] and to avoid comprehensive and time consuming measurements of Jaffé plots which are a prerequisite for the application of the multi-voltage analysis (MVA) or the two voltage analysis (TVA). Many other investigators are concerned with the ion recombination correction factor k_s [5, 6].

Table (I): Ion recombination correction factor (k_s) of the ionization chamber (PTW30006) versus the variation of the nominal dose rate at the reference condition for two photon energies 6 and 10 MV.

Dose Rate in (**M.U./min.**)	Photon energy (**6MV**)		Photon energy (**10MV**)	
	k_s	Standard Deviation	k_s	Standard Deviation
100	1.0008	± 0.00117	1.0020	± 0.00090
300	1.0009	± 0.00041	1.0029	± 0.00040
600	1.0011	± 0.00043	1.0039	± 0.00071
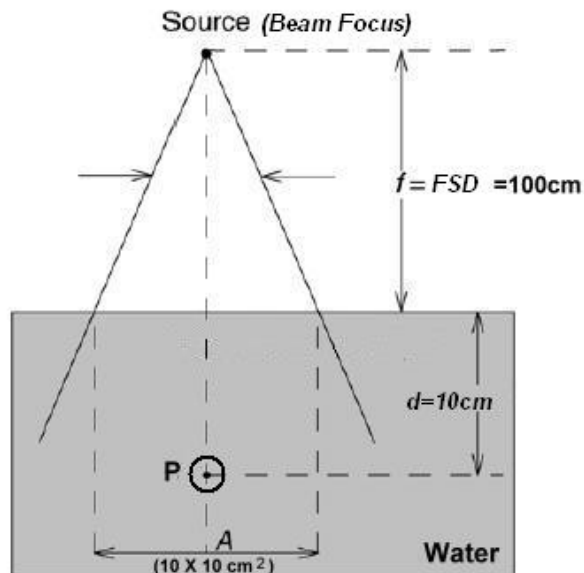
Figure (1): The reference condition of the measurements where field size (A) = 10x10 cm^2 at the distance between the beam focus and water surface (FSD) = 100 cm and the ionization chamber is located at position (P), where the water reference depth (d) = 10 cm.
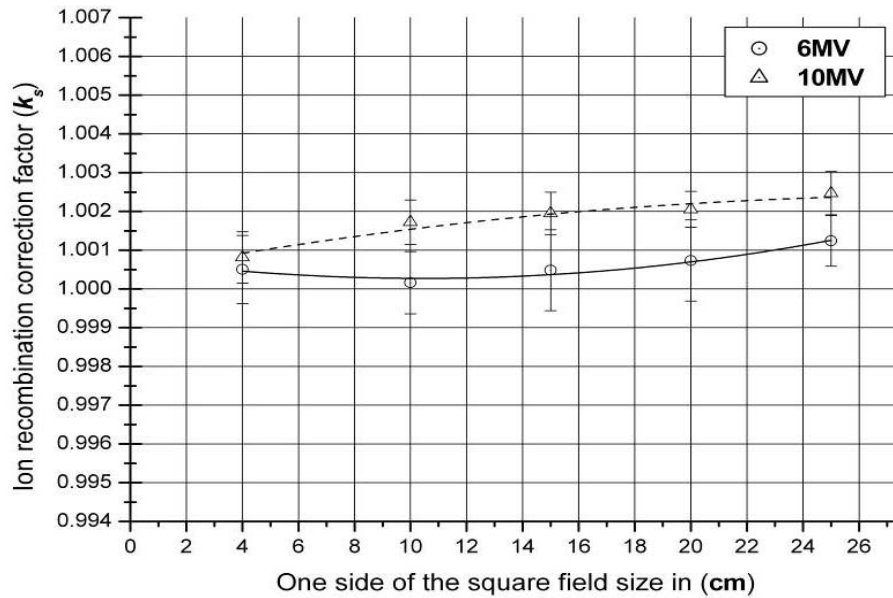
Figure (2): The variation of ion recombination correction factor ks versus the field size at FSD = 100cm and water depth = 10cm for photon energies 6 and 10 MV.
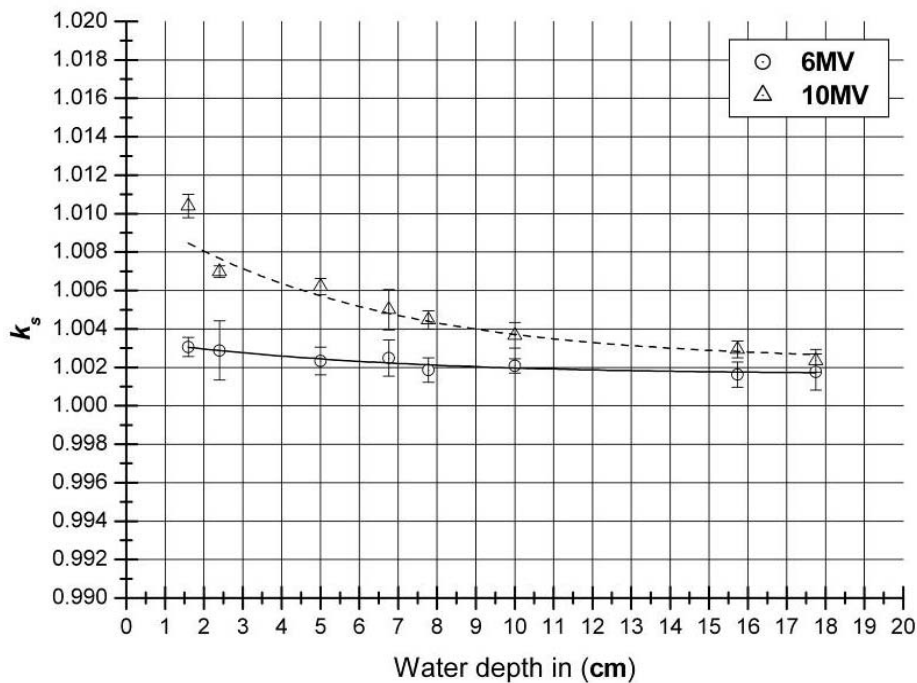
Figure (3): Ion recombination correction factor ks versus the water depth changing of field size=10x10 cm2 at FSD=100cm and for photon energies 6 and 10 MV.

(a)

(b)
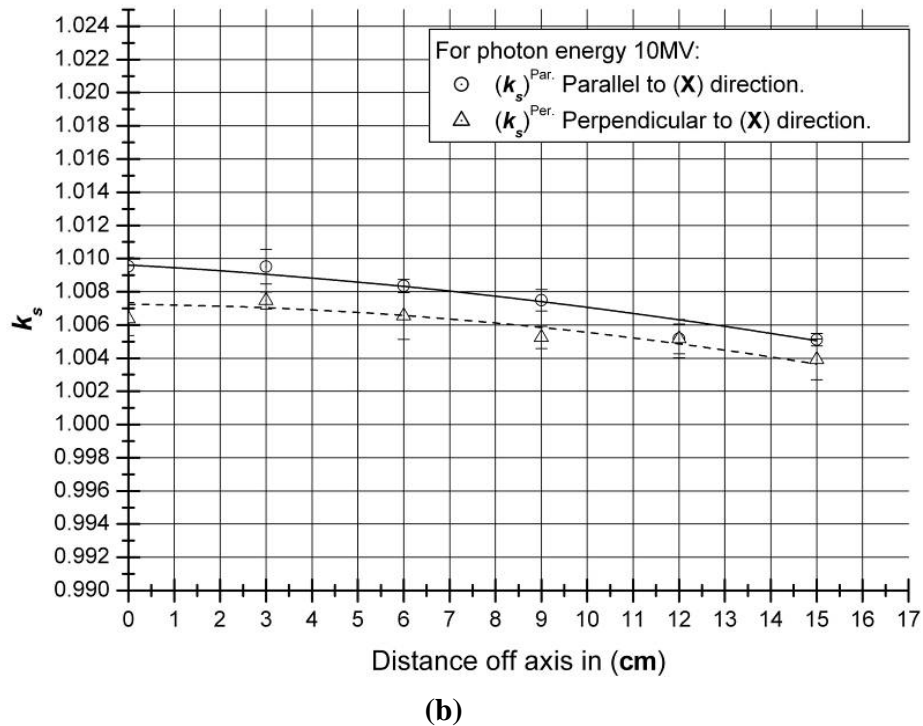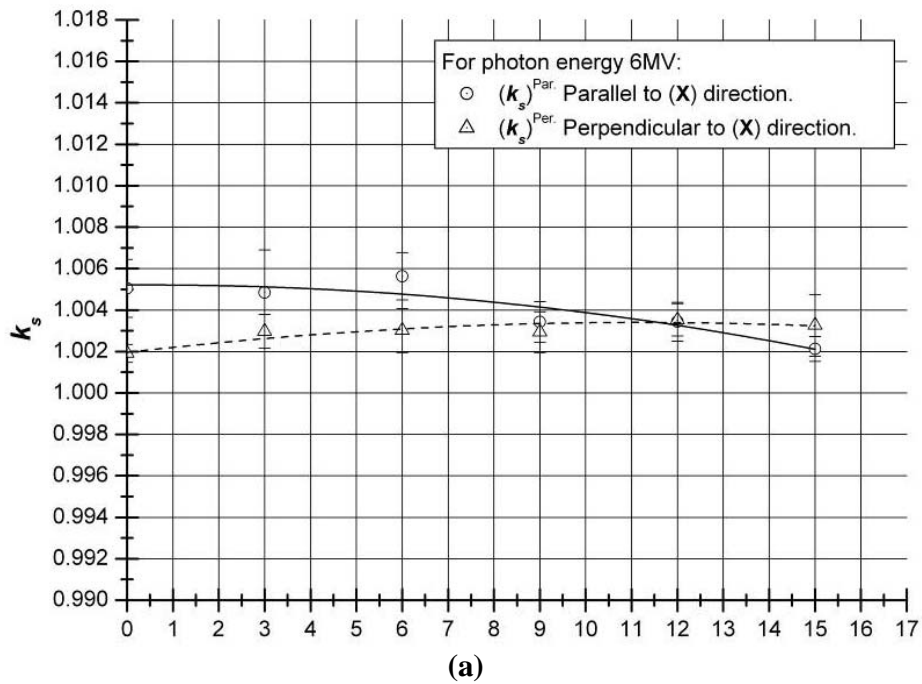
Figure (4) Ion recombination correction factor ks versus the stem length variation in parallel and perpendicular to the (X) axis of the beam plane at FCD = 100cm in the air with suitable build up caps for photon energies: (a) 6MV and (b) 10MV.

4. Conclusions:

To increase the accuracy of the absorbed dose determination at field sizes differ than 10x10 cm^2, using the ionization chamber PTW30006 exposed to pulsed photon beams of energies 6 and 10 MV, it is recommended to measure the ion recombination correction factor (k_s) for each field size. Also, when measuring depth doses, it should account for the change in the ion recombination correction factor (k_s) as a function of depth in phantom. It is better to determine the absorbed dose at low pulse repetition frequency (*p.r.f*) or machine nominal dose rate and if the absorbed dose is determined at the highest (*p.r.f*), a correction must be introduced in the calculation of the dose related to the ion recombination correction factor (k_s) difference at deferent (*p.r.f*s). The amount of the stem length that covered by the radiation field will affect on the ion recombination of the ionization chamber and the value of k_s must be calculated as a function of the stem length.

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Curative effect of basil on liver injury in experimental rats

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Abstract: Forty two albino male rats were classified into six groups. Group I (n=7) served as control (-ve), and animals in groups II–VI CCl4 were induced liver injury. Group II served as control(+ve) and treated groups from III to VI rats received daily oral doses of ursofalk drug , basil ethanolic extract, basil aqua extract, and basil powder. The results revealed that control (+ve) rat group showed a significant decrease in final body weight, body weight gain, food intake & food efficiency ratio (FER); serum total protein, globulin, glutathione transferase (GST) & catalase and liver triglyceride, total lipid, superoxide dismutase (SOD), glutathione peroxidase (GPX) & GST but a significant increase in serum alanine and aspartate aminotransferase , alkaline phosphates, gamma glutamyle peptidase (ALT, AST**,** ALP & GT), total bilirubin & nitrite (NO); albumin/ globulin ratio and liver glycogen, cholesterol & malondialdehyde (MDA) compared with control (-ve) group. All treated groups showed a significant decrease in body weight gain; serum globulin, GST& catalase and liver glycogen but a significant increase in serum ALP, total bilirubin & NO and albumin/ globulin ratio compared with control (- ve) group. Drug group showed a significant increase in serum AST & total bilirubin and liver cholesterol and MDA but a significant decrease in liver triglyceride, total lipid, SOD, GPX, GST compared with control (- ve) group. Basil ethanol extract and basil aqua extract rat showed a significant increase in serum ALT albumin/ globulin ratio and liver cholesterol & MDA and a significant decrease in serum total protein, liver triglyceride while basil powder showed a significant increase in serum ALT, AST, GT and albumin/ globulin ratio and a significant decrease in serum total protein, albumin, liver total lipid and liver SOD compared with control (-ve).

Key word: Basil- Liver injury- Rats.

1-Introduction

Liver is a major metabolic organ affected by various chemicals and toxins. Carbon tetrachloride (CCl4) is a well-known hepatotoxin and induces oxidative stress by the formation of free radicals. Extensive evidence demonstrated that CCl3 and Cl are formed as a result of the metabolic activation of CCl4 which in turn initiate lipid peroxidation process (Abd El-Ghany 2006).

Plants have played a significant role in maintaining human health and improving the quality of human life. Some herbal extracts are known to prevent the oxidative damages in different organs by altering the levels of cytochrome P-450 through their antioxidant properties (Kandasamy et al., 2010).

Ocimum basilicum is an aromatic herb known as African basil and wild basil and is used as an anthelmintic, deodorant, stimulant, anti-inflammatory, cardiotonic, blood purifier, used in skin diseases and as an antipyretic particularly in malarial fevers. Leaves and flowering tops are used for the essential oil which contains eugenol and found to have antibacterial, antiyeast and insecticidal action while extracts showed a hepatoprotective effect by promote liver health by reducing damage due to radiation exposure or environmental pollution or toxicants (Oboh et al., 2009).

The present investigation was undertaken to determine curative effects of concurrent use of basil leaves either extract or powder on CCl4 induced liver injury.

2. Materials and methods

2.1. Chemicals

Carbon tetrachloride was obtained from SIGMA Company for harmaceutical Industries and given 0.5 ml/rat CCl4 by back subcutaneous injection according to Moritz and Pankow (1989). Ursofalk drug is obtained from Minapharm Company. It is a white capsule and contains 250 mg ursodeoxycholic acid used in improvement of liver functions. Rats administered ursofalk drug dissolved in distilled water in dose 10 mg/kg of rat intragastric.

2.2. Basil plant and extracts

Dry plant leaves of basil (*Ocimum basilicum*) were purchased from Agricultural Research Center, Giza, Egypt. Basil leaves were dried with hot air (40–60 C) and grinded to powder which was added to the diet as 5 % of the constituent of fiber. Basil aqua extract was prepared by boiling dried plant with 100 ml distilled water for 15 min. The extract was then filtered with a

clean cotton cloth and the volume of the extract was adjusted to 100 ml by evaporation. Basil ethanolic extract was prepared from basil powdered which was refluxed eight times with an 80% ethanol solution for two times, 1 h each time. The solvent was removed by evaporation under reduced pressure using Rotary Evaporator. Yield of the aqua and ethanol extract were given to rats as 250 mg/kg b.w intragastric.

2.3. Animals and experimental design

The experiment was performed on growing male Sprague dewally rats (n = 42) weighing about 151± g with approval from Helwan Farm of Laboratory Animals were provided with standard diet (Nelson 2000). After 15 days of acclimatization period; they were randomly assigned into six groups. Group I (n=7) served as negative control and animals in groups II–VI were given CCl4 to induce liver injury. Treated groups from III to VI rats received daily oral doses of uroflok drug, basil ethanolic extract, basil aqua extract, and basil powder .The daily food intake and weekly body weight of the individual rat was measured during the entire period of experiment (8 weeks). Food efficiency ratio was calculated according to Chapman et al., (1950). Rats were sacrificed under light chloroform anesthesia at the end of experiment. Blood samples and the liver were collected for biochemical estimation.

2.4. Biochemical estimation

2.4.1. Plasma ALT, AST, GT and ALP

Serum aminotransferase (ALT, AST), gamma glutamyle peptidase (GT) and alkaline phosphates enzymes activity (ALP) were estimated according to Reitman and Frankel (1957), Henry, (1974) and Kind and King (1954), respectively.

2.4.2. Serum total protein, albumin and globulin

Serum total protein, albumin and globulin (G) were estimated following the method of Weichselbaum (1946), Bartholomev and Delany (1966) and Coles (1974), respectively.

2.4.3. Serum glutathione transferase (GST), catalase and nitrite (NO)

Serum GST, catalase and NO were estimated according to Habig et al., (1974), Luck (1965) and Green et al., (1981), respectively.

2.4.4. Liver total lipid, cholesterol, triglyceride and glycogen.

Liver total lipid, cholesterol, triglyceride and glycogen were determined spectrophot- ometrically following the methods of Folch et al., (1957), Abell et al., (1952), Young and Pestaner ,(1975) and Rerup and Lundquist, (1967), respectively.

2.4.5. Liver SOD, GPX, GST and MDA

liver superoxide dismutase (SOD), glutathione peroxidase (GPX), Glutathione-S-transferase (GST) and malondialdehyde (MDA) were determined spectrophotometrically following the methods of Minami and Yoshikawa (1979), Flohe and Gunzler (1984), Habig et al .,(1974) and Placer et al .,(1966), respectively.

2.5. Statistical analysis

The data were analyzed statistically using analysis of variance to compare the means of different treatment groups with that of negative control and positive control groups (Snedecor and Cochran, 1994).

3. Results
3.1. Weekly body weight of rat groups

There was a steady decrease in final body weight, body weight gain food intake and FER of control (+ve) rat group (P<0.05, 0.01&0.001). However, all treated groups showed a significant decrease in body weight gain (P<0.05) compared with control (- ve) group and a significant increase in final body weight, body weight gain, food intake and FER compared with control (+ve) group as given in table (1).

3.2. Serum ALT, AST, GT and ALP

Table (2) showed a significant increase in serum ALT, AST, GT and ALP in control (+ve) rat group (P<0.001) and a significant increase in serum AST in drug, basil aqua extract and basil powder (P<0.05) but a significant increase in ALT in treated groups with basil either ethanolic or aqua and powder (P<0.05) compared with control (-ve) group. All treated groups showed a significant increase in ALP (P<0.05) compared with control (-ve) and a significant decrease in serum ALT, AST, GT and ALP compared with control (+ve) rat group.

3.3. Serum total protein, albumin and globulin

The control (+ve) rat group showed a significant increase in serum total bilirubin and albumin/ globulin ratio (P<0.001&0.01) and a significant decrease in serum total protein and globulin (P<0.001) compared with control (-ve) group. Drug group showed a significant increase in serum total bilirubin (P<0.01) and a significant decrease in serum globulin (P<0.05) compared with control (-ve) but showed a significant decrease in serum total bilirubin and a significant increase in serum total protein, globulin and albumin/ globulin ratio compared with control (+ve). Drug group showed a significant increase in serum total bilirubin (P < 0.01) and a significant decrease in serum globulin (P<0.05) compared with control (-ve) but showed a significant decrease in serum bilirubin and albumin/ globulin ratio and a significant increase in serum total protein and globulin compared with control (+ve).

Basil ethanol extract, basil aqua extract and basil powder rat groups showed a significant increase in serum total bilirubin and albumin/ globulin ratio (P < 0.001,0.01&0.05) and a significant decrease in serum total protein and globulin (P < 0.05&) compared with control (-ve). Basil ethanol extract group showed a significant decrease in serum total bilirubin and albumin/ globulin ratio and a significant increase in serum total protein and globulin while basil aqua extract group showed a significant decrease in serum total bilirubin, albumin and albumin/ globulin ratio but basil powder rat group showed a significant decrease in serum albumin and albumin/ globulin ratio compared with control (+ve) as shown in table (3).

3.4. Serum GST, catalase and NO

Table (4) showed a significant decrease in serum GST and catalase and a significant increase in serum NO (P < 0.05, 0.01&0.001) in control (+ve) and all treated groups compared with control (-ve) but a significant increase in serum GST and catalase and a significant decrease in serum NO in all treated groups compared with control (+ve).

Table (1): Mean values±SD of body weight gain, food intake and food efficiency ratio (FER) of experimental rat groups

Groups Variables	Initial. weight(g)	Final weight(g)	Weight gain (g)	Food intake (g/w)	FER
Control (-ve)	151.33± 3.47 [a]	238.48± 11.41 [a]	87.15± 5.67 [a]	18.49± 1.24 [a]	0.078± 0.002 [a]
Control (+ve)	150.14± 3.24 [a]	199.05± 12.28 [b**]	48.91± 5.01 [c***]	15.45± 1.45 [b*]	0.052± 0.001 [c**]
drug	152.37± 4.21 [a]	229.73± 15.71 [a]	77.36± 7.11 [b*]	18.10± 1.81 [a]	0.071± 0.003 [a]
Basil ethanolic extract	152.44± 4.29 [a]	228.38± 13.14 [a]	75.94± 6.19 [b*]	18.04± 1.77 [a]	0.070± 0.003 [a]
Basil Aqua extract	153.22± 3.21 [a]	227.35± 15.20 [a]	74.13± 7.18 [b*]	18.02± 1.36 [a]	0.068± 0.002 [ab]
Basil powder	151.41± 5.25 [a]	224.70± 16.33 [a]	73.29± 7.03 [b*]	17.80± 2.01 [a]	0.068± 0.001 [ab]

Significant with control group * P<0.05 **   P<0.01 *** P<0.001
Mean values in each column having different superscript (a, b, c, d) are significant

Table (2): The Mean values ± SD of serum ALT, AST, GT and ALP of experimental rat groups

Groups Variables	ALT (μ /ml)	AST (μ /ml)	GT (μ /ml)	ALP (μ /ml)
Control (-ve)	29.91± 3.21 [c]	55.17± 5.12 [c]	8.15± 1.22 [c]	40.27± 5.61 [c]
Control (+ve)	78.11± 6.66 [a***]	102.76± 11.44 [a***]	15.37± 1.14 [a***]	88.98± 8.33 [a***]
drug	35.77± 4.11 [bc]	63.81± 5.99 [b*]	9.88± 1.41 [c]	50.47± 6.17 [b*]
Basil ethanolic extract	38.24± 3.99 [b*]	60.65± 6.11 [bc]	9.11± 1.30 [c]	56.14± 6.03 [b*]
Basil aqua extract	41.33± 4.80 [b*]	66.24± 7.12 [b*]	9.67± 1.21 [c]	61.60± 7.10 [b*]
Basil powder	43.78± 4.71 [b*]	71.30± 7.67 [b*]	11.17± 1.33 [b*]	65.21± 7.30 [b*]

Significant with control group * P<0.05 **   P<0.01 *** P<0.001
Mean values in each column having different superscript (a, b, c, d) are significant

Table (3): The Mean values ± SD of serum bilirubin, total protein, albumin, globulin and albumin/globulin ratio (A/G) of experimental rat groups

Groups / Variables	Bilirubin (mg/dl)	T.protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Albumin /Globulin
Control (-ve)	0.33± 0.01 [e]	7.10± 1.53 [a]	3.31± 0.66 [a]	3.79± 0.55 [a]	0.87± 0.11 [c]
Control (+ve)	1.45± 0.33 [a***]	5.33± 1.10 [c***]	3.25± 0.78 [a]	2.08± 0.53 [c***]	1.56± 0.19 [a**]
drug	0.88± 0.17 [cd**]	6.30± 1.32 [ab]	3.11± 0.45 [ab]	3.19± 0.24 [b*]	0.97± 0.10 [c]
Basil ethanolic extract	0.77± 0.15 [d**]	5.99± 1.13 [b*]	3.01± 0.44 [ab]	2.98± 0.28 [b*]	1.04± 0.13 [b*]
Basil aqua extract	0.99± 0.11 [b**]	5.55± 0.88 [bc*]	2.91± 0.19 [b*]	2.64± 0.35 [bc**]	1.10± 0.18 [b*]
Basil powder	1.03± 0.13 [ab***]	5.45± 0.66 [bc**]	2.85± 0.39 [b*]	2.60± 0.41 [bc**]	1.09± 0.22 [b*]

Significant with control group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each column having different superscript (a, b, c, d) are significant

Table (4): The Mean values ± SD of serum GST, catalase and NO of experimental rat groups

Groups / Variables	GST (μ /ml)	Catalase (μ /ml)	NO (μ mOl/l)
Control (-ve)	261.81± 33.16 [a]	301.41± 45.32 [a]	1.03± 0.16 [a]
Control (+ve)	108.22± 10.27 [d***]	171.20± 20.14 [c***]	9.67± 1.89 [a***]
drug	189.14± 18.33 [c**]	217.33± 31.13 [b*]	3.77± 0.55 [b*]
Basil ethanolic extract	220.35± 28.26 [b*]	235.14± 25.31 [b*]	3.51± 0.67 [b*]
Basil aqua extract	218.82± 27.70 [b*]	230.17± 25.31 [b*]	3.51± 0.67 [b*]
Basil powder	215.41± 30.11 [b*]	233.81± 22.21 [b*]	3.16± 0.73 [b*]

Significant with control group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each column having different superscript (a, b, c, d) are significant

3.5. Liver SOD, GPX, GST and MDA

The control (+ve) rat and drug groups showed a significant decrease in liver SOD, GPX, GST and a significant increase in liver MDA (P< 0.05, 0.01&0.001) compared with control (-ve) group. Basil ethanol extract and basil aqua extract rat groups showed a significant increase in Liver MDA (P<0.05) while basil powder rat group showed a significant decrease in Liver SOD (P<0.05) compared with control (-ve) group. All treated groups showed significant increase in liver SOD, GPX, GST and a significant decrease in liver MDA compared with control (+ve) group as shown in table(5)

Table (5):The Mean values ± SD of some liver glutathione (GSH), malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GPX) of experimental rat groups.

Groups Variables	SOD (μ /mg)	GPX (μ /mg)	GST (mg/g)	MDA (mmol/g)
Control (-ve)	55.91± 6.17 [a]	43.91± 6.01 [a]	4.33± 1.11 [a]	8.21± 1.87 [c]
Control (+ve)	25.89± 3.20 [d***]	21.81± 2.15 [d***]	1.27± 0.12 [d***]	20.17± 2.76 [a***]
drug	37.22± 4.77 [c**]	32.11± 3.02 [c*]	2.77± 0.33 [c*]	11.21± 2.11 [b*]
Basil ethanolic extract	53.41± 5.67 [ab]	39.29± 4.41 [ab]	3.87± 0.54 [ab]	10.14± 1.75 [b*]
Basil aqua extract	50.21± 6.12 [ab]	38.14± 3.82 [ab]	3.67± 0.67 [ab]	10.51± 1.57 [b*]
Basil powder	49.31± 4.19 [b*]	36.35± 4.50 [ab]	3.59± 0.56 [ab]	9.99± 1.44 [bc]

Significant with control group * $P<0.05$ ** $P<0.01$ *** $P<0.001$
Mean values in each column having different superscript (a, b, c, d) are significant

3.6. Liver total lipid, cholesterol, triglyceride and glycogen

Table (6) showed a significant increase in liver glycogen and cholesterol ($P<0.001$) and a significant decrease in liver triglyceride and total lipid ($P<0.01$) in control (+ve). The drug group, basil methanol extract and basil aqua extract rat groups showed a significant increase in liver cholesterol ($P<0.05$) and a significant decrease in liver triglyceride and total lipid ($P<0.05$) but basil powder group showed a significant decrease in liver triglyceride ($P<0.05$) compared with control (-ve).All treated groups showed a significant decrease in liver glycogen and cholesterol and significant increase in liver total lipid compared with control (+ve) rat group.

Table (6): The Mean values ± SD of liver glycogen, cholesterol, total lipids and triglyceride of experimental rat groups

Groups Variables	Glycogen (mg/100g)	Cholesterol (mg/g)	Total lipids (mg/g)	Triglyceride (mg/g)
Control (-ve)	40.11± 5.14 [c]	3.67± 0.33 [c]	2.56± 0.88 [a]	6.11± 1.06 [a]
Control (+ve)	55.16± 6.32 [a***]	6.21± 1.13 [a***]	1.13± 0.46 [c**]	4.01± 0.50 [bc**]
drug	43.40± 4.24 [bc]	4.11± 0.55 [b*]	1.78± 0.55 [b*]	4.99± 0.33 [b*]
Basil ethanolic extract	45.51± 5.14 [bc]	4.57± 0.65 [b*]	1.66± 0.43 [b*]	4.57± 0.47 [b*]
Basil aqua extract	44.12± 4.40 [bc]	4.60± 0.53 [b*]	1.59± 0.37 [b*]	4.87± 0.45 [b*]
Basil powder	38.61± 3.22 [bc]	3.55± 0.54 [bc]	2.01± 0.76 [a]	4.55± 0.34 [b*]

Significant with control group * $P<0.05$ ** $P<0.01$ *** $P<0.001$
Mean values in each column having different superscript (a, b, c, d) are significant.

4-Discussion

It is clear that exposure to CCl4 can cause cellular damages through metabolic activation of those compounds to highly reactive substances such as reactive oxygen species. Carbon tetrachloride causes elevation in ALT and AST .The increase in the level of serum transaminase reflects the liver damage as these enzymes are released in the blood circulation after the administration of hepatotoxin as carbon tetrachloride (Szymonik-Lesiuk et al., 2003). The toxicity is initiated

by formation of a reactive metabolite trichlormethyl radical by microsomal fixed function oxidase which binds covalently to the macromolecules and induces peroxidative degradation of membrane lipids resulting in hepatotoxicity and subsequent increase in serum transaminase (Cabre et al .,2000 and Kandasamy et al., 2010).

The nutritional results were agreed with the fact that basil is a good source of beta carotene, calcium, vitamin C and also contains zinc, manganese and sodium. Volatile oil of basil has estragol, linalool, eugenol, methyl chavicol and small quantities of methyl cinnamate, cineole, and other terpenes, apigenin, luteolin, orientin and vicenin (Samudralwar and Garg 1996). O. sanctum leaves are used in bronchitis, gastric and hepatic disorders. Chewing a couple of leaves before a meal helps to stimulate the appetite and a tea taken after a meal promotes digestion by increasing the flow of gastric juices, while reducing gas and bloating. Leaves of O. sanctum are commonly used in mild indigestion, diminished appetite and malaise (Vats et al., 2004).

The biochemical results were agreed with many previous researches. The Liver enzymatic superoxide dismutase, catalase, glutathione-S-transferase, non-enzymatic antioxidants (reduced glutathione) and lipid peroxidation end product, malondialdehyde levels were significantly modulated by the O. sanctum oil treatment (Geetha and Vasudevan 2004). O. sanctum leaf powder for a month reduced fasting blood sugar, uronic acid ,total amino acids, total cholesterol, triglyceride, phospholipids and total lipids in diabetic rats indicating the hypoglycemic and hypolipidemic effect of O. sanctum (Rai et al., 1997). Fresh leaves lowered total cholesterol, triglyceride, phospholipid and LDL-cholesterol levels and increased HDL-cholesterol in rabbits (Sarkar et al., 1994). O. sanctum water and alcohol leaf extracts showed a significant ability to scavenge free radicals. Both extracts and their fractions inhibit in vitro lipid peroxidation at very low concentrations. In vivo, lipid peroxidation was also inhibited by aqueous extracts of O. sanctum in a dose-dependent manner in male albino rabbits (Suanarunsawat et al., 2010). O. sanctum for 15 days in mice decreased hepatic lipid peroxidation and glucose-6-phosphatase activity, while the activities of endogenous antioxidant enzymes, SOD and CAT were increased (Chintalwar and Chattopadhyay 2005). O. sanctum was found to lower cholesterol, lactate dehydrogenase and alkaline phosphatase levels without affecting blood glucose and urea levels in rats. Ethanolic O. sanctum leaf extract inhibits oxidative stress by modulating xenobiotic-metabolizing enzymes, reducing the extent of lipid and protein oxidation and up-regulating antioxidant defenses (Vats et al., 2004).

On the basis of above results it may be inferred that basil exhibited significant anti hepatotoxic activity

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Effect of thyme powder, extract and oil on carbon tetrachloride-induced liver injury

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Abstract: Forty -two albino male rats, Sprague Dawley strain were randomly classified into six groups (7 rats each). One served as control (-ve) group while the other groups were administered CCL4 to induce liver injury which were control (+ve), silymarin, thyme powder, thyme extract and thyme oil rat groups. The results showed that control (+ve) rat group showed a significant decrease in final body weight , body weight gain ,food efficiency ratio (FER) , blood hemoglobin, packed cell volume & glutathione (GSH) , serum total protein & globulin and liver GSH , superoxide dismutase (SOD), glutathione peroxidase (GPX) ,glycogen and triglyceride. Moreover , showed a significant increase in blood malondialdehyde (MDA) ,serum alanine and aspartate aminotransferase , alkaline phosphates, gamma glutamyle peptidase (ALT, AST**,** ALP & GT) enzymes activity ,total bilirubin ,A/G ratio and liver MDA, cholesterol and total lipid compared with control (-ve) group. Silymarin showed a significant decrease in final weight, hemoglobin, blood GSH, liver GSH, SOD & glycogen and a significant increase in serum AST& MDA and liver cholesterol. Thyme powder showed a significant decrease in final weight, blood GSH & MDA and liver GSH ,SOD and glycogen and a significant increase in serum ALT, AST**,** ALP and GT enzymes activity but thyme oil showed a significant decrease in hemoglobin ,liver glycogen and significant increase in the values of liver cholesterol compared with control (-ve) group. The all treated rat groups showed a significant increase in serum total bilirubin, A/G ratio and liver MDA, triglyceride & total lipid and a significant decrease in body weight gain ,FER, serum globulin and liver GPX compared with control (-ve) group.
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1. Introduction

The liver is the most important organ in terms of biochemical activity in the human body. The liver has a great capacity to detoxify and synthesize useful substances. There are several characteristic pathologies in the livers of patients with liver disease including fatty liver, hepatitis, hepatocirrhosis and liver cancer. Liver fibrosis is the common end stage of most chronic liver diseases regardless of the etiology the early stage of liver fibrosis can be reversed, while liver cirrhosis cannot (Achliya et al., 2004 and Bataller and Brenner, 2005).

Herbs treatments are safe because they are natural and fit into the image of a gentle harmless alternative to conventional medicine. Silymarin is known as milk thistle or Silybum marinum and is a member of the aster family that has been used as a medicinal plant since ancient times. Silymarin might exert beneficial effects in chronic liver diseases through antifibrotic properties. Silymarin interferes with leukotriene formation in Kupffer cell cultures and may thereby inhibit hepatic stellate cell activation, which is a crucial event in fibrogenesis (Stickel and Schuppan 2007 and Cecilia et al., 2009).The valuable medicinal properties of different plants are due to presence of several constituents such as saponines, tannins, alkaloids, alkenyl phenols, glycoalkaloids, flavonoids,

sesquiterpenes lactones, terpenoids and phorbol esters. Among them some are act as synergistic and enhance the bioactivity of other (Kaefer and Milner 2008). Essential oils are natural products extracted from vegetable materials, which can be used as antibacterial, antifungal, antioxidants, and anti-carcinogenic agents or to preserve and give specific flavors to foods (Kruma et al., 2008).

Thyme (*Thymus vulgaris L.*) is belonging to the Lamiacea family and is aromatic native herbs in the Mediterranean region. The leafy parts of thyme and its essential oil have been used in foods for the flavour, aroma and preservation so added to meat, fish and food products and also used as herbal medicinal products. Thymol and carvacrol displayed a concentration dependent antioxidant capacity (Undeger. et al., 2009). The main constituents of essential oil extracted from thyme were borneol, thymol , carvacrol methyl ether , camphene , -humulene and carvacrol (Bounatirou et al., 2007 and Amarowicz et al., 2008). The present investigation was undertaken to study the effect of thyme leaves in form of powder, extract and oil on carbon tetrachloride-induced liver injury.

2. Material and Methods
I-Materials:
1-Carbon tetrachloride (CCL4) and silymarin:

Carbon tetrachloride was obtained from SIGMA Company for Pharmaceutical Industries. The calculated dose for inducing rats liver injuriy was 0.5 ml/rat, administered by back subcutaneous injection according to Moritz and Pankow ,(1989).Silymarin drug was obtained from (CID) Chemical Industries Development. Each capsule contains 140 mg of silymarin and given to rats in dose 50 mg/kg body weight of rats mixed in standard diet (Mourelle et al., 1989).

2- Thyme plant:

Thyme plant *(Thymus Vulgaris)* was obtained from local market. Then dried in dry freezer and crushed into powder then added to standards diet in 5% in substitution of fiber.

3-Experimental rats

Forty two Sprague Dawley strain male rats were purchased from Helwan Farm of Laboratory Animals. The average weight was 110±8 g. The standard diet was performed according to Nelson (2000).

II-Methods:

1- Preparation of thyme extract and thyme oil:

Thyme extract and thyme oil were prepared from thyme powder according to Charles et al., (1993) and Radwan, (1978), respectively. Thyme extract was given to rats at dose 200 mg/kg b.w rat and thyme oil at dose 0.5 ml/kg b.w rat by stomach tube daily.

2-Experimental design:

After adaptation period, rats were divided into one group served as contol (-ve) rat group and five CCL4 induced liver injury groups which were control (+ve) and four treated groups which were silymarine, thyme powder, thyme extract and thyme oil rat groups. During the study period (8 weeks), the daily food intake and weekly body weight gain were recorded. Rats were sacrificed to otain blood and liver. Heparenized blood was used for estimation of hemoglobin, packed cell volume, malondialdehyde

(MDA) and glutathione (GSH) according to Drabkin (1949), Mc Inory (1954), Yagi (1987) and Ellman (1958), respectively. Serum aminotransferase (ALT, AST), alkaline phosphates enzymes activity (ALP), gamma glutamyle peptidase (GT), total protein and albumin, were estimated according to Reitman and Frankel (1957), Kind and King (1954), Henry, (1974),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). A/G ratio was calculated using albumin and globulin values for each individual sample.Livers of rats were analyzed for estimation of GSH, MDA , superoxide dismutase (SOD), glutathione peroxidase (GPX) , glycogen, cholesterol , total lipids and triglyceride according to Reed (1999), Ohkawa et al .,1979 , Beuchamp and Fridovich (1971), Flohe and Gunzler.(1984), Rerup and Lundquist, (1967) ,Abell et al., (1952)., Folch et al.,(1957) and Young and Pestaner ,(1975) ,respectively.

III -Statistical analysis:

Collected data were subjected to analysis according to SPSS program according to Snedecor and Cochran (1967).

3. Results

The statistical data in table (1) showed a significant decrease in final weight, body weight gain and food efficiency ratio ($p<0.001$, 0.01&0.05) in control (+ve), silymarin and thyme powder rat groups but showed a significant decrease in body weight gain and food efficiency ratio ($p<0.05$) in thyme extract and thyme oil rat groups compared with control (-ve) group. Final weight, body weight gain and food efficiency ratio were significantly increase in the all treated rat groups compared control (+ve) rat group.

Table (1): Mean values ± SD of body weight gain, food intake and food efficiency ratio (FER) of control and CCL4 treated rats.

Groups Variables	Control (-ve)	Control (+ve)	Silymarin drug	Thyme powder	Thyme extract	Thyme oil
Initial. weight(g)	110.36± 4.22 [a]	112.41± 4.41 [a]	112.55± 5.31 [a]	110.33± 5.21 [a]	114.20± 4.32 [a]	115.31± 4.28 [a]
Final weight(g)	205.50± 11.12 [a]	157.62± 12.17 [c**]	188.86± 10.21 [b*]	183.54± 10.34 [b*]	196.65± 11.24 [ab]	197.62± 11.28 [ab]
Weight gain (g)	95.14± 5.44 [a]	45.21± 3.24 [d***]	76.31± 6.11 [c**]	73.21± 5.28 [c**]	82.45± 6.27 [bc*]	85.31± 6.60 [b*]
Food intake(g/w)	16.25± 1.71 [a]	14.31± 1.35 [a]	15.33± 1.21 [a]	15.11± 1.40 [a]	15.67± 1.22 [a]	15.11± 1.41 [a]
FER	0.097± 0.002 [a]	0.052± 0.001 [c***]	0.082± 0.003 [b*]	0.080± 0.001 [b*]	0.087± 0.004 [b*]	0.088± 0.002 [b*]

Significant with control group * $P<0.05$ ** $P<0.01$ *** $P<0.001$
Mean values in each raw having different superscript (a, b, c, d) are significant.

Table (2) showed a significant decrease in hemoglobin, packed cell volume and GSH (p<0.01&0.001) and a significant increase in MDA (p<0.001) the control (+ve) rat group and also showed a significant decrease in hemoglobin and GSH (p<0.05) and a significant increase in MDA (p<0.05) in silymarin rat group compared with control (-ve) group. The value of MDA was significantly increased but GSH was significantly decreased (p<0.05) in thyme powder rat group while the value of hemoglobin was significantly decreased (p<0.05) in thyme oil rat group compared with control (-ve) group. Silymarin and thyme oil rat groups showed a significant increase in GSH and a significant decrease in MDA while thyme powder and extract rat groups showed a significant increase in hemoglobin, packed cell volume and GSH and a significant decrease in MDA compared with control (+ve) rat group.

Table (2): The Mean values ± SD of hemoglobin packed cell volume, glutathione (GSH) and malondialdehyde (MDA) in control and CCL4 treated rats.

Groups Variables	Control (-ve)	Control (+ve)	Silymarin drug	Thyme powder	Thyme extract	Thyme oil
Hemoglobin (g/dl)	$14.10\pm$ 1.67^{a}	$11.36\pm$ 1.51^{c**}	$12.30\pm$ 1.14^{c*}	$13.55\pm$ 1.38^{ab}	$14.21\pm$ 1.11^{ab}	$12.55\pm$ 1.35^{c*}
packed cell volume (%)	$38.60\pm$ 5.40^{a}	$31.82\pm$ 3.81^{b**}	$34.21\pm$ 4.25^{ab}	$37.17\pm$ 5.66^{a}	$40.15\pm$ 6.20^{a}	$33.19\pm$ 3.33^{ab}
GSH (n mol/l cells)	$7.30\pm$ 1.60^{a}	$1.72\pm$ 0.13^{d***}	$5.69\pm$ 1.03^{bc*}	$5.40\pm$ 1.10^{bc*}	$6.50\pm$ 1.08^{ab}	$6.36\pm$ 1.19^{ab}
MDA (nmol/ml cells)	$2.11\pm$ 0.15^{c}	$6.20\pm$ 1.22^{a***}	$3.61\pm$ 0.45^{b*}	$4.14\pm$ 0.77^{b*}	$2.51\pm$ 0.15^{c}	$2.66\pm$ 0.44^{c}

Significant with control group * P<0.05 **   P<0.01 *** P<0.001
Mean values in each raw having different superscript (a, b, c, d) are significant.

Table (3) showed a significant increase in serum ALT, AST, ALP and GT enzymes activity (P<0.001&0.01) in control (+ve) and thyme powder (P<0.05) while AST was significantly increased (P<0.05) in silymarin rat group compared with control (-ve) group. Serum ALT, AST, ALP and GT enzymes activity were significantly decreased in all treated rat group compared with control (+ve) rat group.

Table (3): The Mean values ± SD of serum amino transferase (ALT & AST), gamma glutamyle peptidase (GT) and alkaline phosphatase enzymes (ALP), of control and CCL4 treated rats groups

Groups Variables	Control (-ve)	Control (+ve)	Silymarin drug	Thyme powder	Thyme extract	Thyme oil
ALT (µ /ml)	$22.71\pm$ 3.77^{cd}	$67.61\pm$ 9.11^{a**}	$31.19\pm$ 5.60^{bc}	$38.33\pm$ 6.21^{b*}	$28.40\pm$ 4.11^{c}	$27.37\pm$ 4.31^{c}
AST (µ /ml)	$36.36\pm$ 4.22^{c}	$78.99\pm$ 9.62^{a***}	$43.87\pm$ 4.40^{b*}	$44.30\pm$ 6.01^{b*}	$40.17\pm$ 5.55^{bc}	$41.31\pm$ 6.24^{bc}
ALP (µ /ml)	$40.21\pm$ 4.14^{c}	$83.11\pm$ 8.71^{a***}	$38.67\pm$ 5.61^{c}	$45.11\pm$ 4.21^{b*}	$42.71\pm$ 3.21^{c}	$39.99\pm$ 4.01^{c}
GT (µ /ml)	$5.14\pm$ 1.16^{c}	$12.14\pm$ 2.18^{a***}	$7.15\pm$ 1.24^{bc}	$8.22\pm$ 1.55^{b*}	$6.96\pm$ 1.65^{c}	$6.59\pm$ 1.71^{c}

Significant with control group * P<0.05 **   P<0.01 *** P<0.001
Mean values in each raw having different superscript (a, b, c, d) are significant

Table (4) showed a significant increase in serum total bilirubin and A/G ratio (P<0.001) and a significant decrease in serum total protein and globulin (P< 0.01) in control (+ve) rat group. The all treated rat groups showed a significant increase in serum total bilirubin and A/G ratio and a significant decrease in serum globulin (P<0.05) compared with control (-ve) group but showed a significant decrease in serum total bilirubin and A/G ratio and a significant increase in serum total protein and globulin compared with control (+ve) group .

Table (4): The Mean values ± SD of serum bilirubin, total protein, albumin, globulin and albumin/globulin ratio (A/G) of control and CCL4 treated rats groups

Groups Variables	Control (-ve)	Control (+ve)	Silymarin drug	Thyme powder	Thyme extract	Thyme oil
Bilirubin (mg/dl)	0.55± 0.61 [c]	1.87± 0.18 [a***]	0.95± 0.05 [b*]	1.01± 0.12 [b*]	0.89± 0.02 [b*]	0.99± 0.01 [b*]
T.protein (g/dl)	7.57± 1.31 [a]	5.17± 0.88 [b**]	6.67± 1.12 [a]	6.11± 1.19 [a]	6.50± 1.05 [a]	6.49± 0.88 [a]
Albumin (g/dl)	3.49± 0.40 [a]	3.02± 0.33 [a]	3.21± 0.44 [a]	2.95± 0.28 [ab]	3.21± 0.48 [a]	3.13± 0.57 [a]
Globulin (g/dl)	4.04± 0.57 [a]	2.15± 0.23 [c**]	3.46± 0.36 [b*]	3.16± 0.38 [b*]	3.29± 0.55 [b*]	3.36± 0.44 [b*]
A/G ratio	0.86± 0.07 [c]	1.40± 0.13 [a***]	0.92± 0.05 [b*]	0.93± 0.03 [b*]	0.97± 0.02 [b*]	0.93± 0.01 [b*]

Significant with control group * $P<0.05$ ** $P<0.01$ *** $P<0.001$
Mean values in each raw having different superscript (a, b, c, d) are significant.

The data in table (5) showed a significant decrease in the values of liver GSH SOD and GPX ($P<0.001$) and a significant increase in MDA in control (+ve), silymarin and thyme powder rat groups ($P<0.05$ & 0.01) but showed a significant decrease in the values of liver MDA and a significant decrease in GPX in thyme extract and oil rat groups ($P<0.05$) compared with control (-ve) group. Liver GSH SOD and GPX were significantly increased but liver MDA was significantly decreased in all treated rat groups compared with control (+ve) rat group.

Table (5): The Mean values ± SD of some liver glutathione (GSH), malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GPX) in control and CCL4 treated rats

Groups Variables	Control (-ve)	Control (+ve)	Silymarin drug	Thyme powder	Thyme extract	Thyme oil
GSH(mg/g)	9.11± 2.14 [a]	3.17± 0.41 [d***]	6.71± 1.11 [b*]	5.99± 0.78 [bc*]	7.17± 1.20 [ab]	7.11± 1.14 [ab]
MDA(mmol/g)	30.21± 4.41 [d]	100.14± 20.14 [a***]	55.35± 6.17 [b**]	52.16± 5.20 [b**]	48.17± 7.11 [bc*]	45.33± 5.21 [bc*]
SOD(µ/mg)	41.87± 5.11 [a]	20.14± 3.21 [d***]	33.21± 4.55 [c*]	31.81± 3.29 [c*]	37.14± 6.36 [ab]	35.22± 4.33 [abc]
GPX(µ/mg)	60.61± 7.67 [a]	25.67± 3.14 [d***]	35.47± 4.11 [c**]	40.21± 4.57 [b*]	45.41± 5.12 [b*]	46.32± 6.14 [b*]

Significant with control group * $P<0.05$ ** $P<0.01$ *** $P<0.001$
Mean values in each raw having different superscript (a, b, c, d) are significant.

The data in table (6) showed a significant decrease in the values of liver glycogen and triglyceride and a significant increase in the values of liver cholesterol and total lipid in control (+ve) at $P<0.01$ & 0.001 , silymarin and thyme oil at $P<0.01$ while showed a significant decrease in the values of liver glycogen and triglyceride and a significant increase in the value of liver total lipid ($P<0.05$) in thyme powder rat group but a significant decrease in the values of liver triglyceride($P<0.01$) and a significant increase in the values of liver in thyme extract total lipid ($P<0.05$) when compared with control (-ve) group. All treated rat groups showed a significant increase in the values of liver glycogen and triglyceride and a significant decrease in the values of liver cholesterol and total lipid compared with control (+ve) rat group.

Table (6): The Mean values ± SD of liver glycogen, cholesterol, total lipids and triglyceride in control and CCL4 treated rats

Groups Variables	Control (-ve)	Control (+ve)	Silymarine drug	Thyme powder	Thyme extract	Thyme oil
Glycogen (mg/100g)	5.40± 0.44[a]	2.11± 0.14[c**]	4.33± 0.69[b*]	4.81± 0.87[b*]	5.18± 0.68[ab]	4.21± 0.77[b*]
Cholesterol (mg/g)	4.11± 0.48[c]	6.98± 1.11[a***]	5.02± 0.13[b*]	4.55± 0.22[c]	4.36± 0.44[c]	5.11± 0.38[b*]
Total lipids (mg/g)	35.81± 3.01[c]	52.78± 6.01[a***]	45.31± 5.21[b*]	42.16± 5.19[b*]	39.71± 4.29[b*]	44.11± 5.21[b*]
Triglyceride (mg/g)	4.11± 0.58[a]	2.35± 0.11[c**]	3.21± 0.22[b*]	3.17± 0.37[b*]	2.98± 0.18[b**]	3.11± 0.45[b*]

Significant with control group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each raw having different superscript (a, b, c, d) are significant.

4-Disscusion

It is well known that carbon tetrachloride (CCl4) has been widely used in animal models to investigate chemical toxin-induced liver injury. The most remarkable pathological characteristics of CCl4-induced hepatotoxicity are steatosis, fatty liver, cirrhosis and necrosis (Lee. et al 2005). The CCl4 produced damage to liver cells and was followed by the significant increase in serum alanine aminotransferase ALT activity and hepatic lipid peroxidation after 24 h. Increased lipid peroxidation is a mechanism which is commonly suggested to explain the progression of liver damage and the development of fibrosis, and eventually cirrhosis in experimental animals and in alcoholic liver disease (Goldani et al., 2007).Experimental studies demonstrated antioxidant and free radical scavenging properties, improvement of the antioxidative defence by prevention of glutathione depletion, and antifibrotic activity (Basu 2003).

According to several early studies, silymarin has hepatoprotective properties. Silymarin prevents of free-radical damage, stabilization of plasma membranes, and stimulation of new liver cell production (Jacobs et al., 2002). Silymarin acts as an antioxidant and free-radical scavenger that is many times more potent than vitamin E and has also been shown to inhibit lipid peroxidation and to prevent glutathione depletion induced by liver toxins, even increasing total glutathione levels in the liver by 35% over controls. Silymarin has the ability to stimulate protein synthesis resulting in production of new liver cells to replace older and damaged ones (Valenzuela et al. 1985 and Cecilia et al., 2009).

Thymus vulgaris L. has a large number of flavonoids and vitamin E. The main phenolic compounds in thyme are glycuronids of apigenin, luteolin, eriodyctiol, luteolin glycosides, rosmarinic

acid and quercitine. These extracts from different parts of leaves and flowers of this plant are of interest as flavourings, as well as being natural antioxidants for the food industry (Justesen, 2000) Serum hemoglobin concentration was taken as response parameters for the bioavailability of iron. Dry thyme was particularly rich in iron. Iron intake and total iron absorption were highest for the rats fed the dry thyme diet (Abu Jadayil et al., 1999).

The essential oil of thyme plant had harmless effect on liver and kidneys tissues because of inducing little changes in aminotransferase activity in rat plasma and desirable changes in total cholesterol (Hazzit et al., 2006 and Nadia and Nadia 2008). Dietary supplementation of thyme extract to rats was able to reduce the acute hepatotoxicity caused by carbon tetrachloride. In particular, rats fed with thyme essential oil showed higher activities of liver endogenous enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase , as well as an increase of total antioxidant status compared to the control group (Vitaglione et al., 2004). There were significant declines in the superoxide dismutase activities and liver glutathione peroxidase increased significantly in the liver of old rats. There were also significant declines in the total antioxidant status in each tissue examined. A general feature of these various antioxidant parameters measured was that their activities remained higher in rats whose diets were supplemented with thyme oil because of a more favourable antioxidant capacity during their life span (Kuresh et al., 1999 and Kruma et al., 2008).

Thymol inhibited the non-enzymatic lipid peroxidation of normal mice liver homogenate. thymol protects the liver against CCl4 -induced toxicity and the protection may be mediated through its ability to inhibit lipid peroxidation. Thymol acted as a free

radical scavenger of lipid peroxidation in vitro (Aeschobach et al., 1989 and Alam et al., 1999). Volatile oil from thyme was overall the most effective in this protective capacity by reversing the normal trend in polyunsaturated fatty acid metabolism during aging wherein a decrease in levels is concomitant with a reduction in tissue function and integrity (Deans et al., 1993, Youdim and Deans 2000 and Dapkevicius et al., 2002).

Finally the aforementioned results recommended thyme leaves can protect the injury of liver.

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Fruit physical and chemical characteristics at maturity stage of Tommy Atkins, Keitt and Kent mango cultivars grown under Nubariya conditions

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Abstract: The present study was conducted at orchard located at the side of Alexandria desert road (Cairo - Alexandria, Km. 140), at Nubariya region during 2007 and 2008 seasons. The three experimented mango cultivars were Tommy Atkins, Kent and Keitt. The trees of the three studied cultivars are grafted on seeded rootstocks, attained nine years old and grown in sandy Soil .Trees of all mango cvs. Were planted at 3×5 meter apart and subjected to the same horticultural practices .The objective of the present study is evaluating some fruit physical and chemical characteristics at maturity stage of Tommy Atkins, Kent and Keitt mango cultivars grown under Nubariya conditions. The obtained results can be summarized as follows: The observation besides the analysing of some physical and chemical characteristics of the fruits indicated that the physiological maturity was attained in Tommy Atkins cvs. at fruit age 113 day, Kent and Keitt cvs. at fruit age 122 day. Keitt cv. had the highest values of pulp percentage of weight followed by Kent cv. while, Tommy Atkins cv. had the lowest values of pulp percentage of weight. Meanwhile Tommy Atkins cv. had the highest percentage of peel of weight followed by Kent cv., while Keitt cv. had the lowest percentage of peel of weight. Tommy Atkins cv. had the highest percentage of seed of weight followed by Kent cv. while, Keitt cv. had the lowest percentage of seed of weight. Keitt cv. had the highest seed length, followed by Tommy Atkins cv. while, Kent cv. had the lowest values in the two seasons. The highest seed width was Tommy Atkins cv., followed by Kent cv. meanwhile; Keitt cv. had lowest values in the two seasons. Ascorbic acid (vitamin c) percentage was lowest in Kent cv. compared to Tommy Atkins cv. which had the highest value; Keitt cv. had intermediate values in the two seasons. Moisture percentage was almost the same with no significant difference among cvs. Tommy Atkins cv. had the highest moisture percentage, followed by Keitt cv. while, Kent cv. had the lowest moisture percentage in the two seasons. Kent cv. had the highest dry matter content percentage, followed by Tommy Atkins cv. while, Keitt cv. had the lowest value in the two seasons. Total sugars percentage was highest in Kent cv., followed by Keitt cv. while, Tommy Atkins cv. had the lowest total sugars percentage in the two seasons. Tommy Atkins cv. had the highest crude fiber percentage, followed by Keitt cv. while, Kent cv. had the lowest crude fiber percentage in the two seasons.
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1. Introduction

Mango belongs to the family "Anacardiaceae" and is consumed mainly as a fresh fruit or as a juice. Its nutritional value is great and is considered one of the richest sources of vitamins and mineral salts. Besides it contains enough amounts of carbohydrates and proteins.

There are many factors that influence yield, maturity and quality of fruits the, same cultivar can attains different characteristics in different growing conditions. Even in the same region, different environmental conditions at different years can affect maturity and quality of the fruit. (Devilliers, (1998)).

There were great variations in growth, yield as well as physical and chemical properties of the fruits of various mango cultivars grown in different climactic conditions. Abou - El-Az, (1988); Hussein *et al* (1989); Salem (1993); Abd-El Hameed (1996);

Ahmed *et al* (1998) and El-Masry and Galila Said (1998).

The objective of the present study is evaluating some Fruit physical and chemical characteristics at maturity stage of Tommy Atkins, Ket and Keitt mango cultivars grown under Nubariya conditions.

2. Material and Methods

This study was carried out during two successive seasons (2007 and 2008) on three mango cultivars namely Tommy Atkins, Kent, and Keitt at a private orchard located in Nubariya at Alexandria at desert road (km. 140 Cairo – Alex), Beheira governorate, Egypt. The selected trees were about nine years old, budded on seedling rootstocks and planted at 3×5 meters apart, in sandy soil and irrigated by drip irrigation system. Samples of 9 fruits per replicate (9×3 = 27 fruits for each cultivar) were

collected every two weeks. The first fruit sample was collected at fruit age at 77 days in all cultivars. The fruits were collected then washed with tap water, let to dry then put in small plastic boxes in single layer under temperature (25 ± 2 c) and relative humidity (80-85 %) to follow their shrinking. The time of maturity stage was determined by testing some physical and chemical fruit characteristics according to Hussein and Youssef (1972), Subramnyan *et al*. (1975), Mann and Singh (1976), Singh *et al*. (1978), Singh *et al.* (1979) and Roy and Biswas (1985).

1. The physical fruit characteristics
    1. Fruit weight (g.)
    2. Fruit volume (cm.^{3})
    3. Specific gravity (g/cm.^{3})
    4. Flesh firmness (lb/inch^2)
    5. Fruit length (cm.)
    6. Fruit width (cm.)
    7. Peel colour by using colour chart.
    8. Pulp colour by using colour chart**.**
2. The chemical fruit characteristics
    1. Total soluble solids (T.S.S) by using a hand refractometer.
    2. Total Acidity (%) (A.O.A.C, 1985).
    3. T.S.S /Acid ratio.
Fruit characteristics at maturity stage
Nine mango fruits of each mango cultivar at maturity stage were used.
a. The physical characteristics
    1. Pulp weight (%)
    2. Seed weight (%)
    3. Peel weight (%)
    4. Seed length (cm.)
    5. Seed width (cm.)
b. The chemical characteristics
1. Ascorbic acid content (mg / 100 g. fresh weight) (A.O.A.C,1985).
2. Total sugars content was determined colorimetrically in fruit dry weight according to the method of Smith *et al*. (1956).
3. Fruit crude fibers content was determined using the mothed described in the Official Analytical Chemistry (A.O.A.C, 1985).
4. Moisture and Fruit dry matter content .The flesh of fruit samples was cut into small pieces and dried at (60 - 65 c) for 48 h. the moisture and dry matter percentage were calculated using the following equations :
a. Moisture (%)

$$\frac{\text{Weight before drying} - \text{Weight after drying}}{\text{Weight before drying}} \times 100$$

b. Fruit dry matter content (%)

$$\frac{\text{Average dry weight (g.)}}{\text{Average fresh weight (g.)}} \times 100$$

Statistical analysis and comparison among means were made using L.S.D. test at 5 % level according to Steel and Torrie (1980).

3. Results and Discusstion
    Data illustrated in (Table 1)show from the reliable indicators for determining fruit maturity in the three mango cultivars grown under Nubariya region conditions in 2007 and 2008 seasons that the physiological maturity was attained in Tommy Atkins cvs. at fruit age 113 day, Kent and Keitt cvs. at fruit age 122 day.

    Keitt cv. had the highest fruit weight and volume, followed by Kent cv., while Tommy Atkins cv. had the lowest fruit weight and volume. The highest value of specific gravity was found in Keitt cv., while Tommy Atkins cv. had the lowest specific gravity in the two seasons. Keitt cv. had the highest fruit length, followed by Tommy Atkins cv., while Kent cv. had the lowest fruit length. Keitt cv. had the highest fruit width, followed by Kent cv., while Tommy Atkins cv. had the lowest fruit width in the two seasons.

    Peel colour of Tommy Atkins at maturity stage was "Moderate yellow green" + one side colour "dark red" in the two seasons. While, peel colour of Kent fruits was "Moderate yellow green" and peel colour of Keitt fruits was "Moderate yellow green". Pulp colour of Tommy Atkins at maturity stage was "Vivid yellow" While, pulp colour of Kent fruits was "Strong yellow." and pulp colour of Keitt fruits was "Vivid yellow" in the two seasons.

    Total soluble solids percentage was the best in Kent cv. followed by Keitt cv. while, it was relatively low in Tommy Atkins cv. in the two seasons. Kent cv. had the lowest total acidity value followed by Keitt cv. Tommy Atkins cv. had the highest value in the two seasons. Kent cv. had the highest T.S.S / Acid ratio, followed by Keitt cv. while, Tommy Atkins cv. had the lowest value in the two seasons.

1. Physical characteristics at maturity stage
a. Percentage of pulp, peel and seed of fruit fresh weight
    Data in Table (2) show percentage of pulp, peel and seed of fruit fresh weight of the three mango cultivars grown under Nubariya region conditions in 2007 and 2008 seasons.
Percentage of pulp, peel and seed of fruit fresh weight varied significantly according to mango cvs. in two studied seasons.
    Keitt cv. had the highest percentage of pulp of fruit fresh weight (81.60 % in 2007 season and 82.68 % in 2008 season) followed by Kent cv. (77.47 % in 2007 season and 76.27 % in 2008 season) while,

Tommy Atkins cv. had the lowest percentage of pulp (71.70 % in 2007 season and 71.49 % in 2008 season). Meanwhile Tommy Atkins cv. had the highest percentage of peel of fruit fresh weight (15.82 % in 2007 season and 16.11 % in 2008 season) followed by Kent cv. (11.86 % in 2007 season and 12.24 % in 2008 season), while Keitt cv. had the lowest percentage of peel of fruit fresh weight (8.68 % in 2007 season and 7.47 % in 2008 season).

Tommy Atkins cv. had the highest percentage of seed weight (12.48 % in 2007 season and 12.40 % in 2008 season) followed by Kent cv. (10.67 % in 2007 season and 10.30 % in 2008 season), while, Keitt cv. had the lowest percentage of seed weight (9.72 % in 2007 season and 9.85 % in 2008 season).

The above results agree with that obtained by Hussein and Youssef (1972), Hassan *et al*. (2004) and Kudachikar *et al*. (2003) they found that the great

variation in percentage of pulp, peel and seed of fruit fresh weight differed according to mango cultivar.

b. Seed length and width

Data in Table (2) show seed length and width. of the three mango cultivars grown under Nubariya region conditions in 2007 and 2008 seasons. Seed length and width varied significantly according to mango cvs. in two studied seasons. Keitt cv. had the greatest seed length (10.72 cm. in 2007 season and 10.42 cm. In 2008 season) followed by Tommy Atkins cv. (9.77 cm. in 2007 season and 9.48 cm. in 2008 season) while, Kent cv. had the lowest seed length (8.79 cm. in 2007 season and 8.73 cm. in 2008 season).

The highest seed width was recorded in Tommy Atkins cv. (4.41 cm. in 2007 season and 4.35 cm. in 2008 season) followed by Kent cv. (4.26 cm. in 2007 season and 4.20 cm. in 2008 season) meanwhile, Keitt cv. had the lowest seed width (4.13 cm. in 2007 season and 4.12 cm. in 2008 season).

Table 2 . Fruit physical characteristics in maturity stage of the three mango cultivars grown under Nubariya region conditions in 2007 and 2008 seasons.

Cultivars	characteristics				
	Pulp weight (%)	Peel weight (%)	Seed weight (%)	Seed length (%)	Seed width (cm.)
2007 season					
Tommy Atkins	71.70 c	15.82 a	12.48 a	9.77 b	4.41 a
Kent	77.47 b	11.86 b	10.67 b	8.79 c	4.26 ab
Keitt	81.6 a	8.68 b	9.72 c	10.72 a	4.13 b
2008 season					
Tommy Atkins	71.49 c	16.11 a	12.40 a	9.48 b	4.35 a
Kent	79.27 b	12.24 b	10.30 b	8.73 c	4.20 ab
Keitt	82.68 a	7.47 c	9.85 c	10.42 a	4.12 b

Means followed by the same letter (s) in each column are insignificantly at 5% level.

2. Chemical characteristics
a. Ascorbic acid (v.c.)

Data in Table (3) show ascorbic acid (vitamin c) percentage in fruit juice of the three mango cultivars grown under Nubariya region conditions in 2007 and 2008 seasons. Ascorbic acid (vitamin c) percentage varied significantly according to mango cvs. The maximum value was detected in the pulp of mango cv. Tommy Atkins (44.89 % in 2007 season and 40.80 % in 2008 season) in the two seasons, followed by Keitt cv. (41.42 % in 2007 season and 38.60 % in 2008 season) while, Kent cv. had the lowest ascorbic acid content (vitamin c) percentage (37.70 % in 2007 season and 35.99 % in 2008 season).

The results are in full agreement with those of others, who found that vitamin c percentage in fruits of some mango cvs. increased up to maturity, thereafter decreased at ripe stage. Ibrahim *et al.* (1985) reported that the vitamin c fruit content was higher in the fruits of Zebda, Misk and Taimour mango cvs. (at ripe stage) compared with that in fruits of Dabsha, Mabrouka, Alphonso, Bullock's Heart, Hindy Sennara and Baladi varieties. Tawfik (2003) found that the vitamin c content of Tommy Atkins mango cv. was the highest at maturity stage (58.53 mg. / 100 g.fr.w) followed by Keitt mango cv. (48.13 mg /100 g.fr.w) and Ewais mango cv. (45.40 mg. /100g.fr.w), while the lowest vitamin c content was found in Sediek mango cv. (32.60 mg. / 100 g.fr.w). Many works such as Said and El-Masry

(1992), Sharma *et al*. (1999), Mitra *et al*. (2000) and Tawfik (2003) reported that the great variation in

vitamin c content differed according to mango cultivar.

Table 3 . Fruit chemical characteristics in maturity stage of the three mango cultivars grown under Nubariya region conditions in 2007 and 2008 seasons.

Cultivars	characteristics				
	Ascorbic acid (mg. /g. F.wt.)	Total sugars (%	Fiber (%)	Moistue (%)	Fruit Dry Matter (%)
2007 season					
Tommy Atkins	44.89 a	6.70 c	1.18 a	79.78	19.89 b
Kent	37.70 c	9.23 a	0.75 c	77.55	22.45 a
Keitt	41.42 b	8.16 b	0.86 b	79.00	21.00 ab
2008 season					
Tommy Atkins	4.80 a	7.38 c	1.13 a	80.11 a	19.89 b
Kent	35.99 b	9.50 a	0.71 c	75.00 b	25.00 a
Keitt	38.60 ab	8.77 b	0.88 b	79.55 a	20.45 b

Means followed by the same letter (s) in each column are insignificantly at 5% level.

b. Pulp moisture percentage

Data in Table (3) show pulp moisture percentage of the three mango cultivars grown under Nubariya region conditions in 2007 and 2008 seasons. Moisture content percentage varied significantly according to mango cvs. Tommy Atkins cv. had the highest moisture content percentage (79.78 % in 2007 season and 80.11 % in 2008 season) in the two seasons, followed by Keitt cv. (79.00 % in 2007 season and 79.55 % in 2008 season) while, Kent cv. had the lowest moisture content percentage (77.55 % in 2007 season and 75.00 % in 2008 season).

These results are in full agreement with those found by Sobeih and El-Helaly (2002a), they found that the Mabrouka mango cv. fruits recorded the highest moisture percentage in two seasons at harvest stage. Tawfik (2003) reported that the fruit moisture percentage in Tommy Atkins mango cv. was higher than in Keitt cv. at maturity stage (78.3 % and 76.7 % respectively) followed by Sediek cv. (76.44 %), while Ewais mango cv. showed the least value (75.46 %). Sobeih and El-Helaly (2002a) and Tawfik (2003) reported that the great variation in moisture percentage differed according to mango cultivar.

c. Fruit dry matter content percentage.

Data in Table (3) show fruit dry matter content percentage of the three mango cultivars grown under Nubariya region conditions in 2007 and 2008 seasons.

Fruit dry matter content percentage varied significantly according to mango cvs. Kent cv. had the highest dry matter content percentage (22.45 % in 2007 season and 25.00 % in 2008 season) in the two

seasons, followed by Tommy Atkins cv. (19.89 % in two seasons) while, Keitt cv. had the lowest dry matter content percentage in the two seasons (21.00 % in 2007 season and 20.45 % in 2008 season). These results are in full agreement with those found by Sobeih and El-Helaly (2002b) they found that Zebdda fruits recorded the highest value of dry matter at maturity stage followed by Hindy Besinnara cv. while Mabrouka cv. recorded the least value. The same sequence was observed in ripe fruits, 5 days after maturity. Tawfik (2003) found that the dry matter was the highest in Ewais mango cv. (24.54 %) at maturity followed by Sediek cv. (23.56 %) and Keitt cv. (23.23 %) while, the lowest was found in Tommy Atkins cv. (21.67 %).

d. Total sugars percentage

Data in Table (3) show total sugars percentage of the three mango cultivars grown under Nubariya region conditions in 2007 and 2008 seasons. Total sugars percentage varied significantly according to mango cvs. Kent cv. had the highest total sugars percentage in the two seasons (9.23 % in 2007 season and 9.50 % in 2008 season), followed by Keitt cv. (8.16 % in 2007 season and 8.77 % in 2008 season) while, Tommy Atkins cv. had the lowest total sugars percentage (6.70 % in 2007 season and 7.38 % in 2008 season).

These results are in agreement with the findings of Ibrahim *et al*. (1985) Sharma *et al*. (1999) they found that the great variation in total sugars percentage differed according to mango cultivar.

e. Crude fiber percentage

Data in Table (3) show crude fiber percentage of the three mango cultivars grown under

Nubariya region conditions in 2007 and 2008 seasons.

Crude fiber percentage varied significantly according to mango cvs. Tommy Atkins cv. had the highest crude fiber percentage in the two seasons (1.18 % in 2007 season and 1.13 % in 2008 season), followed by Keitt cv. (0.86 % in 2007 season and 0.88 % in 2008 season) while, Kent cv. had the lowest crude fiber percentage (0.75 % in 2007 season and 0.71 % in 2008 season).

The results confirmed the finding of El-Masry (2001) who reported that the percentage of crude fiber of mango fruits was high in some seeded clones and low in other. Sobeih and El-Helaly (2002b) found differences in fruit content of crude fiber percentage among the studied mango cultivars. The highest crude fibers percentage was recorded in Misk mango fruits.

Table 1. Reliable indicators for determining the fruit maturity in the three mango cultivars grown under Nubariya region conditions in 2007 and 2008 seasons.

Cultivars	Characteristics											
	Fruit age (days)	Fruit weight (g)	Fruit volume (cm^3)	Specific gravity (g/cm.^{3})	Flesh firmness s(Lb/inch^2)	Fruit length (cm.)	Fruit width (cm.)	Peel colour	Pulp colour	T.S.S. %	Total Acidity (%)	T.S.S./ Asid ratio
2007 season												
Tommy Atkins	113	443.86	437.75	1.013	33.19	12.48	7.42	Moderate yellow green + dark red	Vivid yellow	8.97	1.31	6.89
Kent	122	478.43	468.27	1.021	26.28	10.22	9.12	Moderate yellow green	Strong yellow	10.23	1.01	10.14
Keitt	122	586.65	573.58	1.022	35.97	12.82	10.41	Moderate yellow green	Vivid yellow	9.85	1.14	8.65
2008 season												
Tommy Atkins	113	433.65	427.34	1.014	33.3	12.32	8.27	Moderate yellow green + dark red	Vivid yellow	8.71	1.37	6.34
Kent	122	45.65	450.65	1.021	26.73	10.10	9.01	Moderate yellow green	Strong yellow	10.14	1.00	10.11
Keitt	122	565.14	565.14	1.022	35.95	12.98	10.43	Moderate yellow green	Vivid yellow	9.70	1.09	8.95

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Effect of Aqueous Extract of Damsissa(*Ambrosia maritima*) on The Biochemical Changes Induced By Potassium Dichromate In Rats

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Abstract: Chromium is a naturally occurring element found in volcanic dust, in earth crust and is widely distributed in air, water, rocks, soil, plants and animals**.** Humans are occupationally, environmentally, or intrinsically (Surgery implants), exposed to high Cr^{6+} concentrations (8.9 mg/m^3, 20mg/L, 890 mg/kg) respectively. The general population may be exposed to Cr $^{6+}$ compounds through inhalation of ambient air , ingestion of water ,or dermal contact with products that contain chromium (VI) compounds such as pressure treated wood**.** The present study aims to evaluate the antioxidant effect of aqueous extract of Damsissa (Ambrosia maritima) against biochemical changes induced by potassium dichromate in rats. The study was conducted on 48 rats which were classified into four equal groups. Group I : untreated animals (control).Group II: Damsissa treated group: rats were orally supplemented with aqueous extract of damsissa at dose of 100 mg/ kg b.wt. for 14days using stomach tube. Group III: Potassium dichromate treated group, animals injected subcutaneously with potassium dichromate at dose of 10 mg/kg b.wt. for fourteen days , then the half number of the animals sacrificed and the remaining animals left without any treatment for seven days(recovery period).Group IV: Combined treatment group: animals were orally administrated with aqueous extract of damsissa by means of stomach tube at dose of 100 mg/kg b.wt. and injected subcutaneous with potassium dichromate at dose of 10 mg/kg for two weeks and the half number of the animals sacrificed and the remaining animals left without any treatment for one week. Six rats from different groups were sacrificed after 14 days and the rest were left for 7 days as a recovery period. The obtained results revealed significant increase in TBARS concentration which was accompanied with significant decrease in GSH content and CAT activity in renal tissue in treated group with potassium dichromate also, significant increase in urea and creatinine was recorded. The serum levels of sodium significantly increased and the levels of potassium significantly decreased as a consequence to the decrease in aldosterone levels. Calcium and estradiol (E2) levels significantly decreased. However, the levels of phosphorous (P), magnesium (Mg) and parathermone hormone (PTH) were significantly increased in animals injected with potassium dichromate. Consecutive administration of aqueous extract of damsissa with potassium dichromate for 14 days revealed significant improvement in the tested parameters. Also, animals injected with potassium dichromate and left without any treatment for one week as a recovery period showed significant improvement in some of the tested parameters. In conclusion, the results demonstrate the protective role of damsissa against oxidative stress and biochemical changes of potassium dichromate.

1. Introduction:

Chromium is a naturally occurring element found in volcanic dust, in earth crust and is widely distributed in air, water, rocks, soil, plants and animals (**Arreola-Mendoza et al.,2006**). The most common forms of chromium in the environment are Cr^0,hexavalent Cr^{6+},or chromate and trivalentCr^{3+},or chromite (**Costa,1997**).Cr^{6+} and Cr^0 are widely used in industrial and chemical processes such as in leather tanning, printing, hair dyes, stainless steel manufacturing, manufacture of pigments, metal finishing (chrome plating),and in wood preservative production (**ATSDR, 2000**). Chromium (VI) compounds also are used in textile dyeing processes,

printing inks, drilling mud's water treatment and chemical synthesis (**HSDB,2003**).In some regions, waste disposal of chromium compounds to the environment contributes to increase its presence and potential toxicity(**Armienta-Hernandez and Rodriguez-Castillo,1995).**

Humans are occupationally (**Proctor et al., 2003**), environmentally (**Kerger et al., 2009**) or intrinsically,(Surgery implants, **Keegan et al.,2008**) exposed to high Cr^{6+} concentrations (8.9 mg/m^3,20mg/L,890 mg/kg) respectively. The general population may be exposed to Cr $^{6+}$ compounds through inhalation of ambient air, ingestion of water, or dermal contact with products that contain

chromium (VI) compounds such as pressure treated wood. People who live near industrial facilities that use chromium(VI) compounds or near chromium waste disposal sites have the greatest potential for exposure (**ATSDR,2000**).Chromium was detected in vegetables, fruits ,grains, cereals, eggs, meat, and fish at concentrations between 20 and 520 μg /kg(**ATSDR,2000**). The main daily dietary intake of chromium was estimated to be less than 0.2 to 0.4μg from air 2.0μg from water and 60μg from food specified color additives may contain chromium as chromates (**ATSDR, 2000**).The organs distribution of hexavalent chromium are more widespread with the kidneys, spleen, liver, lungs and bone accumulating significant concentrations of metal. Blood and tissue samples of human may become highly contaminated by the chromium in needles knives, blenders and other instruments (**WHO, 1988**). It is possible that chromium can accumulate in kidneys and heart as elevated levels have been reported in these organs in chromium platters (**WHO, 1988**).

Important issues in the carcinogenic risk assessment of chromium compounds are whether both trivalent and hexavalent chromium compounds. Workers in chromate production plants where the risk of lung cancer is elevated are exposed to both trivalent and hexavalent chromium compounds(**Gibb and Chen,1989).**

Hexavalent chromium is used in a wide range of industries. Cr-VI from chromate industries and atmospheric emissions contribute to the Cr contamination in the environment (**Banu et al.,2008**).Cr^{6+} compounds are highly toxic. They induced dermatotoxicity, immunotoxicity, neurotoxicity, genotoxicity and carcinogenicity, (**Bagchi et al., 2002**). Nephrotoxicity with increased urinary β-2 microglobulin and acute tubular necrosis has been reported in ferrochromate workers exposed acute and chronically to Cr^{6+} (**Wedeen and Qian, 1991).**

Ambrosia maritima L.(Compositae) an annual herbaceous plant widely distributed throughout the Mediterranean region and Africa. It is well known in Egypt under the name of Damsissa. It acts as antispasmodic, diuretic, and useful in bronchial asthma, spasms, and frequent urination (**Ghazanfar, 1994**). It contains important sesquiterpene lactones and flavonoids which showed molluscicidal effect (**Evans, 1996**).

The most active ingredients of this plant are ambrosin and damsin(**Shoeb and El-Eman,1976**). **Alard et al.,(1991)** fed damsissa leaves to rats as a powder or as an alcoholic extract and did not report any toxicity.Damsissa is not toxic to non target organisms (rats,rabbits,algae and daphnia) **Geerts et al.,**(**1992**). Nowadays, its used in some renal tea due

to it is proved effect in renal colic and expel renal stons(**Saker et al.,2000**).

In view of the increasing interest in the use of medicinal plants as a natural antioxidant. The present study was planned to evaluate the antioxidant effect of aqueous extract of damsissa (*Ambrosia maritima)* against biochemical changes induced by potassium dichromate in rats.

2. Materials And Methods
2.1.1 Chemicals and plant
Potassium dichromate was purchased from Sigma company. Damsissa plant was obtained from the plant farm of the National Organization For Drug Control and Research, Cairo, Egypt. The other chemicals used were in analytical grade.

2.1.2 Aqueous Extract of Damsissa
Fresh damsissa plants were dried in shade, then crushed dried plants by using of (Micro-Feinula-Culotti) MFC. The aqueous extract of damsissa was prepared by dissolving dried plant in deionized distilled water by continuous stirring at room temperature for 24 h. The extract was filtered, evaporated and dryness under vacuum and the residue was stored at 4ºC until use.

2.2.1 Animals
The study was carried out on 48 female albino rats (130-140 g). Rats were obtained from the animal house of the Nuclear Research Center, Egyptian Atomic Energy Authority, Inshas, Egypt. Animals were kept under normal conditions, standard diet and water *ad libtum*.

2.2.2 Experimental design
The animals were classified into four equal groups. Group I : untreated animals (control). Group II: Damsissa treated group: rats were orally supplemented with aqueous extract of damsissa at dose of 100 mg/ kg b.wt. for 14days orally using gastric tube intubation (**Ahmed and Khater,2001**) .Group III :Potassium dichromate treated group: animals injected subcutaneously with potassium dichromate at dose of 10 mg/kg b.wt. for 2 weeks (**Baggett and Berndt,1986**), then the half number of the animals sacrificed and the remaining animals left without any treatment for one week (recovery period).Group IV:Combined treatment group: animals were orally administrated with aqeous extract of damsissa by gavages using stomach tube at dose of 100 mg/kg b.wt. and injected subcutaneous with potassium dichromate at dose of 10 mg/kg for two weeks and the half number of the animals sacrificed and the remaining left without any treatment for one week(recovery period).

Six rats from different groups were sacrificed after 14 days (2 weeks) and the remaining were left for 7 days which represent the recovery period .Blood samples were collected on plain tube to separate the serum and kept frozen at -20 °C until pending the biochemical assays. Kidney was rapidly excised, washed, dried, weighed and homogenized with phosphate buffer pH7.4 and kept frozen until used for the biochemical assays.

2.2.3 Biochemical assays:

Sera samples were collected to determine, urea, creatinine, sodium, potassium, calcium, phosphorous and magnesium colorimetrically using spectrophotometer (Milton Roy Spectronic 1201) using commercial kits purchased from BioMerieux (France). Aldosterone hormone and estradiol (E2) were determined using radioimmunoassay kit purchased from Immunotech A Beckman Coulter Company France. Serum parathyroid hormone (parathermone) (PTH) level was determined using radioimmunoassay kit purchased from Diagnostic Products Corporation (DPC), Los Angeles, California, USA. The day before sacrifice the rats vaginal smear was done to determine the estrous stage so the estradiol level represent the mean levels of eostrous cycle.Lipid peroxidation in kidney tissue was ascertained by the formation of thiobarbituric acid reactive substance (TBARS) according to the method of **Yoshioka et al.,(1979)**. Reduced glutathione content (GSH) and catalase activity (CAT) were determined according to the method described by **Beutler et al.,** (**1963**) and **Bergmeyer et a**l., (**1987**) in renal tissue.

2.3 Statistical analysis

Results were expressed as mean ±standard error (SE) and differences between the groups were determined by ANOVA (One Way Analysis of Variance) according to **Snedecor and Cochran** (**1982**), followed by Duncan multiple rang test (**Duncan,1955**).

3. Results:

As shown in table(1), demonstrated that administration of aqueous extract of damsissa to rats for 14 days showed that the activity of CAT, the content of GSH and the concentration of TBARS in renal tissue were within normal level as compared with the control group. The obtained results showed that treatment with potassium dichromate induced oxidative stress notified by a significant increase in the level of TBARS associated with a significant($P<0.05$) decrease in GSH content and CAT activity as compared to control values throughout the experimental period. Also, as shown in table (2) a significant($P<0.05$)increase was recorded in serum urea and creatinine. Administration of aqueous extract of damsissa to rats during the experimental period caused a significant ameliorative effect in kidney function. The data in table (3) showed that the rats injected with potassium dichromate exhibited a significant decrease in serum potassium and aldosterone levels accompanied with significant($P<0.05$) increase in serum sodium levels as compared to the control group.

Table (1): Effect of aqueous extract of damsissa on lipid peroxidation (TBARS), reduced glutathione (GSH) content and catalase (CAT) activity in renal tissue in different rats groups.

Parameters	Interval time (days)	Groups (n=6)			
		Control	Damsissa	Potassium dichromate	Damsissa +Potassium dichromate
TBARS µmol/g wet tissue	14	17.04±0.66[c]	15.05±1.01[c]	30.37±1.17[a]	21.21±0.19[b]
	Recovery	16.71±1.36[b]	17.72±1.12[b]	26.50±1.16[a]	18.02±1.13[b]
GSH mg/g wet tissue	14	15.21±0.91[a]	15.27±0.20[a]	11.49±0.15[b]	15.53±1.38[a]
	Recovery	15.87±0.59[a]	15.74±0.23[ab]	12.76±0.26[d]	14.27±0.60[ac]
CAT U/g wet tissue	14	17.29±0.30[a]	17.60±0.30[a]	12.93±0.20[c]	15.72±0.13[b]
	Recovery	17.50±0.15[a]	17.08±0.27[ab]	14.79±0.55[c]	16.63±0.28[b]

Data represented as mean ± SE

Means with different superscripts in the same row are significantly different ($P < 0.05$).

Table (2): Effect of aqueous extract of damsissa on serum urea and creatinine levels in different rats groups.

Parameters	Interval time (days)	Groups (n=6)			
		Control	Damsissa	Potassium dichromate	Damsissa +Potassium dichromate
Urea mg/dl	14	48.31±0.24[c]	45.51±0.77[d]	71.20±1.87[a]	54.59±2.66[b]
	Recovery	40.87±1.65[c]	40.08±1.02[c]	51.33±1.11[a]	46.35±1.77[b]
Creatinine mg/ dl	14	0.71±0.01[c]	0.70±0.02[c]	0.91±0.04[a]	0.79±0.03[b]
	Recovery	0.68±0.03[c]	0.65±0.02[c]	0.86±0.01[a]	0.78±0.03[b]

Data represented as mean ± SE
 Means with different superscripts in the same row are significantly different (P < 0.05).

Table (4) showed significant (P<0.05) decrease in calcium and estradiol(E2) levels in animals injected with potassium dichromate as compared to the corresponding control group. Also, data revealed a significant (P<0.05) increase in phosphorous, magnisum and parathermone hormone in animals treated with potassium dichromate.

The data in Tables(3 and 4) showed significant(P<0.05) ameliorative effect in the serum levels of Na$^+$, K$^+$, aldosterone ,Ca,P,Mg,PTH and E2 in the investigated animals treated with aqueous extract of damsissa and potassium dichromate at the same time. Also, the recovery period of potassium dichromate group revealed significant(P<0.05) improvement of some tested parameters but it was more obvious in animals treated with aqueous extract of damsissa.

Table (3): Effect of aqueous extract of damsissa on serum electrolytes levels and aldosterone level in different rats groups.

Parameters	Interval time (days)	Groups (n=6)			
		Control	Damsissa	Potassium dichromate	Damsissa + Potassium dichromate
Na$^+$ mmol/L	14	147.40±0.25[c]	147.43±3.35[c]	193.6±2.09[a]	176.26±1.59[b]
	Recovery	149.48±1.39[c]	150.46±2.75[cb]	176.92±1.59[a]	156.77±1.60[b]
K$^+$ mmol/L	14	5.82±0.29[a]	5.87±0.31[a]	3.69±0.18[c]	4.70±0.41[b]
	Recovery	5.91±0.50[a]	5.71±0.47[a]	3.80±0.22[b]	4.95±0.46[a]
Aldosterone pg/ml	14	297.38±3.75[a]	289.52±3.61[a]	235.95±3.90[c]	270.10±5.99[b]
	Recovery	286.73±2.94[a]	293.60±3.78[a]	272.09±2.57[b]	283.12±4.21[a]

Data represented as mean ± SE
 Means with different superscripts in the same row are significantly different (P < 0.05).

Table (4): Effect of aqueous extract of damsissa on seum levels of calcium(Ca), phosphorous(P), magnesium (Mg), parathermone hormone (PTH) and estradiol hormone (E2)   in different rats groups.

Parameters	Interval time (days)	Groups (n=6)			
		Control	Damsissa	Potassium dichromate	Damsissa +Potassium dichromate
Ca mg/dl	14	7.27±0.28[a]	6.93±0.52[ab]	5.30±0.32[c]	6.23±0.26[b]
	Recovery	6.83±0.26[a]	6.89±0.38[a]	4.47±0.27[c]	5.83±0.25[b]
P mg/dl	14	3.14±0.12[b]	2.97±0.14[b]	3.80±0.23[a]	3.29±0.71[ba]
	Recovery	3.05±0.14[b]	3.10±0.10[b]	3.75±0.27[a]	3.20±0.11[ba]
Mg mg/dl	14	2.23±0.14[b]	2.24±0.17[b]	2.83±0.12[a]	2.30±0.19[b]
	Recovery	2.21±0.12[b]	2.19±0.16[b]	2.69±0.15[a]	2.25±0.12[b]
PTH pg/ml	14	7.89±0.32[c]	8.07±0.40[c]	11.37±0.55[a]	9.19±0.22[b]
	Recovery	8.29±0.19[b]	8.18±0.19[b]	9.68±0.18[a]	8.73±0.27[b]
E2 ng/ml	14	17.81±1.07[ab]	18.78±0.98[a]	13.32±0.52[c]	15.70±0.85[b]
	Recovery	17.90±1.37[a]	17.75±1.19[a]	13.90±0.44[b]	16.41±0.97[a]

Data represented as mean ± SE
Means with different superscripts in the same row are significantly different (P < 0.05).

4. Discussion

The need for economic, less toxic and more common phytoprotective alternative against environmental toxicity, any xenobiotics and heavy metals induced biological hazards to the living cells sequela directed many researches to test different locally available herbs as a natural protective material.

Reactive oxygen species (ROS) produce a wide variety of toxic effects including DNA damage and lipid peroxidation,and therefore the toxic effects of Cr^{6+} may in part be caused by the production of these species(**Arreola-Mendoza et al., 2006**). However, exposure to chromate has been related to oxidative stress due to Cr^{6+} which is a strong inducer of several chromium reactive intermediaries and free oxygen radicals (**Appenroth et al., 2001 and Bagchi et al., 2002**). They provoke the oxidation of macromolecules like DNA and lipids (**Bagchi et al., 1995**) and kidney (**Bosgelmez and Guvendik, 2004**).

The present results indicated significant increase in TBARS in renal tissue as a consequence to potassium dichromate injection. These results became in agreement with (**Arreola-Mendoza et al., 2009**), who reported that owing to the oxidative properties of Cr^{6+} ,its administration led to a rise of lipids oxidative damage visualized by the increase in renal cortical malondealdehyde(MDA) concentration which was evident on day 2 after treatment with potassium dichromate(15mg/kg b.wt.). These indicate that metal such as chromium undergo redox cycling resulting in the production of reactive oxygen species (**Das,2009**). As a consequence of enhanced lipid peroxidation and altered calcium and sulfhydryl homeostasis (**Das et al, 2006).**

Animals administrated with aqueous extract of damsissa and potassium dichromate together had less oxidative damage than that received potassium dichromate alone. These results suggested that aqueous extract of damsissa protect against the oxidative renal damage caused by Cr^{6+}.So, treatment with aqueous extract of damsissa averted oxidative damage probably through its capacity to quickly and efficiently scavenge lipid peroxyl radicals before they attack membrane lipids. In the present study animals left for 7 days after last dose of injection with potassium dichromate without any treatment served as a recovery period in order to follow up the time required to restore the normal levels of the studied parameters. It was observed that TBARS in recovery group decreased. It proved that the renal tissue became capable to regenerate damage tissue. Thus reduction in the TBARS concentrations might be associated to regeneration of renal tissue. The obtained results became in harmony with **Imai et al.,(1996)**, who reported that in a recovering tissue MDA concentration is lower than in a recently injured one.

In biological systems, the soluble forms of Cr^{6+} are absorbed more easily than Cr^{3+} and are reduced to Cr^{3+} via Cr^{5+} by glutathione, ascorbate and hydrogen peroxide (**Aiyar et al., 1991, Stearns et al., 1994**). Once chromium is absorbed, it is distributed in the liver, lung, spleen, kidney, and heart (**Arreola-Mendoza et al.,2006).**

The obtained results recorded significant decrease in GSH content and CAT activity in renal tissue after injection with potassium dichromate. This break down of the GSH and CAT dependent antioxidant defensive system increase the intracellular flux of oxygen free radicals which create an oxidative stress and initiating apoptosis of the cell (**Miesel and Zuber, 1993**). However, GSH is the most abundant non protein sulfhydryl containing compound in plants and constitutes the largest component of the endogenous thiol buffer (**Holmgren et al., 2005**). So, assessment of GSH in biological samples is essential for evaluation of the redox homeostasis and detoxification status of cells in relation to its protective role against oxidative and free radical mediated cell injury (**Rossi et al.,2005**).GSH has diverse cellular functions in addition to its antioxidant properties including enzymatic conjugation through the glutathione-S- transferase family of proteins and nonenzymatic conjugation to cytotoxic compounds. It is kept in its reduced form by the NADPH dependent enzyme, glutathione disulfide reductase. Moreover,GSH may react with H_2O_2 and lipid peroxides by the action of GSH peroxidase to eliminate the reactive intermediates by reduction of hydroperoxides (**Davis et al.,2001**).

In the present study the decrease in GSH content in renal tissue in rats treated with potassium dichromate might be due to enhanced utilization during detoxification process. Also,catalase acts as a preventive antioxidant plays an important role in protection against the deleterious effects of lipid peroxidation(**Pigeolot et al.,1990**). The depletion of GSH content and catalase activity in renal tissue of rats treated with potassium dichromate may be due to the increased utilization of these antioxidants to counter lipid peroxidation production.

Administrations with aqueous extract of damsissa alleviate the production of lipid peroxidation to become nearly the normal levels in renal tissue which supports the view that the plant under the investigation prevents chromate induced depletion of GSH and catalase activity. Phytochemical analyses on *Ambrosia maritima* extract have identified the presence of some coumarins as scopoletin, umbelliferone and isoscopoletin. In addition, other coumarins like isoprimpipinellin, limettin, esculetin and umbelliprenin were also found (**Khalil et al., 1981**). Also, many of the following compounds were isolated from the crude herb include damsin, ambrosin,neoambrosin, parthenin,

hymenin and other pseudoguinolids (**Picman,1986 and Jakupovic et al.,1987).**

Heavy metals are nephrotoxic xenobiotics,that may lead to acute tubular necrosis (**Wang et al.,1994).** Results of the present study revealed that the increase in the degree of injury in kidney caused by potassium dichromate may lead to a significant increase in the resultant urea and creatinine concentration as well as renal TBARS concentration increased and accompanied with significant reduction in GSH content and catalase activity. This result supported by the results of **DE-Ceaurriz and Ban (1991**) who reported that mice administrated with 80 mg/kg b.wt. potassium dichromate revealed damage to about 40-70 % of the proximal tubules of kidney cause renal failure after 8 hours of administration.Previously renal toxicity of Cr^{6+} has been known for a long time (**Bosgelmez and Guvendik,2004**) and its effect as an inducer of oxidative stress has been explored(**Bagchi et al.,1995.**

The increase in urea and creatinine concentration could be considered as an indicator for the elevation of protein catabolic rates (**Guyton.1991**)as well as the depletion of GSH content in renal tissue which enhance utilization of protein resulting in an increase in urea and creatinine levels. However,Cr^{6+} is accumulated in renal cortex(**Wedeen and Qian,1991**) inducing tubular dysfunction(**Wang et al.,1994**) mainly in the proximal tubule(**Arreola-Mendoza et al.,2006**).The elevation in the serum urea and creatinine may be due to injury in the proximal tubular epithelial cells of kidney and sudden fall in glomerular filtration rates(GFR).However, acute renal damage is induced after envieronmental exposure to chromate Cr^{6+} (**Appenroth et al.,2001**).

Animals administrated with aqueous extract of damsissa concurrently with potassium dichromate showed a significant amelioration of the kidney function presented a restoration in both urea and creatinine concentrations almost near the control levels as well as GSH content, CAT activity and TBARS concentration. This may be attributed to the presence of some phytochemicals compounds which had antioxidant properties (**Ahmed and Khater, 2001**).

As a result of treatment with potassium dichromate the transcular absorption of sodium was diminished and urinary losses would also increase (**Arreola-Mendoza et al.,2009**). The abrupt fall of glomerular filtration rate (GFR) could be associated with cells detachment as a result of losses of cell- cell contacts due to obstruction of tubular lumen and changes in ultra filtration pressure (**Arreola-Mendoza et al., 2009**)**.**

The disturbances in Na^+ and K^+ levels induced by potassium dichromate treatment could be attributed to a kind of stress exerted upon the Na^+-K^+ pump mechanism and intern, leads to membrane permeability

imbalance with consequent collapse of calcium homeostasis associated with a decrease in Ca^{2+} level in serum as a result of oxidative stress production as induced in irradiated rats(**Kale and Samul,1987**).The disturbance in Na^+ and K^+ serum levels agitated by potassium dichromate can be elucidated as a kind of stress upon the Na^+ -K^+ pumping mechanism and in turn lead to membrane permeability imbalance.

According to the present finding as illustrated in table (3) the level of aldosterone was significantly decreased under the effect of potassium dichromate treatment. Aldosterone is the principle sodium retaining corticosteroid hormone. It maintains normal fluid balance and circulatory volume. Potassium is also a potent regulator of aldosterone synthesis and its decreased levels decrease aldosterone synthesis where as decreased plasma K^+ levels lower aldosterone synthesis. Because aldosterone promotes Na^+ reabsorbtion by facilitating K^+ and H^+ secretion. This regulatory system is a homeostatic mechanism to maintain normal plasma K^+ level (**West , 1985**).

The kidney function tests seemed to be deteriorated in treated animals with potassium dichromate in the present study, urea and creatinine showed significant increase. However, in the kidney serum calcium is filtered and then completely reabsorbed (**Giovanni et al., 2008**). Renal insufficiency is reflected on increased excretion of calcium with consequent decrease of its serum level.

The obtained results indicated significant increase in parathyroid hormone (PTH) in animals treated with potassium dichromate. The normal calcium level is regulated through three major hormones: parathyroid hormone, thyrocalcitonin and 1,25-dihydroxy vitamin D. The rise of PTH in the present study was attributed to the decline in renal function,calcium absorption efficiency and vitamin D levels(**Allan et al.,2004**).Phosphorous homeostasis is maintained in the kidney by varying the tubular reabsorption of phosphorous. The renal clearance of phosphate is an important regulator of the calcium – phosphate balance in life (**Guyton, 1991**).Several authors reported that the increase of serum phosphorous (P) level is accompanied with hypocalcemia (**Guyton**, **1991**). They attributed these results to the changes in circulating level of parathermone hormone (PTH). These disturbance in Na^+,K^+,Ca, P and Mg may be due to renal tubular defects as a result of Cr^{6+} accumulated in the proximal tubules. **Arreola-Mendoza et al.,(2006)** reported that functional alterations induced by Cr^{6+} were located mostly at the proximal tubules site of action of several xenobiotics including heavy metals.

The results of the present study illustrated that injection with potassium dichromate caused significant decrease in estradiol level (E2).Hexavalent chromium is a reproductive metal toxicant that contraverse the

placental barrier and cause a wide range of fetal effects including ovotoxicity(**Banu et al.,2008**). **Elbetieha and Al-Hamood (1997)** concluded that the ingestion of trivalent and hexavalent chromium compounds by adult male and female mice would cause adverse effects on fertility and reproduction. **Recker et al.,(1996)** observed changes in plasma calcium due to estrogen deficiency and **Uemura et al.,(2000)** found an increase in bone turnover processes as a consequence of estrogen deficiency. In the present work the decrease in calcium is linked to the concomitant decrease in estradiol which maintains calcium level. The administration of aqueous extract of damsissa maintained the levels of calcium, phosphorous and estradiol . However, estrogen exerts beneficial effect by suppression of ROS which in turn stimulate osteoclasts(the cells that reabsorb bone). Thus estrogen might prevent bone loss by enhancing thiol antioxidant defenses in bone through keep the calcium and phosphorous homeostasis balance (**Lean et al., 2003).**

Herbals, as botanical medical treatments have generated a great deal of public controversy in recent years. According to the obtained results administration of aqueous extract of damsissa provide a significant ameliorative effect in renal function and reduce the oxidative stress. The administration of aqueous extract of damsissa revealed significant homeostasis of Na^+,K^+, P,Ca and Mg. Also, recorded normal circulating level of aldosterone, PTH and estradiol hormones. This result revealed that aqueous extract of damsissa has a potential antioxidant effect. However**, Perez et al.,(2004)** reported deleterious renal effects induced byCr^{6+} are partially prevented by antioxidants such as retinoic acid, vitaminC (**Fatima and Mohamood,2007**) and alpha tocopherol (**Arreola-Mendoza et al.,2006**).

In view of the obtained results, it could be concluded that treatment with aqueous extract of damsissa *(Ambrosia maritima)* had the ability to attenuate the possible deleterious effects on renal tissue in populations occupationally or accidentally exposed to chromate Cr^{6+} (potassium dichromate). However, it significantly blunted the increase in lipid peroxidation caused by Cr^{6+}, so it has the potential to enhance endogenous antioxidant status.

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Detection of Greening in Potatoes using Image Processing Techniques

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Abstract: Quality is one of the important factors in marketing of agricultural products. Grading machines have great importance in the quality inspection systems. Most of the current grading machines operate based on machine vision systems to detect blemishes and defects of products, where one image or more are taken for each individual object and the results of processing will decide the quality of the object. One of the major blemishes in potatoes is physiological skin greening, which has negative influence on human health. In this research, a simple machine vision algorithm has been developed in order to detect physiological skin greening of potato tubers rapidly and precisely. The experimental image acquisition setup was consisted of an image capturing box equipped with lighting system, a color CCD camera, and a capturing card. The data set consisted of 25 images of potatoes with physiological skin greening blemishes. Image pre-preprocessing has been carried out to modify the non-uniform distribution of background light intensity. Since potatoes have bright skin, the CCD was saturated in a small part of each image. These parts were eliminated from the images using a relation found between RGB and HSI spaces. The difference between red and green components of RGB space for green parts of potatoes was lower than that of other parts. Finally, the $1.02R - G$ relation was found to be suitable for detection of green parts of potato tubers. The average of error between actual green parts area and estimated green parts area for 25 images was 5.26%.
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Keywords: Automation; machine vision; potato blemish.

1. Introduction

Recently, there are a lot of researches work have been carried out by depending on the computer; in order to reduce the processing time and to provide accurate results. Digital image processing, as a computer based technique, has been extremely used by scientists to solve problems in agriculture (Chen *et al*., 2002). In the case of potato product, most of the research works focused on potato inspection without singulation (Marchant *et al*., 1990; Al-Mallahi *et al*., 2010) and blemish detection (like black dot, silver surface, common scab, etc.) (Barnes *et al*., 2010).

Potatoes contain toxic compounds known as *glycoalkaloids*, of which the most prevalent are *solanine* and *chaconine*. This toxin affects the nervous system, causing weakness and confusion. Exposure to light, physical damage, and age increase *glycoalkaloid* content within the tuber. The highest concentrations occur just underneath the skin. Light exposure causes greening from chlorophyll synthesis, thus giving a visual clue as to areas of the tuber that may have become more toxic (Olsen and Brandt, 2005). Since consumption of green parts of potato is harmful to human, this is very important to develop an inspection system to reject green potatoes during sorting process. Hence, this paper aims to introduce a machine vision algorithm to estimate the potato green surface area.

2. Image Acquisition and Preprocessing

To do experiments 100 potatoes were selected in which 25 of them had the physiological skin greening defect. An image-capturing system was designed to provide an enclosed and uniform light illumination and to obtain standard images from the samples. The size of the capturing chamber was L: 40 cm, W: 40 cm, and H: 40 cm. A sample holder (25 cm × 30 cm) was placed at the bottom of the box and covered by a black fabric to eliminate the shadows. Samples were illuminated using two parallel lamps (with one fluorescent tube in each lamp, model 391 Deluxe, Natural Daylight, 10W, Farhad Lighting Co., Iran) equipped with light diffuser. The two fluorescent tubes (391 mm) were placed 35 cm above and parallel to the sample holder. A color CCD camera (CNB, 560 TV line, model GA4162PF, Korea) was positioned horizontally in the center of the chamber and vertically over the sample holder at a distance of 40 cm. The angle between the camera lens (CCTV Lens, *f*=1.4, model LVA0660D, China)

and the lighting source axis was 90°. The video frames were sent via a TV capture device (Axtrom, XT-TV100, Korea) to a computer (IBM, 2.2 GHz CPU, 160 GB hard disc, and 1 GB RAM) provided with image acquisition and processing toolboxes of MATLAB software (Version R2009a, The MathWorks Inc., MA, USA) to visualize, acquire and process the images directly from the computer.

After image acquisition, some preprocessing operations were carried out on images to segment potatoes from the background. The summarized description of these operations is as following:

1. Obtaining grey images from the RGB space channels.

2. Obtaining binary image of samples using defined threshold values for R and B channels (20<R<40 and 5<B<30).

3. Removing the noise (small external materials with an area under 20 pixels) using erosion operation.

4. Filling the holes in the segmented binary image to obtain an actual binary image using dilation operation.

5. Multiplying the obtained binary images in R, G, and B channels.

6. Obtaining RGB images by combination of grey images obtained from the previous step. Figure 1 shows the results of above operations.
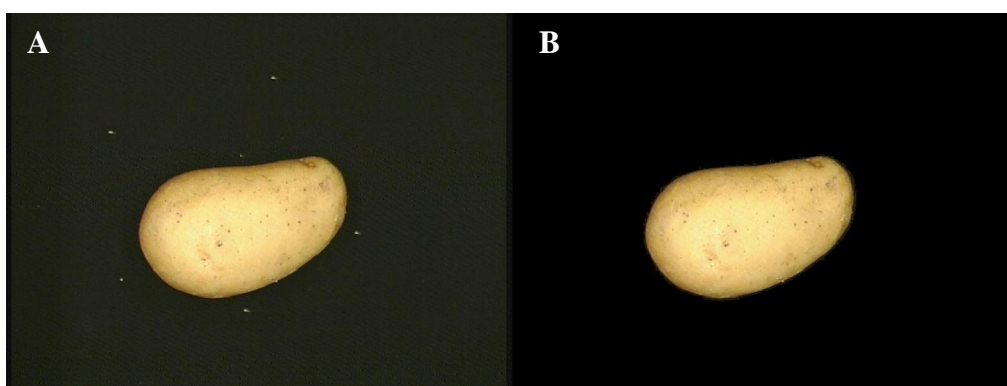
Figure 1. A. Original acquired image and B. Image after preprocessing operations

3. Determination of Greening Degree

To determine the area of green parts, the color specifications of green and non-green parts in the RGB (Red-Green-Blue) space was extracted first (Figure 2-A and B).

According to the Figure 2-A and B, the difference between R and G components for green parts is higher than that for non-green ones. The following relation was found to extract green parts:

$$P_{green\ parts}=1.02\times R - G \qquad (1)$$

Result of relation 1 is shown in Figure 3-B.

During the image analysis, it became clear some parts of potato, which were under intensive light, are extracted with green parts. The reason for this phenomenon is closeness of gray level between R and G components (Figure 2-C). To solve this problem, these parts were extracted separately using the relation found between RGB and HSI (Hue-Saturation-Intensity) spaces as following:

$$P_{saturated\ parts}=1.4\times G - S \qquad (2)$$

Figure 3-C shows the result of above relation. Finally, green parts were obtained by subtraction relation 1 from 2 (Figure 3-D). The area of both whole potato and extracted green parts was computed using *reprops* function in MATLAB. The following relation was used to find the greening degree of potatoes.

$$\text{Degree of greening} = P/t \times 100 \qquad (3)$$

where *P* and *t* are the area of whole potato and green parts, respectively.
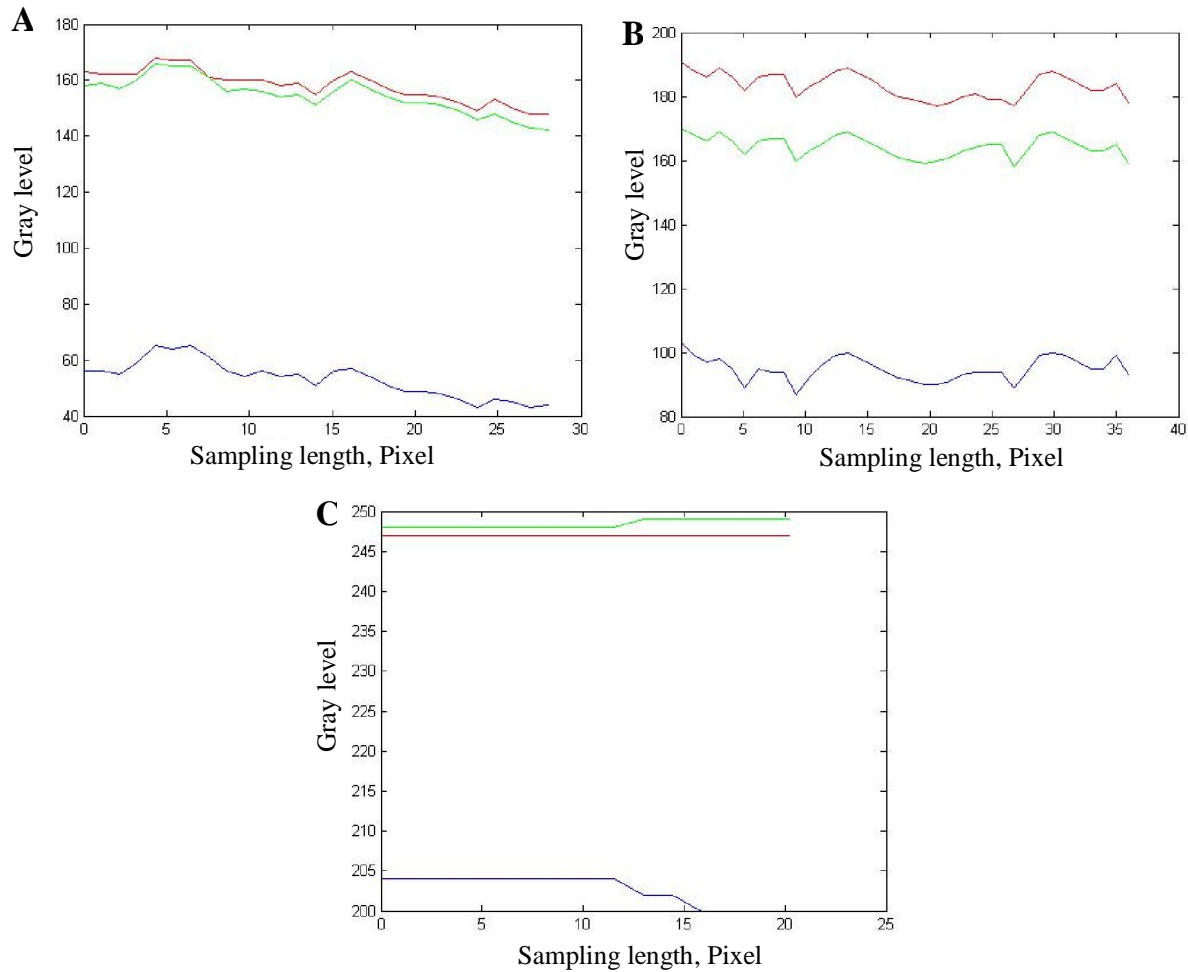
Figure 2. Potato color specification: A. Green parts, B. Non-green parts, and C. Saturated parts

4. Test of proposed algorithm

In order to evaluate the performance of the proposed algorithm, potato images were transmitted to Paint software to substitute green parts by red color (Figure 4). After that the area of red parts was computed using an own script in MATLAB. Performance of the algorithm was evaluated using the following formula:

$$\%\varepsilon = \frac{|A-T|}{T}\times100 \qquad (4)$$

where ε, A, and T are the error value, area of green parts extracted by algorithm, and area of red parts in the test image, respectively.

Figure 5 shows the result of the green parts area of 25 potatoes estimated by the algorithm compared to the actual green area. The results showed that the average error of the algorithm is 5.25%. This shows the algorithm has enough accuracy to be used in sorting systems.

5. Conclusion

Physiological skin greening is an important blemish in potato, which has harmful influence on the human body. To overcome this challenge, the use of machine vision to analyze the greening area of potatoes is suggested. A machine vision based algorithm was proposed in RGB and HIS spaces. Test of the algorithm using comparing the estimated green area by algorithm and actual area of greening showed the potential of the algorithm for its purpose.
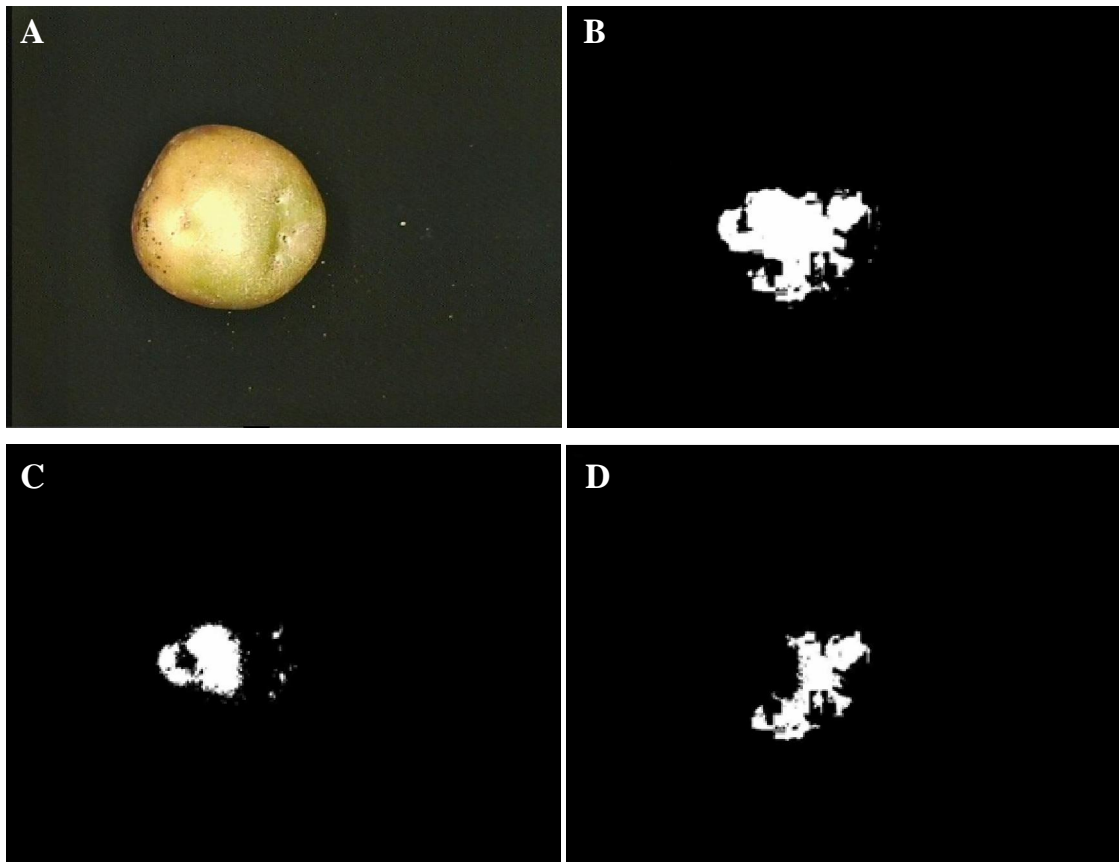
Figure 3. Determination of green parts. A. Original image, B. Extraction of green and saturated parts (result of relation 1), C. Extraction of saturated parts (result of relation 2), and D. Extraction of pure green parts
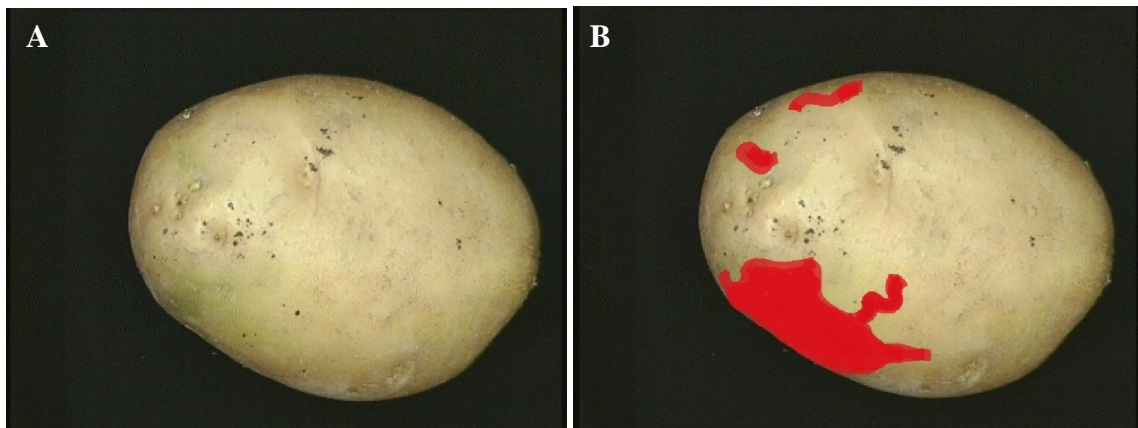
Figure 4. A. Original image of potato with green parts. B. Substitution of green parts by red color
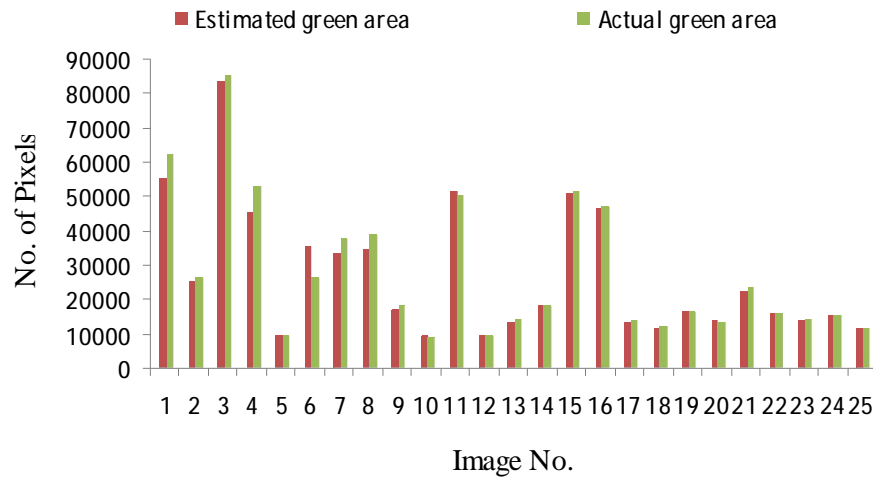
Figure 5. Comparison between estimated (by algorithm) and actual area of green parts

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Effects of irreversible different parameters on performance of air standard Otto cycle

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Abstract: An *irreversible* air standard *Otto cycle* model is proposed in this paper. The performance of an air-standard *Otto cycle* with heat transfer loss and *variable specific heats* of working fluid is analyzed by using finite-time thermodynamics. They are generalized formulas for internal combustion engines because they include the performance characteristic of special cases of Otto engines. The objective of this study is to analyze the effects of heat loss characterized by a percentage of the fuel's energy, *friction* and *variable specific heats* of working fluid on the performance of an air standard *Otto cycle* with a restriction of maximum cycle temperature. A more realistic and precise relationship between the fuel's chemical energy and the *heat leakage* that is based on a pair of inequalities is derived through the resulting temperature. The power output and the working range of the cycle increase with the increase of specific heats of the working fluid, while the efficiency decreases with the increase of specific heats of the working fluid. The *friction* loss has a negative effect on the performance. The results obtained in the present study are of importance to provide good guidance for performance evaluation and improvement of practical Otto engines. [Journal of American Science 2011;7(3):248-254]. (ISSN: 1545-1003).

Keywords: : *Otto cycle, Heat leakage, Friction, Irreversible, Variable specific heat.*

1. Introduction

A series of achievements have been made since finite-time thermodynamics was used to analyze and optimize real heat engines (Bejan, 1996). Preliminary models leading to a qualitative understanding of how engine losses could be reduced are introduced by Mozurkewich and Berry (1982). They are based on the optimal control theory (Chen, Wu, Sun, 1999). The losses are considered to consist of the friction forces in the crank shaft bearings and piston rings, the pressure drop or differentiation effect as the gas flows through the inlet valves, the heat leakage amount from the working fluid to the cylinder walls and also the time loss term containing the burning velocity (Chen, Sun, 2004). In practice, air standard analysis is useful for illustrating the thermodynamic aspects of an engine operation cycle. Meanwhile, it can provide approximate estimates of trends as major engine operating variables change (Hoffman, Watowich, Berry, 1985). Good approximations of power output, thermal efficiency and mep (mean effective pressure) can be expected. For an ideal engine cycle, heat losses do not occur, however, for a real engine cycle, heat losses indeed exist and should not be neglected. It is recognized that heat loss strongly affects the overall performance of the internal combustion engine (Chen, Lin, Luo, Sun, Wu, 2002). If it is neglected, the analysis will just depend on the ideal air standard cycle. Some

attention has been paid to analyzing the effects of heat transfer losses on the performance of internal combustion engines (Brown, Fernandez, Diazpico, 1994).

The heat addition process for an air standard cycle has been widely described as subtraction of an arbitrary heat loss parameter times the average temperature of the heat addition period from the fuel's chemical energy (Orlov, Berry, 1993). That is, the heat transfer to the cylinder walls is assumed to be a linear function of the difference between the average gas and cylinder wall temperatures during the energy release process. However, the heat leakage parameter and the fuel's energy depend on each other. Their valid ranges given in the literature affect the feasibility of air standard cycles (Wang, Chen, Sun, Wu, 2002). If they are selected arbitrarily, they will present unrealistic results and make the air standard cycles unfeasible (Chen, Zheng, Sun, Wu, 2003). There by, the performance analysis of any internal combustion engine can be covered by a more realistic and valid range of the heat loss parameter and the fuel's energy (Chen, Sun, Wu, 2004). Moreover, his study was done without considering the effects of variable specific heats of the working fluid and friction (Ge, Chen, Sun, 2005). In particular, no performance analysis is available in the literature with emphasis on the Otto cycle with considerations of variable specific heats of the working

fluid, friction and heat leakage characterized by a percentage of the fuel's energy (Al-Sarkhi, Jaber, Abuqudais, Probert, 2006). This study is aimed at analyzing these effects (i.e. variable specific heats of working fluid, friction and heat loss characterized by a percentage of the fuel's energy) on the net work output and the indicated thermal efficiency of an air standard Otto cycle (Al-Sarkhi, Jaber, Probert, 2006). In the present study, we relax the assumptions that there are no heat losses during combustion, that there are no friction losses of the piston for the cycle, and that specific heats of the working fluid are constant (Ge, Chen, Sun, Wu, 2006). In other words, heat transfer between the working fluid and the environment through the cylinder wall is considered and characterized by a percentage of the fuel's energy; friction loss of the piston in all the processes of the cycle on the performance is taken into account. Furthermore, we consider the variable specific heats of the working fluid that is significant in practical cycle analysis. The results obtained in the study may offer good guidance for design and operation of the Otto cycle engine (Ozsoysal, 2006).

2. Thermodynamic analysis
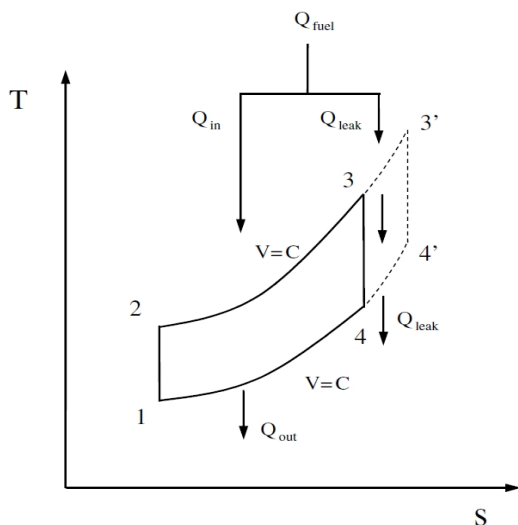
Figure 1. T–s diagram of an air standard Otto cycle model

Fig.1 shows the limitation of the maximum cycle temperature due to heat leakage in the temperature-entropy diagram of an air standard Otto cycle model.

Thermodynamic cycle $1–2–3'–4'–1$ denotes the air standard Otto cycle without heat leakage, while cycle $1–2–3-4-1$ designates the air standard Otto cycle with heat leakage. Process $1–2$ is an isentropic compression from BDC (bottom dead center) to TDC (top dead center). The heat addition takes place in process 2–3, which is isochoric. The isentropic expansion process, 3–4, is the power or expansion stroke.

The cycle is completed by an isochoric heat rejection process, 4–1. The heat added to the working fluid per unit mass is due to combustion. The temperature at the completion of the constant volume combustion (T_3) depends on the heat input due to combustion and the heat leakage through the cylinder wall. In this study, the amount of heat leakage is considered to be a percentage of the delivered fuel's energy (Mozurkewich and Berry, 1982). The fuel's energy then is the sum of the actual fuel energy transferred to the working fluid and the heat leakage through the cylinder walls. If any heat leakage occurs, the maximum cycle temperature (T_3) remains less than that of the no heat leakage case ($T_{3'}$).

When the total energy of the fuel is utilized, the maximum cycle temperature reaches undesirably high levels with regard to structural integrity. Hence, engine designers intend to restrict the maximum cycle temperature. Assuming that the heat engine is operated at the rate of N cycles per second, the total energy of the fuel per second input into the engine can be given by

$$Q_{fuel} = Nm_f Q_{LHV} \qquad (1)$$

And then the heat leakage per second is

$$Q_{leak} = aQ_{fuel} = aNm_f Q_{LHV} \qquad (2)$$

where m_f is the delivered fuel mass into the cylinder, Q_{LHV} is the lower heating value of the fuel and a is an unknown percentage parameter having a value between 0 and 1.

since the total energy of the delivered fuel Q_{fuel} is assumed to be the sum of the heat added to the working fluid Q_{in} and the heat leakage Q_{leak},

$$Q_{in} = Q_{fuel} - Q_{leak} = (1-a)Nm_f Q_{LHV} \qquad (3)$$

In practical internal combustion engine cycles, constant pressure and constant volume specific heats of the working fluid are variable, and these variations will greatly affect the performance of the cycle. it can be assumed that the specific heats of the working fluid are functions of temperature alone and have the following linear forms:

$$C_{pm} = a_p + k_1 T \qquad (4)$$

and

$$C_{vm} = b_v + k_1 T \tag{5}$$

where C_{pm} and C_{vm} are respectively, the specific heats with respect to constant pressure and volume. a_p, b_v and k_1 are constants. Accordingly, the gas constant (R) of the working fluid can be expressed as

$$R = C_{pm} - C_{vm} = a_p - b_v \tag{6}$$

The temperature is restricted as the maximum temperature in the cycle is T_3, and the available energy Q_{in} during the heat addition per second can be written as

$$Q_{in} = Nm_a \int_{T_2}^{T_3} C_{vm} dT = Nm_a \int_{T_2}^{T_3} (b_v + k_1 T) dT$$
$$= Nm_a [b_v (T_3 - T_2) + k_1 bv(T_3^2 - T_2^2)]. \tag{7}$$

Combining Eqs. (3) and (7) yields

$$Nm_a [b_v (T_3 - T_2) + k_1 bv(T_3^2 - T_2^2)] = (1-a) Nm_f Q_{LHV} \tag{8}$$

Dividing Eq. (8) by the amount of air mass m_a, we have

$$a = 1 - \frac{l(m_a/m_f)_s}{Q_{LHV}} [b_v (T_3 - T_2) + k_1 bv(T_3^2 - T_2^2)] \tag{9}$$

Or

$$T_2 = \frac{-b_v + \sqrt{b_v^2 + 2k_1[0.5k_1 T_3^2 + b_v T_3 - (1-a)\frac{Q_{LHV}}{l(m_a/m_f)_s}]}}{k_1} \tag{10}$$

where l is the excess air coefficient defined as $l = (m_a/m_f)/(m_a/m_f)_s$, (m_a/m_f) is the air–fuel ratio and the subscripts a, f, and s, respectively, denote air, fuel and the stoichiometric condition.
The first condition for realizing a feasible cycle is

$T_2 \leq T_3 (= T_{max})$, so that

$$a \leq 1 \tag{11}$$

The upper limit for the percentage of heat leakage is then found as $a_{max} = 1$. The second condition, $T_2 \geq T_1 (= T_{min})$, is utilized to determine the lower limit as follows

$$a \geq 1 - \frac{l(m_a/m_f)_s}{2Q_{LHV}} [k_1(T_3^2 - T_1^2) + 2b_v(T_3 - T_1)] \tag{12}$$

Hence, the minimum value of a is expressed as

$$a_{min} = 1 - \frac{l(m_a/m_f)_s}{2Q_{LHV}} [k_1(T_3^2 - T_1^2) + 2b_v(T_3 - T_1)] \tag{13}$$

The heat rejected per second by the working fluid (Q_{out}) during process $4 \rightarrow 1$ is

$$Q_{out} = Nm_a \int_{T_1}^{T_4} C_{vm} dT = Nm_a \int_{T_1}^{T_4} (b_v + k_1 T) dT$$
$$= Nm_a [b_v(T_4 - T_1) + 0.5k_1(T_4^2 - T_1^2)]. \tag{14}$$

The adiabatic exponent $k = C_{pm}/C_{vm}$ will vary with temperature since both C_{pm} and C_{vm} are dependent on temperature.

Accordingly, the equation often used in reversible adiabatic processes with constant k cannot be used in reversible adiabatic processes with variable k. however, a suitable engineering approximation for reversible adiabatic processes with variable k can be made, i.e. this process can be divided into infinitesimally small processes and for each of these processes, the adiabatic exponent k can be regarded as constant. For instance, for any reversible adiabatic process between states I and II, we can regard the process as consist of numerous infinitesimally small processes with constant k. For any of these processes, when small changes in temperature dT and volume dV of the working fluid take place, the equation for a reversible adiabatic process with variable k can be written as follows:

$$TV^{k-1} = (T+dT)(V+dV)^{k-1} \tag{15}$$

Rearranging Eqs. (4)-(6) and (15), we get the following equation

$$dT/T + [R/(b_v + k_1 T)](dV/V) = 0 \tag{16}$$

Integrating Eq. (16) from state I to state II, we obtain

$$k_1(T_j - T_i) + b_v \ln(T_j/T_i) = -R \ln(V_j/V_i) \tag{17}$$

The compression ratio (g_c) is defined as $g_c = V_1/V_2$. Therefore, the equations for processes $1 \rightarrow 2$ and $3 \rightarrow 4$ are shown, respectively, by the following equations:

$$k_1(T_2 - T_1) + b_v \ln(T_2/T_1) = R \ln g_c \tag{18}$$

and

$$k_1(T_3 - T_4) + b_v \ln(T_3/T_4) = R \ln g_c \tag{19}$$

From Eqs. (7) and (14), the power output without friction losses is given by:

$$P_R = Q_{in} - Q_{out}$$
$$= Nm_a[b_v(T_3 + T_1 - T_2 - T_4) + 0.5k_1(T_3^2 + T_1^2 - T_2^2 - T_4^2)] \quad (20)$$

Every time the piston moves, friction acts to retard the motion. Considering the friction effects on the piston in all the processes of the cycle, we assume a dissipation term represented by a friction force (f_m) that is linearly proportional to the velocity of the piston, which can be written as follows:

$$f_m = -mu = -m\frac{dx}{dt} \quad (21)$$

where m is the coefficient of friction, which takes into account the global losses on the power output, x is the piston's displacement and v is the piston's velocity. Therefore, the power lost due to friction is

$$P_m = f_m v = -m(\frac{dx}{dt})^2 = -mv^2 \quad (22)$$

for a four stroke cycle engine, the total distance the piston travels per cycle is

$$4L = 4(x_1 - x_2) = 4x_2(x_1/x_2 - 1) = 4x_2(g_c - 1) \quad (23)$$

where x_1 and x_2 are the piston's position corresponding to the maximum and minimum volume, respectively, and L is the stroke of the piston. Running at N cycles per second, the mean velocity of the piston is

$$\bar{v} = 4LN \quad (24)$$

Therefore, the net actual power output of the Otto cycle engine can be written as

$$P = P_R - |P_m| = Nm_a[b_v(T_3 + T_1 - T_2 - T_4) +$$
$$0.5k_1(T_3^2 + T_1^2 - T_2^2 - T_4^2)] - 16m[Nx_2(g_c - 1)^2] \quad (25)$$

The efficiency of the Otto cycle engine is expressed by

$$h = \frac{P}{Q_{in}} = \{m_a[b_v(T_3 + T_1 - T_2 - T_4) + 0.5k_1(T_3^2 + T_1^2 - T_2^2 - T_4^2)]$$
$$-16mN[x_2(g_c - 1)]^2\} \times \{m_a[b_v(T_3 - T_2) + 0.5k_1(T_3^2 - T_2^2)]\}^{-1} \quad (26)$$

When T_1, T_3 and g_c are given, T_2 can be obtained from Eq. (18) and T_4 can be found from Eq. (19). Finally, by substituting T_1, T_2, T_3 and T_4 into Eqs. (25) and (26), respectively, the power output and the efficiency of the Otto cycle engine can be obtained. Therefore, the relations between the power output, the efficiency and the compression ratio can be derived.

3. Results and discussion

The following constants and ranges of parameters are used in the calculations: $b_v = 0.6858$-0.8239 kJ/kg K , $m_a = 1.26 \times 10^{-3}$ kg, $T_1 = 300$-400 K , $k_1 = 0.000133 - 0.00034 kJ/kg.K^2$, $x_2 = 0.01$ m , N = 30, $Q_{LHV} = 44000$ kJ/kg and $m = 0.0129$-0.0169 kN s/m . This study focuses on the limitation of the maximum cycle temperature T_3 instead of $T_{3'}$ due to the varying heat leakage conditions. Numerical examples are shown as follows.
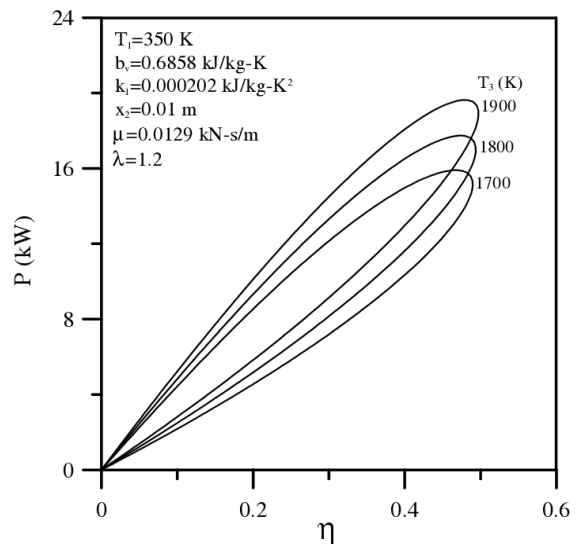
Figure 2. The influence of maximum cycle temperature (T_3) on the power output (P) versus efficiency (h) characteristic curves

We obtain the loop shaped power output versus efficiency curves, which reflect the performance characteristics of a real irreversible Otto cycle engine (Fig 2). It is depicted that the maximum power output, the maximum efficiency, the power at maximum efficiency and the efficiency at maximum power will increase with the increase of T_3.

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efficiency and the efficiency at maximum power will increase with the increase of T_3.
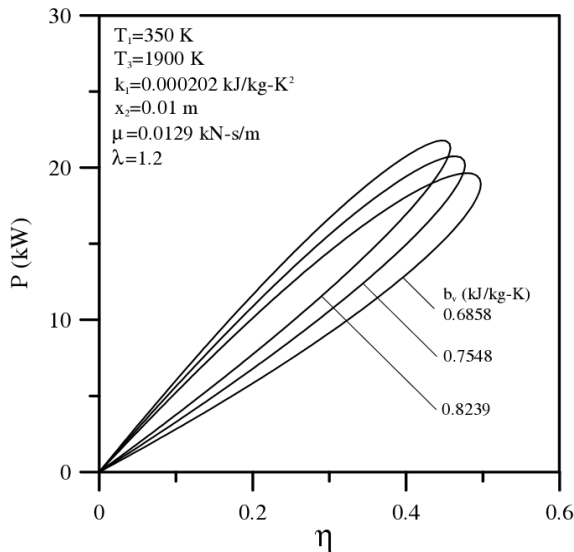
Figure 3. The influence of b_v on the power output (P) versus efficiency (h) characteristic curves

Figs. 3 show the influence of the parameter b_v related to the variable specific heats of the working fluid on the performance of the Otto cycle. For a fixed k_1, a larger b_v corresponds to a greater value of the specific heat with constant volume (C_{vm}) or the specific heat with constant pressure (C_{pm})
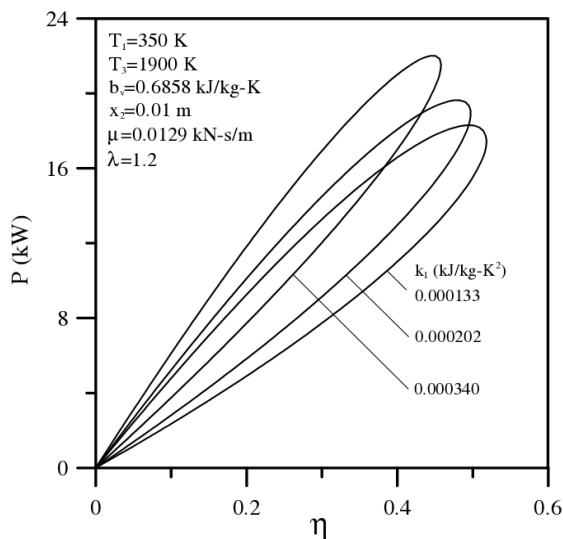
Figure 4. The influence of k_1 on the power output (P) versus efficiency (h) characteristic curves

Figs. 4 represent the influence of the parameter k_1 related to the variable specific heats of the working fluid on the performance of the Otto cycle. For a given b_v, a larger k_1 corresponds to a greater value of the specific heats with constant volume (C_{vm}) or the specific heat with constant pressure (C_{pm}).

With the increase of k_1, the maximum power output and the power at maximum efficiency increase, while the maximum efficiency and the efficiency at maximum power output decrease, as shown in Fig. 4.
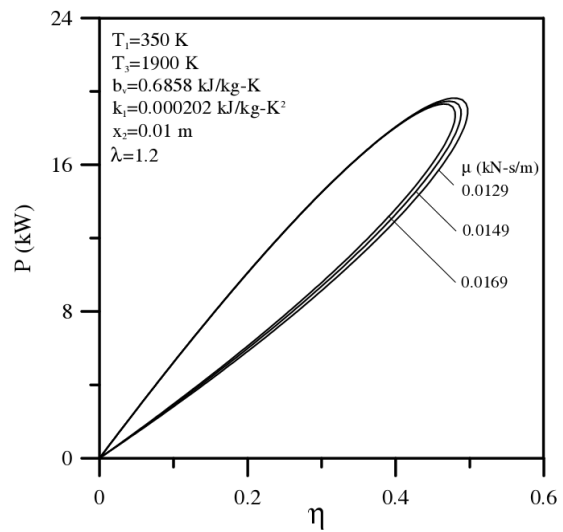
Figure 5. The influence of m on the power output (P) versus efficiency (h) characteristic curves

Figs. 5 show the influence of the friction like term loss (m) on the performance of the Otto cycle. It is clear that the parameter m has a negative effect on the performance. Fig5 shows that the maximum power output, the maximum efficiency, the power at maximum efficiency and the efficiency at maximum power will decrease with the increase of m.
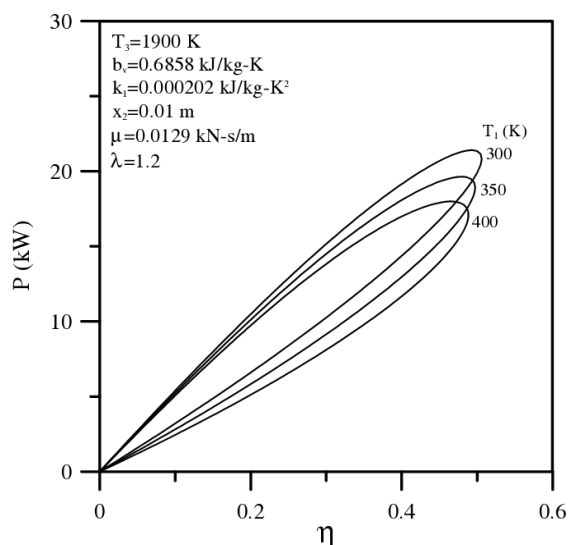
Figure 6. The influence of intake temperature (T_1) on the power output (P) versus efficiency (h) characteristic curves

Fig 6. depict the influence of intake temperature (T_1) on the performance of the Otto cycle. Fig6. It is also found that as T_1 increases, the maximum power output, the maximum efficiency, the efficiency at maximum power output and the power output at maximum efficiency decrease.

4. Conclusions

The effects of heat loss as a percentage of the fuel's energy, friction and variable specific heats of the working fluid on the performance of an Otto engine under the restriction of maximum cycle temperature are presented in this study. The results are summarized as follows.

(1) The maximum power output, the maximum efficiency, the power at maximum efficiency, the efficiency at maximum power and the value of the compression ratio when the power output or the efficiency is maximum increase with the increase of maximum cycle temperature T_3.

(2) The parameters b_v and k_1 related to the variable specific heats of the working fluid have a significant influence on the performance of the Otto cycle. For a fixed k_1 (or b_v), a larger b_v (or k_1) corresponds to a greater value of the specific heats with constant volume (C_{vm}). For a given compression ratio g_c in a feasible range, the power output of the cycle

increase with the increase of the parameter b_v or k_1, nevertheless, the efficiency decreases with the increase of b_v or k_1. Furthermore, with the increase of b_v, the maximum power output and the power at maximum efficiency increase, while the maximum efficiency and the efficiency at maximum power output decrease.

(3) The influence of the friction like term loss m has a negative effect on the performance. Therefore, the maximum power output, the maximum efficiency, the power at maximum efficiency and the efficiency at maximum power will decrease with the increase of m.

(4) The maximum efficiency, the compression ratio at maximum power output and the compression ratio at maximum efficiency of the Otto cycle decrease with the increase of intake temperature T_1. The efficiency at maximum power output and the power output at maximum efficiency decrease with increasing T_1.

(5) It is noteworthy that the effects of heat loss as a percentage of the fuel's energy and friction loss on the performance of an Otto cycle engine with considerations of variable specific heats of working fluid are significant and should be considered in practical cycle analysis. The results obtained in the present study are of importance to provide good guidance for performance evaluation and improvement of practical Otto engines.

In view of the analytical results from this work, we realize that the understanding and development of engines and engine cycles should be further explored by considering a more realistic model with advanced theoretical and numerical techniques. For instance, in air standard analysis, the constant volume heat input process replaces the combustion of the real engine cycle, which takes place at close to constant volume conditions, and exhaust blow down in a real engine is almost, but not quite, constant volume. As expected, the maximum temperature in the cycle will depend on the crank angle at which the exhaust valve opens. Hence, a new type of cycle analysis is needed. In other words, conceiving a new model as a function of crank angle to help understand, correlate, and analyze the relation between the maximum temperature and the crank angle at which the exhaust valve opens in the cycle. Additionally, considering the combined effects of heat loss and friction on the performance of engine cycles, detailed comparisons between this work and numerical analysis (or experiments) are worthy of further study.

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2/19/2011

The Perceptions of Graduate Students about Factors Influencing the Extension of Entrepreneurship Education in College of Agriculture and Natural Resources in Iran

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Abstract: Graduate students at the college of agriculture and natural resources were surveyed in order to explore their perception about the factors influencing the extension of entrepreneurship education in the Science and Research Branch at Islamic Azad University. The methodology used in this study involved a combination of descriptive and quantitative research. The total population was 313 master and doctorate students majoring in agriculture. The results of regression analysis showed that 50% of the variance in the perception of respondents could be explained by tendency toward being successful, being innovative, entrepreneurship education in universities, role of instructor and educational contents.

1. Introduction

Iran has faced a crisis of unemployment among graduates from universities in agriculture majors. Iranian agricultural higher education system currently has to find a comprehensive solution for the employment of these graduates (The Agriculture and Natural Resources Engineering System Organization, 2007).

One strategy that has helped many developed and developing countries to overcome the problem of unemployment, has been the development of entrepreneurship. Entrepreneurial education can play a significant role in changing views of students towards self employment and enhancing their necessary skills, in order to help them to manage a business and eventually prepare them for self employment in labor market (Nelson, 1986).

Oversupply of graduate manpower in agricultural sector, unemployment growth in their community, lack of response or positive feedbacks to the efforts made in recent decade to find a solution for unemployment problem of graduates on one side and on the other hand the necessity to move to competitive market based agriculture created an important ground for paying more attention to entrepreneurship.

Wenneker and Thurik (1999) identify three dimension of entrepreneurship – the condition which leads to entrepreneurship, the attributes and the impacts of entrepreneurship. In regard to individual, the conditions for entrepreneurship are culture and incentives, elements are attitudes, skills and creativity and the impacts are self-realisation and income.

Proposing new ideas based on the role of entrepreneurship in increasing job opportunities, competitiveness, improvement in manpower productivity, technology development, wealth generating and social welfare level and also existence of strong relation between entrepreneurial development and economic growth of the countries have all resulted in a serious consideration of entrepreneurship in new economic theories and have been regarded as a provocative engine in economical social growth and development of countries (Audretsch, 2002; Zoltan, 2006).

This role of entrepreneurship in development of agricultural economy is regarded as one of the major requirement for agricultural development (Smit, 2004). Such situation has brought about an increasing demand in agricultural entrepreneurship education in recent years and has resulted in more emphasize by researchers and government authorities in different countries (McElwee, 2005).

As a result, entrepreneurial education has become a serious necessity for the governments, in order to upgrade the capacities and abilities of young graduates to enter in a competitive job market in agricultural sector (Smit, 2004).

Indeed, the entrepreneurship is a key element in creating employment, a solution to combat the unemployment crisis and a response to community diverse demands. Therefore, it is considered as one of the important fundamental aspects in agricultural development plans (Higgins and Morgan, 2000; Smit, 2004).

Entrepreneurship eduction in universities could enhance the skills of students in areas related to starting a new business.. According to Smilor (1997) and Kilby (1971), entrepreneurial skills refer to those activities, or practical know-how, that are needed to establish and successfully run a business enterprise. These may comprise such areas as finance, accounting, marketing or production. Others want to make a distinction between managerial and entrepreneurial skills.

With a look at the background of education in agricultural sector in Iran, it can be observed that the amount of investment and attention to this issue from different dimensions has never been at a level proportionate to employment criteria. Furthermore, considering available resources, the expansion of this sector in term of frequency, employees and beneficiaries of this sector (3.5 million individuals as beneficiaries) were not enough to fulfill country's demands (Institute of Applied-Scientific Educations, 2002).

In a research, Zamani (2001) has emphasized on establishing self-employment and entrepreneurship mentality among students in the colleges and universities. The findings of study by Streeter et al (2002) show that trend toward entrepreneurial education at Cornell University is strong; the conceptual framework clarifies the different pathways for creating a university wide approach toward entrepreneurship; the radiant model (entrepreneurship out of university) is extremely appealing to students, parents and alumni; the magnet model (entrepreneurship inside university) is easier to administer and present in various methods; the magnet model is simpler to implement, it may lead to conflicts in a long run because the benefits and facilities may not be distributed equally among the university students.

Kuratko (2003) in a study entitled emergence of entrepreneurial education: development, trends and challenges, pointed out the entrepreneurship has emerged and developed over the recent two decades and its recent growth in curricula and programs dedicated to entrepreneurship has been very considerable. The number of faculties and universities which deliver curricula in connection to entrepreneurship has surged to over 1600 in 2005 in comparison to few programs in 1970's. This huge development has resulted in some academic

legislative challenges for entrepreneurship that this article has focused on these trends and challenges of entrepreneurial education in the universities in the 21st century.

UNESCO (2004), in its global prospect of higher education for 21st Century, has described the new universities as: "A place in which the entrepreneurial skills in order to facilitate the graduates' capabilities and promoting them to job producers are developed". In another research entitled conceptual framework for the assessment of the efficiency of entrepreneurial education of programs aimed at entrepreneurship, there is a meaningful relationship between entrepreneurial education and the tendency to entrepreneurship. Knowing the fact that entrepreneurial education of programs can change the entrepreneurship purpose, which is to examine the economic relation of entrepreneurship activity, is fascinating. In this research, the first stage goal is framework development that enables us to explain the programs of entrepreneurial education alongside with the changes in visions and participants' beliefs in the program and then presents the assessment of the impact of entrepreneurial education of programs on participants' goal (Volery and Muller, 2006). The research question for this study is: what are the perceptions of graduate students in the college of agriculture and natural resources about factors influencing the extension of entrepreneurship education in Islamic Azad University, Science and Research Branch?

The purpose of this study was to determine the perceptions of graduate students in the college of agriculture and natural resources about factors influencing the extension of entrepreneurship education in Islamic Azad University, Science and Research Branch. The objectives were as follows: 1) to identify personal characteristics of respondents; 2) to identify factors affecting the extension of entrepreneurship education and 3) to determine the relationship between factors and perception of respondents about the extension of entrepreneurship education.

2. Material and Methods

The methodology used in this study involved a combination of descriptive and quantitative research and included the use of correlation, regression and descriptive analysis as data processing methods. The total population for this study was 1746graduate students at college of agriculture and natural resources at Islamic Azad University, Science and Research Branch, Tehran, Iran (1290 master students, 456 PhD students) and by using Cochran formula, 313 were selected through random sampling method.

Measuring respondent's attitudes towards entrepreneurship extension has been achieved largely though structured questionnaire surveys. The usual questionnaire approach to measure attitude is to include a range of semantic-differential (with good/bad options for example) and Likert items (with agree/disagree options for example) to operationalize the attitude construct. The final questionnaire was divided into several sections. The first section was designed to gather information about personal characteristics of respondents. The second section was designed to measure the attitudes of respondents about their entrepreneurship characteristics. The respondents were asked to indicate their agreements by marking their response on a five point Likert-type scale. The next section explored the role of entrepreneurship education in universities were presented in a 5-point Likert format with responses from 1—completely disagree to 5—completely agree. The last section was designed to identify the most appropriate entrepreneurship extension methods. The variables and their measurement scale are presented in table 1.

Table 1: Variables and their measurement scale

Variables	Measurement Scale
Entrepreneurship characteristics	Five- point Likert
Role of entrepreneurship educations in universities	Five- point Likert
Entrepreneurship extension methods	Five- point Likert
Gender	Categorial
Age	Categorial
Educational Level	Categorial
Marital Status	Categorial
Employment Status	Categorial

Content and face validity were established by a panel of experts consisting of faculty members at Islamic Azad University, Science and Research Branch and some specialists in the Ministry of Agriculture. Minor wording and structuring of the instrument were made based on the recommendation of the panel of experts.

A pilot study was conducted with 30 persons who had not been interviewed before the earlier exercise of determining the reliability of the questionnaire for the study. Computed Cronbach's

Alpha score was 90.0%, which indicated that the questionnaire was highly reliable.

Key dependent variable in the study included entrepreneurship education which was measured by perception of respondents about 14 statements. The independent variables in this research study were tendency toward being successful, accepting risks, being innovative, controlling own destination, being independence, entrepreneurship education in universities, role of instructor, educational contents and entrepreneurship extension methods. For measurement of correlation between the independent variables and the dependent variable correlation coefficients have been utilized and include Pearson test of independence.

3. Results

The results of descriptive statistics indicated that 175 of respondents were male and 217 were single. Majority of students had master degree and more than 180 were employed. Almost half of respondents indicated that they were pursuing a degree in agriculture.

In order to finding the perception of students about the most appropriate methods in entrepreneurship education, respondents were asked to express their views. As can be seen in the table 2, the most appropriate method based on the freedman test was attending in entrepreneurship exhibitions and visiting entrepreneurship centers (n=10.52) and the least important was attending the students entrepreneurship exhibition (n=6.46).

Pearson coefficient was employed for measurement of relationships between the perception of students and factors influencing entrepreneurship extension. Table 3 displays the results which show that there was relationship between perception of respondents and tendency toward being successful, accepting risks, being innovative, controlling own destination, being independence, entrepreneurship education in universities, role of instructor and educational contents.

Table 4 shows the result for regression analysis by stepwise method. Independent variables that were significantly related to perception of students about factors that influence the entrepreneurship extension were subjected to regression analysis. The result indicates that 50% of the variance in the perception of respondents could be explained by tendency toward being successful, being innovative, entrepreneurship education in universities, role of instructor and educational contents.

Table 2: Perception of respondents about the most important methods of entrepreneurship education

Statement	Freedman Test Number	Priority
Entrepreneurship exhibitions and visiting entrepreneurship centers	10.52	1
Students contact with successful entrepreneurs	10.19	2
Seminar and lectures about entrepreneurship	10.18	3
Publication of newsletter about entrepreneurship	9.29	4
Supporting research and students projects about entrepreneurship	9.24	5
E learning programs about entrepreneurship	8.95	6
Holding workshops for converting ideas to economic activities	8.42	7
Establishing entrepreneurship centers in universities	8.18	8
Competition and exhibition about entrepreneurship	7.87	9
Providing books about entrepreneurship for libraries	7.76	10
Publishing scientific journal about entrepreneurship	7.63	11
Workshops about marketing and regulation about entrepreneurship	7.12	12
Showing movies about entrepreneurship	6.59	13
students entrepreneurship exhibition	6.46	14

Table 3: Correlation measures between independent and dependent variable

Independent variable	Dependent variable	R	Sig.
Tendency toward being successful	Entrepreneurship extension	0.182	0.001**
Accepting risk	Entrepreneurship extension	0.137	0.015*
Controlling own destination	Entrepreneurship extension	0.121	0.033*
Being innovative	Entrepreneurship extension	0.174	0.002**
Being independence	Entrepreneurship extension	0.123	0.029*
University entrepreneurship education	Entrepreneurship extension	0.541	0.000**
Instructors	Entrepreneurship extension	0.582	0.000**
Educational Content	Entrepreneurship extension	0.556	0.000**

$**p<0.01$ $*p<0.05$

Table 4: Multivariate Regression Analysis.

Variable	B	BBeta	d	Ssig.
Educational contents (X1)		0.287		0.000
Entrepreneurship education in universities (X2)		0.243		0.000
Instructors(X3)		0.242		0.000
Tendency toward being successful (X4)		0.140		0.001
Being innovative (X5)		0.118		0.006

$R^2=.0.53$

$Y=0.287(X1)+0.243(X2)+0.242(X3)+0.140(X4)+0.118(X5)$

4. Discussions

Entrepreneurial education has a tremendous potential to help in the employment status of students in Iran. The development of entrepreneurial education results in creating more jobs and employment sustainability could be achieved over time. Therefore, certain special factors in developing entrepreneurial education among students in the universities should be identified and need to be carefully examined.

Innovative strategies need to be developed that cater specifically the entrepreneurship educational needs of students. Universities in Iran need to provide practical training in entrepreneurship to their students, to make them more aware of the benefits of entrepreneurship and to address the factors that impact on developing entrepreneurial education.

The findings reflect an important fact, namely that contents of entrepreneurship education would have a positive impact on perception of students about entrepreneurial education. In this regard, a sound educational program is a necessary prerequisite for enhancing the capacity of students to start entrepreneurship activity.

Based on the findings, role of instructors is considered as one of the most important issues. Universities have to ensure that entrepreneurship education should be taught by skillful and experienced instructors especially those who are involved in entrepreneurship activities.

To achieve the goal of entrepreneurship education, sustainability in training students need to be assured over a period of time. Options that provide access to entreprenuership education need to be carefully examined.

Therefore innovative strategies need to be developed that cater specifically to the needs of young students in the universities. In this regard, it is important to help and introduce NGOs and private sector to participate in developing entrepreneurship activities..

The issue is not only the training students about entrepreneurship, but it is equally critical to provide training, tools and guidance to make students aware of what entrepreneurship can do for them, and what they can do with being entrepreneurs.

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2/15/2011

Cigarette Smoking among University Students: Family- related & Personal risk factors

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Abstract: Smoking is considered as an increasing epidemic among youth. This threats with increasing epidemics of chronic non-communicable diseases. This study was performed to reveal a recent estimation of smoking problem among university students in Egypt and to identify possible risk factors related to family life & personal aspects. This cross-sectional study included a representative sample of 1072 Egyptian University students who were interviewed and asked to complete a modified questionnaire derived from the standard questionnaire of National Center for Social and Criminal Research. Obtained result indicated that prevalence of current smoking was 20.2% while ever smoking was 22.1%. In addition, friends were the first ranked motive for smoking followed by family related derives then sense of hopeless future representing 37.2%, 13.8 % and 12.2 % respectively. Personal risk factors for smoking were young age, males gender, studying in theoretical faculties and suffering a chronic health problem. The important family-related risk factors included large number of the family, late order among siblings and living away from the family. In conclusion, this study determined some personal and family- related factors contribute to smoking problem. Dealing with them by coordinated efforts of the family, school, university and government will alleviate smoking problem among youth.

1. Introduction:

Tobacco is the most important cause of preventable death worldwide. It is responsible for death of one in ten adults constituting about 5 million deaths each year. If smoking pattern with which the 21st century started continues, it will cause 10 million deaths each year by 2020. Half the people who smoke - about 650 million people- will eventually be killed by tobacco **(Tobacco Free Initiative, 2005)**.

Health hazards of smoking involve cardiovascular, respiratory, malignant, mental and more problems. Early smoking initiation increases life time duration of smoking and burden of smoking related diseases **(Beaglehole,2007; Egyptian Smoking Prevention Research Institute, 2007& Frost-Pineda et al., 2011)**.

In spite of the great concentration on conducting researches that entail evaluation of smoking cessation interventions **(Mallin, 2002; Wu et al., 2009 & Colby et al., 2010)** ,it is much more important to prevent youth from ever smoke via continuous monitoring of risk factors for smoking among them to modify the modifiable factors and target who have non-modifiable risk factors with health education and smoking prevention programs **(Mukhtar et al., 2006& Baska et al.,2010)**.

University students are at high risk of smoking as they become exposed to greater availability of cigarettes and intimate association with smoking peers. At the same time, they face additional social, emotional and educational challenges when they enter the university **(Abdel Hamid, 2000; Nassar, 2003; Mandil, 2007; Almutairi, 2010& Halperinet al., 2010)** .

Most studies investigated family life effect don't focus on university students but younger categories **(Shamsuddin& Abdul Haris, 2000; O'Callaghan, 2006&** Baska **et al., 2010).** So, this study was done to explore the magnitude of smoking problem among university students and identify personal and family related risk factors to bridge this research gap.

2. Subjects and Methods:

Study design and population:

A cross sectional study was carried out on a sample of university students in Egypt in late 2009.

A representative sample was taken by multistage random technique, starting with selection of a university. Then, faculties were classified into two strata; practical and theoretical and two faculties were taken randomly from each stratum with considering the proportional allocation according to the total number of students in each strata. Lastly, a cluster sampling technique was used to get from 1 to 3 clusters of students from each faculty according to the cluster size and required sample size. Respondents were (1072) out of them (340) students were from practical faculties and (732) students were from theoretical faculties. Non response rate was (2.3%).

Ethical Issues:

The study proposal and instrument were approved by the institution's review board and voluntary informed oral consents were got from students enrolled in the study.

Data Collection:

It was done by 5 trained personnel using an anonymous self-administered questionnaire modified from the standard questionnaire of the National Center for Social and Criminal Researches **(National centre for social and criminal researches, 2002)** after testing its validity and reliability through a pilot study.

The questionnaire included important aspects regarding:
1. Smoking status and full pattern of smoking and its motives among smokers.
2. Socio-demographic characteristics.
3. Familial and personal life profile.

Statistical Analysis:

Collected data were handled using a data base software programs (SPSS version 10 and EPI-INFO 6). Analysis included univariate, bivariate as well as multivariate analytical techniques. Independent variables were analyzed descriptively and in a regression analysis to determine predictors and associated risks. Chi square test and Chi square for trend with corresponding *P*-values were used to test the significance for categorical and ordinal variables respectively. Odds ratio (OR) with 95% confidence intervals (CI) was used whenever possible (i.e. in 2×2 tables and with chi square for trend) to quantify risk, while multiple logistic regression was carried out to identify variables most predictive of taking up the habit of smoking among the surveyed students. P < 0.05 was used as the level of significance.

3. Results:

As shown in Table (1&2); the prevalence of current smokers was (20.2%) while ever smoking students were (22.1%); 30.3% among males and 2.2% among females with quitting rate of 8.44%. All smokers were daily smokers and about their two-thirds were mild to moderate smokers as they expire 20 cigarettes or less per day. A serious finding was that 67% of smokers may add bang to cigarettes. More than (93%) of smokers started smoking before age of twenty including about (60%) at age between 15 and 20 years. Prevalence of smoking among under twenty group is 25.7% versus 16.6% in those over twenty. The first 2 motives for smoking were associated with participation and encouragement by friends followed by family related derives as

negligence, problems or smoking member(s) then gloominess and sense of hopeless future.

On analyzing the socio-demographic characteristics as risk factors for smoking, it was obvious that male students, who are less than 20 years, who study in theoretical faculty and with non-working mothers or mothers working professional occupations are significantly at higher risk for this problem (p<0.05). Insignificant difference was found with residence, marital status, father or mother education and father occupation. (Table 2).

The effect of family life characteristics on smoking is clarified in Table (3); smoking increased significantly with increased number of family members, late order of the student among his siblings, living away from the family and deprivation of mother by her travel (p<0.05).

Personal life factors which were significantly associated with smoking (p<0.05) were increased pocket money, getting money from work alone or from dual sources (family and work), participation in clubs, absence of participation in activities and suffering from a chronic disease (Table 4).

Logistic regression analysis for significant risk factors for smoking revealed that smoking remained significantly (p<0.05) higher among male students, with increased family number, late order in the family, living away from family, young in age, having chronic diseases and studying in theoretical faculties (Table 5).

4. Discussion:

Although smoking is not a new problem, it has become a source of increasing concern due to the increase in realization of its volume, horrible morbidity and mortality. Since the adoption of the Framework Convention for Tobacco Control (FCTC) in 2005, all countries allover the world try to activate and initiate efforts to face smoking, especially among youth **(Centers for Disease Control, 2009& Tobacco free initiative, 2010)**.

Smoking is a great national disaster in Egypt as it impacts health, economy and social aspects of the community. It has become worse and worse in since 1990s as it has become more and more prevalent among youth and teenagers **(Ahmed, 1999; MOHP, 2000; Nassar, 2003& Egyptian Smoking Prevention Research Institute, 2007)**.

This study revealed a prevalence of ever smoking of 22.1% with among male prevalence of 30.3% while current smokers were 20.2%. These percentages are higher than the previously reported figure among a younger age Egyptian adolescents in Global Youth Tobacco Survey, 2005 (13.6% for ever smoking with among males prevalence of 19.6%

Table (1): Smoking prevalence & Some features of smoking pattern

Item	(No)	(%)
Smoking: (among studied sample =1072).		
• Ever smoke	237	22.1
• Current smoker	217	20.2
Cigarettes / day:(among smokers= 237)		
• Less than 10	59	24.9
• 10-20	104	43.9
• more than 20	74	31.2
Age of start : (among smokers= 237)		
• Less than 15	81	34.2
• 15-20	140	59.1
• more than 20	16	6.8
Addition of Bang :(among smokers= 237)		
• Yes	159	67.1
• No	78	32.9
Motives: (among smokers= 237)		
• Participation with friends	88	37.2
• Family negligence or problems	77	32.5
• Feeling of gloominess and emptiness	55	23.2
• Curiosity or to revitalize	15	6.3
• Too much money	2	0.8

Table (2): Smoking & Socio-demographic characteristics

Item	Smoking prevalence (%)	Smokers (N=237) (%)	Non smokers (N=835) (%)	P* value	OR 95% CI
Age:					1.74 (1.28- 2.4)
• Less than 20	25.7	70.0	57.4	0.000*	
• More than 20	16.6	30.0	42.6		
Gender:					19.1 (8.89- 41.1)
• Male	30.3	97.0	63.2	0.000*	
• Female	2.2	3.0	36.8		
Residence:					1.21 (0.85-1.73)
• Urban	23.0	76.8	73.2	0.26	
• Rural	19.7	23.2	26.8		
Faculty:					2.41 (1.66- 3.5)
• Theoretical	26.4	81.4	64.6	0.000*	
• Practical	12.9	18.6	35.4		
Marital status:					
• Not married	22.9	93.2	89.2	0.19	
• Engaged	14.9	5.5	8.9		
• Married	15.8	1.3	1.9		
Father education:					
• Illiterate	25.8	20.3	16.5		
• Primary education.	25.8	22.8	18.6	0.15	
• Secondary education	20.9	26.6	28.5		
• University education	19.1	30.4	36.4		
Father occupation:					
• Unskilled workers	25.6	26.2	21.6		
• Skilled workers	21.4	19.4	20.2	0.49	
• Intermediate	21.5	38.0	39.4		
• Professional	19.9	16.5	18.8		
Mother education:					
• Illiterate	27.1	28.3	21.6		
• Primary education.	22.6	16.9	16.4	0.12	
• Secondary education	18.9	28.7	35.0		
• University education	21.5	26.2	27.1		
Mother occupation:					
• Housewives.	23.6	59.5	55.2	0.045*	
• Intermediate Professional	18.5	29.5	37.0		
	28.6	11.0	7.8		
Total	22.1	100.0	100.0		

* Statistically significant.

Table (3): Comparison of smokers versus non smokers as regard family characters& family life

Item	Smoking prevalence (%)	Smokers (N=237) (%)	Non smokers (N=835) (%)	P* value	OR (95% CI)
Family number:					
• (2-3)	5.1	5.9	31.4	0.0000*	1
• (4-5)	24.2	57.0	50.7		5.97
• (6 or more)	37.0	37.1	18.0		10.98
Students family order:					
• First.	12.9	27.0	51.6	0.0000*	1
• Middle.	28.4	52.3	37.5		2.67
• Last.	35.0	20.7	10.9		3.63
Living:					7.17 (4.07-12.69)
• With family	19.5	83.1	97.2	0.000*	
• Away from family.	63.5	16.9	2.8		
Father alive:					1.31 (0.79- 2.17)
• Yes	21.7	89.5	91.7	0.272	
• No	26.6	10.5	8.3		
Mother alive:					1.51 (0.84- 2.69)
• Yes	21.5	91.6	94.7	0.069	
• No	31.3	8.4	5.3		
Parents traveling:					
• No	21.3	88.2	92.3	0.001*	
• Father	24.0	7.6	6.8		
• Mother	100.0	1.3	0.0		
• Both	50.0	3.0	0.8		
Return: (travelers)		(N=28)	(N=64)		
• Many times/ year	22.7	17.9	26.6		1
• One time/ year	27.1	46.4	54.7	0.1	1.26
• Every more than year	45.5	35.7	18.8		2.83
Total	22.1	100.0	100.0		

* Statistically significant.

Table (4): Comparison of smokers versus non-smokers as regard some personal factors

Item	Smoking prevalence (%)	Smokers (N=172) (%)	Non smokers (N=1028) (%)	P* value	OR 95% CI
Pocket money/month:					
• Less than 100 Pounds	13.6	13.5	24.4	0.000*	
• 100-150 pounds	20.5	37.6	41.4		
• More than 150 pounds	28.9	48.9	34.1		
Source of pocket money:		61.6	80.7		
• Family	17.8	19.4	10.3	0.000*	
• Work	34.8	19.0	9.0		
• Both	37.5				
Participation in Club:					1.58 (1.1-2.3)
• Yes	29.4	19.8	13.5	0.016*	
• No	20.8	80.2	86.5		
Participation in activity:					
• Sport	9.7	11.8	31.1	0.000*	
• Cultural	20.9	5.9	6.3		
• Social	35.7	6.3	3.2		
• No	26.7	75.9	59.3		
Suffering from disease:					4.44 (2.3-8.6)
• Yes	54.1	8.4	2.0	0.000*	
• No	21.0	91.6	98.0		
Total	22.1	100.0	100.0		

* Statistically significant.

Table (5): Logistic regression analysis for important risk factors for smoking

Variable	β coefficient	SE	Wald	p-value
Intercept	0.503	0.937	0.288	0.591
Gender	3.045	0.439	48.206	0.000*
Family number	2.069	0.35	34.9	0.000*
	0.603	0.209	8.308	0.004*
Family order	1.552	0.286	29.417	0.000*
	0.542	0.265	4,193	0.041*
Away from Family	1.98	0.344	33.226	0.000*
Age	0.648	0.195	10.995	0.001*
Disease	1.397	0.465	9.037	0.003*
Faculty	0.795	0.293	7.333	0.007*
Travel parent	1.161	0.622	3.481	0.062
	0.53	0.695	0.582	0.446
Source of money	0.493	0.294	2.812	0.094
	6.179	0.343	0.032	0.0857
Activities	0.464	0.274	2.874	0.09
Club	0. 497	0.298	2.786	0.095
Mother occupation	4.801	0.389	0.015	0.902
	0.344	0.362	0.903	0.342
Income	0.568	0.315	3.263	0.071
	0.187	0.229	0.666	0.414

- * Statistically significant.

- Reference category for each variable was the last one in previous analysis tables [e.g. "Female" for Gender , "6 or more" for Family number …etc].It is not included in the table as it was taken as zero while the other categories were presented in comparison with this reference one.

while current smokers were 4.1%) **(Allam& Abd ElAziz, 2007& Centers for Disease Control, 2009)** denoting that the problem increases on entering the university with decreased control over youth.. Also, The revealed prevalence in the present study is higher than ever smoking prevalence among American adults (19.8%) **(Centers for Disease Control and Prevention, 2007)** and among United Arab Emirates university students (15.1%) **(Mandil, 2007)** denoting the considerable size of smoking problem in Egypt**.**

However, the prevalence of ever smoking of 22.1 % is less than that found some years ago among Egyptian university students (29.4%) **(Abdel Hamid, 2000)**, among 2dry school students in south-east Asia

(33.2%) **(Shamsuddin& Abdul Haris, 2000)** and recently among university students in USA (25%) **(Halperin et al., 2010)**.The explanation of the lower percentage in the present study is most probably the increasing awareness of the horrible consequences of smoking and the increasing religion adherence (Radwan **et al., 2000)**.

In USA, the number of cigarettes smoked per day was less than 1 in 40% less than 10 cigarettes/day in 80% of smoking university students **(Halperin et al., 2010),** while in our study, only 25% of smokers expire less than 10 cigarettes/day. This agrees with **Ahmed and Others (1999)** regarding the fall of cigarette consumption in the United States and

focusing of tobacco companies on overseas markets including Egypt to maintain their profits leading to growing cigarette consumption rate in Egypt.

Agreeing with the difficulty of quitting when starting smoking early **(Robinson, 2003),** quitting rate in the present study was very low (8.4%) compared with 32% found among control group of adults in the study of **Wu and colleagues (2009)**. In an Egyptian study, 44% of smoking university students believed that quitting smoking is not possible at all **(Allam& Abd ElAziz, 2007**) .

In addition, during the present study we found that the age of starting smoking was between 15 and 20 years for about 60% of smokers and less than 15 years in another one third of smokers. This agrees with the gradual decline of the mean age of smoking initiation in Egypt **(El-Salakawy, 1995& Egyptian Smoking Prevention Research Institute, 2007)** and other developing countries **(Shamsuddin& Abdul Haris, 2000& Almutairi, 2010)**.In contrast, among USA university students, 21% started between 15-20 years and 41% started after 20 years old reflecting the decreasing trend of smoking among the teenagers in the developed countries **(SAMHSA, 2005)**.

The serious finding that 67% of smokers in the present study may add bang to cigarettes agrees with the reported strong association between smoking and drug abuse **(Abdel Hamid, 2000; Best et al.,2000& Nassar,2003)**. That's because cigarettes -which are considered legal for adults -are almost always the gateway to drug abuse. So, smoking prevention will - at the same time- prevent other serious problems.

Participation with friends represented the main motive for smoking in more than 37% agreeing with other researches **(Egyptian Smoking Prevention Research Institute, 2007**; **Mandil, 2007; Almutairi, 2010& Halperinet al., 2010)** pointing to the strong influence of peers and friends. So, discussing and supervising the selection of friends among youth is a very vital issue. A higher level of prevention can be provided by family, school and university through care about smoking friends and doing efforts to help them to quit. Family related motives came next and included negligence, presence of continuous problems between parents, presence of a negative role model inside the home in the form of a smoking father or brother agreeing with multiple researches among university and younger students (**Mukhtar et al., 2006; Mandil, 2007; Almutairi, 2010& Baska et al., 2010)**. In 23.2% of smokers, we found that the main motive was feeling of gloominess and hopeless future which were related to the political, economic and social aspects of the community.

Predominantly higher prevalence among males versus females (30.3% versus 2.2% respectively) agrees with other different studies but with great variation in the degree of this predominance according to the cultural difference. In a previous Egyptian study, it was 22% versus 1.7% respectively (**Egyptian Smoking Prevention Research Institute, 2007)** while among Americans, it was 23.9% versus 18.0% respectively **(Centers for Disease Control and Prevention, 2007).** Females constituted (3%) of smokers in our study which is some what less than among United Arab Emirate university students where female smokers constituted 8.9% **(Mandil,2007).** Generally the Arab cultural norm makes it less acceptable for female to smoke and it may be considered as a social stigma. Some cultural norm promotes smoking among men as mentioned for Chinese American **(Wu et al., 2009).**

The significantly higher theoretical faculties' students among smokers than practical faculties' students with increased risk (2.4 times) agrees with previous studies revealed about two times risk (**Abdel Hamid, 2000& Mandil, 2007).** Students studying in theoretical faculties may be less aware of smoking hazards, have more leisure time which expose them more to friends' pressure and may be disappointed with community less appreciation [for] theoretical study. Regarding mother occupation, both housewives and mothers working professional occupations have more smoking sons than mothers with intermediate occupation. Housewives may be busy with the housework and not aware enough with the rapid progression of smoking problem among youth and decreasing age of initiation while mothers with professional occupation may be busy with her profession with no time to speak and share activities with her sons in addition to providing them with much money agreeing with a previous report (**Nassar, 2003**). Absence of statistical association between parents educational level or father occupation with student's smoking status disagrees with among United Arab Emirate university students **(Mandil, 2007**) but agrees with results of 2007Slovakia Global Youth Tobacco Survey (**Baska et al., 2010).**

Regarding family- related factors which is the main concern of this study, It was proved that being a son of a family with large number of kids is a risk factor for smoking with high statistical significance for increasing trend. Also, such significance for increasing trend was found with late order among siblings denoting the importance of family planning to give the sufficient care for all kids in different stages of their life. Our results in this respect are the first report of extension of such influence of having large number of kids on smoking state of sons up to the university age.

Emphasis on the importance of enjoying the family life is also revealed in this study as the

percentage of students living away from their family among smokers is (16.9 %) compared to (2.8%) among non-smokers with high statistical significance and increased risk to 7 times coinciding with other research results regarding the importance of family supervision **(Shamsuddin& Abdul Haris, 2000& Nassar, 2003).** The same explanation of insufficient supervision can be proposed for the effect of absence of mother by travel indicating the particular importance of maternal presence and interest in her sons' and daughters' concerns.

Significance of association between smoking and increasing pocket money and working state of students found with bivariate analysis disappeared with multivariate analysis. This inconsistency agrees with variation in results of previous studies (**Mukhtar et al., 2006; Mandil, 2007& Almutairi, 2010)** as extra-money encourages expending money on cigarettes but alone can't be the motive. Participation in clubs and non participation in activities were significantly higher among smokers and these risk factors were previously found for drug addiction **(Abdel Hamid, 2000).** This is attributed to the two different types of influence as participation in clubs allows long exposure to the effect of peers and friends and gives legal reason for spending a lot of time outside the home while participation in activities- if properly done- enables youth to make use of time in a useful manner. Although the effect of participation in clubs and non participation in activities disappeared in our study on multiple regression analysis, participants in sports and cultural activities had obviously very low smoking prevalence.

The association between adoption of smoking behavior and being diseased with chronic disease whether it was a pain associated disease, skin, endocrinal or psychiatric illness agrees with others **(Smolensk et al., 2009)** and is probably due to the disappointment in some life aspects with the need for stress alleviation which is falsely thought to be got by smoking .

On logistic regression analysis for important risk factors, many factors remain significant with smoking. Male gender, increased number and late order in the family, living away from family, young age, having a chronic disease and studying in theoretical faculty are the most important predictors that should be targeted in smoking prevention activities.

5. Conclusion:

In conclusion, this study directs the attention to the fact that problem of smoking among university students has important contributing family related factors and personal factors in addition to socio-demographic factors. Governmental efforts alone can't face them all as they need coordinated efforts from the family, school and university besides the government.

Recommendations

1- Intensive antismoking program starting from primary schools especially for males.

2- Theoretical faculties students should not only recommended by anti-smoking program but also asked to be antismoking educators. This positive role is always very effective in prevention.

3- Supervision of the behavior of student's peers and friends and use positive peer models for advantageous use of peer pressure.

4- Family planning efforts to got families of a proper number with advising parents to give equal care to late ordered kids are corner stones in smoking prevention.

5- Institutional care for students living away from family with paternal attitude of university staffs.

6- Students with chronic illness should get special care and be supported medically, socially and psychologically besides antismoking efforts.

7- Mother care, not excess pocket money, care about working student and club-participants and encouragement of participation in sport and cultural activities are also important.

Competing interest: None at all

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Effect of Educational Program on Insight into Illness and Attitudes toward Medications among Schizophrenic Patients

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Abstract: This study assessed the impact of the constructed nursing educational program on insight and attitudes toward medications in a sample of schizophrenic patients who were randomly selected. A quasi-experimental design was utilized in this study. The study was conducted in in-patient clinic at Benha governmental hospital for mental health. A total sample of 40 schizophrenic patients (experimental group 20 patients and control group 20 patients) were selected randomly to participate in the study. Three tools were used for data collection, socio-demographic/ medical data sheet, Insight Scale, and Drug Attitude Inventory Scale. Findings of this study proved the effectiveness of the constructed educational program on schizophrenic patients' insight, whereas no effect on patients' attitude toward medications .The total insight and patients' attitude toward medications were not correlated significantly with number of hospital admission, duration of illness and age at onset of the disease. Patients' attitude toward medications didn't correlated significantly with insight at pre, post, and follow-up program. Therefore the study highlight there is a need to conduct educational sessions periodically for the schizophrenic patients, a matter which increase the schizophrenic insight into illness.

1. Introduction

Schizophrenia is a major mental illness that affects approximately 1 % of the population world wide. The cause of schizophrenia remains unknown despite extensive research; however, evidence suggests that genetic factors, early environmental influences and social issues may contribute to the development of this disease (Mueser and McGurk, 2004).

Insight into illness is the ability of the patient to understand the objective circumstances of his illness, its nature and the optimum circumstances that would help in curing it. Patients with schizophrenia often have difficulty identifying their symptoms of illness and recognizing the presence of a mental disorder (Kennedy, et al., 2000 and Jacobson & Jacobson, 2001). Lack of insight of patients with schizophrenia into various aspects of their illness and treatment is an important clinical issue (Goodman et al., 2005). This lack of insight into illness interferes with the relationship between patients and their treating clinicians and reduces patients' willingness to follow through with recommended treatments (Stuart and Laraia , 2001).

A patient with schizophrenia may fail to take his or her medication, as prescribed, for a number of reasons. The patient may be disturbed by medication's side effects, may believe he or she doesn't need the medication, may believe that the medication is poison. Eighty percent of persons with schizophrenia who stop taking their medication have a relapse within a year, compared with 30% who continue their medication and relapse. The most common causes of relapse and re-hospitalization for "revolving-door" inpatients are medication non-adherence and medication non-response (Haynes, et al., 2000) and (Mohr, 2003).

Patient education is a major component of the treatment plan for the patient with schizophrenia, education about their mental and physical health empowers them to collaborate with their clinicians to make rational treatment decisions. Existing options to increase adherence include psychosocial skills training and targeted adherence training (Calkins & Iacono, 2003).

American Psychiatric Association, 2004 added that, administration of medications is critical. The nurse should develop a method with the patient to remind the patient to take medications, teach the patient about the medication, develop methods with the patient to monitor medication response and side effects, and assess if the patient is taking the medication as ordered.

Therefore, this study was conducted to construct an educational nursing program for schizophrenic patients; induce positive changes in the schizophrenic patients as regard insight and attitude toward medications and assess the impact of this

program on patient insight into illness and attitude toward medications.

2. Subjects and Methods
Aim of the Study
Aims of the study are to:

1- Construct an educational nursing program for schizophrenic patients.

2- Induce positive changes in the schizophrenic patients as regard insight into illness and attitude toward medications.

3- Assess the impact of the program on patients insight into illness and attitude toward medications.

Research Design
A quasi-experimental design was used to achieve the study aims

Setting
In-patient departments at Benha governmental hospital for mental health. The mental health services in this hospital are offered through the in-patient as well as the out-patients clinics for all age groups. Care is provided by multidisciplinary team, psychiatrists, nurses, social workers and psychologists.

Sample
A total sample of 40 schizophrenic patients (experimental group 20 and control group 20) were selected randomly to participate in the study.

Some measures were taken to insure privacy and confidentiality such as putting code number for each patient

The sample was recruited according to the following criteria:

- age from 20 to less than 60 years
- inpatients
- chronically ill for more than 6 months
- both sex

Tools for Data Collection
a- Socio-demographic/ medical data sheet
b- Insight Scale
This scale was developed by (Birchwood et al, 1994) and was used to determine the degree to which schizophrenic patients are aware of their illness, their need of treatment and their ability to re-lable experiences. This scale consists of 8 items, each item was scored on a 3-point scale (agree, disagree, and unsure).

c- Hogan Drug Attitude Inventory Scale
This scale was developed by (Hogan & Awad, 1992) to measure subjective response to medication in an effort to obtain a more complete understanding of factors influencing medication compliance. The scale has 15 items that were scored as true and 15 items that were scored as false in the case a fully compliant response. A correct answer to these items was scored as plus 1. An incorrect answer was scored as minus 1. The total score is the sum of pluses and minuses. A positive total score means a compliant response. A negative total score means a non-compliant response. Zero total score means partial compliant response.

Procedure
After official permission from the hospital director, the researcher contacted to the head nurse to explain the purpose and procedure of the study and determine the available time to connect with the patients and demonstrate the educational session. The study sample was selected randomly, then the sample assigned to 20 patients (experimental group) and 20 patients (control group). Experimental group was informed that they will have educating sessions in addition to group activities, while the control group will have the routine activity. Data were collected through interviewing with experimental and control groups at pre, post, and follow up of the program.

- Both groups (control and experimental) were interviewed individually to collect pre-assessment data related to socio-demographic , insight and drug attitude scales for 2 weeks, twice /week.

- 12 sessions, each session was 45 minutes. for 12 weeks one time /week. were provided for the experimental group only with the total hours 9. Each session had its own title and objective according to its content.

- Both groups (control and experimental) were interviewed individually to collect post-assessment data related to insight and drug attitude scales for 2 weeks twice /week.

-Both groups (control and experimental) were departed for 4 weeks.

- Both groups (control and experimental) were interviewed individually to collect follow up-assessment data related to insight and drug attitude scales for 2 weeks twice /week.

Data collection lasted for 22 weeks which started from the beginning of May, till the 10/ 2009

3. Results
Table (1) shows that, 11 (55.0%) from study and 15 (75.0%) from control group were admitted to hospital for more than 3 times, 9 (45.0%) from study and 11 (55.0%) from control were from 20 to less than 30 years old, 9 (45.0%) from study group were diagnosed as residual schizophrenia and 8 (40.0%) from control group were diagnosed as paranoid schizophrenia. The mean duration of illness of the study group is 16.8 years while in control group 13.2 years with standard deviation 10.9 and 9.2 respectively.

Table (2) This table shows that, the difference between patients' attitude toward medications program in study and control groups at pre, post, and follow-up didn't show statistically significant difference (p=0.45 and 0.69 respectively).

Table (3) shows no statistically significant difference between " ability to re-lable experience in the study group and control group at pre, post, and at follow-up(p=0.4) (p=0.09) (p=0.056) respectively.

Regards to "awareness of illness", at pre program there was no statistically significant differences between study group and control group (p=0.5). At post program, there was a highly statistically significant differences between study group and control group (p=0.008). At follow-up there was no statistically significant differences between study group and control group (p=0.12).

Regarding "insight into need of treatment" , there was no statistically significant difference between study and control group at pre, post, and at follow-up(p=0.50) (p=0.114) (p=0.11) respectively.

Generally total insight at preprogram didn't show statistically significant difference between study and control group (p=1.0). However, at post and follow-up program the difference between study and control groups was statistically significant p=0.04 and 0.007respectively.

Table (4) revealed that, total insight and total patients' attitude toward medications were not correlated significantly with number of hospital admission at pre, post, and follow-up program (p>0.05).

Table (5) shows that, total insight and total patients' attitude toward medications were not correlated significantly with duration of illness at pre, post, and follow-up program (p>0.05).

Table (6) shows that, total insight and total patients' attitude toward medications were not correlated significantly with age at onset of the disease at pre, post, and follow-up program (p>0.05).

Table (7) revealed that, patients' attitude toward medications didn't correlate significantly with insight at pre, post, and follow-up program (p>0.05).

4. Discussion
Medical Characteristics of Patients in Study and Control Groups.

Nearly half of patients in study group and more than half of patients in control group, age of onset of disease ranged from 20 to less than 30 years. This finding indicates no statistical significant difference between study and control groups. This may be due to the age at onset of schizophrenia usually occurs early in life – in adolescence or young adulthood and the disease is a progressive and disabling condition. This result is consistent with

Boyed (2002) who found that, the schizophrenia begins earlier than the age of 25 years.

The findings of the present study revealed that total insight and total patients' attitude toward medications didn't correlated significantly with age at onset of the disease at pre, post, and follow-up program. This result is consistent with Mintz et al.,(2003) who found no significant correlation between lack of insight and age at illness onset.

In relation to duration of illness, there was no statistical significant difference between study and control groups. This result contradicted with El-Nady(2003) who found a statistical significant differences between the control and experimental groups in relation to the duration of illness. In this respect, Osman (1990) reported that, long stay of chronic patients (duration of illness 15 years or more) had more frequency and initiations of interactions. This may be due to the fact that patients might have achieved a state of institutionalism in which they became so adapted on the life within the hospital and found their own contacted and relationships within this system. Osman added that because of the lack of community mental health services in Egypt, psychiatric hospitals tend to keep schizophrenic patients especially the chronic ones for long periods.

The findings of the current study revealed that total insight and total patients' attitude toward medications didn't correlate significantly with duration of illness. This result is consistent with Mintz et al.,(2003) who found no significant correlation between lack of insight and duration of illness. Also, McEvoy et al., (1989) failed to identify any relationship between the degree of insight and severity of psychopathology. Further, the changes in insight scores during hospitalization did not vary consistently with changes in acute psychopathology. The only significant finding was that there was an overall relationship between insight and clinical outcome in schizophrenia. The authors concluded that the deficiency in insight could not be explained on the basis of psychopathology. David et al., (1992) also reported a moderate correlation between insight and severity of illness in schizophrenics in a subsequent study. Amador et al., (1993) identified moderate correlation between insight and course of illness.

Concerning number of hospital admission, the current results found that, more than half of patients in study group and three quarters of patients in control group were admitted to hospital for more than three times in their life. There was no any statistical significant difference between two groups. This result indicated that the schizophrenia is episodic and patients' ability to adjust with a disease stressors is decreased. Also, this result indicates that patients

were noncompliant with medication regimens and lack of insight. This might be due to the lack of social support, the rejection of the patient by family and the patients' dissatisfaction with relations which are significant predictors of relapse and re-hospitalization. In addition side effects of medications which were prescribed for the treatment of schizophrenia are often unpleasant for the patient and are likely to decrease compliance with doctors' recommendations and consequently re-hospitalization.

This result is consistent with El-Kayal (2002) who pointed out that, the majority of her studied sample was previously admitted more than five times, due to lack of family support which increases relapses among those patients. This result also agreed with Elnady (2003) who found no significant statistical difference between the control and the experimental groups regarding to number of hospitalization.

However, this result is inconsistent with Stuart et al., (2001)who found a significant difference between cases and controls regarding to number of relapses and hospitalizations where patients in the case group experienced less relapses and even less hospitalization than in the control group.

The findings of the current study revealed that, total insight and total patients' attitude toward medications didn't correlated significantly with number of hospital admission. This result is consistent with Mintz et al.,(2003) who stated that, no significant correlation between lack of insight and the number of hospitalization. In contrary, Amador et al., (1993) identified moderate correlation between insight and number of hospitalization and better insight among patients with more hospitalization.

The lack of statistical significant difference in medical characteristics between study and control group means that all of these factors are recessive that didn't affect the intervention program.

Comparison between Control and Study Group In Relation to Patients' Attitude toward Medications and Insight at Different Study Periods
The finding of the present study revealed that there was no statistical significant difference between study and control group at pre, post, and follow-up program regards to patients' attitude toward medications.

This result may be due to sample selection from inpatient units and thus compliance increased as the patients compelled to take medications. Also, decreased size of the sample and increased percent of compliance at pre program lead to lack of significant difference. Also, this result can be interpreted as psycho-educational interventions focused primarily

on dissemination of knowledge about schizophrenia, treatment, and medication without focusing on attitudinal and behavioral change to achieve medication adherence.

This result is consistent with Kempt et al., (1996) who found that, improvement of attitude based on gain knowledge didn't lead to change in attitude. Also, Gilmer et al., (2004) reported a lack of significant differences in compliance between the study and control group. However Glynn et al.,(2002)reported significant group differences in compliance immediately after the intervention but a lack of difference at follow-up one year later.

These results disagree with Kempt et al., (1996) who found a significant improvement in compliance and attitude towards psychotropic medication as compared to controls. Also, Lacro et al., (2002) tested compliance therapy after a combination of cognitive approaches and motivational interviewing to enhance medication compliance and formed sustained gains in medication compliance over 18 months after hospital discharge and better measurement of insight and attitudes towards medication.

On the other hand Bak et al., (2003) noted that hospitalized psychotic inpatients tended to focus on secondary benefits of medication, such as keeping them out of the hospital as well as allowing them to work and maintain significant relationships. The more important of these benefits were the adherent of the patient to treatment. One major long-term problem was coined by Weiden et al., (2004) as an awakening experience following a patient's first severe psychotic episode, marked improvement may occur. However, there may then be a realization by the patient that there is something wrong with him or her. This may lead to a post-psychotic depression whereby the patient may finally understand that he or she suffers from a severe and debilitating illness. The consequences of these realizations may include suicidal ideation or behavior. Such an awakening may lead to a denial and outright refusal to take antipsychotics.

Among insight into illness, "ability to re-lable experience", represented about two thirds of patients in study group and four fifths of patients in control group and the difference didn't prove any statistical significance at pre program. At post program, more than three quarters of patients in study group and half patients in control group were able to re-lable experience. Where as, at follow-up (after 2 months) program, more than four fifths of patients in study group and nearly half of patients in control group were able to reliable experience and didn't show statistical significant difference. Generally "ability to re-lable experience" didn't show statistical significant

difference at pre, post, and follow-up program in study and control group.

Regarding awareness of illness two fifths of patients in study group and one quarter of patients in control group were aware to their illness at pre program. The difference didn't prove any statistically significant difference. At post program, more than two thirds of patients in study group and more than one quarter of patients in control group were aware to their illness. There was highly statistically significant difference between two groups. This may be due to increased percent of awareness of illness in study group as a result to educational program. At follow-up program two thirds of patients in study group and more than one third of patients in control group were aware to their illness. The difference didn't prove any statistical significant difference.

Concerning "insight into the need of treatment", the current study revealed that, there was no statistical significant difference between study and control group at pre, post, and follow-up program

This result can be interpreted as many patients have cognitive distortions concerning medication use. They often feel there is something wrong with them if they need medication (they are psychological misfits), they may feel if they have to take medication, they are morally weak and the medication is simply a crutch and doesn't do anything for the life problems they need to deal with. Patients may also worry about the medication controlling their thoughts and actions. This result is consistent with Lacro et al., (2002) who explored reasons for medication non adherence. For the patient, the meaning of taking medication determines his or her attitude in taking such medication. If the patient perceives that the medication can help, he or she will take it.

Total insight didn't statistically significantly differ between study and control group at pre, post, and follow-up program. However, at post program and follow-up the difference between two groups was statistically significant. Also, the current study revealed that, total insight among pre, post and follow-up program in study group was statistically significantly different. However, in control group, there was no statistical significant difference among pre, post, and follow-up program regards to total insight. This result can be interpreted as a result of educational program that were concerned of improving insight by educating patients about schizophrenia and accepting their mental disorder. These results go on line with Macpherson et al., (1996) and Kempt et al., (1996) who tested compliance therapy, a combination of cognitive approaches and motivational interviewing to enhance medication adherence. Intervention sessions encouraged patients to articulate their beliefs and ambivalence about antipsychotic medication while focusing on adaptive behaviors and the importance of staying well. Therapists helped patients connect indirect benefits of medication, such as improved personal relations, medication adherence and symptom reduction. In patients who received compliance therapy demonstrated sustained gains in medication adherence over 18 months after hospital discharge and better measures of insight and attitude toward treatment.

The finding of the present study revealed that Patients' attitude toward medications didn't correlated significantly with insight at pre, post, and follow-up program. These results go on line with Martha et al.,(2002) who found no significant differences between the three groups of patients in previous adherence to prescribed medication regimens, symptoms, functional level, or insight into illness .He also added that attitudes may be more positive in patients who recognize therapeutic drug effects .

On the other hand Freudenreich et at., (2004) concluded that greater insight into illness, and better social functioning would be associated with better attitudes toward psychiatric medication.

5. Conclusion

Findings of this study proved the effectiveness of the constructed educational program on schizophrenic patients' insight. The educational program has no effect on patients' attitude toward medications .The total insight and total attitude toward medications were not correlated significantly with number of hospital admission, duration of illness, age at onset of the disease. Patients' attitude toward medications didn't correlate significantly with insight at pre, post, and follow-up program.

6. Recommendation

- Psychiatric nurses need to conduct educational sessions periodically for the schizophrenic patients, a matter which increases their insight into illness.
- Further study should be done to study the effect of educational program on insight and attitudes toward medications using a large sample of schizophrenics and long duration of educational program.
- Further study should be done on schizophrenics who are treated with atypical antipsychotics, typical antipsychotics, or mixed medication regimens to assess the insight into illness and attitude toward medication

Total number of the study groups in the study periods

	Study group	Control group
Pre program	20	20
Post program	19	17
Follow-up	18	17

Table 1: Medical characteristics of patients in the study groups

Items	Study group (No=20) No %		Control group (No=20) No %		X2 test	P- value
Age at onset of the disease						
< 20 years	3	15.0	0	0.0		
20-<30	9	45.0	11	55.0		
30- <40	4	20.0	7	35.0	5.21	0.26
40 - <50	3	15.0	2	10.0		
50 or more	1	5.0	0	0.0		
Diagnosis						
-Residual schizophrenia	9	45.0	7	35.0		
-Disorganized	1	5.0	1	5.0		
-Undifferentiated	7	35.0	4	20.0	3.34	0.34
-Paranoid	3	15.0	8	40.0		
-catatonic	0	0.0	0	0.0		
Duration of illness (years) $(x \pm SD)$	16.85± 10.9		13.20 ± 9.2		t-test 1.14	0.26
No of hospital admission						
Once	2	10.0	0	0.0		
Twice	2	10.0	1	5.0	3.06	0.38
Three times	5	25.0	4	20.0		
More than three	11	55.0	15	75.0		

Table 2: Frequency distribution of patients' attitudes toward medications at pre, post, and Follow-up in the study groups

patients' attitudes toward medications	Study group No	%	Control group No	%	X2	p-value
Pre program:						
-Noncompliant	5	25.0	8	40.0		
- Partial compliant	0	0.0	2	10.0	3.6	0.11
-Compliant	15	75.0	10	50.0		
Post program:						
Noncompliant	3	15.8	7	41.2	Fisher	
-Compliant	16	84.2	10	58.8	exact test	0.13
Follow-up:						
Noncompliant	3	16.7	6	35.3	Fisher	
-Compliant	15	83.3	11	64.7	exact test	0.26
(p-value)	0.45		0.69			

Table 3a: Frequency distribution of insight into illness at pre, post, and follow-up in the study groups.

of insight into illness	Study group		Control group		X2	p- value
	No	%	No	%		
Ability to relable experience:						
Pre program						
- unable	7	35.0	4	20.0	1.12	0.4
- able	13	65.0	16	80.0		
Post program						
- unable	4	21.1	8	47.1	2.73	0.09
- able	15	78.7	9	52.9		
Follow-up						
- unable	3	16.7	8	47.1	3.74	0.056
- able	15	83.3	9	52.9		
(p-value)	0.31		0.12			
Awareness of illness						
Pre program						
- unaware	12	60.0	15	75.0	1.02	0.50
- aware	8	40.0	5	25.0		
Post program						
- unaware	5	26.3	12	70.6	7.05	0.008**
- aware	14	73.7	5	29.4		
Follow-up						
- unaware	7	38.9	11	64.7	2.33	0.12
- aware	11	61.1	6	35.3		
(p-value)	0.12		0.24			

(*)statistically significant at P<0.05 (**) highly statistically significant at P<0.01

Table 3b: Frequency distribution of insight into illness at pre, post, and follow-up in the study groups (con).

Of insight into illness	Study group		Control group		X2	p- value
	No	%	No	%		
Insight into need of treatment						
Pre program						
- Lack of Insight	5	25.0	8	40.0	1.02	0.50
- Insightful	15	75.0	12	60.0		
Post program						
- Lack of Insight	2	10.5	6	35.3		0.114
- Insightful	17	89.5	11	64.7		
Follow-up						
- Lack of Insight	2	11.1	6	35.3		
- Insightful	16	88.9	11	64.7		0.11
(p-value)	0.24		0.71			
Total of insight scale						
Pre program						
- Lack of insight	8	40.0	8	40.0	0.000	
- Insightful	12	60.0	12	60.0		1.00
Post program						
- Lack of insight	4	21.1	8	47.1	6.15	
- Insightful	15	78.9	9	52.9		0.04*
Follow-up						
- Lack of insight	1	9.1	8	47.1		
- Insightful	17	90.9	9	52.9		0.007**
(p-value)	0.050*		0.95			

(*) statistically significant at P<0.05   (**) highly statistically significant at P<0.01

Table 4: Correlation between number of hospital admission and insight into illness and patients' attitudes toward medications in the study groups at pre, post and follow-up

Items	Number of hospital admission		
	Pre program r (P)	Post program R (P)	Follow-up R (P)
Total insight	0.30 (0.19)	-0.12 (0.60)	-0.36 (0.13)
patients' attitudes toward medications	-0.06 (0.78)	-0.01 (0.95)	-0.35 (0.16)

Table 5: Correlation between duration of illness and insight into illness and patients' attitudes toward medications in the study groups at pre, post and follow-up

Items	Duration of illness		
	Pre program r (P)	Post program R (P)	Follow-up R(P)
Total insight	0.08 (0.73)	-0.11 (0.62)	-0.44 (0.06)
patients' attitudes toward medications	-0.10 (0.67)	-0.14 (0.55)	-0.23 (0.35)

Table 6: Correlation between age at onset of the disease and insight into illness and patients' attitudes toward medications in the study groups at pre, post and follow-up

Items	Age at onset of the disease		
	Pre program r (P)	Post program R (P)	Follow-up R (P)
Total insight	-0.26 (0.25)	0.04 (0.86)	0.24 (0.33)
patients' attitudes toward medications	0.25 (0.28)	-0.01 (0.95)	0.14 (0.58)

Table 7: Correlation between insight into illness and patients' attitudes toward medications in the study groups at pre, post, and follow-up

Items	Insight		
	Pre program r (p)	Post program r (p)	Follow-up r(p)
patients' attitudes toward medications	-0.23 (0.33)	0.35 (0.13)	0.33 (0.17)

(*) statistically significant at $P<0.05$

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-Methyl Crotonitrile in Synthesis of Some New Compounds and Evaluation of Their Herbicidal Efficiency

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Abstract: In an effort to establish new candidates with improved antiherbal   activities we report here the synthesis and herbicidal evaluation of various series of -methyl crotonitrile benzoxazoles, -(benzoxazole-2-yl)- -cycloalkylidene crotonitrile (**3**) and 3-(benzoxazole-2-yl)-2-mercapto-4- methyl-6-pyridinethione (**10**) together with the synthesis of some substituted benzoxazolyl anilines(**5,7,9**).The herbicidal evaluation of these compounds was carried out on wheat as pattern for monocotyledonous plants under laboratory conditions. Three plant parameters, seed germination, root and shoot growth of wheat seeds were taken as indicators for the herbicidal efficiency of the newly synthesized compounds. The most active compounds that showed an observable inhibition effect on the process of germination, root and shoot growth or one of them were (**3**),(**5b**),(**5c**),(**9a**) and (**9c**) so that, they were rescreened by a serial of concentrations to stand on the most potent derivative. Their EC_{50} values were calculated and showed that compound (**9a**) was the most potent and greatly inhibited shoot growth (EC_{50}, 1.4mg/ml).
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Keywords: -Methylcrotonitriles, cycloalkylidenecrotonitrile pyridinethione, benzoxazolyl anilines, wheat, monocotyledonous plants, growth parameters and herbicidal efficiency.

Introduction.

In the last few years various 2-substituted benzoxazole derivatives were studied extensively for their antiviral [1-7], antimicrobial [8-15], antibacterial [16], antifungal [17-26], and antiherbal activity[27-30].In addition to the previously mentioned applications. It was reported that the presence of this heterocycle in any compound supports the ability to expect that the whole compound is biologically active, for example ethyl (R)–2–[4–(6-chloro–2–benzoxazolyl) oxy] phenoxy propanoate, which contain benzoxazole moiety as shown from its structure, is one of the most commonly used selective herbicides (I, fig. 1). Furthermore the cyano function is included in the structure of some pesticides like cymoxanil (II. Fig.1),chlorothalonil(III,fig.1),dichiobenil(IV,fig.1) and bromoxynil(V,fig.1)[31].

I
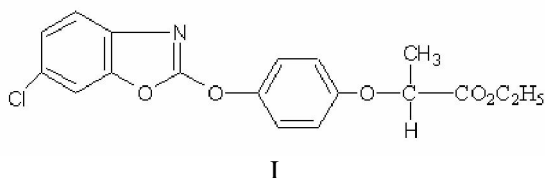
Moreover, it was also reported that many benzoxazole derivatives like (VI,VII,fig.2) control wide spectrum of weeds in rice paddies for long time [32] and benzoxazole (VIII,fig.2) that completely controls barnyard grass [33].

VI
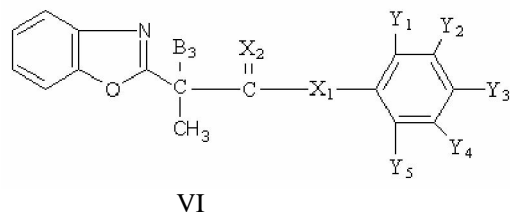
Y1-Y5= H,halo,(un)substituted
  alkyl,alkoxy,alkylthio,cyano,SH.
  X1,X2=O,S
  B3= H,Alkyl,CN

VII
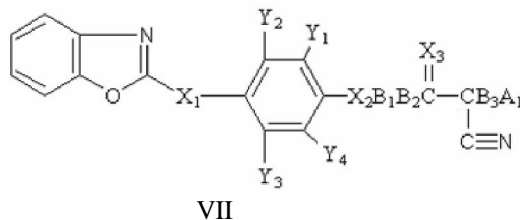
Y1-Y4= H,halo, alkyl
X1,X2,X3=O,S
B1,B2,B3=Alkyl,alkenyl,alkynyl.
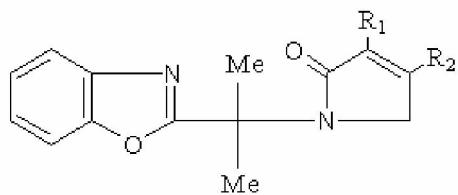
VIII
R1=Ph.
R2=Me.
Fig.2

In view of the above mentioned findings and as continuation of our effort to identify new candidates, that may be of value in designing new, potent, selective antiherbal agents, we report in the present work the synthesis of some related new 2-substited benzoxazoles that comprise both benzoxazoles and cyano groups in their framework in order to investigate their herbicidal efficiency.

The synthesized compounds include phenyl or substituted phenyl, aniline carbonitrile, aniline carboxylic ester, (thio) amide aniline and pyridinethione groups linked to benzoxazole moiety through one or two atom spacer (compounds 2,3,5,7,9 and 10). These compounds are considered as related structures to the previously reported (I)as they contain the same heterocycle, so it was found that the synthesis and study of the herbicidal activity of some new 2-cyanomethyl benzoxazole derivatives is a subject of great interest hoping that these new compounds could be applied as new herbicidal agents in the field of pest control.

Results and discussion

1-Chemistry part

The target compounds were synthesized as outlined in Schemes 1 and 2. Condensation of crotonitrile (**1**) with aldehydes in alkaline medium afforded an isolable product identified as -(Benzoxazole-2`-yl)- -styrenylcrotonitrile (**2a-d**). The products that were found to be directed to the opposite positions (trans) to the benzoxazole ring while, the other expected isomer due to the attack from the cis position to the benzoxazole ring was excluded due to the expected steric factors with the heterocyclic ring system. Both elemental and spectral data of the obtained compounds are consistent with the assigned structure (c.f. Experimental). In analogy -(Benzoxazole-2`-yl)- -ycloalkylidene crotonitrile (**3**) was prepared by reacting (**1**) with cyclo hexanone in ethanol and piperidine as a basic catalyst.

-Methyl Crotonitrile (**1**) on treatment with , -unsaturated nitriles (**4**)in ethanol and ppiperidine under

reflux resulted in the formation of aniline derivatives which were formulated as 5-Aryl-6-cyano-3-methyl-2-(benzoxazole-2`-yl)aniline (**5a-c**).Structural elucidation of derivatives (**5a-d**) was carried out by different ways as elemental analysis, spectroscopic analysis as well as chemical ways. Whereas IR spectra of all obtained compounds revealed a new absorption bands at 3350 and $3217cm^{-1}$ region due to the formation of NH_2 functional group. Moreover ^{1}H-NMR (DMSO-d6) of compound (**5a**) showed signals at 2.50 (s,3H,CH3), 5.37 (s,2H,NH_2) and 6.90-7.91 (m,9H,Ar-H). Furthermore the structures of anilines (**5a-c**) was confirmed chemically through the reaction of compound (**2d**)with malononitrile in the presence of catalytic amount of piperidine under reflux to afford aniline derivative (**5d**), which was confirmed by m.p, mixed m.p, IR spectra that revealed the new absorption bands at $33730,3437cm^{-1}$ region due to the formation of NH2 functional group. Moreover, mass spectrum of the compound showed the expected molecular ion peak at m/z=355 with relative abundance of 3.2 corresponding to the correct molecular formula.

Similarly -Methyl Crotonitrile (**1**) was reacted with arylidene ethyl -cyanocinnamate (**6**) in ethanol in the presence of piperidine as a basic catalyst under reflux to afford aniline derivatives (**7a-c**). The structures of compounds (**7a-c**) were confirmed by elemental analysis, spectroscopic analysis as well as chemical ways. Whereas IR spectra of all obtained compounds revealed a new absorption bands at $3425cm^{-1}$ region due to the formation of NH_2 group and at $1741cm_{-1}$ due to the presence of C=O group. Also ^{1}H-NMR (DMSO-d6) of derivative (**7a**) showed signals at 1.30(s, 3H,CH_3ester), 3.1(s,3H,CH_3), 4.3(q,2H,CH_2 ester), 7.3 (s,3H,thiophene-H), 8.04-8.19 (m,5H,Ar-H) and 8.57(s,2H,NH_2) In addition to that , the structure of anilines (**7a-c**) was confirmed chemically by reacting crotonitrile derivative (**2c**) with ethyl cyano acetate in ethanol and piperidine to afford aniline derivative (7d). The structure of compound (**7d**) was confirmed upon the compatible elemental analyses and spectral data (c.f. Experimental).

In analogy -Methyl Crotonitrile (**1**) on treatment with , -unsaturated nitriles (**8**) in ethanol in the presence of piperidine as a basic catalyst afforded aniline derivatives (**9a,b**) Structural elucidation of derivatives (**9a,b**) was carried out by different ways as elemental analysis, spectroscopic data as well chemical ways. Whereas, IR spectra of all obtained compounds revealed a new absorption band at $3186cm^{-1}$ region due to the formation of NH_2 functional group. Also structure (**9**) was confirmed by the presence characteristic signal at 6.1-6.7(br.s, 4H, 2NH_2) due to the presence of two NH_2 groups, mass spectrum of compound (**9a**) showed the expected molecular ion peak at m/z=402 with relative abundance 33.0

corresponding to the correct molecular formula. Refluxing crotonitrile derivative (**2c**) with cyano thio acetamide in ethanol and piperidine gave aniline derivative (**9c**) which was confirmed by elemental analysis and spectroscopic data (c.f. Experimental).

In addition, -Methyl Crotonitrile (**1**) was reacted with carbon disulfide in alkaline medium to yield 3-(Benzoxazole-2`-yl)-2-mercapto-4-methyl-6-

pyridinethione (**10**), the product that was obtained by addition of the active methylene of crotonitrile (**1**) to C=S to form a non isolable acyclic intermediate which, in turn, undergoes cyclization to form pyridinethione (**10**). Both elemental and spectral data of compound (**10**) provided satisfying evidences for the proposed structure.
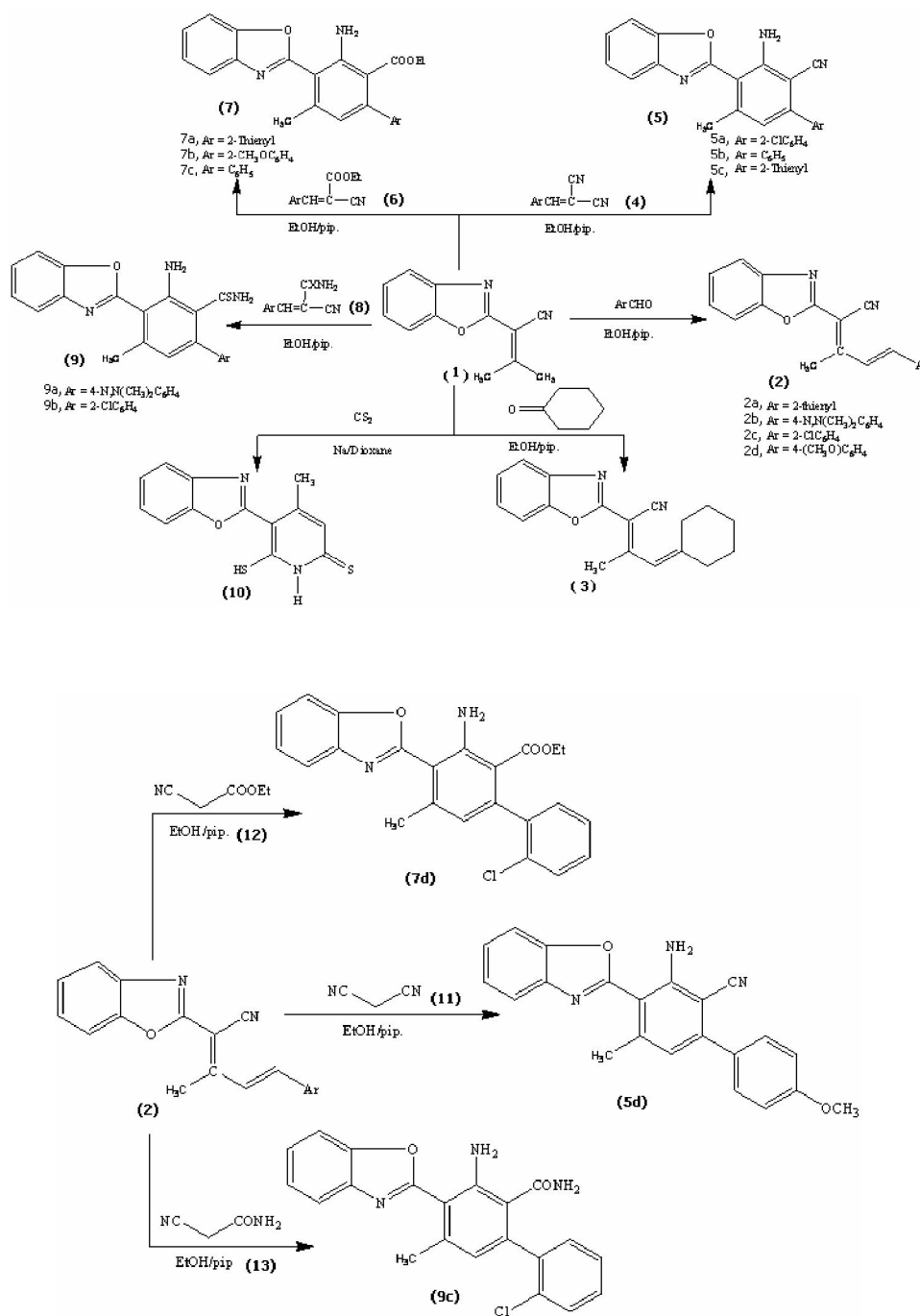
EXPERMENTAL

All melting points are uncorrected and were determined on an electric melting point (Gallenkamp) 9200 A apparatus. IR spectra were recorded (KBr) on pye Unicam SP-1000 Spectrophotometer. 1H–NMR spectra were obtained from Varian Gemini 200 MHz spectrometer and chemical shifts are expressed in (ppm) using TMS as internal reference. Mass spectra were recorded on a GCMS–QP 1000 mass spectrometer opening at 70 eV. Microanalytical data were obtained from the micro analytical data center at Cairo University.

-(Benzoxazole-2`-yl)- -styrenylcrotonitrile (2a-d).

To a solution of compound (**1a**) (1.98 g, 0.01 mole), in ethanol (40 ml) containing catalytic amount of piperidine (0.5 ml, 0.02 mole), aromatic or heterocyclic aldhyde (0.01 mole) was added. The reaction mixture was heated under reflux for 5 hrs. The solid product precipitated was collected by filtration, washed with ethanol, dried and crystallized from a suitable solvent to give styrenyl crotonitrile derivatives (**2a-d**).

(**2a**) yield (2.63 g, 85%); (DMF); mp 172-74ºC. IR (cm^{-1}): 3098 (CH-arom.), 2924 (CH-aliph.), 2195 (CN), 1650, 1606 (C=C), 1531 (C=N), 1115 (C-O-C); 1H-NMR (DMSO–d6): 2.50 (s, 2H, CH3), 6.09-7.02 (m, 3H, thiophene -H), 7.02-7.96 (m, 6H, Ar-H). MS m/z (%): 294 (M+ + 2, 20.0), 292 (M+ , not detected), 251 (28.0), 91 (32), 65 (100). Ana. Calcd. for C17H12N2OS (292.35): C 69.84, H 4.14, N 9.58, S 10.97. Found: C 69.62, H 4.08, N 9.44, S 10.63%.

(**2b**) yield (3.12 g, 90%); (EtOH/H2O); mp 178-80ºC. IR (cm^{-1}): 3060 (CH-arom), 2931 (CH-aliph..), 2203 (CN), 1590 (C=C), 1517 (C=N), 1037 (C-O-C). Ana. Calcd. for C21H19N3O (329.4): C 76.57, H 5.81, N 12.76. Found: C 76.34, H 5.59, N 12.63%.

(**17c**) yield (3.19 g, 94%); (EtOH/H2O); mp 155-57ºC. IR (cm^{-1}): 3061 (CH-arom), 2968 (CH-aliph.), 2201 (CN), 1519 (C=N), 1590 (C=C), 1040 (C-O-C). Ana. Calcd. for C19H13ClN2O (320.77): C 71.14, H 4.08, Cl 11.05, N 8.73. Found: C 71.06, H 3.99, Cl 10.97, N 8.65%.

(**2d**) yield (2.73 g, 82%); (EtOH/ H2O); mp 192-94C. IR (cm^{-1}): 3065 (CH-arom), 2930 (CH–aliph.), 2198 (CN), 1600 (C=C), 1510 (C=N), 1029 (C-O-C). Ana. Calcd. for C20H16N2O2 (316.35): C 75.93, H 5.10, N 8.86. Found: C 75.65, H 5.12, N 8.64%.

-(Benzoxazole-2`-yl)- -ycloalkylidene crotonitrile (3).

To a solution of compound (**1a**) (1.98 g, 0.01 mole), in ethanol (40 ml) catalytic amount of piperidine (0.5 ml, 0.02 mole), cyclohexanone (1.96 g, 0.01 mole) was added. The reaction mixture was refluxed for 5 hrs, cooled, poured into ice/ cold water mixture and neutralized with dilute HCl. The solid product which precipitate was collected by filtration, washed was water, dried and crystallized from acetonitrile to afford alkylidene derivative (**3**).

(**3**) yield (2.75 g, 93%); (Acetonitrile); mp 168-70ºC. IR (cm^{-1}): 2934 (CH-aliph.), 2200 (CN), 1617 (C=C), 1590 (C=N), 1034 (C-O-C). MS m/z (%): 281 (M+ + 3, 0.1), 280 (M+ + 2, 3.0), 279 (M+ + 1, 10.0), 278 (M+, not detected), 250 (2.0), 167 (28.5), 149 (100.0), 132 (7.0), 104 (11.0), 57 (28.5). Ana. Calcd. for C18H18N2O (278.35): C 77.67, H 6.52, N 10.06. Found: C 77.52, H 6.43, N 9.79%.

5-Aryl-6-cyano-3-methyl-2-(benzoxazole-2`-yl)aniline (5a-d).
Method A

To a solution of compound (1a) (1.98 g, 0.01 mole), in ethanol (40 ml) containing catalytic amount of piperidine (0.5 ml, 0.02 mole), arylidene malononitrile (**4**) (0.01 mole) was added. The reaction mixture was heated under reflux for 10 hrs, cooled, then the solid product precipitated was collected by filtration, washed by ethanol, dried and crystallized from proper solvent to give aniline derivatives (**5a-c**).

Method B

-Stytenyl crotonitrile (**2d**) (3.16 g, 0.01 mole), malono-nitrile (**11**) (0.66 g, 0.01 mole) and catalytic amount of piperidine (0.5 ml, 0.02 mole) was heated under reflux for 10 hrs. The reaction mixture was left to cool at room temperature, poured into an acidified crushed ice, filtered off and the finally obtained solid product was crystallized from ethanol to afford aniline derivative (**5d**).

(**5a**) yield (3.47 g, 90%); (EtOH/H2O); mp 163-65ºC. IR (cm^{-1}): 3350, 3217 (NH2), 2937 (CH–aliph.), 2206 (CN), 1617 (C=C), 1517 (C=N), 1042 (C-O-C). 1H-NMR (DMSO–d6): 2.50 (s, 3H, CH3), 5.37 (s, 2H, NH2), 6.90–7.91 (m, 9H, Ar-H). MS m/z (%): 359 (M+, not detected), 356 (M+ -3, 39.1), 315 (43.5), 199 (56.5), 129 (13.5), 121 (34.8), 95 (60.9), 53 (100). Ana. Calcd. for C21H14ClN3O (359.81): C 70.10, H 3.92, Cl 9.85, N 11.68. Found: C 69.87, H 3.84, Cl 9.77, N 11.62%.

(**5b**) yield (3.02 g, 86%); (EtOH/H2O); mp 192-94ºC. IR (cm^{-1}): 3435, 3335 (NH2), 2208 (CN), 1618 (C=C), 1030 (C-O-C). Ana. Calcd. for C21H15N3O (325.36):

C 77.52, H 4.56, N 12.91. Found: C 77.47, H 4.53, N 12.84%.

(**5c**) yield (2.79 g, 78%); (DMF); mp 144-46ºC. IR (cm^{-1}): 3420, 3328 (NH2), 3094 (CH-arom.), 2932 (CH-aliph.), 2207 (CN), 1585 (C=C), 1038 (C-O-C). Ana. Calcd. for C19H13N3OS (331.39): C 68.86, H 3.95, N 12.68, S 9.68. Found: C 68.53, H 3.84, N 12.72, S 9.75%.

(**5d**) yield (2.67 g, 70%); (EtOH/H2O); mp 152-54ºC. IR (cm^{-1}): 3730, 3437 (NH2), 2932 (CH-aliph.), 2208 (CN), 1609 (C=C), 1029 (C-O-C). 1H-NMR (DMSO-d6): 2.50 (s, 3H, CH3), 3.46 (s, 3H, OCH3), 5.36 (s, 2H, NH2), 6.81-6.91 (m, 9H, Ar-H). MS m/z (%): 355 (M+, 3.2), 354 (M+ -1, 32.3), 317 (19.4), 245 (35.5), 183 (40.3), 158 (24.2), 123 (59.7), 91 (85.5). Ana. Calcd. for C22H17N3O2 (355.39): C 74.35, H 4.82, N 11.82. Found: C 74.21, H 4.68, N 11.72%.

5-Aryl-6-ethoxycarbonyl-3-methyl-2-(benzoxazole-2`-yl) aniline (7a-d).
Method A

A mixture of -methyl crotonitrile (**1a**) (1.98 g, 0.01 mole), arylidene ethyl -cyanocinnamate (**6**) (0.01 mole), catalytic amount of piperidine (0.5 ml, 0.02 mole), in ethanol (40 ml) was heated under reflux for 10 hrs. The reaction mixture was left a side at room temperature to cool, poured into an acidified crushed ice and filtered off. The obtained solid product was crystallized from suitable solvent to afford aniline derivatives (**7a-c**).

Method B

To a solution of –styrenyl crotonitrile (**2c**) (3.2 g, 0.01 mole), in ethanol (40 ml) containing catalytic amount of piperidine (0.5 ml, 0.02 mole), ethyl cyanoacetate (**12**) (1.13 g, 0.01 mole) was added. The reaction mixture was heated under reflux for 10 hrs, left to cool at room temperature, poured into an acidified crushed ice. The solid formed product was filtered off and crystallized from a proper solvent to afford the corresponding aniline derivative (**7d**).

(**7a**) yield (3.24 g, 80%); (EtOH/H2O); mp 125 -27ºC. IR (cm^{-1}): 3425 (NH2), 2928 (CH-aliph.), 1741 (C=O), 1514 (C=N), 1095 (C-O-C). ^{1}H-NMR (DMSO-d$_6$): 1.30 (s, 3H, CH$_3$ ester), 3.1 (s, 3H, CH$_3$), 4.3 (q, 2H, CH$_2$ ester), 7.3 (s, 3H, thiophene-H), 8.04 (m, 5H, Ar-H) and 8.57 (s, 2H, NH$_2$). MS m/z (%): 381 (M$^+$ + 3, 13.3), 380 (M$^+$ + 2, 16.7), 379 (M$^+$ + 1, 18.3), 378 (M$^+$, not detected), 343 (10.0), 294 (18.3), 241 (81.7), 176 (43.3), 135 (100.0), and 114 (11.7). Ana. Calcd. for

C$_{21}$H$_{18}$N$_2$O$_3$S (378.44): C 66.65, H 4.79, N 7.40, S 8.47. Found: C 66.51, H 4.68, N 7.21, S 8.32%.

(**7b**) yield (3.5 g, 82%); (EtOH/H2O); mp 121-23ºC. IR (cm^{-1}): 3425 (NH$_2$), 2933 (CH-aliph.), 1740 (C=O), 1069 (C=C), 1513 (C=N), 1030 (C-O-C). Ana. Calcd. for C24H22N2O4 (402.44): C 71.63, H 5.51, N 6.96. Found: C 71.49, H 5.32, N 6.78%.

(**7c**) yield (3.7 g, 94%); (EtOH/H2O); mp 147-49ºC. IR (cm^{-1}): 3364 (NH2), 3063(CH-arom.), 2933 (CH-aliph.), 1740 (C=O), 1616 (C=C), 1517 (C=N), 1026 (C-O-C). Ana. Calcd. for C23H20N2O3 (372.42): C 74.18, H 5.41, N 7.52. Found: C 74.04, H 5.32, N 7.41%.

(**7d**) yield (3.4 g, 80%); (EtOH/H2O); mp 123-25ºC. IR (cm^{-1}): 3392 (NH2), 2934 (CH-aliph.), 1742 (C=O), 1611 (C=C), 1514 (C=N), 1035 (C-O-C). MS m/z (%): 407 (M+, 4.9), 325 (20.6), 280 (14.7), 245 (43.1), 209 (33.3), 172 (73.5), 127 (48.0), 63 (100). Ana. Calcd. for C23H19ClN2O3 (406.86): C 67.90, H 4.71, Cl 8.71, N 6.89. Found: C 67.58, H 4.62, Cl 8.63, N 6.73%.

5-Aryl-3-methyl-6-(thio)amide-2-(benzoxazole-2`-yl)aniline (9a-c).
Method A

To a solution of -methyl crotonitrile (**1a**) (1.98g, 0.01 mole), in ethanol (40 ml) containing catalytic amount of piperidine (0.5 ml, 0.02 mole), , -unsaturated nitrile (**8**) (0.01 mole) was added and the reaction was heated under reflux for 10 hrs. The reaction mixture was left a side at room temperature to cool, poured into an acidified crushed ice and the precipitated solid product was filtered off and crystallized from a suitable solvent to afford aniline derivatives (**9a,b**).

Method B

-Styrenyl crotonitrile (**1c**) (3.2 g, 0.01 mole), cyano actamide (**13**) (1.0g, 0.01 mole) , catalytic amount of piperidine (0.5 ml, 0.02 mole) was heated under reflux for 10 hrs. The reaction mixture was left to cool at room temperature, poured into an acidified crushed ice and the formed solid product was filtered off and crystallized from isopropyl alcohol to give aniline derivative (**9c**).

(**9a**) yield (3.4 g, 80%); (EtOH); mp 197-99ºC. IR (cm^{-1}): 3186 (NH$_2$), 2965 (CH-aliph.), 1617 (C=C), 1514 (C=N), 1241 (C=S), 1037 (C-O-C). 1H-NMR (DMSO-d6): 2.39, 2.50 (2s, 2CH3, N (CH3)2), 3.2 (s, 3H, CH3), 6.1-6.7 (br.s, 4H, 2NH2), and 7.4-7.8 (m, 9H, Ar-H). MS m/z (%): 404 (M+ + 2, 2.0), 403 (M+ + 1, 8.0), 402 (M+, 33.0), 387 (6.0), 373 (100.0), 356 (80.0), 345 (20.0), 318 (18.0), 283 (22.0), 254 (75.0),

226 (50.0). Ana. Calcd. for C23H22N4OS (402.51): C 68.63, H 5.51, N 13.92, S 7.96. Found: C 68.42, H 5.31, N 13.83, S 7.78%.

(**9b**) yield (3.6 g, 88%); (EtOH); mp 210-12ºC. IR (cm^{-1}): 3328, 3198 (NH2), 3063 (CH-arom.), 2935 (CH-aliph.), 1614 (C=C), 1520 (C=N), 1241 (C=S), 1039 (C-O-C). Ana. Calcd. for C21H16ClN3OS (393.89): C 64.03, H 4.09, Cl 9.00, N 10.67, S 8.14. Found: C 63.95, H 3.92, Cl 8.92, N 10.52, S 8.04%.

(**9c**) yield (3.4 g 90%); (isopropyl alcohol); mp 188-90ºC. IR (cm^{-1}): 3401 (NH2), 3022 (CH-arom.), 2916 (CH-aliph.), 1666 (C=O), 1606 (C=C), 1569 (C=N), 1035 (C-O-C). MS m/z (%): 380 (M+ + 1, 0.3), 379 (M+, 0.4), 367 (2.0), 352 (5.0), 279 (10.0), 245 (100), 216 (9.0), 190 (7.0), 158 (6.0), 78 (10.0), 63 (20.0). Ana. Calcd. for C21H16ClN3O2 (377.82): C 66.76, H 4.27, Cl 9.37, N 11.12. Found: C 66.62, H4.11, C l9.12, N 11.06%.

3-(Benzoxazole-2`-yl)-2-mercapto-4-methyl-6-pyridinethione (10)

To a mixture of compound (**1a**) (1.98 g, 0.01 mole) in sodium/dioxane [prepared from sodium metal (0.23 g, 0.01 mole) dissolved in day dioxane], carbon disulfide (1.5 ml, 0.01 mole) was added gradually. The reaction mixture was heated under reflux for 8 hrs and cooled. The reaction mixture was evaporated under reduced pressure and the residue was triturated with crushed ice and neutralized with dilute HCl. The separated solid products were filtered off and crystallized from dioxane to afford pyridine thione (**10**).

(**10**) yield (3.2 g, 92%); mp 137-39ºC. IR (cm^{-1}): 3416 (NH), 3056 (CH-arom.), 2915 (CH-aliph.), 1601 (C=C), 1548 (C=N), 1242 (C=S), 1038 (C-O-C). 1H-NMR (DMSO-d6); 3.3, (s, 3H, CH3), 6.85 (s, 1H, 5H), 7.8-8.06 (m, 5H, Ar-H) and 9.95 (s, 1H, pyridine-NH). MS m/z (%): 277 (M+ + 3, 0.2), 276 (M+ + 2, 0.4), 275 (M+ + 1, 7.5), 274 (M+, 0.2), 260 (9.0), 223 (40.0), 198 (60.0), 158 (80.0), 133 (100.0), 78 (30.0), 63 (96.0). Ana. Calcd for. C$_{13}$H$_{10}$N$_2$OS$_2$ (274.36): C

65.91, H 3.67, N 10.21, S 23.37. Found: C 65.78, H 3.54, N 10.11, S 23.26%.

2- Biological study

The herbicidal evaluation of the newly synthesized compounds on wheat as pattern for monocotyledonous plants.

Preliminary test :-

The preliminary study of the herbicidal efficiency of the newly synthesized compounds was carried out on wheat as pattern for monocotyledonous plants with concentration of 2000 ppm for each compound under laboratory conditions (table 1). Three plant parameters, seed germination, root and shoot growth were taken as indicators for the herbicidal efficiency of the tested compounds. According to the obtained data all tested compounds caused changes to growth parameters of wheat. These changes were as activation or inhibition of growth parameters. Activation was recorded in compounds (**2a**), (**2d**), (**5a**) and (**10**). These compounds activated all or some growth parameters of wheat. On contrast the other tested compounds inhibited all growth parameters of wheat. So the later compounds were considered as effective compounds On the other hand most of these compounds recorded high inhibition effect on shoot growth followed by root then germination.

From another point of view compounds that recorded inhibition percentages greater than 22, 39, and 41 on germination, root and shoot growth respectively were considered as candidate compounds. The descending order of the promising compounds on germination was (9a), (**3**), (**5b**) and (**9c**), while it was (**9a**), (**9c**), (**7d**), (**3**) and (**7c**) in case of root growth. On the other hand compound (**9a**) showed the highest inhibition effect on shoot growth followed by (**9c**), (**5c**), (**7d**) and (**3**) respectively.

The variation in effect between the different derivatives of the same compound could be attributed to substitution in each case, for example compounds (**2a**), (**2c**) and (**2d**) inhibited germination as a result to the presence of thienyl, chloro phenyl and methoxy groups but this effect was changed to activation in compound (**2b**) due to the presence of amino phenyl group.

Preliminary evaluation of synthesized compounds on wheat as pattern for monocotyledonous plants with concentration of 2000 ppm.

Compound	germination	root growth	Shoot growth
2a	15.2	18.7	24.5
2b	12.3	-29.0	26.7
2c	2.6	8.7	32.1
2d	0	-2.5	-.97
3	30.3	41.9	42.9
5a	-4.88	-21.7	-13.2
5b	0	27	28
5c	26.7	33.8	47.3
5d	15.2	24.3	21.5
7a	15.8	15.5	31.9
7b	15.2	9.3	0
7c	18.6	40.96	36.7
7d	18.6	49.4	44.97
9a	34.8	51.0	59.9
9b	15.2	8.3	14.7
9c	23.3	50.4	53.3
10	-0.69	-37.9	5.99

Compounds (**3**), (**5b**), (**9a**) and (**9c**) that were considered as herbicidal active ingredients were re-evaluated with a serial of concentrations to determine their EC50 values (table 2). Generally all tested compounds showed a regration relation between the tested concentration and shoot growth, in contrast the above result was not observed with germination and root growth except compound (3) that showed an inhibition for root growth.

Table (2): Effect of candidate compounds (5b, 9a, 9c, 3) on shoot growth of wheat.

Concentration (ppm)	% of Inhibition of shoot growth of compounds			
	5b	**9a**	**9c**	**3**
250	5.7	-	-	-
500	10.6	-	-	-
1000	18.5	10.6	14.7	-
1250	20.9	-	-	-
1500	24.3	15.2	17.2	30.4
2000	-	-	-	-
2500	31.6	-	-	-
4000	-	28.8	22.7	-
5000	44.1	-	-	-
8000	-	39.8	27.5	52.8
10000	57.2	-	-	-
16000	-	52.7	32.7	56.4
2000	-	-	-	57.4
32000	-	65.7	38.3	59.9
64000	-	-	44.1	-
128000	-	-	54.0	-
EC50	6.2mg/ml	1.4mg/ml	125mg/ml	5.01mg/ml
Slope	1.1	1.1	.5	.3
Toxicity index	22.5	100	1.1	27

* EC_{50} is the effective concentration that inhibits 50% of the sample under study.

Data in table **(2)** showed that there is a positive relation ship between the tested concentration and their percentage of inhibition, depending on EC_{50} values, compound **(9a)** was found to be the most effective against the shoot growth of wheat followed by **(3)**, **(5b)** and **(9c)** by EC_{50} values 1.4, 5.01, 6.2 and 125 mg/ml, respectively, according to slope values, compounds **(5b)** and **(9a)** possess the same slope value 1.1, this result may be due to the ability of both compounds to act with the same mode of action. Also the slope of both compounds was sharper than compounds **(9c)** and **(3)** that recorded a flattest slope with small values 0.5 and 0.3.

Depending on toxicity index compound **(9a)** was found to be the most effective followed by **(3)**, **(5b)** and **(9c)** with toxicity index values 100, 27, 22.5 and 1.1, respectively.

Conclusion

1- All tested compounds showed change in growth of wheat (germination, root and shoot growth).
2- These changes were activation effect in compounds (**2a**), (**2d**), (**5a**) and (**10**) or inhibition effect in the other compounds.
3- The highest inhibition effect was recorded against shoot followed by root growth then germination.
4- The variation in effect between the different derivatives of the same compound could be attributed to substitution in each case.
5- Depending on the percentages of inhibition compounds (**3**), (**5b**), (**5c**) and (**9a**) were considered as promising compounds and were re-evaluated with a serial of concentrations to determine their EC_{50} values.
6- Rgration relation was recorded with all promising compounds against shoot growth, whereas this relation was not found with other growth parameters except compound (**3**) with root growth.
7- The descending order of inhibition of the promising compounds depending on their EC50 values were (**9a**), (**3**), (**5b**) and (**9c**).

Experimental
Evaluation of Herbicidal Efficiency of the newly synthesized compounds.
Under laboratory conditions.

- Seed germination, root and shoot growth inhibition were carried out according to the procedure described by Powel and Spencer [34], some modifications were made for this work as described below.
- Serial concentrations from each compound were prepared by dissolving it in dimethyl sulfoxide and dilution with water. The calculated amount from each concentration was pipetted on thirty seeds of wheat or cucumber as a test plant and agitated to coat the seed surface. Each ten seeds were transferred to Petri dish (90 mm diameter), lined with a dry filter paper and left at 25°C without led to grant solvent evaporation. After that, 6 ml distilled water was pipetted on the filter paper, Petri dish was sealed with (PVC) electrical insulting tape. After complete germination of control (Petri dishes containing untreated seeds), the number of germinated and non germinated seeds and radical length were recorded. Three replicates were done for each treatment [35,36].

Under green house conditions:

Compounds that showed an observable inhibition effect on germination, root and shoot growth were considered as candidate compounds and tested under green house conditions to ensure the obtained results from treating seeds in Petri dishes under laboratory conditions. Three plastic pots for each compound were filled till their lower surface by sand, teen wheat seeds were planted in each pot and filled with water, left untill wheat seeds grown up then the three pots were spayed by the calculated concentration for each compound, left for about ten days, irrigated with water daily according to need, then compared with untreated pots [37].

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Phenolic Metabolites from *Acacia nilotica* Flowers and Evaluation of its Free Radical Scavenging Activity

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Abstract: The study of the chemical constituents of the flowers of *Acacia nilotica* has resulted in the isolation and characterization of nine compounds. These compounds were identified as catechin (**1**), catechin 7-*O*- gallate (**2**), gallic acid (**3**), naringenin 7-*O*-β- glucopyranoside (**4**), quercetin 3-*O*- β- glucoside (2→1) glucopyranoside (**5**), quercetin 3-*O*- β-glucopyranoside (**6**), chalconaringenin 4'-*O*-β-glucopyranoside (**7**), naringenin (**8**) and quercetin (**9**), which were isolated for the first time from *Acacia nilotica* flowers. These compounds were individually identified by spectroscopic analyses and were compared with reported data. The total amount of phenolic compounds of the aqueous methanol extract and fractions was determined by ultraviolet (UV) spectrometry and calculated as gallic acid equivalents. The antioxidant potential of *Acacia nilotica* extract and fractions has been investigated by DPPH radical scavenging assay.
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Keywords: *Acacia nilotica*; phenolic compounds; antioxidant activity; DPPH

1. Introduction:

Free radical reactions, especially with participation of oxidative radicals, have been shown to be involved in many biological processes that cause damage to lipids, proteins, membranes and nucleic acids, thus giving rise to a variety of diseases (Datta et al., 2000). Reactive oxygen species (ROS) have been recognized as playing an important role in the initiation and/or progression of various diseases such as atherosclerosis, inflammatory injury, cancer and cardiovascular diseases (Halliwell, 1997). Many antioxidant compounds, naturally occurring from plant sources, have been identified as a free radical or active oxygen scavengers (Zheng and Wang, 2001). Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their side effects such as carcinogenicity (Ito et al., 1983). Natural antioxidants have the capacity to improve food quality and stability and can also act as nutraceuticals to terminate free radical chain reactions in biological systems, and thus may provide additional health benefits to consumers. The antioxidant activities of plants are mainly due to the presence of secondary metabolites of the phenolic compounds, such as simple phenolic acids, flavonoids, isoflavonoids, hydrolyzable tannins and condensed tannins (Chung et al., 1998; Pietta, 2000). There have been numerous studies on the biological activities of phenolics, which are potent antioxidants and free radical scavengers (Kahkonen et al., 1999, Sugihara et al., 1999, Toyokuni et al., 2003).

Acacia is a cosmopolitan genus containing more than 1350 species that found in African and the Middle Eastern countries, with monotypic genus. This genus contains variety of bioactive components such as phenolic acids (Singh et al. 2009a), alkaloids (Clement et al., 1997), terpenes (Mujoo et al., 2001), tannins (Readel et al., 2001) and flavonoids (Fourie et al., 1974) which are responsible for numerous boilogical and pharmacological properties like hypoglycaemic, anti-inflammatory, antibacterial, antiplatelet, aggregatory, antihypertensive, analgesic, anticancer, and anti-atherosclerotic due to their strong antioxidant and free radical scavenging activities (Chopra et al., 1999; Singh et al., 2009b).

Plants have played a major role in the introduction of new therapeutic agents. A medicinal plant, *Galega officinalis*, led to the discovery and synthesis of metformin (Cusi and Defronzo, 1998). It is our opinion that instead of random search of plants, a selective search based on traditional knowledge would be more focused and productive and certainly more economic. To the best of our knowledge, there is no scientific literature on phenolic constituents and antioxidant activity of *Acacia nilotica* flowers. The present study deals with the isolation and identifycation of phenolic metabolites from the flowers of *Acacia nilotica* and evaluation of its free radical scavenging activity**.**

2. Materials and Methods:
General

NMR experiments were performed on a Bruker AMX 400 and 500 instruments with standard pulse sequences operating at 400, 500 MHz in ^{1}H NMR and 100, 125 MHz in ^{13}C NMR. Chemical shifts are given in δ values (ppm) using tetramethylsilane as the internal standard and DMSO-d_6 as solvent at room temperature. HRESI-MS was taken on a Micromass Autospec (70 eV) spectrometer. UV spectral data was measured on a Shimadzu 240 spectrometer in MeOH. Paper chromatography Whatman 1, using solvent systems A (15% AcOH) and B (n-BuOH: AcOH: H_2O, 4:1:5, upper layer). Compounds were visualized by exposure to UV light (365 nm), before and after spraying with $AlCl_3$ and Naturestoff-polyethylene glycol reagents.

Plant material

Flowers of *A. nilotica* were collected in March 2009 from the Upper Egypt. Identification of the plants was confirmed by Prof. Dr. Ibrahium El-Garf, Department of Botany, Faculty of Science, Cairo, University and comparison with herbarium specimens. Voucher Specimens were kept in herbarium, Department of Botany, Faculty of Science, Cairo, University (Boulos, 1999).

Extraction and isolation

The flowers of *A. nilotica* (1 Kg) were defatted with $CHCl_3$ (3 x 1 L) and extracted with CH_3OH: H_2O (7:3; 5 x 3 L) at room temperature. The combined extracts were filtered, evaporated under reduced pressure and lyophilized (80 g). The dry extract was loaded on a polyamide 6S column chromatography (80 x 3 cm). The column was eluted with H_2O, and then H_2O-EtOH mixtures of decreasing polarity and 10 fractions (1 L, each) were collected. The major phenolic fractions obtained were combined into five fractions after chromatographic analysis. Fraction A (2.5 g) was fractionated by column chromatography on Sephadex LH-20 with aqueous EtOH (0- 70%) for elution to give compounds **1** (17 mg) and **3** (25 mg). Fraction B (3 g) was subjected to column chromatography on cellulose and n-BuOH saturated with H_2O as an eluent to give two major subfractions, then each of them was separately fractionated on a Sephadex LH-20 to yield pure samples **6** (22 mg) and **5** (18 mg). Using the same procedure fraction C (2.8 g) and fraction D (2.4 g) gave chromatographically pure samples **4** (15 mg) **2** (25 mg) and **7** (15 mg). Fraction E (1.5 g) was chromatography on Sephadex LH-20 using aqueous acetone (0- 25%) for elution to give pure sample **8** (20 mg) and **9** (25 mg).

Determination of total phenolics concentration

The concentration of total phenolics of the plant extract and fractions was determined according to the method described by (Kumar et al., 2008). Gallic acid was used as standard. Briefly, a mixture of 100 μL of plant extract (100 μg mL^{-1}), 500 μL of Folin-Ciocalteu reagent and 1.5 mL of Na_2CO_3 (20 %) was shaken and diluted up to 10 mL with water. After 2 hours, the absorbance was measured at 765 nm (using a spectrophotometer. All determinations were carried out in triplicate. The total phenolic concentration was expressed as gallic acid equivalents (GAE).

Determination of Total flavonoids concentration

Total flavonoid concentration of plant extract and fractions was determined according to the reported procedure by (Kumaran and Karunakaran, 2007). 100 μL of plant extract (10 mg mL^{-1}) in methanol was mixed with 100 μL of 20 % $AlCl_3$ in methanol and a drop of acetic acid, and then diluted to 5 mL with methanol. The absorbance was measured at 415 nm after 40 min against the blank. The blank consisted of all reagents and solvent without $AlCl_3$. All determinations were carried out in triplicate. The total flavonoid concentration was expressed as rutin equivalents (RE).

Antioxidant activity
Free radical scavenging activity by DPPH• (1, 1-diphenyl -2-picryl hydrazyl).

The hydrogen atom-or-electron donation ability of the total extract of *A. nilotica* and fractions was measured from the bleaching of the purple colored methanol solution of DPPH•. This spectrophotometric assay uses the stable radical, 1, 1-diphenyl-picrylhydrazyl (DPPH•), as a reagent (Amarowicz et al., 2004). Different concentrations of the total extract and fractions (0.5-20 μg/ml) were mixed with 1.86 ml distilled water and then added to 1.5 ml of 0.1 mM DPPH• in methanol, and final volume adjusted up to 3.5 ml with distilled water. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min, and then the absorbance was measured at 517 nm using spectrophotometer (Shimadzu UV-VIS Recording 2401 PC, Japan.). Distilled water was used as blank. The absorbance of distilled water and DPPH• without samples was measured as control. The radical-scavenging activities of samples, expressed as percentage inhibition of DPPH•, were calculated according to the formula:

$I (\%) = [(A_C - A_S)/A_C] \times 100$

Where: A_C and A_S are the absorbance of the control and sample, respectively. The IC_{50} value represented the concentration of the total extract of *A. nilotica* and fractions that caused 50% inhibition of DPPH•.

Hydrogen peroxide (H$_2$O$_2$) scavenging activity

The hydrogen peroxide scavenging of fraction C assay was carried out according to the method of Ruch et al. (1989). The principle of this method is that, there is a decrease in absorbance of H$_2$O$_2$ upon oxidation of H$_2$O$_2$. A solution of 40 mM H$_2$O$_2$ was prepared in 0.1 M phosphate buffer (pH 7.4). Different concentration of 1-30 µg/ml of fraction C was added to 0.6 ml of H$_2$O$_2$ solution (40 mM) and phosphate buffer (pH 7.4) was added up to a final volume of 4 ml. Absorbance of H$_2$O$_2$ at 230 nm was determined after 10 min against a blank solution contained the phosphate buffer without H$_2$O$_2$, using spectrophotometer and ascorbic acid (1-30 µg/ml) was used as the reference compound.

The percentage of scavenged [H$_2$O$_2$] = [(A$_c$-A$_t$)/A$_c$] x 100

Where: A$_c$ was the absorbance of the control and A$_t$ was the absorbance in the presence of the standard sample or fraction C.

Reducing power

Total reducing capacity of fraction C of *A. nilotica* extract was determined according to the method of (Shi and Dalal, 1991). One ml of fraction C at different concentrations (0.5-20 µg/ml) were mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide [K$_3$ Fe (CN)$_6$] (1%). The mixture was incubated at 50 °C for 20 min., and then a portion (2.5 ml) of trichloroacetic acid TCA (10%) was added to mixture, which was centrifuged for 10 min at 1000 x g. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and 0.5 ml FeCl$_3$ (0.1%). Then the absorbance was measured at 700 nm. Ascorbic acid (0.5-20 µg/ml) was used as the reference compound.

Chemical Characterization of the some isolated compounds

catechin 7-*O*- gallate (2): white amorphous powder UV λ$_{max}$ (MeOH) nm: 216, 268; ^{1}H NMR spectral data (400 MHz, DMSO-*d6*) δ 7.06 (2H, *s*, H-2'',6'' galloyl), 6.76 (1H, *d*, *J* = 1.5 Hz, H-2')**,** 6.72 (1H, *d*, *J* = 7.8 Hz, H-5'), 6.64 (1H, *dd*, *J* = 7.8,1.5 Hz, H-6'), 6.2 (1H, *d*, *J* = 2.1 Hz, H-6), 6.14 (1H, *d*, *J* = 2.1 Hz, H-8), 4.65 (1H, *d*, *J* = 7.2 Hz, H-2), 3.93 (1H, *m*, H-3), 2.75 (1H, *dd*, *J* = 16.8, 5.1 Hz, H-4α), 2.50 (1H, *dd, J* = 16.8, 7.8 Hz, H-4β), ^{13}C NMR : 156.71 (C-5), 155.74 (C-9), 150.39 (C-7), 146.56 (C-3'), 145.53 (C-4'), 130.84 (C-1'), 118.85 (C-6'), 115.78 (C-5'), 115.02 (C-2'),106.34 (C-10), 101.26 (C-6), 100.99 (C-8), 81.75 (C-2), 66.39 (C-3), 28.18 (C-4); galloyl moiety: 165.04 (C-7''), 146.35 (C-3'',C-5''), 139.76 (C-4''), 120.07 (C-1''), 109.66 (C-2'',6'').

Naringenin-7-O-β-D-glucoside (4): A pale yellow, UV λ$_{max}$ (MeOH) nm: 286, 332sh; ^{1}H NMR spectral data (400 MHz, DMSO-*d6*) δ 7.32 (2H, *d*, *J* = 8.3 Hz, H-2',6'), 6.80 (2H, *d*, *J* = 8.3 Hz, H-3',5'), 6.40 (1H, *d*, *J* = 1.8 Hz, H-8), 6.09 (1H, *d*, *J* = 1.8 Hz, H-6), 5.39 (1H, *dd*, *J* = 12.8, 2.2 Hz, H-2), 3.06 (1H, *dd*, *J* = 17.1, 12.8 Hz, H-3ax), 2.63 (1H, *dd*, *J* = 17.1, 2.2 Hz, H-3eq); 4.72 (1H, *d*, *J* = 7.2 Hz, H-1'') 3..21-3.76 (m, the rest sugar of glucose); ^{13}C NMR :190.43 (C-4), 165.26 (C-7), 164.58 (C-5), 161.03 (C-9), 158.02 (C-4'), 129.30 (C-1'), 128.62 (C-2',6'), 115.51 (C-3',5'), 105.83 (C-10), 99.25 (C-6), 98.11 (C-8), 78.52 (C-2), 44.87 (C-3); 103.80 (C-1''), 77.92 (C-5''), 75.99 (C-3''), 73.83 (C-2''), 70.02 (C-4''), 61.11 (C-6'').

Quercetin 3-*O*-β-D-glucopyranosyl (1→2)-β-D-glucopyranoside (5): yellow amorphous powder, UV λ$_{max}$ nm (MeOH) 256, 268^{sh}, 298^{sh}, 355;. ^{1}H-NMR (DMSO-d6) 7.67 (1H, *dd, J* = 8.4, 2.2 Hz, H-6'), 7.61 (1H, *d, J* = 2.2 Hz, H-2'), 6.92 (1H, *d, J* = 8.4 Hz, H-5'), 6.44 (1H, *d, J* = 1.8 Hz, H-8) , 6.24 (1H, *d, J* = 1.8 Hz, H-6), 5.75 (1H, *d, J* = 7.12 Hz, H-1'', glc), 4.82 (1H, *d, J* = 7.7 Hz, H-1''' glc) ^{13}C NMR: δ 156.94 (C-2), 133.79 (C-3), 177.88 (C-4), 157.14 (C-5), 99.21 (C-6), 164.61 (C-7), 94.13 (C-8), 156.94 (C-9), 104.47 (C-10), 122.11 (C-1'), 115.74 (C-2'), 145.25 (C-3'), 148.92 (C-4'), 116.72 (C-5'), 121.69 (C-6'), 103.22 (C-1''), 82.31 (C-2''), 76.39 (C-3''), 72.35 (C-4''), 76.35 (C-5''), 61.24 (C-6''), 101.25 (C-1'''), 73.21 (C-2'''), 76.32 (C-3'''), 70.06 (C-4'''), 77.76 (C-5'''), 61.21 (C-6''').

Quercetin-3-*O*-β-D-glucopyranoside (6): yellow amorphous powder; UV λ$_{max}$ nm: (MeOH), 253, 263^{sh}, 294 ^{sh}, 352; ^{1}H NMR (DMSO-*d$_6$*): δ 7.53 (1H, *d*, *J* = 2.1 Hz, H-2'), 7.67 (*1H, dd*, *J* = 2.1 Hz and 8.6 Hz, H-6'), 6.82 (1H, *d*, *J* = 8.6 Hz, H-5'), 6.40 (1H, *d*, *J* = 1.8 Hz, H-8), 6.20 (1H, *d*, *J* = 1.8 Hz, H-6), 5.37 (1H, *d*, *J* = 7.6 Hz, H-1'', glc), 3.28-3.65 (m, the rest proton of glucose protons). ^{13}C NMR: δ 156.80 (C-2), 133.60 (C-3), 177.50 (C-4), 161.60 (C-5), 98.90 (C-6), 164.60 (C-7), 93.80 (C-8), 156.60 (C-9), 104.00 (C-10), 121.60 (C-1'), 115.80 (C-2'), 145.80 (C-3'), 148.80 (C-4'), 116.20 (C-5'), 122.00 (C-6'), 101.20 (C-1''), 71.60 (C-2''), 74.40 (C-3''), 70.02 (C-4''), 77.70 (C-5''), 61.50 (C-6'').

chalconaringenin 4'-*O*-β-glucopyranoside (7): yellow amorphous powder; UV λ$_{max}$ nm: MeOH, 266, 319^{sh} 369**;** ^{1}H NMR (DMSO-*d$_6$*): 7.98 (1H, *d*, *J* = 15.5 Hz, H-β), 7.64 (2H, *d*, *J* = 8.2 Hz, H-2,6), 7.61 (1H, *d, J* = 15.5 Hz, H-α), 6.82 (2H, *d*, *J* = 8.2, H-3,5), 6.16 (1H, *d*, *J* = 2.0 Hz, H-5'), 5.96 (1H, *d, J* = 2.0 Hz, H-3'), 5.07 (1H, d, *J* = 7.4 Hz, H-1''), 3.3-3.72 (m, rest proton of glc). ^{13}C NMR: δ: 192.37

(C=O), 143.07 (C-α), 124.57 (C-β), 126.59 (C-1), 131.15 (C-2, 6), 116.31 (C-3, 5), 160.64 (C-4), 105.80 (C-1'), 165.28 (C-2'), 97.32 (C-3'), 166.44 (C-4'), 95.05 (C-5'), 160.23 (C-6'), 100.76 (C-1''), 74.03 (C-2''), 77.12 (C-3''), 69.80 (C-4''), 77.68 (C-5''), 60.83 (C-6'').

3. Results and Discussion:

The flowers extract of *A. nilotica* was fractionated by polyamide 6S column chromatography to give several fractions, which were further chromatogramphed on Sepadex LH 20 and cellulose to afford two condensed tannins (**1**, **2**), one phenolic acid (**3**), three flavonoids (**5**, **6**, **9**) two flavanones (**4**, **8**) and one chalcone glucoside (**7**) (Fig.1). The compounds were identified catechin (**1**), catechin 7-*O*- gallate (**2**) (Malan and Pienaar, 1987), gallic acid (**3**), naringenin 7-*O*-β- glucopyranoside (**4**), quercetin 3-*O*- β-glucoside (2→1) glucopyranoside (**5**), quercetin 3-*O*-β-glucopyranoside (**6**), chalconaringenin 4'-*O*-β-glucopyranoside (**7**), naringenin (**8**) and quercetin (**9**) by comparison of their 1D and 2D NMR spectral data with the reported data in the literature (Agrawal 1989; Harborne and Baxter,1999).

Plant phenolics possess the ability to scavenge both active oxygen species and electrophiles (Robards et al. 1999). Plant phenolic compounds, including flavonoids, tannins and phenolic acids exhibit a strong antioxidant activity. The total phenolics and flavonoids concentration in the extract and fractions of *A. nilotica* is shown in table 1. Among the extract and fractions, the fraction C is the higher concentration of phenolics and flavonoids content.

Table 1: Total concentration of phenolic and flavonoid compounds from total extract and fractions

Sample	TPC (mg GAE g^{-1} plant extract)	TFC (mg RE g^{-1} plant extract)
Total extract	292.99±5.55	25.2±1.12
Fraction A	259.80±6.40	20.55±0.95
Fraction B	101.17±1.52	19.10±0.29
Fraction C	342.03±1.64	30.33±1.42
Fraction D	164.97±4.02	11.83±0.24
Fraction E	185.31±4.52	15.84±0.65

Values are mean ± SD of three determination, n=3.

Antioxidant activity
1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity.

Fig 2 represents the radical scavenging activity of the extract from *A. nilotica* and fractions. The antioxidant activity of *A. nilotica* flowers extract and fractions (A-E) were tested by measuring their capacity to scavenge DPPH radical. The fraction C extract showed the highest antioxidant activity with

an IC$_{50}$ value of 3.32 μg /mL, while total extract 9.71 μg /mL and the standard antioxidant ascorbic acid showed an IC$_{50}$ value of 2.12μg /mL. The tested samples reduced the stable radical DPPH to the yellow-colored diphenylpicrylhydrazine. DPPH radicals have been widely used to evaluate the anti-oxidant properties of natural products as well as plant extracts (Wang et al., 2003).

Reactive oxygen species (ROS) are continuously produced by the body's normal oxygen usage such as respiration and some cell mediated immune functions. ROS include free radicals such as superoxide anion radicals (O$_2^{•-}$), hydroxyl radicals (OH$^•$) and non-free-radical species such as hydrogen peroxide (H$_2$O$_2$) and singlet oxygen (^{1}O$_2$) (Farombi and Fakoya, 2005; Glc, 2006). It is increasingly being realized that many of common diseases are due to the "oxidative stress" that results from an imbalance between formation and neutralization of prooxidants. In this respect, ROS play an important role related to the degenerative or pathological processes of various serious diseases, such as aging (Burns et al., 2001), cancer, coronary heart disease, Alzheimer's disease (Smith et al., 1996; Diaz et al., 1997), neurodegenerative disorders, atherosclerosis, cataracts, and inflammation (Aruoma, 1998).

Recently, interest has increased in naturally-occurring antioxidants that can be used to protect human beings from oxidative stress damage (Scalbert et al., 2005), because these natural antioxidants avoid undesired health problems that may arise from the use of synthetic antioxidants, which may have toxic effects (Aruoma et al., 1992).

The model of scavenging the stable DPPH radical is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. The effect of antioxidant on DPPH radical scavenging was thought to be due to their hydrogen donating ability. Substances capable of donating electrons/hydrogen atoms are able to convert DPPH radical into their non-radical form 1,1-diphenyl-2- picrylhydrazine. Positive DPPH test suggests that the samples were free radical scavengers. In the present study, the scavenging effect of the total extract and fractions on DPPH radical was compared. DPPH$^•$ was reduced with the increasing of total extract of *A. nilotica* and fractions concentration in a concentration-dependent manner and a higher DPPH radical-scavenging activity is associated with a lower IC$_{50}$ value. The total extract and fractions from the *A. nilotica* flowers showed potent antioxidant activity. Among the extract and fractions, the fraction C exhibits the best antioxidant performance. Phenolics concentration of the total extract and fractions was responsible for the antioxidant

activity. Extract and fractions from the *A. nilotica* flowers might be valuable antioxidant natural sources for both the medical and food industry.

1   R = H   **2**   R = G

5   R = glucoside (2→1) glucopyranoside   **6** R = glucoside   **9** R = He

4   R = glucoside   ,   **8**   R= H

7

Fig 1
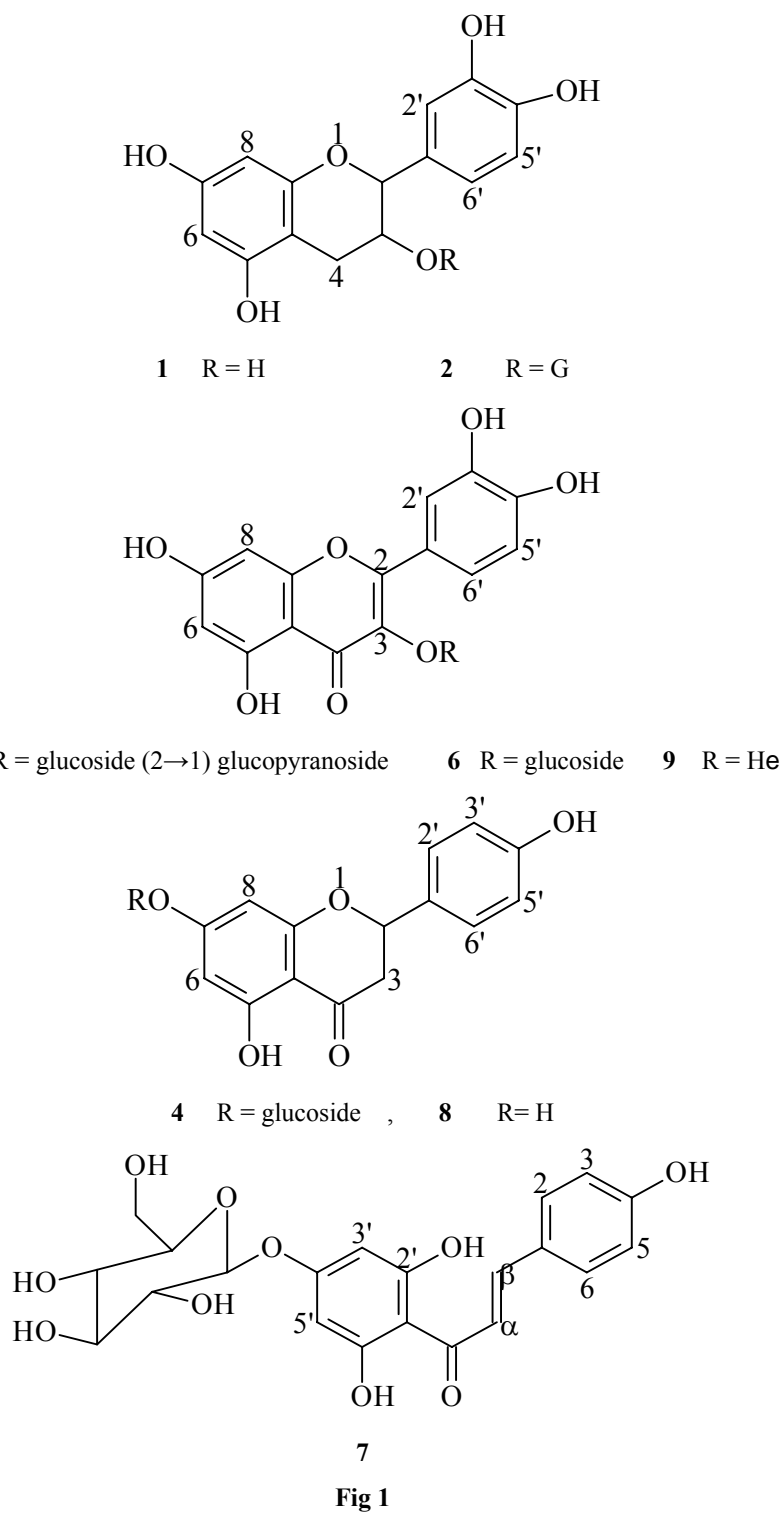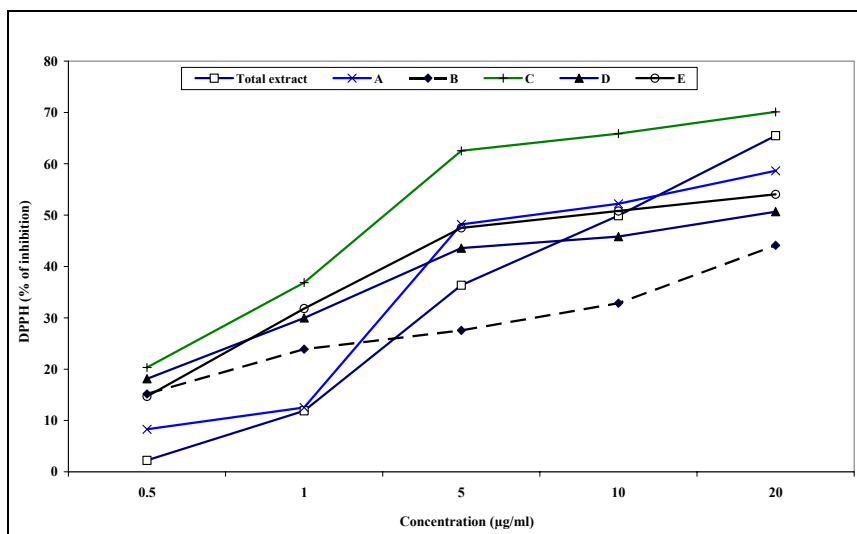
Fig 2: DPPH radical scavenging assay of total extract and fractions (A-E)

Hydrogen peroxide (H₂O₂) scavenging activity

The hydrogen peroxide scavenging ability of fraction C of *A. nilotica* extract and ascorbic acid is shown in Fig (3). Hydrogen peroxide scavenging activity of fraction C at 30 µg/ml was found to be 77.19% and for ascorbic acid at the same concentration was 96.55 %. The IC_{50} value of fraction C was found to be 18.05 µg/ml and for ascorbic acid was 5.75 µg/ml, respectively. The fraction was capable of scavenging H_2O_2 in a concentration dependant manner.

Hydrogen peroxide is a weak oxidizing agent that inactivates a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly; once inside the cell, it can probably react with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radicals and this may be the origin of many of its toxic effects (Miller et al., 1993). From the results, fraction C was capable of scavenging H_2O_2 in a concentration dependant manner. These results suggest that Fraction C can be a better antioxidant for removing H_2O_2 and thus protecting living or food systems.

Reducing power

As shown in Fig (4), fraction C and ascorbic acid had significant inhibition of reducing power with increasing concentration in the range of 0.5-20 µg/ml. At the concentration of 0.5 µg/ml, the OD values of fraction C and ascorbic acid were 0.19, 0.11 but at concentration 20 µg/ml, the OD values 0.29, 0.34, respectively.

The reducing power reflects the electron donating capacity of bioactive compounds, is associated with antioxidant activity. Antioxidant can be reductants and inactive of oxidants. The reducing capacity of a compound can be measured by the direct reduction of $Fe[(CN)_6]_3$ to $Fe[(CN)_6]_2$. Addition of free Fe^{3+} to the reduced product leads to the formation of intense Perl's Prussian blue complex, $Fe_4[Fe(CN^-)_6]_3$, which has a strong absorbance at 700nm. The reducing ability of a compound greatly depends on the presence of reductones, which have exhibit antioxidative potential by breaking the free radical chain by donating a hydrogen atom (Pin-Der, 1998). In this regarded, increase in Fe^{3+} to Fe^{2+} transformation in presence of test sample implies that sample is electron donor and thus can cause reduction of the oxidized intermediates of lipid peroxidation process. In this assay, the yellow color of the test solution changes to various shades of green and blue depending on the reducing power of antioxidant samples (Karimi, et al., 2010). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the antioxidant activity of an antioxidant compound have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging.
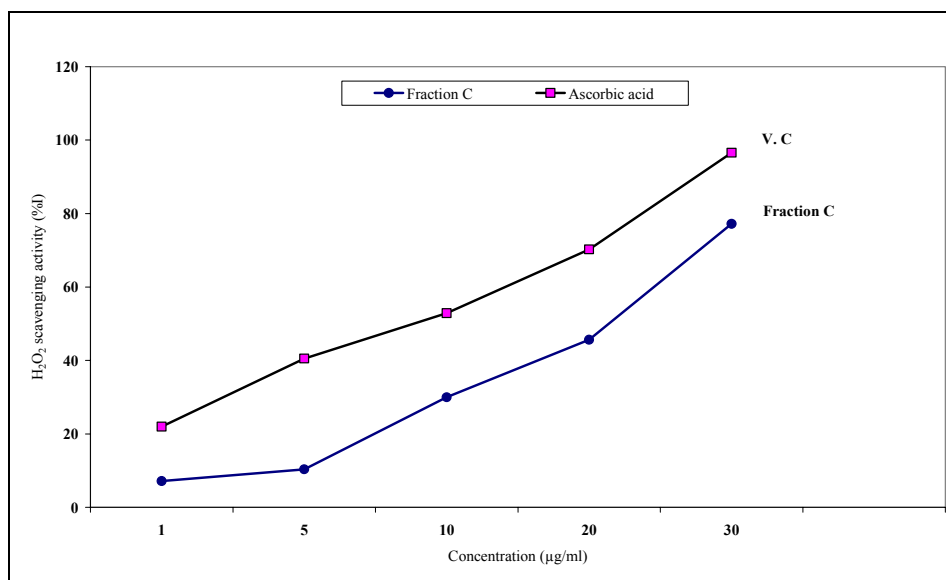
Fig 3: Hydrogen peroxide scavenging activity (H_2O_2) of fraction C and ascorbic acid. Values are mean ± SD.
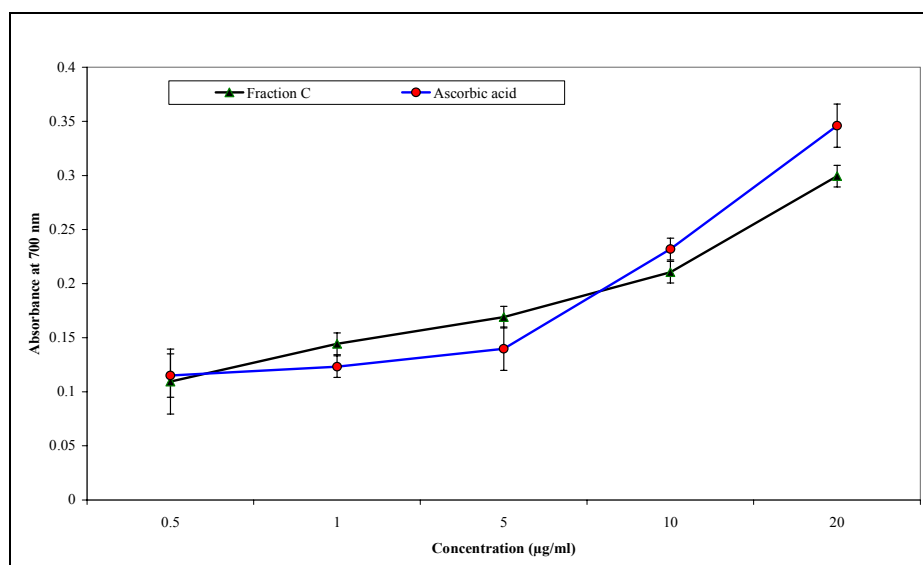
Fig 4: Reducing power of fraction C and ascorbic acid. Values are mean ± SD.

4. Conclusion

   In this work, phenolic constituents have been shown to possess various biological properties related to antioxidant mechanism. Thus, in the present study, the antioxidant potential of *A. nilotica* flowers and its fractions may be attributed to the presence of phenolic compounds and the other constituents present there in.

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Effect of Hyperthermia at Different Ages and Mode of Recovery on the Chromosomal Aberrations and Biological Parameters in Female Rats.

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Abstract: The present study was designed to investigate the various biological changes induced by hyperthermia (at 42°C) in female rats and the mode of recovery at 1, 6 , 24 & 72 hrs at different ages (2, 6 , 12 & 24 months). Biological parameters studied were RBCs, WBCs, Hb count, B% & T% lymphocytes. IgG & IgA and serum activities of T3 & T4, the HSP70. Besides, the chromosomal aberrations test and micronucleus formation were investigated in female rats. In attempt to find out the interaction between age and hyperthermia in such parameters in normal female rats. Female Albino rats of four different ages 2,6,12 and 24 month .We have studied the thermal kinetics of whole body hyperthermia (WBH, at 42°C) and its thermal late effect at 1, 6, 24 and 72 hours post WBH in rats. The results revealed that highly significant increases of WBC's, B%, IgG and HSP70 at 1 till 72hr post WBH in aged 2 and 6 months. On the other hand, WBH caused a significant decrease in each RBC's, T3 & T4 at 6 till 72 hrs post WBH. As well as, the count of Hb decreased in age 2 month at 1 till 72 hrs post the heat exposure but increased at 1 & 6 hr in 6 months aged post WBH then decreased at 24 hr & 72 hr post WBH. T% lymphocyte count significantly (p< 0.05) decreased at 1 hr post WBH and increased at 6 hr & 24 hr then decreased again at 72 hrs post WBH in ages 2 & 6 months. IgA level significantly increased in 6 aged rats at 1, 6 & 24 hrs post WBH then decreased at 72 hr below the control value post WBH. The results revealed that WBH caused a significant increase of B% lymphocyte, Hb and IgA at 1 & 72 hr post WBH in age 12 & 24 months, except Hb in 12 month decreased at 72 hr post heat exposure. On the other hand, T% lymphocyte, RBC's , IgG and serum T3 &T4 decreased at 1 & 72 hr post heat exposure except IgG level increased at 72 hr post WBH . The level of HSP70 increased significantly at 1 till 24 hr post WBH in 12 month and reached to the control value at 72 hr post WBH. On the contrary, HSP70 decreased significantly at 1hr in aged rats (24 month), then increased significantly at 6 and 24 hr post heat exposure then decreased below the control value at 72 hr post WBH. With respect to chromosomal aberrations positive responses were observed at all ages but in different frequencies and recover may occur at 72 h for the all except young ages (2 month) which needed more time to completely recover., in the micronucleus test, we observed positive responses in all ages at 24h only, while at 72h the mean frequencies of micronucleated poly chromatic erythrocytes (MNPCEs) were within the vehicle control group at all ages except 2 month which increased significantly than control group. The results suggest that hyperthermia can induce both chromosomal aberrations and micronucleus formation.

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Key words: Hyperthermia –HSP70, chromosomal aberration -micronucleus

1. Introduction:

Whole body hyperthermia (WBH), as a treatment modality, shows promising results in the management of advanced and refractory cancer in combination with chemotherapy (Ismail- Zade *et al.*, 2002). Stress is a sudden environmental change that induces damage at the molecular, cellular and organism level (Soti and Peter, 2007).Macromolecules such as proteins are continuously exposed to potential damaging agents that can cause loss of molecular function and depletion of cell populations over the lifetime of essential organs. One of the key homeostatic responses involved in maintaining longevity is the induction of heat shock proteins (HSPs), a conserved reaction to damaged intracellular proteins(Calderwood *et al.*, 2009) Damage to macromolecules is characteristic of aging and degenerative diseases. Beyond DNA damage, protein damage may not only a consequence, but also a causal factor in cellular malfunction. Damage may induce misfolding, and the aggregating oligomeric species may gain a novel toxic property, severely compromising cellular function (Dobson, 2003) .The relationship between thermal resistance and expression of inducible heat shock proteins, especially Hsp70, depends on the species and temperature treatments (Bahrndorff *et al.*, 2009). The major stress of life is oxidative stress. Thus, it is not

surprising that oxidized protein level increases with aging of all animal species (Stadtman, 2004).

The heat shock response is a highly conserved "stress response" mechanism used by cells to protect themselves from potentially damaging insults. It often involves the upregulated expression of chaperone and heat shock proteins (HSPs) to prevent damage and aggregation at the proteome level (Michael *et al.*, 2007).Induction of heat shock protein (Hsp) expression appears to correlate with a cytoprotective effect in cultured cells and with improved healing of damaged tissues in animal models and in humans. This family of proteins can also serve as indicators of thermal stress (Rodwell *et al.*, 2008). Area of focus has been the family of highly conserved stress proteins known as heat shock proteins (HSPs). Some of the well-studied HSPs in mammals are those with molecular masses of 70 kDa, and these ubiquitous proteins include the highly heat inducible 72 kDa protein (Hsp70). There is substantial evidence showing that Hsp70 plays a critical role in providing cellular protection against the adverse effects of a wide array of stress and toxic conditions (Kregel, 2002).. Interestingly, investigators have also shown that the ability to induce Hsp70 under a variety of physiologically-relevant conditions, including hyperthermic challenge, is reduced with aging (Jin *et al.*, 2004). Hsp70 appears to be regulated at both transcriptional and translational levels (Vivinus *et al.*, 2001). Zhang *et al.* 2003 demonstrate that the reduction in stress tolerance that accompanies aging is associated with a complex set of integrated alterations in hepatic steady-state levels of ROS, macromolecular damage, redox buffering, and transcription factor regulation. Heat stress proteins (HSP) are induced by a variety of stimuli. They contribute to maintaining the metabolic and structural integrity of the cell, as a protective response to external stresses. A raised body temperature raises the metabolic rate and makes the immune response more efficient (Broom, 2007). Levels of protein Hsp70 were not detectable in the control treatment and 2 h after hardening treatment. However, levels of Hsp70 gradually increased thereafter and at 4 and 6 h after the hardening treatment expression was elevated but with large variation (Bahrndorff *et al.*, 2009). Several authors reported either a decrease or no change in the number of RBC, s in animals subjected to high environmental temperature (Terajima *et al.*, 2000). As well as these authors reported the destruction of red blood cells at 42ºC. Lee and Korean (1987) observed a slight decrease in the Hb content of rabbits exposed to heat stress (37–80% RH) for 2-4 hrs daily for two weeks. They also found a decrease in total leukocytes count and a significant decrease in

lymphocytes without a statistically significant change in monocyte count. Whereas, Kamal et al. (1992) reported a significant increase in WBC, s count in heat stressed mature rats. Mostafa *et al.* (2005) reported that WBH caused significantly decrease in T3 and T4 hormones in rabbits after immediately heat exposure and after 24 hrs. post WBH. Furthermore, they observed that WBH caused a significant decrease below the control values in each of RBC, s, Hb and total WBC's counts. Following after the 24 hrs recovery period. The WBC's was significantly higher while T3, T4, RBC's , Hb and lymphocytes values were significantly lower than in the acute WBH group.

Hyperthermia exerts numerous effects on mammalian cells including division delay, cell killing and chromosomal aberrations when cells are heated in the S-phase (Dewey *et al.,* 1990). Also, Takahashi *et al.* (2004) suggested that heat-induced DNA double strand breaks contribute to heat-induced cell killing because heat treatment induces histone γH2AX-containing foci. Such foci have been associated with double strand breaks induced by ionizing radiation, other agents, and other stresses. (Capetillo *et al.,* 2004). DNA synthesis is inhibited by heat, dependent on the time and temperature of heating (Warters *et al.,* 1984).

2. Materials and Methods:

One hundred female Albino rats at 2, 6, 12 and 24 months of age (20 animals from each age) were purchased from the Animal House of the National Research Centre, Dokki, Egypt. Food and water were provided *ad libitum* under standard condition of light, humidity and temperature. After a period of acclimation the room environmental temperature and relative humidity (RH) will be recorded twice daily using Thermohygrometer.

Whole body hyperthermia (WBH) Treatment:

A plastic cage (45 x 25 x 20 cm), conscious and unrestrained. Rectal temperature (Tr) was continuously monitored on a digital display and was established over a 30-min control period for each rat, followed by a heating protocol. An infrared lamp was positioned 40 cm above each rat and either raised or lowered to obtain an ambient temperature of 42ºC. Movement of the lamp permitted a constant heating rate (0.06 ºC/min) to be attained. Heating was terminated when Tr reached 42ºC, but was then commenced at an appropriate time to maintain Tr at 42ºC for one hour. At the end of this period, the thermistor probe was removed and rats were allowed to passively cool in a cage at room temperature. The rectal temperature (Tr) of the rats will be recorded using a thermocouple (Cole Parmer type T.

thermocouple thermometer) connected with a rectal probe, which will be inserted 8 cm beyond the anal sphincter. When Tr reaches (42 C), directly blood samples will be collected from orbital venous plexus of experimental animals. The animals will be allowed recovering at room temperature and blood samples will be taken after 1,6,24 and 72 hrs post WBH. The blood of each animal was divided into two essay tubes, one heparinzed and the other wasn't; the serum in this last tube was obtained through centrifugation at 3000rpm for 15 min., the obtained serum was kept at –20°C for subsequent analyses of IgG, IgM using ELISA kit from Life Diagnostics according to Salauze et al. (1994) and HSP70 by ELISA kit from Stress Biotechnologies were investigated in female rats. Besides, serum activities of total T_3 & T_4 by radioimmunoassay kit purchased from (Institute of Isotopes Ltd. Budapest). The non-clotted blood collected was used for the determination of red blood cell (RBC' s) and white blood cell (WBC' s) counts were determined using a haemocytometer. Hemoglobin (Hb) concentration was measured by the Cyanmaethmoglobin method, B% & T% lymphocytes.

Bone marrow chromosomal aberrations assay:

After heat exposure, the animals were injected intraperitoneally (i.p) with 0.5 ml colchicines (0.5% mg/kg b.w.) one hour and 30 minutes before killing to block the cells in metaphase. Animals were sacrificed and chromosomes of bone marrow were prepared by using methodology of Yosida and Amano (1965) with slight modifications. The cells in both femurs were collected by pushing 2-3 times of 0.075M KCL into the marrow cavity, left in hypotonic KCL for 35 min. and then centrifuged at 1000 rpm for 8 min. The supernatant was removed and the pellet was resuspended and fixed in methyl: acetic acid (3:1). Centrifugation and fixation were repeated five times at 20 min. intervals. The pellet was resuspended in a small volume of fixative. Finally, the cells were dropped on a clean wet slide and stained with 5% Giemsa stain. 50 metaphases examined from each animal under 1000X magnification to determine the frequencies of chromosome damage cells. The classification of aberrations was carried out as described by Venitt and Parry, 1984 and in the international system for Cytogenetic Nomenclature (ISCN) (Cohen *et al.*, 1993).

Bone marrow micronucleus assay:

The micronucleus test was performed according to Heddle (1973) and Schmid (1975). After heat exposure, animals were killed by cervical dislocation. Both femurs were dissected and bone marrow was flushed from the femoral cavity with fetal calf serum and the cells were centrifuged at 1000 rpm for 5 min. The pellets were suspended and smeared on a clean dry slide. The slides were fixed by methanol and stained with Giemsa stain. The micronuclei (MN) were analyzed under a microscope with 1000X magnification using oil immersion. About 1000 polychromatic erythrocytes (PCEs.) were scored for each treated animal and control. To count the number of micronucluated poly chromatic erythrocytes (MNPCEs).

Statistical analysis:

All results are expressed as mean ± SE. All values were expressed as mean ± SE. Statistical analysis was performed with two way analysis of variance (ANOVA) followed by Duncan's test using SPSS program 17.0. *P* values < 0.05 were considered to be statistically significant.

3. Results:

Results of the present study are presented in tables (1-4). It demonstrated the effect of WBH 42 ° C in young, middle and aged rats (2, 6, 12 and 24 months) on hematological indices which manifested a significant decrease of WBC's in the 6 months, rats before heat value of WBC' s increased significantly after 1 hr post WBH in all groups. The highest value was showed in 2 month old rats. The count of WBC' s reached to the control values at 24 &72 hrs post WBH in 12 month old rats. On the contrary, reached below the control values in 24 month old rats as shown as in table (1a). The data demonstrated in table (1a) shows the count of RBC' s decreased significantly in all different ages' rats at 1 hr till 72 hrs post WBH except in 2 and 24 months' rats. The count of RBC' s in that both groups increased significantly at 24hr and 6hrs respectively, post WBH. The count of T% lymphocytes decreased significantly in all groups after 1 hr post WBH. This decrease was continues till the end of experiment 72 hr in 24 months group but in 2 & 6 month old rats , the count of T% lymphocytes increased significantly at 6 & 24 hrs post WBH, then decrease at 72 hr post heat exposure(Table 1a). On the other hand, WBH caused significantly increased of B% in all experimental groups at 1hr till 72 hrs post WBH. The maximum increase was recorded in 12 month old rats at 6 &72 hrs post heat exposure.

WBH revealed significant increase of IgG in 2 & 6 month old rats but decreased significantly in 6 & 24 month old rats after 1 hr post WBH (Table 1a). Then IgG values showed increase at 6 &24 hrs after heat exposure in aged rats (24 months). This parameter fluctuates down and up in 12 months old rats. WBH significantly increased IgA in rats 6, 12 & 24 months at 1 till 72 hrs post heat exposure, the only decrease showed at 72 hr post WBH in 6 month old

rats (Table 1b). The value of IgA in the group 2 month old rats was significantly (P< 0.05) higher than that in 6, 12 & 24 month old rats by 30.08%, 13.69% and 29.12%, respectively (Table 2). The results revealed that WBH increased significantly Hb count as compared to control values in 6, 12& 24 months at 1 hr post heat exposure (13.76 ± 0.61, 12.23 ± 1.55 g/dl and 14.34 ± 0.40 g/dl, respectively). While Hb content showed a significant decrease in 2 month old rats at 1 till 72 hr post WBH. However, the increase of Hb level in aged rats (24 months) continued at 1 till 72 hr post WBH, decreased at 72 hr in 6 & 12 months. In the present study, WBH caused a significant increase of HSP70 at 1hr post WBH in 2 month old rats and this increase was higher than that of 6,12 &24 months by 49.71%, 20% & 78%, respectively (Table 1b) . Then HSP70 decreased at 6, 24 & 72 hrs post WBH in 2 month old rats group,1.26 ± 0.43, 2.46 ± 0.99 and 1.40 ± 0.35, respectively (Table1b) . This decrease was lower than that in 12 month by 139.68%, 30% & 87.14%, respectively, but still higher than control value. The highest decrease of HSP70 was recorded at 6 hr post WBH in aged group (24 months), this decrease was 188.89% lower than that in 2 month old rats. Moreover, WBH caused significant decreases of total T3 &T4 in all groups at 1 hr till 72 hrs post heat exposure except in 2 & 6 months showed that T3 approached to the control values at 72 hr post WBH (Table 1b).

Effect of WBH on chromosomal aberrations of bone marrow cells:

Our experiment was demonstrated the effect of hyperthermia on chromosomal aberrations in female rats at different ages (2, 6, 12 and 24 months) and different times (1, 6, 24 and 72 hours) after heat stress. These data are presented in table (3). It can be seen that heat induced various types of structural chromosomal aberrations which consisted of gaps, breaks, deletions, fragments, centromeric attenuations and endomitosis. Numerical aberrations resulted in hypoploidy and hyperploidy only. When heat was applied on rats at 2 month of age, mean values of total chromosomal aberrations were statistically increased (P<0.05) in animals sacrificed 1, 6, 24 and 72 hrs after heat stress than those of control (Table 3). While 72 h group statistically decreased than 6 and 24 hours groups means that at 72 h the aberrations begin to recover. Also, the number of abnormal metaphases significantly increased in all groups than control.

In female rats at 6 month of age, there were significant (at P<0.05) increased in the total chromosomal aberrations of animals sacrificed 1, 6, 24 and 72 hrs than control. The value observed 72 hr after heat exposure was within the vehicle control group and thus not considered of biological

significance. Abnormal metaphases significant increased than control in all groups except 72 hr post WBH in which the increasing had no significant.

The data obtained from female rats at 12 month old rats recorded that there were significant (at P<0.05) increased in the total chromosomal aberrations of rats sacrificed 1, 6 and 24 hours after heat shock when compared with control (Table3). While, there was non-significant difference between the frequencies of the total aberrations induced by heat at 72 hr and that observed in the control. The abnormal metaphases decreased at 72 hr group and returned to the control.

In addition, heat caused significant (P< 0.05) increase in the mean values of total chromosomal aberrations of 1, 6 and 24 hrs than those of control at 24 month of age. In contrary, 72 hr showed significant (P< 0.05) decrease in the frequencies of total aberrations than those of other groups and this value was within the vehicle control group and thus not considered of biological significance. Abnormal metaphases reached the control value at 72 hr after treatment (Table 3).

At 1 hr after heat shock, there were no significance differences between 2 and 6 month of age in the total chromosomal aberrations, while between these and other two groups there were statistically significant difference. Young age (2 months) were more affected than adult (6 month) which have less frequencies than old age rats (12 and 24 months). 72 hrs showed that 2 month old rats there were increased statistically than other groups. Means that young age need more time than other groups to recover. The number of abnormal metaphases increases with increasing the age and begin to recover at 72 hrs after heat stress in all ages except 2 month old rats which need more time to completely recover.

Effect of WBH on micronucleus formation of bone marrow cells:

Heat stress was tested for induction of micronucleus in bone marrow of female rats (Table 4). The number of MNPCEs unaffected rats at 1 and 6 hours after heat stress non- significantly differ than control of all ages (2, 6, 12 and 24 months of age). At 24 hr after heat stress, the number of micronucleus was significantly (P<0.05) increased for 2, 6, 12 and 24 months of age than those of control. Also, there were significantly decrease in the number of MNPCE's at 6 month of age than the all. At 72 hr post treatment, the mean frequencies of MNPCE's at 6, 12 and 24 months of age (2.0±0.41, 2.25±0.25 and 2.75±0.48%, respectively) were within the vehicle control group (2.0±0.41%). On the other hand, there was a significant increase in 2 month old rats (4.50±0.65%) than control group (2.0±0.41%).

Table 1a: Effect of WBH at 42 ° C and its late effects on various biological parameters in 2,6,12 and 24 months old rats.

Age	Time	Parameters				
		WBC's (X10³/cmm)	RBC's (X10⁶/cmm)	T lymphocytes (%)	B lymphocytes (%)	IgG (ng/ml)
2 month	Control	5.36±1.57^{efg}	3.46±0.56^{bc}	23.62±3.20^{fg}	16.59±1.61^{i}	826.60±67.92^{h}
	1 hr	14.51±1.58^{a}	2.01±0.47^{g}	23.15±3.34^{fgh}	21.31±2.91^{ghi}	1436.40±220.18^{b}
	6 hr	6.41±1.62^{defg}	2.56±0.28^{ef}	25.50±1.79^{ef}	32.54±3.74^{b}	1078.50±53.12^{fg}
	24 hr	8.88±1.83^{c}	3.94±0.76^{a}	26.91±1.58^{de}	33.48±8.87^{b}	1233.40±152.21^{cdef}
	72 hr	6.67±1.42^{defg}	2.42±0.39^{efg}	20.31±1.28^{h}	26.73±4.52^{defg}	1315.60±220.75^{bcde}
6 months	Control	4.99±0.23^{g}	3.88±0.51^{ab}	28.08±6.01^{bcde}	17.10±2.05^{i}	813.10±36.78^{h}
	1 hr	7.54±2.23^{cd}	2.02±0.16^{g}	20.30±2.40^{h}	27.33±7.63^{bcdef}	942.60±61.88^{gh}
	6 hr	6.78±1.45^{def}	3.13±0.35^{cd}	31.08±2.21^{ab}	26.27±5.03^{efg}	957.70±103.96^{gh}
	24 hr	10.94±2.43^{b}	2.50±0.26^{ef}	30.14±4.36^{abc}	28.44±5.47^{bcdef}	1382.50±169.69^{bc}
	72 hr	11.61±2.22^{b}	2.31±0.43^{fg}	27.19±4.44^{cde}	31.89±5.42^{bcde}	1717.80±157.15^{a}
12 months	Control	7.87±2.00^{cd}	3.85±0.50^{ab}	23.95±3.59^{fg}	23.82±5.33^{fgh}	1226.20±113.70^{cdef}
	1 hr	8.85±1.53^{c}	2.53±0.37^{ef}	21.44±2.53^{gh}	29.32±4.^{85bcdef}	1153.40±251.30^{ef}
	6 hr	13.99±1.50^{a}	3.17±0.59$_{cd}$	22.85±1.66^{fgh}	42.69±13.67^{a}	1304.10±255.40^{bcde}
	24 hr	7.78±1.25^{cd}	3.14±0.53$_{cd}$	30.56±4.41^{ab}	19.78±3.47^{hi}	1203.50±254.48^{def}
	72 hr	7.58±1.60^{cd}	3.19±0.70$_{cd}$	27.35±2.90^{cde}	43.19±4.93^{a}	1308.00±148.72^{bcde}
24 months	Control	11.33±1.20^{b}	3.73±0.31^{ab}	31.67±1.24^{a}	27.12±4.89^{cdefg}	1179.00±141.45^{def}
	1hr	5.05±0.76^{fg}	2.06±0.17^{g}	22.55±2.14^{fgh}	33.29±5.89^{bc}	941.50±89.72^{gh}
	6 hr	13.55±3.10^{a}	4.16±0.30^{a}	22.64±1.36^{fgh}	27.63±8.08^{bcdef}	1350.00±223.56^{bcd}
	24 hr	11.35±2.49^{b}	3.49±0.45^{bc}	28.74±1.76^{abcd}	39.19±3.62^{a}	1309.00±150.36^{bcde}
	72 hr	6.83±1.19^{de}	2.78±0.36^{de}	28.57±4.40^{abcde}	30.91±5.27^{bcde}	978.20±150.36^{gh}

Data were expressed as mean ± S.E
Means with different superscript letters are significantly different (P<0.05)

Table 1b: Effect of WBH at 42 ° C and its late effects on various biological parameters in 2, 6, 12 and 24 months old rats

Age	Time	Parameters				
		IgA (ng/ml)	Hb (g/dl)	HSP70 (ng/ml)	T4 (µg/dl)	T3 (ng/dl)
2 month	Control	93.10±11.22^{efgh}	13.75±0.55^{a}	1.01±0.24^{ghi}	5.74±0.43^{a}	81.75±2.10^{a}
	1 hr	80.50±7.29^{ij}	7.73±0.36^{i}	3.50±0.63^{ab}	5.2±0.37^{ab}	78.53±1.30^{ab}
	6 hr	101.20±11.91^{cde}	11.51±2.17^{cde}	1.26±0.43^{fghi}	4.50±0.41^{c}	72.41±1.96^{b}
	24 hr	83.10±6.76^{hij}	11.12±1.52^{cdef}	2.46±0.99^{de}	5.31±0.64^{ab}	58.62±3.4^{c}
	72 hr	114.70±7.79^{a}	10.45±1.13^{efg}	1.49±0.35	5.40±0.42^{a}	53.70±5.1^{d}
6 months	Control	90.00±2.40^{fghi}	13.02±0.44^{ab}	1.56±0.43^{fg}	5.37±0.50^{a}	75.50±2.42^{a}
	1 hr	113.40±2.27^{ab}	13.76±0.61^{a}	1.76±0.94^{f}	4.84±0.43^{b}	64.35±3.70^{b}
	6 hr	94.50±15.39^{defg}	14.32±0.78^{a}	0.87±0.06^{hi}	4.51±0.47^{bc}	65.80±2.92^{b}
	24 hr	104.20±4.24^{bcd}	10.72±1.92^{defg}	2.79±0.43^{cde}	5.25±0.41^{a}	73.30±3.34^{ab}
	72 hr	80.20±9.44^{ij}	9.12±1.19^{h}	2.31±0.88^{e}	5.18±0.46^{a}	74.10±3.42^{ab}
12 months	Control	68.30±14.84^{k}	11.76±1.72^{bcde}	2.60±0.45^{de}	4.92±0.24^{ab}	71.78±1.48^{b}
	1 hr	85.40±12.05^{ghij}	12.23±1.55^{bc}	2.77±0.25^{cde}	3.87±0.68^{efg}	68.22±5.74^{bc}
	6 hr	106.80±6.96^{abc}	9.72±0.86^{gh}	3.02±0.45^{bcd}	3.61±0.74^{efg}	54.40±2.99^{d}
	24 hr	90.30±13.78^{fgh}i	12.01±1.73^{bcd}	3.22±0.76^{abc}	2.91±0.22^{fg}	55.20±3.45^{d}
	72 hr	99.00±9.97^{cdef}	11.11±2.13^{cdef}	2.62±0.88^{de}	3.75±0.47^{efg}	48.75±3.14^{e}
24 months	Control	53.70±14.48^{l}	9.08±0.62^{h}	1.78±0.46^{f}	5.5±0.40^{a}	74.43±1.32^{a}
	1 hr	94.00±10.60^{efg}	14.34±0.40^{a}	0.77±0.11^{i}	4.81±0.65^{ab}	71.80±2.27^{b}
	6 hr	92.20±12.98^{efgh}	9.98±2.22^{fgh}	3.64±0.47^{a}	4.77±0.64^{ab}	65.35±5.21^{bc}
	24 hr	78.30±10.15^{j}	10.55±1.56^{efg}	2.37±0.76^{e}	4.30±0.82^{d}	59.10±4.09^{c}
	72 hr	81.30±7.90^{ij}	13.94±0.76^{a}	1.04±0.26^{ghi}	4.55±0.64^{c}	44.75±6.07^{e}

Data were expressed as mean ± S.E
Means with different superscript letters are significantly different (P<0.05)

Table 2: Effect of WBH at 42 ° C and its late effects on various biological parameters in 2, 6, 12 and 24 months old rats(% change).

Age	Time	Parameters									
		WBC's	RBC's	T (%)	B (%)	IgG (mg/dl)	IgA (pg/ml)	Hb (g/dl)	HSP70 (ng/ml)	T3 (ng/dl)	T4 (µg/dl)
6 months	Control	6.90	-12.14	-18.88	-3.07	1.63	3.33	5.31	-54.46	6.45	7.65
	1 hr	48.04	-0.49	12.31	-28.25	34.38	-40.87	-78.0	49.71	6.92	18.06
	6 hr	-5.77	-22.27	-21.88	19.26	11.20	6.62	-24.41	30.95	-0.22	9.13
	24 hr	-23.20	36.55	-12.33	15.05	-12.09	-25.39	3.60	-13.41	1.13	-25.04
	72 hr	-74.10	4.55	-33.87	-19.30	-30.57	26.64	12.73	-65.00	4.07	-37.99
12 months	Control	-46.83	-11.27	-1.39	-43.58	-48.34	-6.09	14.47	-157.43	14.29	12.19
	1 hr	39.01	-25.87	7.39	-37.59	19.70	-5.53	-85.21	20.86	25.58	13.13
	6 hr	118.25	-23.83	11.18	-31.19	-20.92	-8.66	15.56	-139.68	19.77	24.87
	24 hr	12.39	20.30	-13.56	40.92	2.42	42.32	-8.00	-30.89	45.20	22.89
	72 hr	-13.64	-31.82	-34.66	-61.58	0.58	-16.77	-6.32	-87.14	30.56	9.22
24 months	Control	-111.38	-7.80	-34.08	-63.47	-42.63	8.89	33.96	-76.24	4.18	8.95
	1hr	65.15	-2.94	2.59	-56.22	34.45	5.78	-85.51	78.00	7.50	8.57
	6 hr	-111.39	-62.50	11.22	15.09	-25.17	30.08	13.29	-188.89	-6.00	9.75
	24 hr	-27.82	11.42	-6.80	-17.05	-6.13	13.96	5.13	3.66	19.02	-0.82
	72 hr	-2.40	-14.88	-40.67	-15.64	25.65	29.12	-33.40	25.71	15.74	16.67

Table (3): Mean values of chromosomal aberrations after heat exposure in female rat bone marrow cells at different ages.

Age	Time	Abnormal metaphases	Types and no. of chromosomal aberrations						Total chromosomal aberrations	Total aberrations excluding gaps
			Gap	break	Del &frag	CA	End	Numerical aberrations		
2m	control	11±0.41[hi]	5	7	6	13	5	8	11.0±0.41[j]	9.75±0.63[ij]
	1h	16.25±0.85[f]	6	3	16	27	0	24	19.0±0.41[gh]	17.5±0.50[fg]
	6h	26.50±1.04[b]	16	3	19	32	11	37	29.5±0.29[abcd]	25.5±0.29[c]
	24h	23.75±1.93[c]	21	5	21	24	5	34	27.5±0.29[bcd]	22.25±0.63[d]
	72h	19.50±0.65[de]	16	0	0	15	18	29	19.75±0.48[fg]	15.75±0.25[g]
6m	control	9.50±0.87[i]	5	5	7	12	4	6	9.75±0.63[j]	8.5±0.87[j]
	1h	18.0±0.41[ef]	6	0	6	27	23	12	18.5±0.50[ghi]	17.0±0.41[fg]
	6h	27.50±1.26[b]	9	5	20	40	11	35	30.0±0.0[abc]	27.75±0.25[bc]
	24h	21.0±0.58[d]	3	0	8	45	11	20	21.75±0.25[efg]	21.0±0.41[d]
	72h	10.75±0.25[hi]	4	0	7	17	7	8	10.75±0.25[j]	9.75±1.63[ij]
12m	control	13.50±0.29[g]	8	4	2	12	5	19	13.50±0.29[ij]	11.50±0.29[hi]
	1h	24.0±0.41[c]	21	4	15	28	11	19	24.50±0.50[ef]	19.25±0.48[ef]
	6h	32.50±0.29[a]	8	8	22	59	13	26	34.0±0.41[a]	32.0±0.41[a]
	24h	28.0±0.71[b]	11	3	24	53	8	12	28.75±0.48[abcd]	26.0±0.41[bc]
	72h	12.0±0.41[gh]	11	0	0	19	5	13	12.0±0.41[j]	9.25±0.63[ij]
24m	control	11.75±0.48[ghi]	3	3	3	15	13	11	12.0±0.41[j]	11.25±0.48[hi]
	1h	28.50±0.65[b]	13	5	29	42	16	19	31.0±0.41[ab]	27.75±0.75[bc]
	6h	31.0±0.41[a]	16	0	24	58	11	21	32.50±0.29[a]	31.0±2.68[a]
	24h	28.50±0.29[b]	9	4	32	41	8	18	30.50±0.29[ab]	28.25±0.25[b]
	72h	14.0±0.41[g]	7	0	7	14	14	12	14.50±0.50[hij]	12.75±0.48[h]

Data were expressed as mean ± S.E

 Means with different superscript letters are significantly different (P<0.05)

Del: deletion, Frag: fragment, CA: centromeric attenuation, End: endomitosis

Table (4): Mean values of micronucleated polychromatic erythrocytes after heat exposure in female rat bone marrow cells at different ages

times ages	Mean values of MNPCEs based on 1000 PCEs assessed per animal				
	Control	1h	6h	24h	72h
2 m	2.0 ± 0.41^f	1.75 ± 0.48^f	2.25 ± 0.48^{ef}	6.75 ± 0.48^c	4.50 ± 0.65^d
6m	2.0 ± 0.41^f	2.0 ± 0.41^f	2.0 ± 0.41^f	$4.0\pm0.0.41^{de}$	2.0 ± 0.41^f
12m	2.0 ± 0.41^f	2.25 ± 0.75^{ef}	2.0 ± 0.41^f	9.0 ± 0.41^b	2.25 ± 0.25^{ef}
24m	2.0 ± 0.41^f	2.25 ± 0.75^{ef}	2.25 ± 0.63^{ef}	12.0 ± 1.23^a	2.75 ± 0.48^{ef}

Data were expressed as mean ± S.E;   Means with different superscript letters are significantly different (P<0.05).

4. Discussions:

In animals and humans, some physiological and biochemical adaptations could occur to protect essential cell functions against heat stress and to permit a rapid recovery from moderate hyperthermic damage (Hales *et al.*, 1996). However, each tissue and organ has a different sensitivity for sustaining thermal injury (Ando *et al.,* 1994). The biochemical impacts of heat stress on different ages to know the ability of tissues to repair them need to be evaluated. In the present study, the whole body hyperthermia induced increase of WBC's in all different ages after WBH at 1hr till 72 hr except in the aged group (24 months), WBC decreased significantly at 1hr post WBH then increased at 6 hr and approached to control value at 24 hrs post heat stress. This decrease may be due to aging which physiologically decrease WBC's count in aged rats. Also in most mesenchymal tissues a subcompartment of multipotent progenitor cells is responsible for the maintenance and repair of the tissue following stress with increasing age, the ability of tissues to repair themselves is diminished, which may be due to reduced functional capacity of the progenitor cells (Stolzing and Scutt ,2006). While WBH caused significantly decreased of RBC in all groups after I hr post the treatment. The significant decrease in the numbers of RBC count is in agreement with the results of Terajima *et al.(*2000) and Mostafa *et al.* (2005). The decreased numbers of RBCs may be due to accumulation of blood in different organs as a result of internal hemorrhage as was confirmed by the histopathological alterations observed in liver, kidney and heart in previous studies (Hassan , 2006; Abd El- Samea *et al.,* 2007 and Mostafa *et al.,* 2009). These effects might have been also attributed to thrombocytopenia or to decreased blood platelets combined with direct leading to extravasation and escape of red blood cells into the tissue spaces (Mostafa *et al.,* 2005). Another possible reason for the observed decrease of RBCs count could have been the direct hemolytic effect of hyperthermia on the cell membrane resulting in increased membrane fluidity and consequently cell fragility(Zaidi *et al.,* 2002) However, Olayemi and Nottidge (2007) reported that the values of RBC, Hb and WBC were similar in the young and adult New

Zealand rabbit. The data in the present study revealed a significant increase of Hb level in all groups after 1 hr post WBH except in 2 month's group Hb content decreased in this group till the end of the experiment (72 hrs). The present study showed a significant decrease of T lymphocytes percentage in all groups of the experiment after 1hr post WBH which may be due to the stimulating effect of heat on lymphoid organs (Mostafa, 2005).This decrease continued till the end of experiment in group (24 months) , these results are in accordance with the results observed by Ahlers *et al*.(1998) , who established that the number of lymphocytes decreased significantly from 37°C to 42°C . This effect was mainly caused by a significant decrease of the absolute T4-Cell count and a slight decrease of the T8-Cell count with a resulting significant decrease of T-Cells. In addition, IL2-Receptor expression on T-Cells, as a marker for activation, decreased significantly .It seems remarkable, that these effects were reversible in a very short time-period after decrease of temperature.

Induction of heat shock protein (Hsp) expression appears to correlate with a cytoprotective effect in cultured cells and with improved healing of damaged tissues in animal models and in humans (Christian *et al.,* 2002). As well as, the current study was to investigate the impact of both hyperthermia and aging on *in vivo* Hsp70 regulation in response to heat stress. The current results demonstrated that both young animals (2 months) are capable of inducing Hsp70 protein in the early phases of recovery1 hr after a heat challenge. However, at 1 hr and later time points of recovery, old animals (24 months) failed to maintain the high Hsp70 protein levels that were noted in their young counterparts. These results are in accordance with those of Singh *et al.* (2006) and Soti and Csermely (2006). Our results indicate that both 6 & 12 month groups were capable of up-regulating Hsp70 level at 24 & 72 hrs following heat stress, while levels of HSP70 in old rats (24 month) decreased significantly at 1 & 72 hours after heat stress. The decrease in HSP70 levels could potentially result from either lowered transcriptional abilities or accelerated mRNA degradation in old rats (Zhang *et al.,* 2006). Nevertheless, Singh *et al.* (2006) speculated that age dependent decline in the

ability of peripheral blood mononuclear cells to respond to heat stress in terms of HSP70 induction. Hall *et al.* (2000), reported that the inducible HSP70 response in the cytoplasm and nucleus was markedly reduced with age at several time points over a 48hrs recovery period, although senescent rats were able to strongly express HSP70 early in recovery. Older animals had extensive zone-specific liver injury, which corresponded to the diminished HSP70 response observed in these regions, and a significant reduction in thermotolerance compared with their young counterparts (Hall *et al.,* 2000). The process of aging has been associated with increased oxidative damage to macromolecules such as lipids, proteins and DNA in a wide array of tissue types in many eukaryotic species (Dobson, 2003). Therefore, an alteration in the ability of cells to express heat shock proteins could be physiologically important in aging because all living organisms show a reduced ability to respond to stress with increasing age (Vasilaki *et al.,* 2002 and Jin *et al.,* 2004). We have shown that the induction of HSP70 expression by heat shock is reduced approximately 188.89% at 6 hr. of recovery in an aged group (24 month old rats) as compared to that in 2 month old group. Other investigators have also shown that the induction of HSP70 expression by heat shock as well as other stresses declines significantly with age in a variety of tissues from rats as well as mononuclear cells from human subjects (Stolzin *et al.,* 2008). Therefore, it appears that a reduced ability to express HSP70 in response to stress may be a common phenomenon underlying the aging process (Heydari *et al.,* 2000). Induction of heat shock proteins including HSP70 that gives a cytoprotective effect against further stress. However, HSP70 induction is attenuated in aged cells. The lower HSP70-levels may contribute to the impaired stress response seen in the aged, and to the higher rates of chronic wounds in aged, which arise from repeated ischemia-reperfusion injury (Andrea *et al.,* 2006). The prevalence of anabolic activity can trigger the increased basal production of HSP70 in young animals (Maiello *et al.,* 1998). Aging is accompanied by a decay of self-defensive mechanisms and by an accumulation of damages at the molecular, cellular, and organic level as a result of a constant exposure to adverse environmental stresses (Shao *et al.,* 2007). The impaired response to heat stress observed in old rats could be caused by a failure to induce heat shock proteins (HSP70) (Tandara *et al.,* 2006). Because HSP70 is believed to promote the correct refolding of denatured or unfolded proteins damaged by stress and can also act as a chaperone in the ubiquitin–proteasome degradation pathway (Riezman 2004), the failure of senescent organisms to properly induce the expression of HSP70 and other HSPs would lead to the accumulation of damaged proteins that may be toxic to the cell (Riezman, 2004).

The data in the present study revealed a significant increase of IgG in both groups 2 & 6 months after 1 hr till 72 hrs the end of the experiment. On the contrary IgG level in remainder two groups 12 & 24 months showed a fluctuated decrease and increase. On the other hand, WBH induced a significant increase of IgA in 6, 12 and 24 months of age, after 1hr post heat exposure. This increase still even 72 hr post WBH in both groups 12 & 24 months. On the contrary, Hietala *et al.* (2006) reported that hyperthermia has no changes occurred in immunoglobulins or cell-mediated immunity. As well as, Koga *et al.* (2006) reported that total-body hyperthermia (TBHT) therapy may lead to a reduction in the immune response of cancer patients because of the immediate effect of heat on lymphocytes, the authors studied the immunity of advanced cancer patients receiving combined TBHT and anticancer chemotherapy. A decrease was found in their lymphocyte blastogenesis and lymphocyte rosette formation, IgG. These parameters returned to their pretreatment levels at 1 week after completion of TBHT therapy. This result indicates that there is no necessity for giving special consideration to a reduction of cell-mediated immunity in TBHT therapy. Further, Salauze *et al.,* (2004) indicated that both IgM and IgG levels increase gradually with age. By contrast Watt *et a.* (1986) were observed infants under 6 months failed to respond by the production of IgG antibodies, although increases in IgA and IgM levels.

The endocrine system reacts to the change in the environmental temperature, resulting in change in delivery of thyroid hormones and glucocorticoids (Hardy 1974) .Thyroid hormones are known to play an important role in adaptive thermogenesis(Arieli and Chint 1986). In the present study there was a dramatic reciprocal change in T3 and T4 during the heat stress of WBH in all groups of the experiment. Although T3 approached to the control value at 72 hr post heat stress in 2 months group and at 24hr in 6 months group. The decrease of T3 and T4 hormones is in agreement with other studies (Ashour *et al.*, 1995; Mostafa *et al.,* 2005). The reduction of T3 and T4 may be due to the direct effect of high temperature on the thermal receptors, which stimulate the hypothalamus to depress its secretion of TSH releasing hormone(TRH) and consequently the pituitary TSH and thyroid T3 and T4 secretion (Mustafa *et al.,* 2008). This decrease conversion of T4 to T3 and faster utilization of T3 by the respiratory muscles to speed up ventilation in order to enhance energy production (Bianco *et al.,* 2005).

The influence of recovery for 72 in 12 & 24 months groups. So, there is fairly good agreement among authors that the thyroid gland activity in different species increases at moderate cold environment and decreases at high temperature.

There are few reports on the relationship between chromosome aberrations and high body temperature, although there are many in vitro reports of the induction of chromosome aberration by heat treatment (Coss *et al.,* 1982 and Yamada *et al.,* 1989). Here, we demonstrated the effect of hyperthermia on the induction of chromosomal aberrations and micronuclei of bone marrow cells in female albino rats. The results accomplished in this work demonstrated that exposure to hyperthermic treatment (42 °C) for 1 hour caused chromosomal aberrations and micronucleus of bone marrow cells at all times but in different frequencies. Our results in agreement with Waissenbourn and Obe, (1992) who reported that structural chromosome aberrations (breaks, stickiness, fragmentation) were observed at 41.5°C and 43 °C respectively . On the other hand, exposure to heat stress in Muscovy ducks leads to negative effects on some physiological, immunological responses and chromosomal aberrations (El-Badry, *et al.,* 2009). Similar findings were obtained in broiler chickens during high temperature exposure (Zhou *et al.,* 1998). These aberrations may because hyperthermia produces little DNA damage (Jorritsma and Konings, 1993). Heat may induce DNA base damage indirectly via protein damage (Takahashi, 2004) and changes in enzyme complexes for DNA synthesis and repair (Streffer, 1995). Our results demonstrated that at 24 hrs, young age (2 months) have more frequencies of chromosomal aberrations than adult (6 months) which have less frequencies than old age (12 and 24 months). 72 hours group showed that there were no significant differences between all ages except 2 months which increased statistically than other groups. This mean that young age more affected and need more time than others to recover. This may due to the potential thermotolerance cannot be incorporated into the developing mechanisms of thermoregulation (El-Badry *et al.,* 2009). Laszlo, 1988 suggested that there is a relationship between thermotolerrance and heat shock proteins (hsp) synthesis rate. Furthermore, it has been explained that heat shock proteins bind to chromosomes following heat stress and they take part in chromosome condensation and on recovery induce damages like chromatid stickiness. Mamon and Kutskova, 1993 added that high temperature has a role in inducing damages of mitotic chromosomes in Drosophila melanogaster. From the present results, it was observed that adults were more resistance to heat

stress than young and old ages due to potential thermotolerance can incorporat into the developing mechanisms of thermoregulation (Rotwell, 1992). On the other hand, some reports indicate no clear correlation between the frequency of chromosome aberrations and age in the occupationally exposed individuals (Monfared *et al.,* 2003).

Our results also finding that heat shock led to the formation of micronucleus at 24 hours after heat treatment in all ages but more frequencies at 24 months of age. These mean values not differ significantly than control in 1 and 6 hours and recovered at 72 hours after heat at all ages except at 2 months of age. Our findings are in good agreement with other investigators such as Asanami and Shimono, 1997 and Asanami *et al.,* 2001 they observed positive responses of micronucleus at 31, 33 and 40°C for 24h and 42°C for 2h. Results suggested that in Chinese hamster cells line, hyperthermic conditions can induce both chromosome aberration and micronuclei. The micronucleus is based on observations that chromosome fragments and/or entire chromosomes separated (lagging) from the main group at anaphase of mitosis tend to be excluded from a daughter cell nucleus at the telophase stage of mitosis. These chromosomes or fragments are often transformed into micronuclei in the cytoplasm of the daughter cell. As this micronucleus formation may occur spontaneously (not under mutagen influence) at a low rate of incidence, a specific cell type known to have just undergone mitosis under mutagen influence is needed. The mammalian erythrocyte offers this opportunity and has the added advantage of not having a main nucleus so micronuclei can be easily observed. After the last mitotic cycle in the bone marrow, mammalian erythroblasts expel their nuclei but retain any micronuclei and take on a different staining ability. These erythroblasts are polychromatic and stain blue for a period of about 24 hours, then become normochromatic or red staining (Chrisman and Baumgartner, 1980). This may give interpretation for our results that micronucleus significantly increased only at 24 hours after heat stress. Also, MHLW, count the number of micronucleated PCEs in 2000 PCEs of bone marrow cells at 24 and 48 hrs after the administration.

In conclusion, aging and hyperthermia have a synergistic effect on hematological indices as well as, HSP70 that may be essential for surviving and recovering from thermal injury in aged animals. Also, exposure to hyperthermic treatment of 42°C for 1 hr induced chromosomal aberrations and micronucleus formation in female rat bone marrow cells, and recovered at 72 hrs after heat treatment in adults and old ages but in young, it take more time to recover.

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Health risk assessment of workers exposed to heavy metals in cement kiln dust (CKD)

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Abstract: Cement kiln dust (CKD) like cement itself is not considered to be hazardous material under EPA regulations. However, this does not mean that CKD does not contain anything that could pose a hazard to the environment. Therefore, the objectives of this paper were to: 1) evaluate the concentration of six hazardous metals; arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), nickel (Ni) and zinc (Zn) in CKD. 2) Carry out health risk screening analysis for occupational exposure in the cement plants. CKD samples were collected from the biggest three companies for Portland cement production, which are located at Helwan governorate south of Cairo, Egypt. In the present study concentrations of the six metals were measured using Atomic Absorption Spectrometry techniques. The obtained average concentrations were 35.95; 30.17; 15.44; 12.49; 1.27; and1.02 for Cr, Zn, Ni, Pb, As and Cd, respectively. The average daily and lifetime average daily doses for each metal were calculated to evaluate the health risk assessment (HRA) among workers exposed to hazardous metals detected in CKD. Moreover, the results of the current work showed that Cr represents a high risk in the three cement plants comparing to the others measured ones. It might be attributed to high content of this metal in CKD and its carcinogenicity characters.

1. Introduction

The rising problem of air pollution over the last few decades as a consequence of the various developmental activities, especially in cement industry and transportation, is catching attention of the policymakers and common man in Egypt. Despite some effective steps have already been taken in Egypt to tackle the air pollution problem, Egypt is still suffering from this problem. Cement industry is one of the biggest dust producing industries in Egypt. One kilogram of cement manufactured daily generates about 0.07 kilogram of dust in the atmosphere (Hindy and Attia, 1988). Cement dust and associated chemicals can spread over a large area through wind and rain, becoming accumulated in lichens, plants, animals and soils, and consequently, negatively affecting environment and human health. (Carreras and Pignata, 2002; Grantz et al., 2003; Schuhmacher et al. 2009; Al-Saleh et al. 2010).

Cement plants are important emission sources of pollution of both organic and inorganic chemicals, and produce an input of metals. There are about 17 trace elements in cement dust; antimony, arsenic, lead, cadmium, chromium, cobalt, copper, manganese, nickel, thallium, tin, vanadium, zinc, beryllium, selenium, tellurium and mercury (Hg) (Achternbosch and Bräutigam, 2001). As, Cd, Hg and Pb, are known neurotoxins to which workers can be exposed (Quandt et al., 2010). Lead and some species of Cd, Cr and Ni compounds are classified as human carcinogens (International Agency of Research on Cancer (IARC), 1993; Das et al., 1997; Grimsrud et al., 2005). Arsenic is known to cause skin lesions and cancer of the brain, liver, kidneys, and stomach (Tchounwou et al., 2004; Yoshida et al., 2004; Yoon et al., 2010).

Metals are diverse substances, with different properties and characteristics, considered important in Life Cycle Impact Assessment (LCIA) because of their toxicity to humans and ecosystems (Pizzol et al., 2010). Several metals, such as Pb, Cd, Cr and cobalt are considered hazardous contaminants that can accumulate in the human body, with a relatively long half-life (Onder and Dursun, 2006). For example, Cd has a half-life of 10 yr in the human body (Salt et al., 1995). Women are more susceptible to the adverse effects of Cd and have higher body burdens due to the long half-life of Cd and increased dietary absorption of Cd in

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menstruating women (Mijal and Holzman, 2010). Cd is long-lived in the body and low-level cumulative exposure has been associated with changes in renal function and bone metabolism (Mijal and Holzman, 2010).

Some of the heavy metals emitted by cement industry are known to be toxic for human and plants, even at low concentrations (Forstner and Wittmann, 1983; Kabata-Pendias and Mukherjee, 2007). For instance, Pb low level exposure can be harmful to enzyme systems, brain and blood production for human body. While high Pb level may affect blood Pb level and intelligence (Li et al., 2001; Sezgin et al., 2003). While long-term exposure to Pb can increase the probability of mentally retarded children and slow down the mentality development of children (Ahmed and Ishiga, 2006). Exposure to Cr results in the increased production of reactive oxygen species (ROS), lipid peroxidation, and enhances the excretion of urinary lipid metabolites (Bagchi et al., 2002; Caylak et al., 2007 and 2008).

Moreover, the wildlife populations around the cement plant also might be experiencing toxic metal effects. For example, Bobak, (2000) and Yang et al. (2003) found that cement dust air pollution has an impact on an adverse pregnancy outcome (preterm delivery) in many counties. The prevalence of delivery of preterm birth infants was significantly higher in mothers living within 0-2 km of a cement plant than in mothers living within 2-4 km (Yang et al., 2003). Concentrations of Pb and Zn in top soils were influenced by the cement plant faraway 18 km (Bermudez et al., 2010; Al-Saleha et al. 2010).

Heavy metals in cement raw materials and fuels may be discharged through three separate streams. These include emissions from a kiln through exhaust stacks, incorporation into CKD and cement clinker; these latter two solids are continuously withdrawn from the kiln (Guo et al., 1996). CKD is a similar, in appearance, to Portland cement, and it is fine-grained particulate material composed of oxidized, anhydrous, micron-sized particles collected from electrostatic precipitators during the high temperature production of clinker. 75% of CKD is fine particles that consider within the respirable range (Siddique, 2006).

Exposure to hazardous air pollutants (HAPs) at sufficient concentrations and durations may increase cancer risk or cause toxicity to the immune, neurological, reproductive, developmental, and respiratory systems (Roels et al., 1992; National Toxics Program, 1993; Tam and Neumann, 2004). It is usually estimated by measurement of the airborne pollutants or by biomonitoring. Metal in hepatitis patients with chronic hepatitis (B, C) and healthy control was higher at Egyptian polluted areas (Edfu) than an unpolluted area (Daraw) (Rashed et al., 2010). Exposure to HAPs may pose both short-term and long-term health risks. It concerns workers and subpopulations living in urban and industrial areas or in houses built on abandoned contaminated industrial sites (Grandjean et al., 1988a; Heinzow et al., 1991). Mothers and their newborns might jeopardize the health risk of heavy metals even they are non-occupational (Bermudez et al., 2010; Al-Saleha et al. 2010).

Exposure to CKD has long been associated with respiratory symptoms and varying degrees of airway obstruction for exposure (Yang *et al.,* 1996; Noor *et al.,* 2000). Exposure to CKD may result in DNA damage and lipid peroxidation through oxidative stress owing to the existence of heavy metals and silica particles, which is a principal compound of CKD (Siddique, 2006; Liu *et al.,* 2009). Therefore, the present work aimed to: 1) evaluate the concentration of some metals (As, Cd, Cr, Ni, Pb and Zn) in CKD collected from the biggest three companies for Portland cement production in Helwan, Egypt; 2) present the scientific knowledge of the possible health risk of these metals on occupational exposure to CKD.

2 .Materials and methods

The present study was executed in three Portland cement production companies located in Helwan governorate south of Cairo, Egypt. Helwan Portland Cement Company and Qawmia Cement Company locate inside Helwan industrial area, about 27 km to the south-east of Cairo. Whereas Tura Portland Cement Company locates 13 km the north of Helwan industrial area. The qualifications of the companies are exhibited in Table 1.

Table (1) Qualification of the investigated companies for Portland cement production.

Parameter	Cement company		
	Tura	Helwan	Qawmia
Established date	1927	1929	1956
Production date	1929	1930	1960
Total area in million m^2	10	1	2.2
Number of quarries	69	548	72
No. of productions lines	9	10	2
Number of workers	2500	4000	3000
The regular used fuel	Mazout*	Mazout	Mazout
The secondary used fuel	natural gas	natural gas	natural gas
First annual production in million ton	0.16	0.1	0.3
Annual production in million ton	3.0	4	2.4
CKD damping area in million m^2	0.34	2.7	0.36
Total discarded CKD in million ton	0.3	0.4	0.24

Mazout*:   The cheapest type of heavy oil in Egypt
Sources: Web site of the three Egyptian cement plants and EEAA (2005).

2.2 Sampling and analytical procedures

CKD samples were taken from the center of two different hoppers of ESPs installed on cement dry kilns through three consecutive days of September, 2009, for each company. Each investigated CKD sample represented a mixture of the six samples gathered from the individual company. CKD samples were kept all time in a dessicator then samples were dried in an oven at 95 ^{O}C for two days and then size differentiated physically by sieving. The size fraction < 20 μm of CKD was then chemically analyzed. A fixed portion of CKD sample (0.05 gm; < 20 μm) was completely decomposed by hydrochloric, nitric and hydrofluoric acid mixture according to the procedure described by El-Ghandour *et al.* (1982). The concentrations of As, Cd, Cr, Pb, Ni and Zn were determined by Atomic Absorption Spectrometry (Perkin Elmer) model 3300 manufactured by U.S. Instrument Division, Norwolk, CT 06859 USA.

2.3 Human health risk assessment

The risk estimates generated using the risk model, as a general case in risk assessment, are presented in numbers, which look very similar to both cancer risk and non-cancer toxic risk estimates. Fundamentally, "Carcinogenic Risks" are statement of probability and represent a judgment of how likely is the specified exposure will lead to cancer in the exposed individuals. Cancer risk is expressed as the likelihood of occurring cancer due to pollutant intake through different routes (e.g. ingestion, inhalation, dermal) over the entire exposure duration and parameters which presented in Table 2. The carcinogenic risk was calculated by multiplying the estimated dose by the cancer potency factor. In contrast, the estimates for "Non-Cancer Toxic Risks" are numbers on a scale that reflect whether the exposure was larger or smaller than a specified 'safe' level of exposure. This critical level represents the threshold below which assumed adverse effects will not occur. Non-cancer risk is expressed by the hazard quotient (HQ), which compare the exposure to the RfD/RfC (Reference Dose/Reference Concentration) (Han *et al.*, 1998).

Table 2   Exposure parameters used to generate exposure estimates for adults

parameter	Exposure estimate
General Parameters	
Body Weight	70.00 kg
Lifetime	70.00 years
Exposure Period	30 years
Specific Parameters	
Event Frequency	350 events/per year
Event Duration	8 hours/per event
Oral Amount Ingested	100 mg/event
Oral Fraction concentration	100%
Inhalation Breathing rate	2.2 m^3/hour

2.4 Model parameters and risk estimate

In the present study, human exposure and risks were assessed by using *Risk*Assistant* multimedia model, the description of this model is written elsewhere (Hassanien *et al.,* 1999). Briefly, the computer software (Risk*Assistant, 1997) is a powerful set of tools and databases for estimating the health risk of various chemicals in the environment, in particular, settings. It was first developed by the Hampshire Research Institute. The most updated version of Risk*Assistant for Windows (1995) offers more features for a variety of assessors to perform risk assessments, especially at the local level, where risk occurs. Default input parameters provided by the model are used, whenever possible, instead of a site-specific information. Default parameters for calculating exposure have been extracted from the USEPA exposure factor's handbook (Konz *et al.,* 1989; USEPA, 1991).

It was assumed that exposure frequency, exposure levels and exposure duration do not change in a lifetime and that the human activities remain the same. It is apparent that the probability of this is extremely small. To illustrate this, people spend only a fraction of their lifetime in one location (Paustenbach *et al.,* 1992). The main exposure pathway for this research study was through the direct ingestion and inhalation of the contaminated cement dust. Many EPA risk assessments have assumed an adult soil ingestion rate of 100 mg/day.

3. Results and discussion

Figure 1 presents concentration of the six metals (mg/kg) determined in CKD collected from the three Egyptian companies for Portland cement production. The following conclusions can be extracted; concentration of Cr was the predominant (30.7, 33.9 and 43.4 mg/kg) followed by Zn (26.7, 27.8, and 36.0 mg/kg), Ni (14.9, 15.5, and 16.0 mg/kg) and Pb averaged 12.5 mg/kg at Helwan, Tura and Qawmia, respectively. While As and Cd concentrations were less abundant with concentration averaged 1.27 and 1.02 mg/kg, respectively. As recorded the same value at all sites, concentration of all pollutants was higher at Qawmia compared to the other areas, and Cr was the highest value (43.35 mg/kg) comparing to the others measured ones.

Concentration of Cd and Cr were higher than the values (0.3 and 25.0 mg/kg, respectively) reported in the similar study done by Achternbosch *et al.* (2003) in the raw meal used in Portland cement production. Meanwhile, concentration of Zn, Ni, Pb, and As were lower than the values (53.0, 25, 22, 20, 5, and 0.3 mg/kg, respectively) founded in the raw meal in the same study.

The increase in Cd and Cr concentration may be attributed to their emission during the fossil fuel fired in the kiln which contains trace amounts of heavy metals (Serclerat and Moszkowicz, 1997). While the decrease in other metals concentrations could be related to the high temperature inside the kiln. Where the row meal is must be heated up to 1450˚C which is an emergency for cement manufacturing operation. Thus an increase in metal evaporation would be expected especially some metals consider semi-volatile metal, such as lead (Dellinger *et al.,* 1993; Sarotim *et al.,* 1994). It might be also due to a difference in the specification of the raw meal used in Egyptian cement plants.

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The sum average of the studied metals represented 0.01% by weight. This ratio is in close to the corresponding value found in cement plants that alternative waste derived fuels (WDF) was not used (similar to our case) nor other hazardous waste feed rates to the kiln and reported by Dellinger *et al.* (1993); Sarotim *et al.* (1994); Siddique (2006). Average concentration of Cr, Ni, Pb, and Cd for the three plants was in agreement with the range from three regions of the United States for CKD which were between (11.5 – 81.7; 6.9–39; 5.1–1490; and 0.89–80.7 mg/kg, respectively). While the average concentration of As was less than corresponding range (2.1- 20.3 mg/kg) at the same three American regions (EPA, 1993).

The low metal concentration, particularly of As and Cd does not deny or moderate its health a hazardous effects. In some cases, low-level exposures can be more harmful than high-level exposures of the same pollutant (Schmidt, 2001). In addition, there is still a potential for long-term contamination since heavy metal is known to be an accumulation within biological systems (Waly *et al.,* 2007). Interest in the biological effects of toxic metals such as Pb, Cr, Ni and the metalloid arsenic has increased since 1985. The reason is that large amounts of toxic and carcinogenic elements have been released into the environment, particularly in industrial areas (Vahter, 1986; Järup *et al.,* 1989; World Bureau of Metal Statistics, 1990; American Bureau of Metal Statistics, 1991). As is one of the worst cancer-causing chemicals distributed widely in the environment from both natural and anthropogenic sources, (Moon *et al.,*

2004; Wang and Duan, 2009). In soil As can a pose risk to human health either by ingestion via the food chain or through secondary pollution of air and water due to dust and leaching loss (Zeng *et al.,* 2008; Wu and Chen, 2010).

Estimated average daily dose (ADD) and lifetime daily dose (LADD) of inhaled and orally ingested heavy metals by adult workers besides the total carcinogenic risk (CR) and total hazard quotient (HQ) in the three cement plants are presented in Table 3. The assumption upon which these values are based is explained in the methodology. ADD values of dust oral ingestion of Cd, Zn, Ni and Cr are high at Qawmia, while ADD corresponding values for Pb and As are equal in all sites. The highest values of ADD among all data recorded were reported for Zn and Cr metals. Regarding LADD ingestion of dust is a bit different from ADD. LADD values of Pb and Ni were similar in all sites. The corresponding values of Cd, Zn and Cr were higher at Qawmia. The results of total exposures to these metals during the daily exposure were used for computing the associated cancer risk is summarized in Table 3. The Table clarifies CR and HQ conducted for all pollutants had the same value at the three cement companies. This can be attributed to the very close amount of investigated pollutants, the same method of cement manufacturing and technology were used, and applying the same condition.
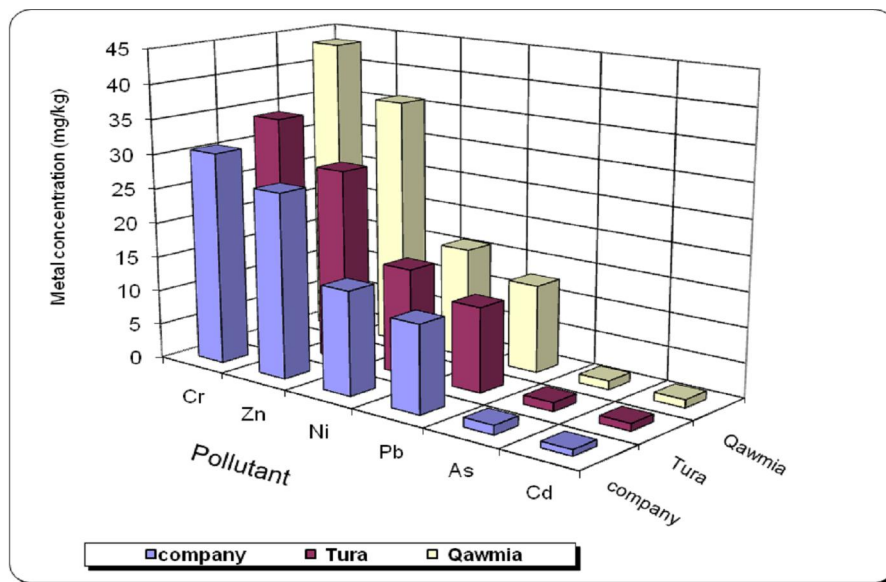
Figure (1) Metal concentrations (mg/kg) detected in CKD collected from the three Egyptian Companies for Portland cement production.

Table (3) The estimated average daily dose (ADD) and lifetime average daily dose (LADD) in mg/kg/d of metals detected in CKD of the three Egyptian companies of Portland cement production.

Company	Parameter	Metals					
		Cr	Zn	Ni	Pb	As	Cd
Qawmia	ADD (mg/kg/d)	5.90E-05	4.90E-05	2.20E-05	1.70E-05	2.00E-06	2.00E-06
	LADD (mg/kg/d)	2.50E-05	2.10E-05	9.00E-06	7.00E-06	7.30E-07	6.80E-07
	CR	3.00E-06					
	HQ	0.02					
Tura	ADD (mg/kg/d)	4.60E-05	3.80E-05	2.10E-05	1.70E-05	2.00E-06	1.00E-06
	LADD (mg/kg/d)	2.00E-05	1.60E-05	9.00E-06	7.00E-06	7.30E-07	6.20E-07
	CR	3.00E-06					
	HQ	0.02					
Helwan	ADD (mg/kg/d)	4.20E-06	3.70E-05	2.00E-05	1.70E-05	2.00E-06	1.00E-06
	LADD (mg/kg/d)	1.80E-05	1.60E-05	9.00E-06	7.00E-06	7.70E-07	5.10E-07
	CR	3.00E-06					
	HQ	0.02					

CR: Total Carcinogenic Risk from all pollutants;   HQ: Total Hazard Quient from all pollutants.

Uncertainty analysis

Uncertainties exist in the risk assessment of exposure could be due to many factors such as uncertainties in measurement analysis (Fritz and Schenk, 1987), variations in exposure values assigned to examined population (Wallace, 1991), and variations in concentrations from place to another (Kim *et al.,* 2002). While chemical analysis uncertainties can be arisen from the number of possibilities such as poor sampling, incomplete samples extraction and weighting errors, the potency calculations are an important factor responsible for uncertainties in assigned exposure values. Furthermore, uncertainties in the general extrapolation to toxicity information are responsible for the highest uncertainty (Asante-Duah, 2002).

In general, the basic process and hazards including CKD and associated metal concentration is still the most important occupational hazards in the cement industry. In Egypt most exposures to these metals are from CKD sources and its uncontrolled damping close to the company. Rather, the primary sources during most processes of a cement manufacturing operation in plant which are close to them too.

5. Recommendation

Undoubtedly, extermination of air pollution source roots is better and chipper than its impact treatments. So reutilization of CKD is a feasible solution to mitigate air pollution levels in Egypt. The handy and convenient usage of CKD may be in highway establishment as well as mineral filler in asphalt concrete mixes. Fortunately, Egypt has recently witnessed a great interest in enhancing and upgrading the highway and main road networks to cover all the country, and it is estimated to have reached to double during the next decade.

6. Conclusion

In light of this study, cement plant workers would be under the threat of heavy metal health risk. It is of even greater concern if the carcinogenic metal concentration in cement dust reaches an amount that cannot be ignored. Consequently, the results of the current work showed that Cr represents the high risk in the three cement plants comparing to the others measured ones due to its high content in CKD and its carcinogenicity character. Transportation of the big amounts of CKD into the damping area, and water required to granulate it cost the company a lot of unprofitable money and effort. Besides CKD occupies a large area which can be used in other modern projects and investment with high economic profit. Briefly, CKD poses a very challenging task of safe handling, proper disposal and utilization. Therefore, environmental concerns related to Portland cement production, emission and disposal of CKD must be progressively signficant.

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2/15/2011

The Direct Inversion of λ/μ from Elastic Impedance

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Abstract: Elastic impedance (EI) contains valuable information that can be used in reservoir rock as fluid and lithology identification. To get more understanding about the reservoir properties, EI can be reformulated according to the Gray's approximation, in which lamé parameters and density can be successfully extracted. λ/μ, the most sensitive parameter to variations in rocks properties going from shale to gas sand, is often derived indirectly from lamé parameters. On real seismic data often affected by noises, However, This procedure may poses the numerical computation that can introduce cumulative errors in the inverted results. To avoid these ambiguities, the gray's approximation is reformulated introducing the ratio λ/μ. the application of this equation to synthetic and real data show that the inverted results are more stable and less ambiguous than that from conventional procedure, and thus can recover reservoir information very well.

1. Introduction

The variation of amplitude with offset (AVO) is a powerful tool to distinguish rocks containing gas and oil. From the most popular approximation of the Zoeppritz equations, Gray et al (1999), proposed the new equations expressing the change in AVO in terms of the fundamental elastic rock properties (bulk modulus, lamé modulus and shear modulus). So far there are several approximations of PP reflection coefficient that are often used in AVO analysis and inversion (Castagna, 1994, Gray, 2000). Due to the wavelet variation with offset which requires a special correction (wavelet stretching correction) in order to perform AVO inversion, it has recently become popular to perform elastic impedance inversion (Cambois, 2000) and extract the elastic parameters from the EI. Following the same derivation procedure proposed by Connolly (1998), Wang et al (2008) derived the new elastic impedance in terms of lamé parameters and density, and thus inverted lamé parameters and density from this EI .laboratories measurements shown that the moduli ratio, λ/μ are by far the most sensitive parameter to variations in rocks properties going from shale to gas sand (Goodway, 1997) .Since today, the moduli ratio λ/μ is always computed indirectly after the inversion of lamé parameters. On real seismic data often affected by noises, However, This procedure may poses the numerical computation that can introduce cumulative errors in the inverted results. To avoid this ambiguity, the gray's approximation is reformulated introducing the parameter λ/μ.

Using the same derivation procedure as in Connolly (1998), the new elastic impedance in terms of the ratio moduli λ/μ, lame's modulus, and density is derived, the application of this equation to synthetic and real data show that the inverted results are more stable and less ambiguous than that from conventional procedure, and thus can recover reservoir information very well.

2. Material and Methods
2.0 Methods
2.1 Gray approximation and its reformulation

From the well known linearization of the Zoeppritz equations for P-wave reflectivity, introduced by Aki and Richard (1980), Gray et al (1999) proposed an approximation in terms of lamé parameters and density.

$$R_{PP}(\theta) = \frac{\sec^2\theta}{4}\left[1 - 2\left(\frac{\beta}{\alpha}\right)^2\right]\frac{\Delta\lambda}{\lambda} + \left(\frac{\beta}{\alpha}\right)^2\left[\frac{\sec^2\theta}{2} - 2\sin^2\theta\right]\frac{\Delta\mu}{\mu} + \frac{1}{4}\left[1 - \tan^2\theta\right]\frac{\Delta\rho}{\rho}$$

$$(1)$$

Where R is the reflectivity at the incidence angle θ, λ, μ and ρ are the Lamé constant, the shear modulus and the density, respectively.

The formula above has an advantage over previous linearization of the Zoeppritz equations in fact that the fundamental elastic rock properties (bulk modulus, lamé modulus and shear modulus) can be inverted directly (Gray, 2000).In incorporating the moduli ratio λ/μ into Gray approximation, one can derive this parameter directly.

Assuming $K=\left(\dfrac{\beta}{\alpha}\right)^2$ is a constant, and $Q=\dfrac{\lambda}{\mu}$,

$$\frac{\Delta Q}{Q} = \frac{\Delta \lambda}{\lambda} - \frac{\Delta \mu}{\mu}$$

Gray's approximation is reformulated as:

$$R_{PP}(\theta) = \left[\frac{\sec^2\theta}{4} - 2K\sin^2\theta\right]\frac{\Delta\lambda}{\lambda} + \left(\frac{\beta}{\alpha}\right)^2\left[2K\sin^2\theta - \frac{1}{2}K\sec^2\theta\right]\frac{\Delta Q}{Q}$$
$$+ \frac{1}{4}\left[1 - \tan^2\theta\right]\frac{\Delta\rho}{\rho} \qquad (2)$$

The amplitudes computed using equation (2) overlies the results of existing AVO approximations.
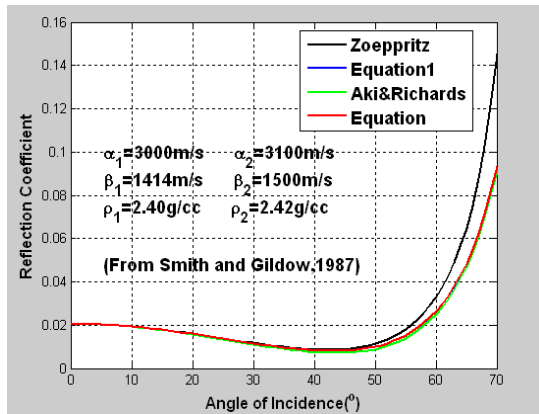
Figure1. A comparison of angle reflection coefficients, $R_{PP}(\theta)$ obtained from equation (2) to those obtained from equation (1), exact Zoeppritz equations and Aki & Richard AVO approximation.

2.2 Elastic impedance

The reflection coefficient $R_{PP}(\theta)$ can be expressed in the same form as the normal incidence AI as:

$$R_{PP}(\theta) = \frac{EI_{i+1} - EI_i}{EI_{i+1} + EI_i} \approx \frac{1}{2}\ln\frac{EI_{i+1}}{EI_i} \qquad (3)$$

Where EI_{i+1} is elastic impedance of the lower layer and EI_i is elastic impedance of the upper layer.

Using the same guidelines of previous work (Connolly, 1999) in deriving the EI equation, one can derive the new elastic impedance as:

$$EI_{new}(\theta) = \lambda^{\frac{\sec^2\theta}{2} - 4K\sin^2\theta} Q^{4K\sin^2\theta - K\sec^2\theta} \rho^{1 - \frac{1}{2}\sec^2\theta} \qquad (4)$$

To control the variation of elastic impedance values versus angles, one can incorporate normalization parameters which are the average values along the entire log. This reference values can reduce the dimensionality of the equation (4).

$$EI_{new}(\theta) = A_0\left[\frac{\lambda}{\lambda_0}\right]^a\left[\frac{Q}{Q_0}\right]^b\left[\frac{\rho}{\rho_0}\right]^c$$

With $A_0 = \left[8\lambda_0\mu_0\rho_0^2\right]^{0.25}$, $a = \dfrac{\sec^2\theta}{2} - 4K\sin^2\theta$

$b = 4K\sin^2\theta - K\sec^2\theta$, $c = 1 - \dfrac{1}{2}\sec^2\theta$

where A_0, λ_0, μ_0 and ρ_0 are references values of P-impedance, Lamé constant, shear modulus and the density, respectively. It can be shown when the angle of incidence equals zero that EI (0) from equation (5) is equal to acoustic impedance.

2.3 Elastic Impedance Inversion steps

The success of any EI-inversion is largely depended on the quality of seismic traces, the wavelet estimation and the low frequency model which can be estimated in several manners (the most popular being the integration of well logs, interpreted seismic horizons and seismic velocities).

Quality of seismic trace

It well known that removing totally undesirable signal (noise) from seismic data is a thorny question that has been studying so long. To improve signal to noise ratio, offset gathers can be transform into limited angle gather stacks. Three limited angle stack corresponding to near, middle and far angles are created in this paper.

Wavelet estimation

From statistical way (i.e. from seismic data) or from well logging, wavelet must be extracted separately at each limited angle stacks.

Inversion

Post stack inversion methods are used to transform seismic limited angle stack into relative elastic impedances.

Low frequency model

From equation (5), the pseudo elastic impedance logs are computed to constrain the inversion of the seismic limited angle stacks. At well location, seismic limited angle stack and the EI log corresponding to the same incident angle are extrapolated via interpreted horizons to build low frequency model. The latter and the relative elastic

impedance are added up to get the absolute elastic impedance section.

2.4 Extraction of Lamé constant, ratio modulus and the density from elastic impedance

In literature, there exist several algorithms aiming to estimate elastic parameters from elastic impedance. Lu et al (2004) have been shown the most common used in which extraction results are largely affected by the K values. To avoid this dependency, lamé parameters can be extracted as follows: from well logging, considering one angle of incidence and three sampling times (t1, t2, t3), the coefficients in the above equation are computed. The same procedure is used to the second and third incidence angles.

$$\ln\{ EI_{new}(\theta, t_1)/A_0\} = a\ln\frac{\lambda}{\lambda_0}(t_1) + b\ln\frac{Q}{Q_0}(t_1) + c\ln\frac{\rho}{\rho_0}(t_1)$$
(6)

Once the coefficients (9 in total) are obtained, using the linear system with 3 equations (each equation corresponding to an incidence angle), it easy to extract Lamé constant, ratio modulus and the density.

3.0 Well log data synthetic results

To evaluate the effectiveness of the method, one creates synthetic EI data for three angles using equation (5). The lamé parameters logs are computed using P-wave and S-wave velocities. Under free noise, lamé constant λ and the ratio moduli λ/μ (both inverted from equation (5) and indirectly from elastic impedance based on gray approximation) can be successfully recovered (figure 2).

The method is also tested with synthetic noise data. Considering the same data as previously and adding 7% random noise to the seismic reflection, recursive inversion is performed on this data. It's clear in the figures below, that the inversion of λ log from equation (5) is better than from elastic impedance based on gray approximation. From equation (5), the inversion errors related to λ/μ log are not more pronounced than those derived from the inversion based on Gray-approximation .

4. Application to real data

The real data is from a demo dataset distributed with the Hampson-Russell (H-R) inversion package. The 2 D prestack seismic data is inverted to give lamé constant λ , the ratio moduli (λ/μ and the density. When comparing figure (4) and figure (5), it is clear that the new method highlights more better the presence of gas than the method based on gray approximation.

Based on rock physics, incompressibility is

the resistance to a change in volume caused by a change in pressure, and can distinguish lithology effects to fluid effects. While rigidity is the resistance to shear stress, thus it is the lithology indicator. The ratio of the two lamé parameters, named lamé moduli ratio λ/μ, shows a low value at 640 ms, indicating the presence of gas.
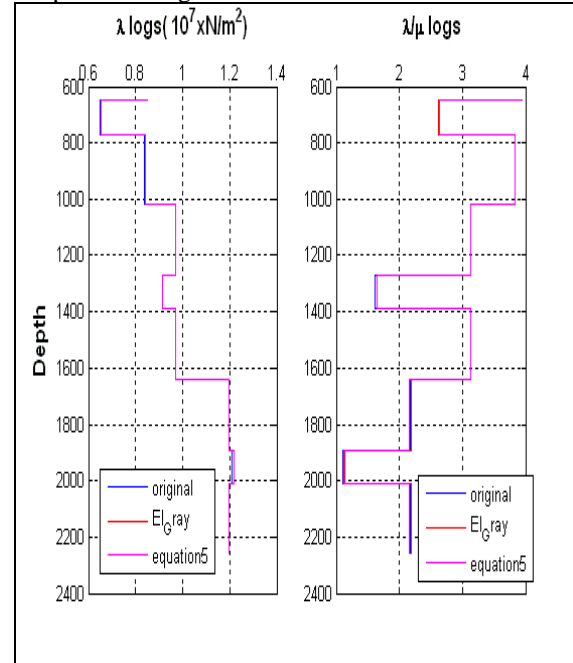
Figure2. Elastic parameters inverted from synthetic EI (equation 5 and elastic impedance based on gray approximation). Left: Lamé constant curves, Right: ratio moduli λ/μ curves. The original log is in blue, λ and λ/μ logs from equation (5) are in magenta, and λ and λ/μ logs from elastic impedance based on gray approximation are in red.

5. Conclusion

In order to derive the lamé ratio moduli, λ/μ directly, the approximation of gray is modified including this parameter. The new approximation in terms of constant lamé, lamé ratio moduli, and density is tested on synthetic and real data. The Synthetic elastic impedance data generated using synthetic well log show that the inversion errors related to λ/μ log are not more pronounced than those derived from the inversion based on Gray-approximation. In addition, the inversion results of real prestack seismic data from a demo dataset distributed with the Hampson-Russell (H-R) inversion packages show that the new method can highlights better the presence of gas than the method based on gray approximation.
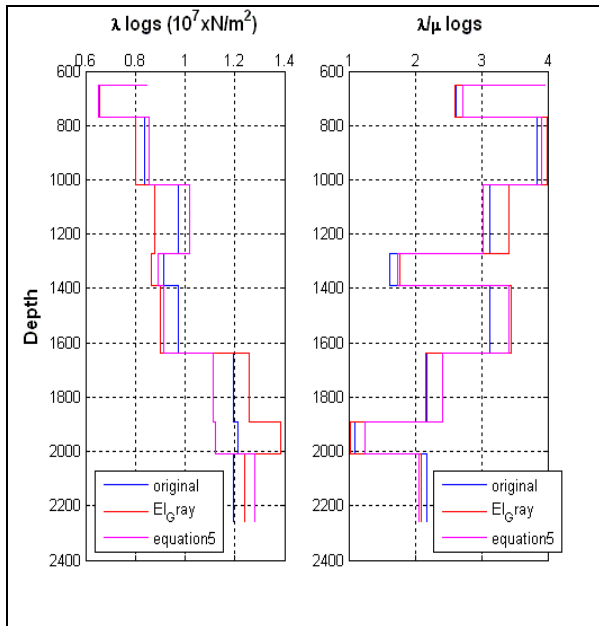
Figure 3: Elastic parameters inverted from synthetic EI (equation 5 and elastic impedance based on gray approximation) using noisy data. Left: Lamé constant curves, Right: ratio moduli λ/μ curves.The original log is in blue, λ and λ/μ logs from equation (5) are in magenta, and λ and λ/μ logs from elastic impedance based on gray approximation are in red.
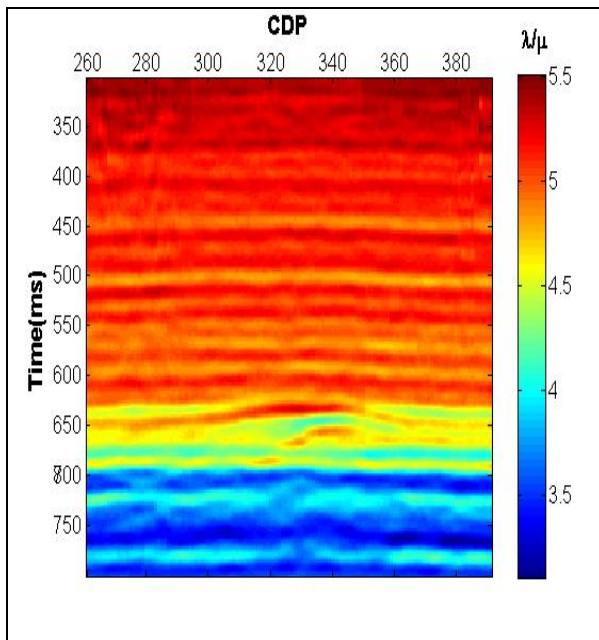
Figure4. λ/μ Profile obtained from elastic impedance based on gray approximation.
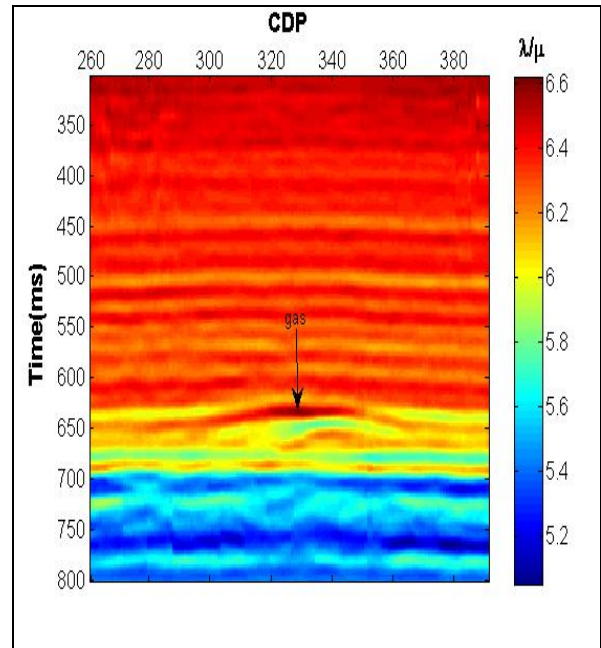
Figure5. λ/μ Profile obtained from the new method.

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Relationship between coping styles and level of depression among depressed patients

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Abstract: Depressed patients perceive stress more readily than non-depressed persons, and they struggle to cope with their problematic situations, and their depressive symptoms. Changing maladaptive coping style to adaptive one is a crucial aspect of nursing role through treatment of depression. The aim of this study is to identify relationship between depressed patients coping styles and their level of depression. Total sample of 150 depressed patients of both sexes aged between 21-60 years were selected conveniently from outpatient clinics in El Maamoura Hospital for Psychiatric Medicine and Ras El-Teen General Hospital in Alexandria. Two tools were used to collect the necessary data: the first one is the Ways of Coping Questionnaire" (WOC) to assess thoughts and actions that individuals use to cope with stressful encounters of every day living. Second tool is Beck Depression Inventory is (BDI). to identify or confirm the presence of depressive symptoms and measure their severity. The results showed positive correlation between level of depression and each of emotion focused coping, confrontive coping, accepting responsibility, and escape avoidance coping styles, while negative correlation was found between level of depression and each of problem focused coping, self controlling coping, planful problem solving, and positive reappraisal coping styles.

1. Introduction

Depression refers to a cluster of symptoms that include, anhedonia, emotional flatness or emptiness with diurnal variation, depressed mood, changes in sleep and appetite, and the cognitive set of futility and hopelessness. These symptoms lead to reduce patient`s ability to perform even normal daily activity. Depression comes by various terms, including major, melancholic, unipolar, and dysthymia [1-3].

Depression ranks as one of the major health problems of today. Millions of patients suffering from some form of this disorder crowd the psychiatric and general hospitals, the outpatient clinics, and the offices of private practitioners. The toll exacted by depression extends not only to the devastation of the well-being of individual patients, but also to their families, their wider social and occupational contacts and to the health care system [4,5].

Depression is one of the most disabling disorders in the world. It causes more disability than ischemic heart disease or cerebrovascular disease, and it is expected to replace cancer as the second leading cause of morbidity within the next decade. Moreover the onset of depression becomes earlier today than in past decades [6-8].

Depression has no single cause. It results from a combination of neourobiological and psychosocial factors which are extremely complex[2,9]. Stressful experience is one of the important factors which play a significant role to make persons vulnerable to become depressed, especially when combination with other neourobiological and psychosocial factors occur. Just as stressful events play a role in the initial episode of depression, they can play a role in recurrences of new episodes [10-12].

Depressed patients are trapped by low self esteem, hopelessness, lack of inherent capacities, and helplessness. They appraise harm or threat more readily than nondepressed persons in demanding encounters. So it is more difficult for them to use adaptive coping styles to meet the demands of stressors, which lead to increase the negative impact of stress on the clinical course of depression and decrease the choices of treatment [13-16].Depression coping responses may be influenced by the severity of the depression symptoms experienced , the complexity of the personal and social problems experienced , the exposure to stressful life events, and the availability of healthy coping resources [17-19].

Depression coping is defined as dynamic human responses to complex symptoms of depression and symptom related problems in psychosocial functioning [20]. According to Lazarus and Folkman (1984), coping is defined as "the cognitive, behavioral and emotional efforts to manage particular external and/or internal demands that are appraised as taxing or exceeding the resources of the person"[21].

Coping theories discussed coping as a dynamic process that is strongly influenced by situational factors or as a trait which is stable across

situations [22]. One response to the trait-situation debate was the development of the interactionist position, which postulates that all behaviors are a function of both the person's traits and the situation [23]. Coping styles and coping transactions are really two sides of the same coin, with transactions involving the application of one's style in a particular situation [24]. Coping theories suggested that there are adaptive as well as maladaptive coping styles for stress [25]. Problem-focused coping is directed at managing or changing the problem that causes stress. It is considered as adaptive for controllable events, and maladaptive for uncontrollable ones. While Emotion-focused coping, however, aims at regulating the emotional response to the problem. It is considered as an adaptive way of coping for uncontrollable events [26-28].

Changing maladaptive styles of coping is an important target of depression treatment. People with chronic depression must learn to cope with their illness and its treatment while striving to live a meaningful, productive life [29,30]. Changing maladaptive coping style to adaptive one is a crucial aspect of nursing role, which requires integration of major neurobiological and psychosocial concepts to understand the clients perception of stressors, assessing present and past coping behaviors, identifying available resources for coping, and teaching adaptive coping styles [2].

Moreover recovery from depression as well as relapse prevention depend on accurate nursing process which leads to increase patient`s ability to cope with their negative thinking, low self esteem, social isolation and other depressive symptoms, and to catch early signs of relapse [4,29-32]. Accordingly, the aim of this study is to identify the coping styles used by patients with depression when they encounter problematic situations, and to describe its relation to patients` level of depression.

2. Material and Methods
Materials:
    Design: A descriptive correlational design was followed in this study.

    Setting: This study was conducted in two psychiatric outpatient clinics in Alexandria:
 1) The outpatient clinic of El Maamoura Hospital for Psychiatric Medicine in Alexandria,
 2) The outpatient clinic of Ras El-Teen General Hospital.

    Subjects : A convenient sample of 150 psychiatric patients of both sexes, medically diagnosed as having depression was selected from the previous outpatient clinics. Patients fulfilled the following inclusion criteria:
1. Being older than 20 years.
2. With no co morbidity .

3. Willing to participate in the study.
4. Able to complete an interview.

Tools:
Tool (I): "The Ways of Coping Questionnaire" (WOC):
    This tool was developed by Folkman and Lazarus (1980) to assess thoughts and actions that individuals use to cope with stressful encounters of every day living. The ways of coping questionnaire contains 66 items rated on a likert -type scale that ranges from 0 (does not apply or not used) to 3(used a great deal). There are two classification for this scale, the first one contain thirteen items contribute to the summed raw total score for problem focused coping , and 29 items contribute to the summed raw total score for emotion focused coping [33]. The second classification contain fifty items which represent the following subscales: confrontive coping (six items), distancing (six items), self controlling (seven items), seeking social support (six items), accepting responsibility (four items), escape-avoidance (eight items), planful problem solving (six items), and positive reappraisal (seven items) [34]. In two classification, there are some items were deleted because it did not load clearly to any one factor [33,34].

Tool (II): Beck Depression Inventory (BDI):
    The Beck Depression Inventory was given to identify or confirm the presence of depressive symptoms and measure their severity. The BDI is a 21-item self report measure of the severity of depressive symptoms. Each of the BDI items is rated on a likert-type scale from 0-3, yielding a range of scores from a minimum of 0 to maximum of 63. According to BDI manual, the severity of depressive symptoms is indicated from the total score. A score from 0-13 indicates minimal depressive symptoms; 14-19 indicates mild symptoms; 20-28 indicates moderate symptoms; and 29-63 indicates severe depressive symptoms [19].

    In addition, a sociodemographic and clinical data interview schedule was added. It included patient`s characteristics as: age, sex, marital status, level of education, economic status, birth order, family size, place of residence, living situation, and monthly income . And clinical data as: history of illness, history of previous hospitalization, treatment taken, family history, disease duration, and systemic diseases.

Method:
- Permission to carry out the study was obtained from the responsible authorities of El Maamoura Hospital for Psychiatric Medicine, and Ras El-Teen General Hospital.

- The Ways of Coping Questionnaire and the Beck Depression Inventory were translated into Arabic Language by the researcher.
- The Ways of Coping Questionnaire and the Beck Depression Inventory were tested for content validity by a Jury of nine experts in the field of psychiatric nursing and psychiatric medicine, six of them were staff members in the department of psychiatric nursing and mental health in the Faculty of Nursing, three of them were from psychiatric medicine department in the Faculty of Medicine, and necessary correction were done accordingly.
- A Reliability test for "the Ways of Coping Questionnaire" and "Beck Depression Inventory" was done on 20 psychiatric patients with depression (which were excluded from the actual study) by using Cronbach`s coefficient alpha which gave an internal consistency of (= 0.907) for the ways of coping questionnaire which was highly acceptable, and (= 0.786) for Beck Depression Inventory which was also acceptable.
- Fifteen depressed patients were selected conveniently from outpatient clinic of El Maamoura hospital to check and ensure the clarity and applicability of the Ways of Coping Questionnaire and Beck Depression Inventory (and they were also excluded from the actual study). Some changes in the wording of the Ways of coping Questionnaire and Beck Depression Inventory were done accordingly, and then the tools were put into final form.
- Confidentiality and privacy were assured.
- The researcher visited each outpatient clinic 6 days per week.
- The researcher determined patients who were diagnosed as having depression through checking patient files.
- The researcher explained the aim of the study to every selected patient in the study.
- The written and verbal informed consent was obtained.
- The researcher interviewed each selected depressed patient individually to collect necessary information about socio-demographic characteristics, level of depression and coping styles, by using socio-demographic and clinical data interview schedule, Beck Depression Inventory and Ways of Coping Questionnaire respectively. Each patient interview was fulfilled through 20-45 minutes.
- The data collection covered a period of 4 months and a half (from 15th of January 2010 to 30th of May 2010).

Statistical analysis:
• Data were coded, computerized and then analyzed using the Statistical Package for Social Science (SPSS)

software package version 13.0. the percentage, mean, and standard deviation were used to describe data.
• Count and percentage: used for describing and summarizing the qualitative data.
• Student t-test of significance: used to assess whether the means of two independent groups are statistically different from each other.
• ANOVA test of significance: used to assess whether the means of three or more independent groups are statistically different from each other.
• Welch-ANOVA test of significance: used when ANOVA test is not valid i.e. when different groups have different variances.
• Pearson correlation coefficient: used to quantify the association between two parametric measurement variables .
• The 0.05 level was used as the cut off value for statistical significance to assess significance of the results.

3. Results
Table (1):
It shows the distribution of depressed patients according to their socio-demographic characteristics. It was noted that the majority of the studied patients (79.3%) were from El-maamoura outpatient clinic, while 20.7% were from Ras El-Teen outpatient clinic. The ages of the studied depressed patients ranged from 20 to more than 50 years with a mean age of 38.4 ± 10.53 years. It was noted that nearly one third of the studied depressed patients (31.3%) are aged between 40 to less than 50 years, 29.3% of them were in the age group of 30 to less than 40 years, followed by 22.7% of them who were in the age group 20 to less than 30 years.

As regard patient's sex, the sample was distributed as follows; sixty percent of them were males and forty percent females. Concerning their marital status more than half of the subjects (53.3%) were married while 36.7% were single, and the rest either were widow (6.7%) or divorced (3.3%).

Concerning educational level, 30.6% of the studied patients were illiterate or just read and write, while 34% had university or postgraduate education, 28.7% had primary or preparatory education, and only 6.7% had secondary education.

Pertaining to the place of residence, the table shows that 67.3% of the subjects lived in urban areas, while 32.7% lived in rural areas. Concerning working status, sixty percent of the patients were employed, while forty percent of them were not employed.

Table (2) illustrates the distribution of the studied depressed patients according to their clinical characteristics. In relation to family history, it was found that the majority of the studied patients had no

family history of psychiatric illness (68.5%), while 31.5% had the family history.

Regarding duration of having depression, 66.7% of patients had depression from five years and more, while 14.7% had depression from one to two years, 16.7% from three to four years, and only 2% suffered from depression from less than one year. The mean of depression duration was 3.46 ± 1.61 years. As regard the type of psychotropic drugs prescribed to patients, 52% of patients had antidepressants medication and 48% of them had antidepressants medication with other types of psychotropic drugs, as anxiolytics and mood stabilizers.

In relation to the number of psychiatric hospitalization, 44.7% of patients were not hospitalized at all. While 26% were hospitalized one to two times, 14.7% were hospitalized three to four times, as well as five times and more. The mean according to number of past hospitalization of the studied depressed patients was 1.05 ± 1.21 times.

minimal depressive symptoms (15.3%). Knowing that the scoring range of Beck Depression Inventory was (0-63).

Table (4): In relation to problem focused coping, the table shows that the Problem focused coping mean score for the studied depressed patients was 18.63 ± 8.42. More than half of the studied patients (50.7%) used problem focused coping quite a bit to cope with stressful encounters, while 26.7% used it in somewhat degree, 22% used it in a great deal degree, and only 0.7% of the studied patients did not use it at all.

Concerning emotion focused coping, the table shows that the emotion focused coping mean score for the studied depressed patients was 50.28 ± 11.346. The majority of studied depressed patients (68.7%) used emotion focused coping quite a bit, while 27.3% used it in a great deal degree, and only (4%) used it in somewhat degree.

Table (1): Socio-demographic characteristics			
Variable		Frequency Total (150)	Percent %
Patients` distribution	EL-maamoura	119	79.3
	Ras El-Teen	31	20.7
Age	20-	34	22.7
	30-	44	29.3
	40-	47	31.3
	50+	25	16.7
Mean ± SD		38.4 ± 10.53	
Sex	Male	90	60
	Female	60	40
Marital status	Single	55	36.7
	Married	80	53.3
	Divorced	5	3.3
	Widow	10	6.7
Level of education	Illiterate / Read and write	46	30.6
	Primary / preparatory	43	28.7
	Secondary	10	6.7
	University / Postgraduate	51	34
Mean ± SD		5.61 ± 2.01	
Place of residence	Rural	49	32.7
	Urban	101	67.3
Working status	Working	60	40
	Not working	90	60

Significant at *P 0.05

Table (3): Shows the distribution of depressed patients according to their level of depression. It reveals that the Beck Depression Inventory mean score for the studied patients was 31.13 ± 16.76. More than half of studied patients (51.3%) had severe degree of depressive symptoms , while 15.3% of had moderate depressive symptoms, and the rest of the studied patients had either mild depressive symptoms (18%) or

Table (2): Clinical characteristics of the studied patients			
Variable		Frequency Total(150)	Percent %
Family history	Yes	47	31.5
	No	102	68.5
Disease duration	Less than 1 year	3	2
	1-2 years	22	14.7
	3-4 years	25	16.7
	5+ years	100	66.7
Mean ± SD		3.46 ± 1.61	
Psychotropic drugs	antidepressants	78	52
	Antidepressants with other psychotropic drugs	72	48.0
Number of hospitalization	0	67	44.7
	1-2	39	26
	3-4	22	14.7
	5+	22	14.7
Mean ± SD		1.05 ± 1.21	

* Significant at *P 0.05

Table (3): Distribution of depressed patients according to their level of depression:

Level of depression	Frequency (n=150)	Percent %
Minimal depressive symptoms (0-13)	23	15.3
Mild depressive symptoms (14-19)	27	18.0
Moderate depressive symptoms (20-28)	23	15.3
Severe depressive symptoms (29-63)	77	51.3
Total Mean score (Mean ± SD)	31.13 ± 16.76	

* Significant at *P 0.05

Table (4): distribution of studied depressed patients according to problem and emotion focused coping styles:

Coping style	Degree	Frequency Total (150)	Percent %
Problem focused coping (0-39)	not used (Zero score)	1	0.7
	Used somewhat (1-13)	40	26.7
	Used quite a bit (14-26)	76	50.7
	Used a great deal (27-39)	33	22
Total Mean Score (Mean ± SD)	18.63 ± 8.429		
Emotion focused coping (0-87)	not used (Zero score)	0	0
	Used somewhat (1-29)	6	4
	Used quite a bit (30-58)	103	68.7
	Used a great deal (59-87)	41	27.3
Total Mean Score (Mean ± SD)	50.28 ± 11.346		

* Significant at *P 0.05

Table (5): describes the eight coping styles used by the studied patients. This table shows that the majority of the studied patients (62%) used "confrontive coping" quite a bit, while 20.7% used it somewhat, and 17.3% used it a great deal. This table shows that the Confrontive coping mean score of the studied subjects was 9.42 ± 3.28.

As regard "distancing" coping style, more than half of the studied sample (54.7%) used distancing coping style quite a bit to deal with stressful encounters, while 26.7% used it somewhat, while only 18% used it a great deal. Distancing coping mean score for studied patients was 9.01 ± 3.79.

Concerning "self controlling" coping style, about half of the studied depressed patients used self controlling through stressful situations in quite a bit degree, while 38% used it somewhat, (12%) used it a great deal, only (1.3%) did not use this style of coping. Self controlling coping mean score for studied subjects was 9.29 ± 4.16.

As for "seeking social support" coping, the highest percentage of depressed patients (43.3%) used this style of coping quite a bit, 29.3% used it a great deal, while 26.7% used it somewhat, and only 0.7% did not use it at all. Seeking social support coping mean score for studied subjects was 9.77 ± 4.40.

It was noted that the majority of studied depressed patients (44%) used accepting responsibility coping style a great deal, while 41.3% used it quite a bit, 14% used it somewhat, and only (0.7%) did not use it. Accepting responsibility coping mean score for studied subjects was 7.72 ± 2.66.

It is clear that 41.3% of studied depressed patients used "escape-avoidance" coping style quite a bit to relief stress, while 40.7% used it a great deal, and the lowest percentage was in patients who used it in somewhat degree. Escape-avoidance coping mean score for studied subjects was 14.22 ± 5.17.With respect to "planful problem solving" coping style, the majority of the studied patients used this style of coping quite a bit, 36% used it somewhat, (22%) used it a great deal, and only 1.3% did not use it. Planful problem solving coping mean score for studied depressed patients was 8.161 ± 4.60.

In relation to positive reappraisal coping style, 38% of the studied depressed patients used it quite a bit, while about one third (32%) of them used it somewhat, 28.7% used it in a great deal degree, and only 1.3% did not use this style of coping. Positive reappraisal coping mean score for studied depressed patients was10.86 ± 5.40.

Table (6): Pearson correlation coefficient revealed that, problem focused coping style was negatively and significantly correlated to level of depression in moderate degree (r =-0.401, P=0.000*) . While, emotion focused coping style was positively and significantly correlated to level of depression in moderate degree (r = 0.288, P = 0.000*).

When looking specifically at the correlation between depression and eight coping styles, Pearson correlation coefficient showed that, the greater use of escape avoidance coping was found to be significantly and positively correlated to greater depression in moderate degree (r=0.597, p=0.000*). Greater accepting responsibility and confrontive coping, showed a trend toward significant positive correlation in moderate degree with level of depression (r = 0.284, p=0.000*; r=0.229, p=0.005* respectively), while self controlling, planful problem solving and positive reappraisal were negatively correlated with greater depression in moderate degree. (r=-0.352, P=0.000*; r=-0.380, P=0.000*; r=-0.420, P=0.000* respectively). Furthermore, results of correlations between coping styles and depression showed that distancing coping and seeking social support were not correlated to level of depression (r=0.042, p=0.613; r=-0.056, P=0.493) respectively.

Table (7): Shows the relations between depressed patients age and their problem and emotion focused coping styles, the table revealed that there are no statistical significant difference both problem and emotion focused coping styles mean scores as a function of age groups (F = 0.923   P = 0.431 ; F = 1.897, p = 0.133 respectively). Furthermore, it is clear that the higher mean score for problem focused and

emotion focused coping was in age 30 to less than 40 years (20.27 ± 8.976).

Table (8): Shows the relations between age and eight coping styles, it is clear that there are statistical significant differences between both distancing and accepting responsibility coping styles as a function of depressed patients age (F = 3.19, P = 0.025* ; F = 3.162, P = 0.026* respectively). The highest mean score for distancing coping style was in age 50 and more (11.00±4.301).

The results revealed that there are increasing in mean score for confrontive coping style with age. Regarding self controlling, seeking social support, accepting responsibility, planful problem solving, and positive reappraisal coping styles means, the highest

mean was in age 30 to less than 40 years (10.30 ± 5.000, 10.86 ± 4.095, 8.61 ± 2.223, 9.02 ± 4.678, 11.39 ± 5.735 respectively). As for escape-avoidance coping means, the highest mean score was in age 50 years and more (14.84 ± 4.589).

Table (9): shows the relation between level of education of depressed patients and their both problem and emotion focused coping, the table shows that there are statistical significant relation between patients` level of education and their problem focused coping style (F = 3.538, P =0.016*). No statistical significant relationship was found between patients` level of education and emotion focused coping style (F = 1.023, P = 0.393).

Table (5): distribution of the subjects according to the eight coping styles			
Coping style	degree	Frequency (n=150)	Percent
Confrontive coping (0-18)	not used	0	0
	Used somewhat	31	20.7
	Used quite a bit	93	62.0
	Used a great deal	26	17.3
Mean ± SD	9.42 ± 3.28		
Distancing coping (0-18)	not used	1	0.7
	Used somewhat	40	26.7
	Used quite a bit	82	54.7
	Used a great deal	27	18
Mean ± SD	9.01± 3.79		
Self controlling (0-21)	not used	2	1.3
	Used somewhat	57	38
	Used quite a bit	73	48.7
	Used a great deal	18	12
Mean ± SD	9.29 ± 4.16		
Seeking social support (0-18)	not used	1	0.7
	Used somewhat	40	26.7
	Used quite a bit	65	43.3
	Used a great deal	44	29.3
Mean ± SD	9.77 ± 4.40		
Accepting responsibility (0-12)	not used	1	0.7
	Used somewhat	21	14
	Used quite a bit	62	41.3
	Used a great deal	66	44
Mean ± SD	7.72 ± 2.66		
Escape avoidance (0-24)	not used	0	0
	Used somewhat	27	18
	Used quite a bit	62	41.3
	Used a great deal	61	40.7
Mean ± SD	14.22 ± 5.17		
Planful problem solving (0-18)	not used	2	1.3
	Used somewhat	54	36
	Used quite a bit	61	40.7
	Used a great deal	33	22
Mean ± SD	8.161 ± 4.60		
Positive reappraisal (0-21)	not used	2	1.3
	Used somewhat	48	32
	Used quite a bit	57	38
	Used a great deal	43	28.7
Mean ± SD	10.86 ± 5.40		

* Significant at *P 0.05

Table (6): Correlation between level of depression and coping styles:

		level of depression	problem focused coping	emotion focused coping	confrontive coping	distancing coping	self controlling coping	seeking social support	accepting responsibility	escape-avoidance	planful problem solving	positive reappraisal
level of depression	R	1	-0.401	0.288	0.229	0.042	-0.352	-0.056	0.284	0.597	-0.380	-0.420
	P		0.000**	0.000**	0.005**	0.613	0.000**	0.493	0.000**	0.000**	0.000**	0.000**
problem focused coping	R		1	0.336	0.275	0.256	0.725	0.519	0.141	-0.230	0.891	0.777
	P			0.000**	0.001**	0.002**	0.000**	0.000**	0.086	0.005**	0.000**	0.000**
emotion focused coping	R			1	0.500	0.591	0.312	0.427	0.646	0.652	0.319	0.390
	P				0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**
confrontive coping	R				1	0.264	0.165	0.210	0.411	0.382	0.249	0.226
	P					0.001**	0.043*	0.010**	0.000**	0.000**	0.002**	0.005**
distancing coping	R					1	0.159	0.152	0.285	0.253	0.272	0.347
	P						0.052	0.064	0.000**	0.002**	0.001**	0.000**
self controlling coping	R						1	0.294	0.036	-0.153	0.568	0.651
	P							0.000**	0.659	0.062	0.000**	0.000**
seeking social support	R							1	0.286	0.066	0.255	0.300
	P								0.000**	0.423	0.002**	0.000**
accepting responsibility	R								1	0.440	0.151	0.146
	P									0.000**	0.065	0.076
escape-avoidance	R									1	-.0195	-0.180
	P										0.017**	0.028**
planful problem solving	R										1	0.770
	P											0.000**
positive reappraisal	R											1

Table (7): Relations between depressed patients age and their problem and emotion focused coping styles:

Age /years	Problem focused coping	Emotion focused coping
	Mean ± SD	Mean ± SD
20- (n=34)	18.65 ± 8.876	46.79 ± 12.656
30- (n=44)	20.27 ± 8.976	52.59 ± 11.659
40- (n=47)	17.45 ± 7.796	49.81 ± 9.879
50+ (n=25)	17.92 ± 7.984	51.84 ± 10.881
Total (n=150)	18.63 ± 8.429	50.28 ± 11.346
Significance level	F = 0.923 P = 0.431	F = 1.897 p = 0.133

Table (8): Relations between age and eight coping styles in studied depressed patients:

Age /years	confrontive coping	distancing coping	self controlling coping	seeking social support	accepting responsibility	escape-avoidance	planful problem solving	positive reappraisal
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
20- (n=34)	8.71±3.416	8.18±3.503	9.12±3.859	9.76±4.749	6.85 ± 2.851	13.56±5.428	8.59±4.356	10.74±5.701
30- (n=44)	9.73±3.637	9.00±3.894	10.30±5.000	10.86±4.095	8.61±2.223	14.48±5.634	9.02±4.678	11.39±5.735
40- (n=47)	9.55±2.910	8.55±3.348	8.74±3.608	9.36±4.198	7.45±2.780	14.13±4.933	8.06±4.613	10.68±5.251
50+ (n=25)	9.60±3.175	11.00±4.301	8.76±3.811	8.60±4.637	7.84±2.561	14.84±4.589	8.92±4.974	10.44±4.883
Total (150)	9.42±3.286	9.01±3.798	9.29±4.162	9.77±4.402	7.72±2.665	14.22±5.175	8.61±4.608	10.86±5.401
Significance level	F = 0.710 P = 0.547	F = 3.19 P = 0.025*	F= 1.287 P=0.281	F= 1.650 P =0.180	F = 3.162 P = 0.026*	F = 0.341 P =0.795	F = 0.371 P = 0.774	F = 0.210 P = 0.890

Table (9): relation between level of education of depressed patients and their both problem and emotion focused coping:

Level of education	problem focused coping	emotion focused coping
	Mean ±SD.	Mean ±SD.
Illiterate /read and write (n= 46)	16.17±7.153	51.80±8.120
Primary / preparatory (n=43)	17.63±8.944	48.70±11.141
Secondary (n=10)	21.10±9.620	46.60±13.492
University / postgraduate (n=51)	21.20±8.215	50.96±13.407
Total (n=150)	18.63±8.429	50.28±11.346
Significance level	F = 3.538 P =0.016*	F = 1.023 P = 0.393

* Significant at *P 0.05

Table (10): Shows the relations between level of education and eight coping styles. According to this table, there are statistical significant difference between level of education and planful problem solving coping (F = 3.250, P = 0.024). Table (10) showed that mean score of planful problem solving coping among patients who have (University / postgraduate) education was higher than planful problem solving mean score among patients who have (Illiterate /read and write) education (9.94±4.492, 7.33±4.227) respectively. Furthermore, it was noted from table (10) that there are highly statistical significant relation between level of education and positive reappraisal coping style (F = 4.582, P=0.004**).

Table (10) showed that mean score of positive reappraisal coping among patients who have (University / postgraduate) education was higher than both positive reappraisal mean score among patients who have (Illiterate /read and write) education and patients who have (Primary / preparatory) education (12.92±5.059, 9.35±5.087 respectively), accordingly patients who have (University / postgraduate) education use positive reappraisal coping style more than patients who have (Illiterate /read and write) education and patients who have (Primary / preparatory) education. In addition there are gradual increasing in mean score of positive reappraisal with increase level of education.

Table (10): Relations between level of education and eight coping styles:

Level of education	confrontive coping	distancing coping	self controlling coping	seeking social support	accepting responsibility	escape-avoidance	planful problem solving	positive reappraisal
	Mean ±SD.	Mean ±SD.	Mean ±SD.	Mean ±SD.	Mean ±SD.	Mean ±SD.	Mean ±SD.	Mean ±SD.
Illiterate /read and write (n = 46)	9.83±2.791	8.93±3.890	8.93±3.849	9.33±4.784	8.37±2.245	15.50±3.793	7.33±4.227	9.35±5.087
Primary / preparatory (n=43)	9.28±3.142	8.21±3.622	8.53±4.008	9.81±3.990	7.12±2.422	14.16±4.624	8.07±4.600	9.84±5.580
Secondary (n=10)	9.10±3.755	10.60±5.147	9.10±5.626	8.70±4.547	6.70±3.093	12.00±6.037	10.00±5.416	11.70±4.945
University / postgraduate (n=51)	9.24±3.755	9.43±3.511	10.27±4.181	10.33±4.394	7.84±3.009	13.55±6.275	9.94±4.492	12.92±5.059
Total (n=150)	9.42±3.286	9.01±3.798	9.29±4.162	9.77±4.402	7.72±2.665	14.22±5.175	8.61±4.608	10.86±5.401
Significance level *P 0.05	F = 0.341 P = 0.795	F = 1.450 P = 0.231	F = 1.559 P = 0.202	F = 0.628 P = 0.598	F = 2.460 P = 0.078	F = 2.015 P = 0.128	F = 3.250 P = 0.024*	F = 4.582 P =0.004**

4. Discussion

Depressed patients are trapped by low self esteem, hopelessness, lack of inherent capacities, and helplessness. They appraise harm or threat more readily than non-depressed persons in demanding encounters. So it is more difficult for them to use adaptive coping styles to meet the demands of stressors. This leads to increase the negative impact of stress on the clinical course of depression and decreases the choices of treatment [13-15,18]. Changing maladaptive styles of coping is an important target of depression treatment. People with chronic depression must learn to cope with their illness and its treatment while striving to live a meaningful, productive life [29,30]. Hence, the present study was carried out to identify coping styles among depressed patients, and its relation with the level of depression, with the hope of increasing nurses awareness about both adaptive and maladaptive coping styles among depressed patients, to increase effectiveness of depression treatment and prevention of suicidal risk.

The results of the current study showed that about half of the studied patients had a severe degree of depressive symptoms. These findings may be related to

the use of emotional focused coping styles by the majority of the studied patients. The emotional coping style includes self blame which is positively correlated with psychological distress and depression [35,36]. Moreover, this severe degree of depressive symptoms was found to be related to the use of maladaptive coping styles, such as escape-avoidance coping style in the present study. Other studies revealed that depressed patients may use less planful problem solving, even if the problems are solvable, and they do not seek social support, but they prefer loneliness and blaming themselves [37,38].

It is worth noting that the mean score regarding the level of depression was higher in females than in males (34.80 ± 16.36, 28.68 ± 16.67 respectively). This may be attributed to the fact that females are more emotionally reactive than males, due to hormonal changes that occur during the menstrual cycle, postpartum period and menopause [39-41]. In addition, it may be related to females motherhood instincts' which leads females to be highly emotional by nature. This result was in agreement with a descriptive epidemiological studies reviewed by Hankin and Abramson (2001) who examined the effect of gender difference on depression , they found that females were preponderant in depression [42]. On the same line, Hankin et al. (2007) found that females exhibit greater levels of depressive symptoms than males [43]. While a study done by Stangler and Printzs (1980) showed that depression was not correlated with gender difference [44].

However, in the present study the majority of the subjects were males, which is in contrast with many studies that explored depression [45-47]. This limited females sample size may be due to the eastern culture that restricts females to seek out psychiatric treatment without obtaining their husband`s permission, or permission of the male authority figure in the family.

According to the findings of the current study, it has been noted that there are statistical significant relationships between level of education and problem focused coping style, it is clear that increase in the level of education will be accompanied by increase in use of problem focused coping style. This may be related to the effect of the educational level on the analytical abilities, the increasing of the educational level will be accompanied by increasing in analyzing and planning abilities [48]. Also planful problem solving and positive reappraisal were significantly related to depressed patient`s level of education. It may be related to that highly educated patients may have more awareness about their problems.

Regarding the coping styles of the studied patients, the present study revealed that depressed patients used emotion focused coping more than problem focused coping when they face problematic situations. This finding may be attributed to neuroticism personality trait that characterizes the personality of depressed patients. Theories of personality suggested that individuals high in neuroticism (emotionality) tend to report more emotion focused coping styles [49]. Individuals with neuroticism personality trait have enduring tendency to experience negative emotional states, they respond more poorly to environmental stress, and are more likely to interpret ordinary situations as threatening, and minor frustrations as hopelessly difficult [50]. Individuals who score high on neuroticism trait are more likely than the average to experience guilt feeling and depressed mood. Neuroticism is also a risk factor for "internalizing" mental disorders such as phobia, depression, panic disorder, and other anxiety disorders [50].

The current study revealed that, a positive moderate correlation was found between emotion focused coping and level of depression. The use of emotion focused coping styles will increase the level of depressive symptoms among depressed patients, as they tend to regulate their emotions more than analyze and solve their problematic situations. In other words when depressed patients are faced by problematic situations, they have more anxiety and more negative emotions, So they try to regulate these emotions by avoiding the stressors cognitively or behaviorally. Avoidance will be effective for a short period of time, but the negative effect of the stressor is still existing, and it will cause depression again [51].

In this respect, Tremblay and King (1994) and Endler and Parker (1990) found that there is a positive correlation between level of depression and emotion focused coping [35,52]. They rationalize their results by the fact that some of the reactions in emotion-oriented coping include "blame the self for being too emotional", "get angry", and "become tense", and the maladaptive flavour of these reactions perhaps contributes to negative self-statements, and therefore to increase the depression [35, 52]. Similarly, Goodwin (2006) found that increase use of emotion focused coping leads to increase the likelihood of depression [53].

Moreover, the results of the current study found that, the majority of the studied patients tend to use less problem focused coping style, also a statistical significant negative moderate correlation was found between the level of depression and problem focused coping style. This may be due to lacking of analytical abilities and concentration among depressed patients that lead them to expend more time and efforts to gather and analyze information about the problematic situations [4]. Consequently, they cannot be able to try out new problem solving skills.

Many studies concluded that depressed patients tend to use less problem focused coping style through their stressful encounters [35,54-57]. Moreover,

Studies carried out by Folkman and Lazarus (1986), Rosenberg et al. (1987) , Greenglass et al (2006), and vitaliano et al. (1992) found that the severity of depressive symptoms among depressed patients were negatively correlated with planful problem solving coping style [16,56,58,59]. Also, Carnazzo (2000) suggested that depressed patients have a significantly lower planful problem solving score [55].

Based on the results of the current study, Problem focused coping style has a positive effect on reducing depressive symptoms. When depressed patients succeed in solving their problems, it gives them a type of reward which increases their self esteem, which in turn has a positive effect on depressive symptoms. Thus increasing the self esteem level leads to decrease the level of depressive symptoms, such as guilt feeling, depressed mood and so on [60,61]. Another interpretation may be that when depressed patients use problem focused coping style, they put their negative energies toward their problem solving instead of toward negative thinking and self blame, thus distracting the patients away from their negative thoughts and decreasing depressive symptoms [4]. Moreover coping theories suggested that solving or managing a problem satisfactorily is one of the best ways of managing emotions. Accordingly when depressed patients succeed in managing their problematic situations, they have less negative emotions and less depressive symptoms [49].

In this respect, Vandam et.al (2003) found that when individuals are taught to systematically investigate their problems, gather information about these problems, develop a plan to address the problem, and to execute and evaluate their plan, it is effective in reducing depressive symptoms [62].

Regarding the adaptability of coping styles, previous researches suggested that problem focused coping style is considered as adaptive in controllable situations and is considered as maladaptive in uncontrollable ones. While emotion focused coping style is considered as maladaptive in controllable situations and adaptive in uncontrollable ones [63]. Moreover there are many studies which proved that adaptive coping styles lead to decrease the severity of depressive symptoms [37,38,64].

The results of the present study showed that confrontive, distancing, seeking social support, positive reappraisal, self controlling, escape-avoidance, and planful problem solving coping styles were used in "quite a bit" degree , only accepting responsibility coping style was used in "great deal" degree. In addition, the findings of the current study indicated that, patients with higher level of depressive symptoms reported more use of confrontive coping and escape avoidance coping styles, but they reported less use of self controlling coping style.   On the same line,

Rosenberg et al. (1987) found that people with higher levels of depression reported more use of emotion focused coping, confrontive coping, self control, and escape avoidance coping, than those who reported fewer symptoms of depression [56].   This study supported the results of the current study for emotion focused, confrontive and escape avoidance coping styles, but not for self controlling coping style [56]. Similarly, Folkman and Lazarus (1986) found that depressed patients with severe depressive symptoms use more confrontive and escape avoidance coping styles than those with lower degrees of depression [16]. Also, Coyne et al. (1981) found that escape avoidance coping style was significantly higher among depressed patients [65]. However ,Turner et al. (1992) found a negative relationship between depression and escape-avoidance coping [66].

Concerning social support the present findings revealed that 43.3% of the studied subjects used seeking social support in "quite a bit" degree, also it has been noted that there are no significant correlation between level of depression and seeking social support coping style. This result may be related to the fact that the majority of the studied patients lived with their family and more than half of them were married. These patients' characteristics are considered social resources to seek social support . [Coyne et al. (1981)] and [Folkman and Lazarus (1986)] found that there are significantly greater use of seeking social support among depressed patients [16,65]. While, Carnazzo (2000) found that depressed patients did not report seeking social support any more than non-depressed patients [55].

Regarding "accepting responsibility" coping style, the present results revealed that there is a positive correlation between the level of depression and accepting responsibility coping style. This may be due to high degree of patient`s self criticism, as one of the types of self punishment, that will increase the severity of depression. Also the severity of depression may be increased when depressed patients   put high level of standards to achieve their responsibilities and then failed in achieving it. Furthermore it may be related to the use of negative thinking by depressed patients such as should statements, which leads to high level of self punishment as they are unable to achieve what they say [34,67].   Folkman and Lazarus (1986) reported that patients with more depressive symptoms used more accepting responsibility coping style than other coping styles [16].

As for distancing coping style, the present findings reported that more than half of the subjects use distancing coping in "quite a bit degree". Also, the current study showed that distancing coping style was positively correlated with escape avoidance, accepting responsibility, planful problem solving, positive

reappraisal, problem focused and emotion focused coping styles. However the current findings did not reveal significant relation between level of depression and distancing coping style among depressed patients. Carnazzo (2000) concluded that depressed patients use less of distancing coping through stressful encounters which is considered inconsistent with the present findings [55].

Moreover the present findings revealed that nearly half of the studied patients used self controlling coping style in "quite a bit degree". Also, a negative correlation was found between self controlling coping style and patient's level of depression. Folkman and Lazarus (1986) supported the present findings, they found that depressed patients use more of self controlling coping style while they are struggling to face their stressors [16]. Carnazzo (2000) reported that depressed patients used less of self controlling coping style, which is in contradiction with the present findings [55].

Regarding positive reappraisal coping style, the present results found that the highest percentage of the subjects used positive reappraisal coping style in quite a "bit degree". Also the present findings showed that increasing use of positive reappraisal coping will be accompanied by decrease in the level of depressive symptoms among depressed patients. This may be attributed to the current results which suggested that positive reappraisal coping style is positively correlated with problem focused and seeking social support coping styles. Furthermore, when depressed patients reappraise negative stressful situation in a positive way, this leads to positive thoughts about the stressful situation, which in turn leads to a positive effect on the depressed patient`s mood, and accordingly the level of depression will decrease. This result was supported by the findings of Jones (2007), who showed that participants who used more of positive reappraisal coping style had less depression [68].

By studying the relations between socio-demographic variables and coping styles, the present findings shows that age was significantly related to two styles of coping, which are distancing and accepting responsibility. In relation to the distancing coping styles, results of the present study revealed that there are statistical significant relationship between age and distancing coping style, depressed patients in age group 50 and more used distancing as a way of coping more than other age groups (the highest mean score (11.00±4.30) was found in age group 50 and more) . This result may be related to the fact that older people become stressed more readily than younger ones because of their lack of capacities which are related to their age, so they use many styles of emotional coping such as distancing to decrease their internal anxiety level. This result can be explained in the light of traditional ego psychology which suggested that older men become more passive than younger ones, accordingly they use distancing coping style when they confront stressful encounter [69]. Also the present findings are supported by Vasquez and Winner (2002), who found that older adults use more passive coping than younger ones [70]. Folkman and Lazarus et.al (1987) found that younger subjects use less distancing coping style than older ones [71]. Similarly, Aldwin (1991) found negative relationship between age and the reported use of escapist coping styles which support the result of the current study. This result was inconsistent with two studies, the study done by Billings and Moos (1981), and the study done by Yamadaa et al. (2003) who did not find any relationship between this age group and distancing coping style [72,73].

Regarding accepting responsibility coping style, the current results revealed that there was a statistical significant relation between age and accepting responsibility coping style. The depressed patients who are in middle adulthood stage used accepting responsibility coping style more than other age groups. The rationale of this result may be due to the fact that middle adulthood stage characteristics are competence, maturity, responsibility and stability [74]. Accordingly depressed patients who aged between 30 to less than 40 years are mature enough to take their own role to solve their problematic situations. While depressed patients who become elder, are mature enough but they have inferiority feeling which is generated from their lack of capacities due to old age changes, like health problems, and changes in daily life, memory changes, and financial situations , and they perceive stressors as not changeable, so they may become more dependent in relation to their responsibilities. [67]

The results of the current study found that there are no relationship between age and confrontive, seeking social support, planful problem solving and positive reappraisal coping styles. The study done by Folkman and Lazarus et.al (1987) found that younger subjects use more confrontive, seeking social support, and planful problem solving coping styles than did the older subjects, while the older subjects use of distancing, acceptance of responsibility, and positive reappraisal coping styles, more than the younger subjects did [71].

5. Conclusion:

The present findings explored coping styles among depressed patients and its relation to level of depression. Based on these findings, it can be concluded that emotion focused coping is the most style used by depressed patients to relief anxiety, and negative emotions. Emotion focused coping is maladaptive way to cope with stress which depressed

patients face, because it lead to increase level of depression. Problem focused , self controlling, planful problem solving ,and positive reappraisal coping styles are effective ways to relieve level of depression. They can be developed to be parts of the nursing intervention by teaching patients these styles of coping with their stressful encounters, to decrease their level of depression, and to prevent them from suicidal behaviors.

Recommendations

Based on the results of the current study need the following recommendations are suggested:

- Implementation of Psycho-educational programs for depressed patients which aim to:
- Educate them about adaptive and maladaptive coping styles.
- Increase their awareness about coping styles which decrease their level of depressive symptoms.
- Increase patient's awareness about their stressors and its causes, are recommended.
- Conferences and workshops about coping styles are recommended for nurses to increase their awareness about the importance of patient`s coping on the effectiveness of depression treatment.
- Adaptive coping styles such as planful problem solving, positive reaapraisal, self controlling coping styles may be part of nursing process in psychiatric nursing clinical practice to decrease level of depressive symptoms.
- Family education about coping styles and its relation to depressive symptoms, should be implemented.
- Further future researches should be done to increase understanding of the complexity of coping styles among depressed patients.
- Include the different coping styles in the assessment tool to determine their use by the patients and to evaluate them on admission.

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Bioremediation the Toxic Effect of Mercury-Exposure in Nile Tilapia (*Oreochromis Niloticus*) by using *Lemna gibba L*

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Abstract: The effect of mercury (Hg) toxicity, its impact on histopathological changes, the median lethal concentration (LC_{50}-96 h) and the bioremediation effect of *Lemna gibba L* to Nile tilapia, *Oreochromis niloticus*, were investigated through semi-static acute toxicity test developed with mercury chloride ($HgCl_2$).). Fingerlings (2.76±0.21 cm and 0.51±0.12 g) were kept during 96 hours in 5-liter glass aquaria, according to the following mercury concentrations, set up in three replicates: 0.00 (control), 0.037, 0.185, 0.370, 0.740, 0.925 mg Hg L^{-1}. The value of LC_{50}-96h was estimated in 0.240 mg Hg L^{-1}.
This study indicated that:1) Hg poisoning caused structural damage in the fish organs ,2) *Lemna gibba L* (weed and extract) were effective in removing Hg from water and reducing Hg bioaccumulation in liver and muscular tissues of fish , 3) The addition of *Lemna gibba L*-extract reduced significantly (*P<0.05*) the Hg level uptake as compared to fish exposed to Hg alone and 4) Addition of *Lemna gibba L* remediated the toxic effect of Hg and provided protection against the degenerative action of Hg. (*Oreochromis Niloticus*) **by using *Lemna gibba L*.** Journal of American Science 2011;7(3):336-343]. (ISSN: 1545-1003). http://www.americanscience.org .

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1. Introduction:

The aquatic environment is constantly exposed to various pollutants, and the group of heavy metals has been the focus of many studies with deep concern. Some particular heavy metals, such as mercury (Hg), are especially included in investigations due to their high toxicity. This element is classified as one of the most toxic metals, which are introduced into the natural environment by human interferences (Buhl, 1997). Inorganic mercury is the most common form of the metal released in the environment by industries, presenting a stronger acute effect on fish tissues than that of the organic form of mercury (Sunderland and Chmura, 2000). Some papers have reported situations where high mercury levels were detected in water, mainly nearby gold extraction locations (Maurice-Bourgoin *et al.,* 2000; Dolbec *et al.,* 2001) and industrial zones (Kime, 1998; Sunderland and Chmura, 2000). Consequently, aquaculture is vulnerable to this pollutant, since supplied ponds water generally comes from rivers, dams or other sources that can possibly be contaminated by mercury. Fish contaminated by Hg suffers pathological alterations, with consequent inhibition of metabolic processes, hematological changes, and decline in fertility and survival (Micryakov and Lapirova, 1997).

Most aquatic organisms have the capability of concentrating metals by feeding and metabolic processes, which can lead to accumulation of high concentrations of metals in their tissues. Metals interact with legends in proteins particularly, enzymes and may inhibit their biochemical and physiological activities (Passow *et al.*, 1961).

Metal bioaccumulation can occur via complexation, coordination, chelating, ion exchange and other processes of greater or lesser specificity. It is a strictly aggressive process in which metal ions are sequestered by metal binding site in the interior of the cell. The removal of toxic elements from contaminated water has potential advantages over the conventional treatment process. The reduction of toxic elements like cadmium and mercury in aquatic environments is needed by any acceptable method. The most widely used technique for the removal of toxic elements involves the process of neutralization and metal hydroxide precipitation (Hiemesh & Mahadevaswamy, 1994). The use of aquatic plants in water ecosystems and terrestrial plants in hydroponic systems has high potential to clean up the metal contaminated water through phytoextraction and

phytostabilization. Phytostabilization utilizes the plant production of compounds, which immobilize contaminants at the entrance of roots. An example of this method is where root exudates cause the precipitation of metals and reducing their bioavailability. Phytodegradation (also known as phytotransformation) is the enzyme-catalysed metabolism of contaminants, typically organics, within plant tissues. The enzymes are usually dehalogenases, oxygenases and reductases (Black, 1995).

Biosorption potential of *Prosopis juliflora* seed powder (PJSP) for lead (Pb) from aqueous solution has been investigated by Jayaram and Prasad (2009) where they found that the maximum Pb (II) adsorbed was found to be 40.322 mg/g and the adsorption process was spontaneous and exothermic in nature. Removal of certain heavy metals from waste water by *Lemna gibba L.* has been reported by Kwan & Smith (1991); Buckley (1994); Miranda & IIangovan (1996) and Wafaa (2007).

In the present study, short and long-term bioassays were designed to evaluate the influence of *Lemna gibba L-* plant and/or its extract on the reduction of mercury in water as well as to investigate the reducing effect of *Lemna gibba L* on some histopathological alterations induced by Hg exposure on Nile tilapia (*Oreochromis niloticus*).

2. Materials and Methods:

Fish culture management

Healthy *Oreochromis niloticus* fingerlings were collected in Marsh 2010, from ponds of the Central Laboratory for Aquaculture Research at Abbassa, Abo-Hammad, and Sharkia, Egypt (belonging to a single population) .They were collected locally and confined to large plastic aquaria bearing tap water for up to 7 days in the laboratory for acclimation.

Mercury chloride

Technical grade mercury chloride (99% purity) was obtained from El-Nasr Chemical Company (Cairo, Egypt) and prepared in aquatic solution to provide the required concentrations of mercury. Control test without mercury was performed.

Determination of LC_{50}

Acute Toxicity Assays

The stock solution (370 mg Hg L^{-1}) was prepared by dissolving a calculated quantity of active ingredient (0.5 g $HgCl_2$ in 1,000 mL of dechlorinated tap water). A series of five concentrations of Hg was prepared by adding a calculated volume from the stocky solution into test containers, considering the

equivalent on mercury (Hg). Therefore, nominal concentrations were: 0.037, 0.185, 0.370, 0.740, and 0.925 mg Hg L^{-1} (range determined by preliminary tests). One container was kept as unexposed control group. Test was carried out with three simultaneous replicates. No food was supplied during the experiment. Test solutions were replaced by fresh ones of the same respective concentrations every 24 h until 96 h of testing, according to the renewal method recommended in APHA (1998).

The bioassay was conducted in Marsh 2010, Laboratory, Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University, with controlled conditions of water temperature (24.40±2.25 ^{o}C) and photoperiod (10L: 14D cycle). The used fish species was Nile tilapia, *Oreochromis niloticus*. Fingerlings with a mean weight of 0.51±0.12 g and mean total length of 2.76±0.21 cm. The acclimatization period was of 7 days, in a 50-L glass aquarium. During this period, fish were fed a dry commercial food (pellets with 25% of crude protein). Afterwards, fingerlings were transferred to 5-L glass aquaria, which were internally covered with a plastic film to prevent contamination by residues from previous experiments. Plastic film was also placed on the top of the aquarium to prevent evaporation. Air pumps and individual air stone diffusers provided aeration. The experiment was carried out at a stocking density of 10 fish/aquarium.

Mortalities were recorded at 24, 48, 72 and 96 h of exposure, and dead fish were removed regularly from the test solutions. The data obtained were statistically analyzed using the Trimmed Spearman Karber method (Hamilton *et al.,* 1977) for estimating the median lethal concentration (LC_{50}), and 1/100 of the LC_{50}-96 h was taken as the safe Hg concentration (Sprague, 1971).

The tested weed

The duckweed species used was *Lemna gibba L* which was taken from Ganabiet-Tersa drain, Giza, Egypt. The duckweed was acclimatized to the laboratory conditions for one week before starting the experiments.

Plant extracts

Dried plant materials were extracted twice with 50% and 100% methanol as well as 50% and 100% acetone in v/v proportions (200 ml/5g plant) for 2 hrs with constant stirring. The collected filtered extracts were dried in a rotary evaporator (Büchi: Rotavapor-R114 and water bath B-481) at 40ºC under reduced pressure (Ghobrial *et al.*, 2009).

Mercury reduction

Tilapias were distributed randomly in 50-Litres rectangular fiberglass aquaria filled with well-aerated tap water (pH 6.5–7.0) at a rate of 10 fish / aquarium. Dissolved oxygen in each tank was maintained at close to saturation by aeration. The temperature in each aquarium was maintained at $24\pm1.5°C$ by means of thermostats. The photoperiod was 10L: 14D cycle. These aquaria were divided into five groups with three replicates each per group. The first group was free from Hg and *Lemna gibba L* and maintained as a control. The second group was exposed to 0.0 6 mg of $HgCl_2$ only (Equivalent to 1/4 96 h LC_{50}). The third, fourth and fifth groups were exposed to 0.0 6 mg $HgCl_2$ L^{-1} and 0.1, 1 and 0.1 plus 1 g L^{-1} extract, plant and extract plus plant of *Lemna gibba L*, respectively .

Fish were fed frequently on a diet containing 25% crude protein at a rate of 2-3% of live body weight twice daily for 7 and 25 days. Siphoning three quarters aquariums was done every day for waste removal and replacing it by an equal volume of water containing the same concentration of Hg and *Lemna gibba L.* Dead fish were removed and recorded daily.

Hg Residue
Preparation and Analysis of Mercury
Water Samples

The analysis of water samples was carried out according to A.P.H.A. (1992). The water samples were filtered through $0.45\mu l$ membrane filter. The required volume (100 ml) of the filtrate was collected to measure mercury levels in water samples by using Air/Acetylene Flame Atomic Absorption Spectrophotometer (UNICAM 696 AA Spectrometer).

Fish Samples

The measurement of the mercury concentration in examined fish samples was carried out at minimal temperature for all fish samples where 0.5 gram macerated fish tissues was digested according to the technique described by Diaz-Ravina *et al*. (1994). About 5 ml stannous chloride solution were added to the obtained solutions to reduce mercury to elemental form and then analyzed by using Flameless Atomic Absorption Spectrophotometer equipped with "MHS" mercury hydride system "Cold Vapour Technique".

Histopathological examination

Tissue specimens from fresh NileTilapia were taken (liver and muscles) and fixed in 15 % buffered neutral formalin. They were processed to obtain five micron thick paraffin sections then stained with Hematoxylin and Eosin (Bancroft *et al.*, 1996) and examined under light microscope.

Statistical analysis

The obtained data were subjected to analysis of variance according to Snedecor & Cochran (1982). Differences between means were done at the 5% probability level, using Duncan's new multiple range test (Duncan, 1955).

Exposure Time (hour)	LC50 (mg Hg L^{-1})	95% Confident Limit (mg Hg L^{-1})
24	0.48	0.38 ñ 0.46
48	0.39	0.32 ñ 0.53
72	0.29	0.24 ñ 0.33
96	0.24	0.19 ñ 0.24

3. Results:

Table 1. Mean mortality (%) of Nile tilapia, *Oreochromis niloticus*, and expressed according to different exposure times to mercury concentrations.

Concentration (mg Hg L^{-1})	Time (hour)			
	24	48	72	96
0.00 (control)	10	10	12	15
0.037	0	15	22	15
0.185	3.33	20	30	40
0.370	28.6	40	90	90
0.740	92.3	100	100	98
0.925	100	100	100	100

Three replicates

Table 2.*LC50* and Confident Limit

The value of LC_{50-96h} was estimated in 0.240 mg Hg L^{-1}.

Table 3.LC50 of mercury in Tilapias fish species.

Reference	Species	LC_{50-96h}
Ishikawa *et al*. (2007)	*Oreochromis niloticus*	0.220
Charuwan-Somsiri (1982)	*Oreochromis niloticus*	3.710
Ramamurthi *et al*. (1982)	*Tilapia mossambicus*	0.739
Present study	*Oreochromis niloticus*	0.240

Histopathological alterations

The histopathology of different Tilapia tissues revealed that there are several histopathological changes in different Tilapia organs as shown in our figures.
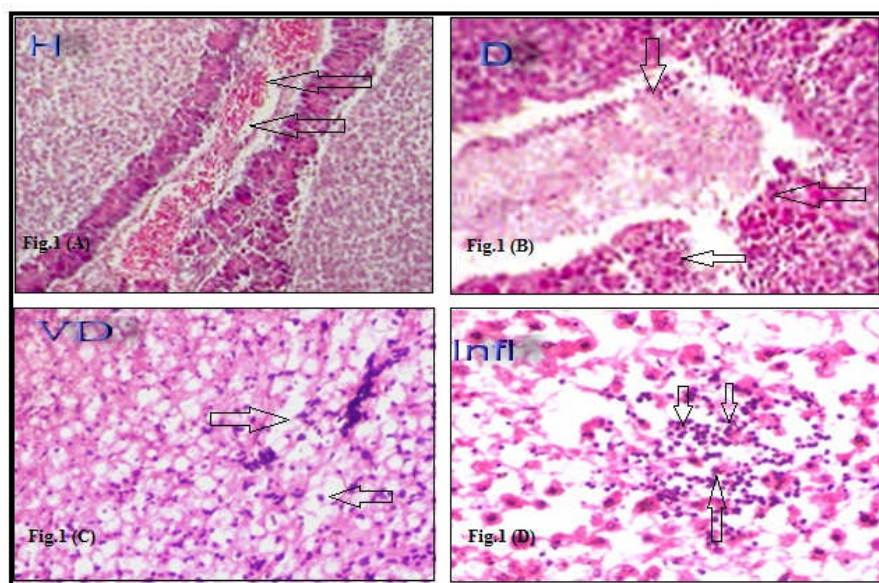
Figure 1.

A. Liver of tilapia treated with mercury showing intravascular haemolysis is seen in blood vessels and sinusoids (arrows) (H & E X 400).

B. Liver of tilapia treated with mercury showing degeneration of the hepatocytes with nuclear pyknosis and necrosed hepatocytes (arrows) (H & E X 400).

C. Liver of tilapia treated with mercury showing dissociation of hepatocytes with individual hepatocellular necrosis and focal mononuclear cell aggregation (H & E 400 X). (arrows) (H & E X 400).

D. Liver of tilapia treated with mercury showing lecucytic infiltration (arrows) (H & E X 400).
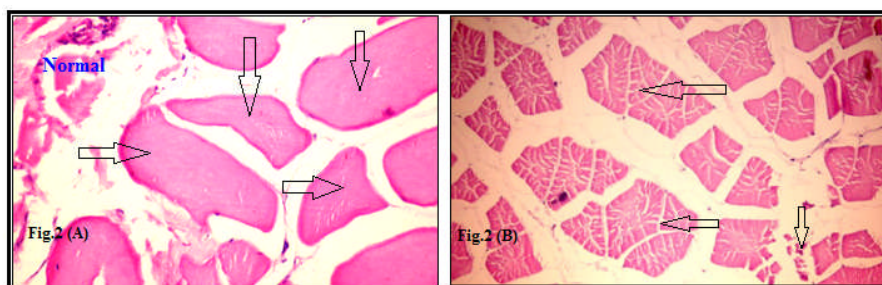
Figure 2.
A. Muscles of tilapia treated with mercury showing healthy muscular tissue (H & E X 400).
B. Muscles of tilapia treated with mercury showing hyalinised muscles tissue (arrows) (H & E X 400).

Table 4: Changes in mercury residue in water (mg Hg L^{-1}), liver and muscles (mg Hg g^{-1} dry weigh) of Nile tilapia *(O. niloticus)* exposed to Hg with and without *Lemna gibba L* plant

Group	Water	Liver		Muscles	
Days		7	25	7	25
Control (metal free water)	0.002±0.0002[a]	0.003±0.001[a]	0.0040±0.001[a]	0.0021±0.0004[a]	0.008±0.0007[a]
Mercury alone (0.06 mg) L^{-1}	0.060±0.009[b]	0.0550±0.008[b]	0.0588±0.0054[b]	0.0485±0.001[b]	0.097±0.0018[b]
Mercury (0.06 mg)+0.1g ext. L^{-1}	0.04 ±0.027[b]	0.0 32±0.009[b]	0.0200±0.008[b]	0.0143±0.0086[a]	00.068±0.018[b]
Mercury (0.06 mg)+1g P L^{-1}	0.038 ±0.008[c]	0.050±0.015[c]	0.039 ±0.002[c]	0.0180±0.008[b]	0.0383±0.007[b]
Mercury (0.06 mg)+0.1g ext. L^{-1}+1g P L^{-1}	0.0120 ±0.005[d]	0.0210 ±0.001[d]	0.0020 ±0.005[d]	0.0026±0.0006[a]	0.0021±0.0006[a]

The same letter in the same column is not significantly different at P<0.05.

The first group was free from Hg and *Lemna gibba L* and maintained as a control.

The second groups were exposed to 0.06 mg of HgCl$_2$ only (Equivalent to 1/4 96 h LC$_{50}$).

The third was exposed to 0.06 mg Hg L^{-1} and 0.1 extract.

The fourth group was exposed to 0.06 mg Hg L^{-1} and 1 g L^{-1} *Lemna gibba L* plant.

The fifth group was exposed to 0.06 mg Hg L^{-1} + 0.1g extract L^1 +1 g L^{-1}*Lemna gibba L* plant.

4. Discussion:

Physical and chemical variables analyzed in the test solutions showed no statistical differences among the range of five concentrations, neither between concentration range and control group. The average values for these variables were: temperature, 25.40±2.45 $^{°}$C; pH, 7.20±0.50; electric conductivity, 83.58±2.04 ìS cm^{-1}; hardness, 44.96±1.30 mg CaCO$_3$ L^{-1}; alkalinity, 29.45±1.35 mg CaCO$_3$ L^{-1}; and total ammonia, 1.72±0.22 mg L^{-1}. All of these variables results were in conformity to the standards that are recommended in APHA (1998) for toxicity tests.

Mortalities recorded along 96-hours exposure are registered in Table 1. The values of LC$_{50}$ determined for Nile tilapia in the present study, according to the different exposure times, are shown in Table 2. The LC$_{50}$-96h was compared with the results from other studies developed on mercury toxicity to fish, Table 3. Ramamurthi *et al.* (1982) and Charuwan-Somsiri (1982) estimated higher LC$_{50}$-96h for *Tilapia mossambica* and *Oreochromis niloticus,* respectively. The higher values obtained by Ramamurthi *et al.* (1982) and Charuwan-Somsiri (1982) may be attributed to some differences in standard techniques that were adopted in their experiments, such as the larger size of the test-organisms (3.5 cm) used by Charuwan-Somsiri (1982). According to Buhl (1997) and Boening (2000), older and larger aquatic organisms are more resistant to toxicants.

The LC$_{50}$ determined in the present study (LC$_{50}$-96h, 0.240 mg Hg L^{-1}) was very similar to those reported to Ishikawa *et al.* (2007) who estimated similar LC$_{50}$-96h for *Tilapia Oreochromis niloticus* (LC$_{50}$-96h, 0.220 mg Hg L^{-1}) and other fish groups, such as *Cyprinus carpio* and *Roccus americanus* (Rehwoldt *et al.* 1972),

Varichorhinus barbatulus (V. barbatus), Variocorhinus barbatulus (V. barbatus) and *Zacco barbata* (Shyong and Chen, 2000), *Ptychocheilus lucius* (Buhl, 1997).

Histopathological alterations

Liver of tilapia treated with mercury showed degeneration of the hepatocytes with nuclear pyknosis in the majority of the cells as well as the accumulation of the metal binding proteins in their nuclei. Intravascular hemolysis is seen in blood vessels and sinusoids with necrosed hepatocytes (Figures 1: A, B, C, D).

Muscular tissues degeneration in muscle bundles with aggregations of inflammatory cells (leucocytic infiltration) between them with focal areas of necrosis, atrophy and edema of muscle bundles as well as splitting of muscle fibers and hyalinized muscles tissue were seen (Figure 2: A, B).

Histopathological biomarkers have been largely used in fish to identify and evaluate the toxic effects of pollutants exposure (Rabitto *et al*., 2005; Oliveira Ribeiro *et al*., 2006). The presence of necrosis is in fact one of the most visible damages in tissues affected by a pollutant (Rabitto *et al*., 2005). According to Manahan (1991) the occurrence of necrosis is also a consequence of enzymatic inhibition, damages in the cellular membrane integrity, and disturbances in the synthesis of proteins and carbohydrate metabolism.

Pandey *et al.* (1994) described alteration in liver and intestine of *Liza parsia* exposed to HgCl$_2$ (0.2 mg Hg L^{-1}) for 15 days. Similarly, Oliveira-Ribeiro *et al.* (2002) reported serious injuries in gill and olfactory epithelium of *Salvelinus alpinus* exposed to 0.15 mg Hg L^{-1}. According to Allen (1994), the exposure of *Orechromis aureus* to 0.5 mg Hg L-1 caused a raise in the number of

leucocyte and erythrocyte within 24 hours. Gill and Pant (1985) also related hematological anomalies in *Barbus conchonius* exposed to 0.18 mg Hg L-1 in acute test.

Hg Bioaccumulation

The highest bioaccumulation of mercury was observed in the organs mainly implicated in metal intoxication and so it was higher in the liver followed by muscles.

Addition of *Lemna gibba L*-extract to the Hg polluted media reduced significantly ($P<0.05$) the Hg level in aquarium's water as compared to that of Hg alone. Hg concentration in water exposed Hg alone was 0.06 mg Hg L^{-1} and declined significantly ($P<0.05$) to 0.04, 0.038 and 0.0120 mg L^{-1} with 0.1, 1 and 0.1 plus 1 g L^{-1} extract, weed and extract plus weed of *Lemna gibba L*, respectively. The highest amount of Hg residue was found in the liver after 7 days of exposure. The uptake of Hg in the liver of fish exposed to Hg alone was 0.0550 and 0.0588 mg g^{-1} dry weight for 7 and 25 days, respectively. It declined significantly to 0.0 32, 0.050 and 0.0210, 0.0200 , 0.039 and 0.0020 mg g^{-1} dry weight in fish group exposed to Hg with 0.1, 1 and 0.1 plus 1 g L^{-1} extract, weed and extract plus weed of *Lemna gibba L* at 7 and 25 days, respectively. Similar trends were observed in fish muscles.

The present results indicate that *Lemna gibba L* weed and extract are effective in removing Hg from water and reducing Hg bioaccumulation in Tilapia fish, Table 4. The addition of *Lemna gibba L*-extract reduced significantly ($P<0.05$) the Hg level in water and the metal uptake as compared to fish exposed to Hg alone. Hg concentration in water was 0.06 mg L^{-1} and it decreased significantly ($P< 0.05$). Hg accumulation in liver and muscles of fish exposed to Hg alone was higher than that of *Lemna gibba L*-extract treatment group.

These results suggest that *Lemna gibba L weed and/or* extract could chelate Hg ions producing a stable complex, thus reducing the chance for metal uptake by tissues. These results are in agreement with Santschi (1988) who reported that any agent that can remove Hg from water helps to reduce the bioaccumulation of this metal in fish.

The addition of *Lemna gibba L*-extract reduces the toxic effect of Hg in Tilapia fish which indicating the capability of *Lemna gibba L*-extract to chelate Hg from the media. Subsequently, the Hg toxicity was reduced. These results are in agreement with those recorded by Jayaram & Prasad (2009) who observed the biosorption potential of *Prosopis juliflora* seed powder (PJSP) for Pb (II) from aqueous solution at pH 6.0 and Kaoud *et al.,*(2011) who found the removal of cadmium (Cd) from aqueous solution by the addition of *Lemna gibba L*-extract and/or the

plant. Findings in fish also indicated that degenerative changes were less when *Lemna gibba L*-extract or the weed and their extract were added to the rearing water.

The present study shows that addition of *Lemna gibba L-* weed and/or its extract to Hg contaminated media reduced significantly the Hg level in the water and helped to eliminate metal from the fish body and in turn improved the biochemical parameters as compared to fish exposed to Hg alone.

Finally, we could conclude that Hg poisoning cause structural damage in Tilapia liver and muscles. It is also demonstrated that *Lemna gibba L*-extract, weed or the weed plus the extract provided protection against the degenerative action of Hg and increased the chance of tissue regeneration.

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Evaluation of Quaternary aquifer for agricultural purposes in northwest Sinai, Egypt

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Abstract: Northwest Sinai is characterized by a semiarid climate and due to insufficient surface water resources; Quaternary aquifer is the main water supply. The Quaternary groundwater exists under free water table conditions with water level ranges between -3.7 m and 10 m. The groundwater flow is concentric around El-Salam Canal as a result of its closure and over-pumping of groundwater. In order to evaluate the suitability of Quaternary aquifer for irrigation purposes, the chemical characteristics have been investigated in fifty samples collected from tube wells and dug wells. The total dissolved solids (TDS) range from 692 mg/l to 9384 mg/l; indicating fresh to saline water classes. Sodium, chloride and sulphate ions display a nearly linear increase with increasing salinity. The main groundwater genetic types are $CaCl_2$ and $MgCl_2$, reflecting the marine water affinity. Such waters are mostly unsuitable for irrigation under a normal condition and further action for salinity control is required in remediating such a problem. Also, the poor irrigation water can be managed by improving irrigation management technologies and using salt tolerance plants.

Introduction:

The Sinai Peninsula covers an area of about 61,000 km^2 in the northeastern side of Egypt. The study area is located in the northwest corner of Sinai Peninsula, bordered from the north by the Mediterranean Sea and from the west by the Suez Canal (Fig. 1). The northern Sinai coast is located within the rainy belt of Egypt; while the aridity increases generally to the south (El-Ghazawi, 1989). Rainfall is scarce and varies from place to place and increases in the northeastern direction, ranging from about 30 mm/year in the southwest at Isamilia to about 300 mm/year in the northeast at Rafah. The annual rainfall in northwest Sinai varies between 36 to 54.8 mm and the total quantity of rainfall generally increases northward (El-Sheikh, 2008). The rainfall has a direct contribution to groundwater recharge in the sand dune aquifer north Sinai, particularly El-Arish−Rafah area (El-Ghazawi, 1989). The northern Sinai is occupied by sand dunes that mostly acting as water bearing formation, where the groundwater exists as a thin layer above the main saline water (El-Shazly *et al.,* 1974). The salinity values of Quaternary aquifer between Rommana and Bir El-Abd region range from 1876 to 7937 mg/l. This variation is due to the variation of the annual rainfall (Eweida *et al.,* 1992). The sources of high salinity in the area between Baloza and Rommana can be attributed to evaporation, the dissolution of evaporites, salt water intrusion and the influence of brines (Groschke, 2010). The total dissolved solids (TDS) of groundwater in El-Tineh plain and its vicinities increase in the direction of groundwater flow and range from 2450 mg/l to 16940 mg/l (Deiab, 1998). The salinity of surface and subsoil water in northwest Sinai is a mixture of meteoric and marine water due to salt water intrusion from the Mediterranean Sea and leaching processes of the lagoonal deposits (Deiab, 1998). Mohamed (2007) found that the danger of sea water intrusion on the Quaternary aquifer may not permit exploiting north Sinai as an industrial district for Bir El-Abd and its surroundings as was planned before.

Much of the arable land in the area would eventually be irrigated with Nile River water through the El-Salam Canal after blending with agricultural drainage water in a ratio of about 1:1 to reach TDS not more than 1000-1200 mg/l to be suitable for cultivated crops (Hafez, 2005). The estimated reclamation area in East Suez Canal is about 400,000 feddans (feddan= 4200 m^2) divided to 50,000 feddans for El-Tina plain area, 75,000 feddans south El-Qantara Shark area, 70,000 feddans for Rabaa area, 86,000 feddans for Bir El-Abd area, 33,000 feddans for El-Mazar and Midan areas, and 85,000 feddans for Alsir and Qwareer areas (Hafez, 2005).

The objective of the present study is to assess the chemical groundwater composition and its suitability for agricultural uses in northwest Sinai. To achieve this goal the collected samples were analyzed for the major constituents, nitrogen (nitrate and nitrite) and phosphate and different chemical indices are calculated.
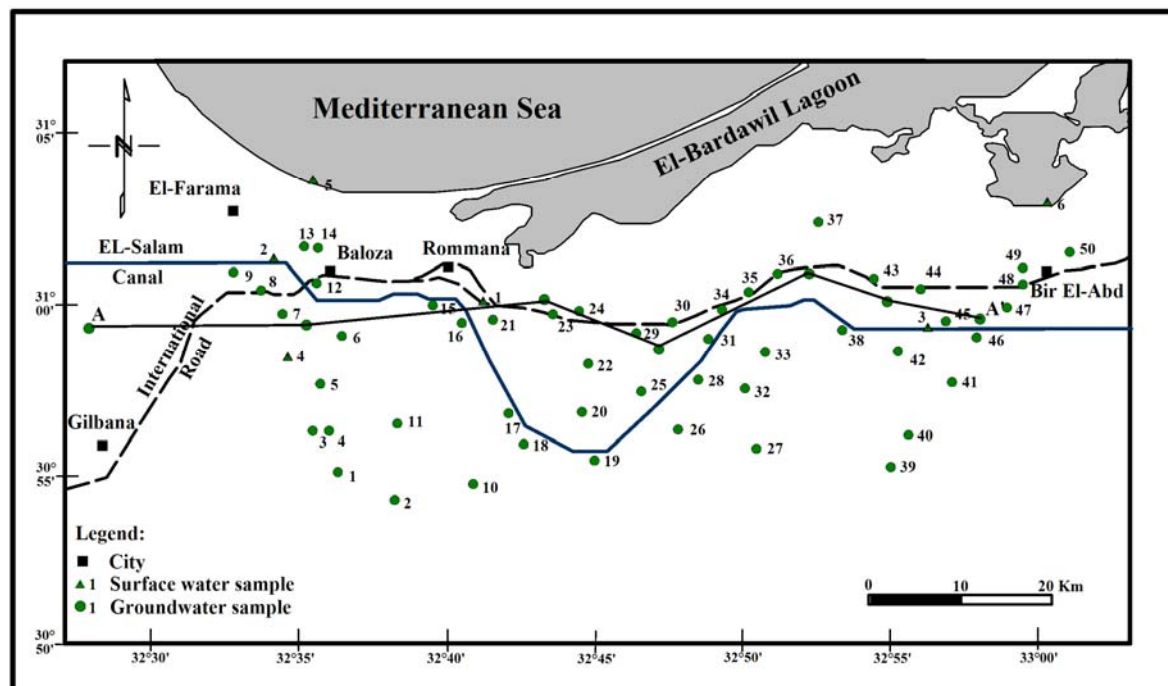
Fig. (1): Location map of the collected surface- and ground-water samples, northwest Sinai, Egypt (April, 2010).

Methodology:

Fifty groundwater samples from wells tapping the Quaternary sandy aquifer were collected in addition to five surface water samples in April 2010 (Fig. 1). Unstable parameters such as pH and electrical conductivity (EC) were measured in the field using pH meter (CONSORT P903, after Richards, 1954) and EC meter (Cyberscan Conductivity Meter CON 100). Major ions were analyzed in the laboratory. Calcium and magnesium contents were determined by EDTA titration using Eriochromeblack-T as indicator (Jackson, 1958). Sodium and potassium contents were determined using Flame Photometer (Rhoades, 1982). Carbonate and bicarbonate contents were measured by acid-base titration (Nelson, 1982). Sulphate was measured using a turbidity method (Adams, 1990). Chloride concentration was measured by silver nitrate titration using Volhard's method. Nitrate and nitrite ions were determined colorimetrically by using UV/visible Spectrophotometer (Harrison and Perry, 1986). Phosphate was determined colorimetrically based on hydroquinone method described by Snel and Snel (1967).

Geomorphologic and geologic setting:

Northwest Sinai is located within the semi-arid belt of Egypt. This aridity is manifested by the occurrence of sand dunes and sand sheets, salt marshes and ponds as well as lack of vegetation. Geomorphologically, northwestern Sinai embodies five distinctive units; 1) coastal area, which includes old shore, coastal sand dunes, the strand plain of successive beach ridges with intervening runnels recording shoreline progradation during Late Holocene, and deltaic plain covered by mouth bar and distributary channel fill sands (El-Asmar, 1999); 2) El-Bardawil Lagoon; 3) aeolian sand which covers the majority of north Sinai and consists of wind-laid sediments including aeolian siliclastic sabkhas (Assal, 1999); 4) mobile sand dunes; and 5) salt marshes and sabkhas (inland sabkhas and coastal sabkhas).

Geologically, northwestern Sinai is covered by Quaternary deposits. The Pleistocene deposits include: Sahl El-Tineh Formation which consists of a mixture of black and white sands with silt, Al-Qantara Formation which consists of sand and grits with minor clay interbeds, coquina deposits, fanglomerates, and alluvial hamadah deposits (Geological Survey of Egypt, 1992). According to GSE (1992), the Holocene deposits are classified into: coastal sand dunes which extend parallel to the Mediterranean Sea coast, inland sand dunes and sheets that cover large areas of northwestern Sinai

(the main water bearing formation for groundwater), coastal and inland sabkhas, and interdunal playa deposits; consist of fine sand and silt associated with evaporates (Deiab, 1998).

Hydrological setting:

The Quaternary deposits constitute the important water-bearing formations in the northwestern Sinai area. These deposits consist mainly of loose sand with few clay intercalations (Fig. 2). The thickness of such deposits increases towards the west. The Quaternary groundwater exists under free water table condition (Fig. 2). The depth to water varies from 0.5 m in the northwest to 9.1 m in the southeast. It is principally controlled by the surface topography, lithology and recharge. The water table ranges between -3.7 m to 10 m in the northeast corner where steep gradient is observed (Fig. 3). A remarkable local groundwater flow is detected in two locations; the first is concentric around El-Salam Canal to the east of Baloza and south El Farama sector, while the second is recorded to the south of Rommana where extensive parts of land are waterlogged forming salt marshes and salty ponds, e.g. Rommana pond. The

amount of rainfall recharging the Quaternary groundwater aquifer within the period 2005-2006 is about 8.67 million m^3 (El-Sheihk, 2008).

Geochemical properties of Quaternary aquifer:

The source of recharge, type of sediment, and groundwater flow are mainly affecting the geochemical characteristics of the Quaternary aquifer in northwest Sinai. From the chemical analyses given in Tables (1 & 2), the following properties could be deduced:

The pH values of groundwater mostly reflect slightly alkaline condition. The total dissolved solids (TDS) range from 692 mg/l to 9384 mg/l (Table 3). The salinity decreases at some patches located along El-Qantara Shark/El-Arish Road. The Quaternary groundwater along this road is affected by seepage from municipal water. Most of the Quaternary groundwater samples belong to brackish and saline classes; only 8% of the samples are fresh water (less than 1500 mg/l) according to Chebotarev's classification (1955).
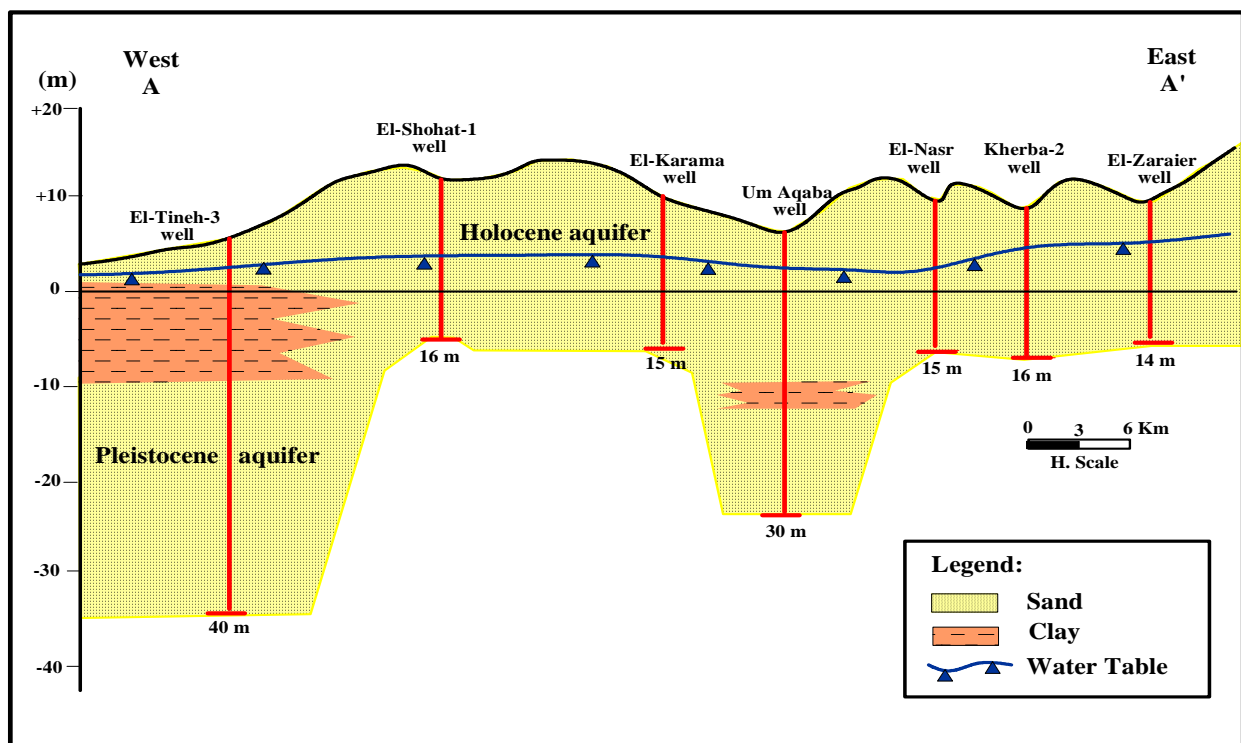
Fig. (2): West-East hydrogeological cross section (modified after El-Osta, 2000), the locations of these wells are shown in Fig. (1).
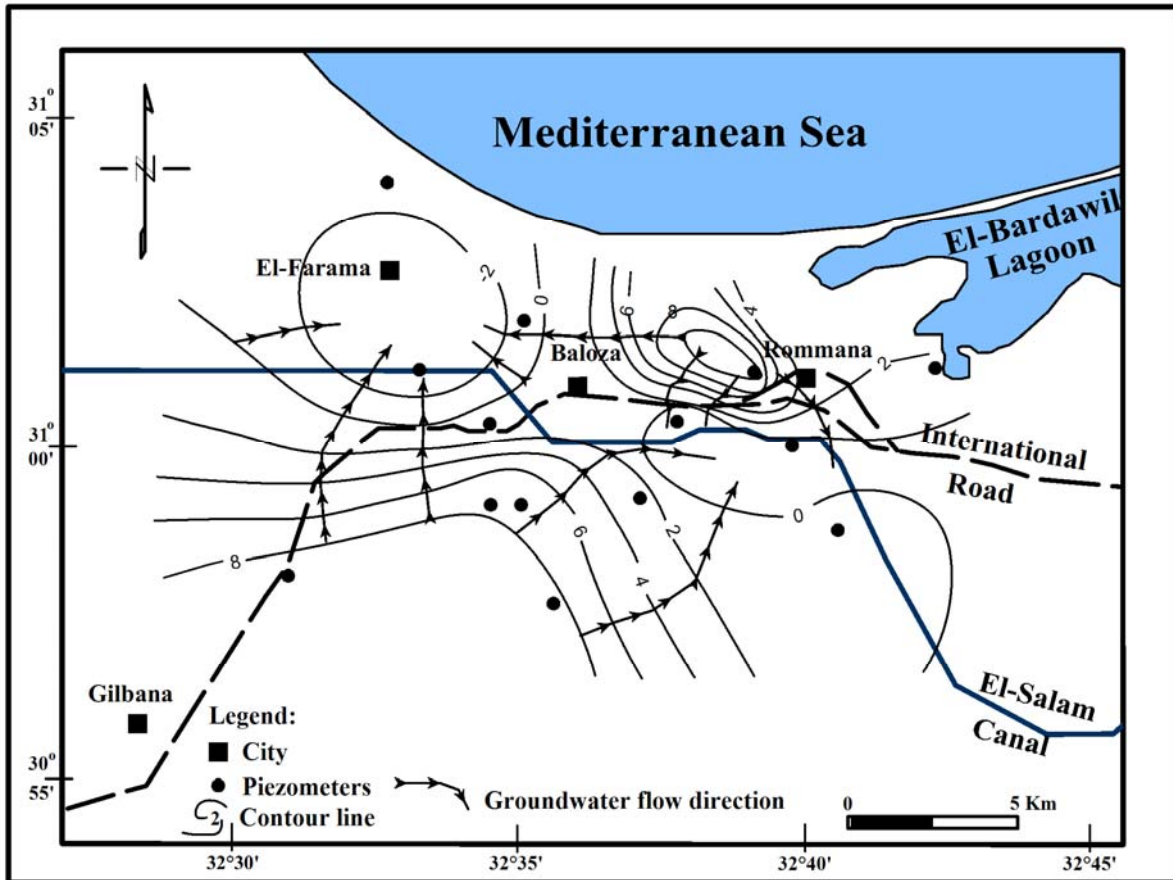
Fig. (3): Water table contour map of Baloza-Rommana area (April, 2009) with groundwater flow direction.

Potassium represents the least dominant cation; ranges from about 3 to 80 mg/l with an average of about 21 mg/l (Fig. 4 & Table 3). Sodium represents the dominant cation in the majority of the analyzed groundwater samples; varies between 86 and 2902 mg/l with an average of 1121 mg/l (Fig. 4 & Table 3). The highest Na^+ concentrations are recorded near the Mediterranean Sea. Sodium, chloride and sulphate ions display a nearly linear increase with increasing salinity of Quaternary groundwater (Fig. 5).

Magnesium ranges between 4 and 571 mg/l. The high values of magnesium reflect the abundance of magnesium salts that formed by marine water intrusion (mainly $MgCl_2$ & $MgSO_4$ salts). Calcium ranges between 20 and 1002 mg/l. Calcium

concentrations increase generally towards northeast direction due to leaching and solubility processes of calcium carbonate rich deposits (Kurkar) due east. Similar conclusions have been suggested by El-Osta (2000).

Chloride ranges between 68 and 4756 mg/l. The highest concentrations are recorded near the Mediterranean Sea due to sea water encroachment. Sulphate content ranges between 108 and 1340 mg/l. In addition to salt water encroachment, the high values of sulphate may be due to dissolution of sulphate-bearing sediments and input from sulphate fertilizers applied in new reclaimed lands. Bicarbonate content ranges between 121 and 416 mg/l with an average of 201 mg/l (Table 3).

Table (1): Results of chemical analyses for the surface water samples in the area Northwest Sinai, Egypt (April, 2010).

| Ser. No. | Locality | EC | TDS | pH | Cations | | | | Anions | | | | NO_2^- &NO_3^- | PO_4^{-3} | Water parameters | | | | |
					K^+	Na^+	Mg^{++}	Ca^{++}	Cl^-	SO_4^{--}	CO_3^{--}	HCO_3^-			SAR	Na^+ %	MH	RSC	TH
1	Municipal water (mosque in 6th October village)	1.02	952	8.24	12.9	174	55	40	238	197	0.0	235	1.22	1.40	4.2	52.6	69.2	-2.7	325
2	El-Salam Canal at pumping station No. (5)	2.90	2518	9.29	46.0	656	68	148	1208	257	66.0	134	0.08	0.35	11.2	66.8	43.0	-8.6	650
3	El-Salam Canal at pumping station No. (6)	2.20	1549	8.97	26.5	532	8	40	500	302	19.8	141	0.44	1.40	20.0	87.4	25.1	0.3	134
4	Water logged area at Baloza El-Shohat road	38.20	23160	7.32	114.9	6999	717	521	11572	2968	0.0	269	0.05	1.75	46.7	77.6	69.4	-80.6	4251
5	Mediterranean Sea.	48.20	34252	8.07	938.5	9749	1434	440	18631	2857	0.0	201	0.17	0.35	50.7	72.1	84.3	-137.0	7000
6	El-Bardawil Lagoon.	57.20	37416	7.95	24.6	12948	371.9	721	19740	3368	0.0	242	0.29	1.05	97.5	89.3	45.9	-62.7	3332

Table (2): Results of chemical analyses for the groundwater samples of the Quaternary aquifer in the area Northwest Sinai, Egypt (April, 2010).

| Ser. No. | Locality | EC | TDS | pH | Cations | | | | Anions | | | | NO_2^- &NO_3^- | PO_4^{-3} | Water parameters | | | | |
					K^+	Na^+	Mg^{++}	Ca^{++}	Cl^-	SO_4^{--}	CO_3^{--}	HCO_3^-			SAR	Na^+ %	MH	RSC	TH
1	Mohamed Abd Al-Rahman	5.92	4296	7.92	17.2	1091	95	341	1906	686	0.0	161	0.39	1.05	13.5	65.2	31.4	-22.2	1241
2	Farm south El-Shohat road	5.78	3808	7.34	15.3	894	109	293	1702	660	0.0	134	0.07	0.70	11.3	61.8	38.1	-21.4	1181
3	El-Akharsa (1)	9.02	5783	7.26	20.0	1534	340	100	3063	584	0.0	141	0.86	1.05	16.4	66.6	84.8	-30.7	1650
4	El-Akharsa (2)	6.08	4505	7.14	18.6	1210	103	301	2042	629	0.0	201	0.90	0.70	15.3	68.7	36.1	-20.2	1176
5	El-Shohat	8.81	6697	7.08	18.6	1961	255	80	3403	784	0.0	195	0.00	0.70	24.1	77.0	84.0	-21.8	1250
6	Amr Tawfik	7.27	3799	7.50	15.3	614	316	301	1702	718	0.0	134	0.15	1.05	5.9	39.2	63.4	-38.8	2051
7	Ali Selim	6.32	4385	7.54	41.5	1190	67	220	2042	556	0.0	268	1.05	0.70	18.0	74.6	33.3	-12.1	825
8	Masoud Abo El-Sood (1)	1.97	1574	7.74	21.5	451	34	60	400	225	0.0	383	1.76	1.05	11.5	75.5	48.2	0.5	290
9	Masoud Abo El-Sood (2)	0.81	820	7.47	19.3	86	12	116	68	143	0.0	376	2.16	1.40	2.0	33.8	14.7	-0.6	340
10	Salama Nasaar	4.93	4443	7.60	20.1	931	272	252	2246	600	0.0	121	0.35	1.05	9.7	53.3	64.0	-33.0	1751
11	Fathy	7.22	3895	7.42	14.4	759	122	441	1634	724	0.0	201	0.25	1.05	8.2	50.5	31.2	-28.7	1601
12	Hanan Kamal	19.75	9384	7.44	80.4	2902	58	481	4756	905	0.0	201	0.81	2.10	33.2	80.3	16.6	-25.6	1441
13	Yasser El-Sayd (1)	5.25	3549	7.87	38.3	631	168	333	1787	324	0.0	268	10.03	0.70	7.0	46.6	45.3	-26.0	1521
14	Yasser El-Sayd (2)	10.56	6566	8.19	56.5	2179	56	200	3403	470	0.0	201	2.85	1.05	35.0	85.5	31.5	-11.3	730
15	Ebrahem Salem	16.57	8697	7.00	27.2	2591	407	20	4254	1270	0.0	128	3.34	1.75	27.1	76.2	97.1	-32.4	1725
16	Mohamed El-Atar	6.32	4242	6.90	15.7	635	365	361	2042	622	0.0	201	0.95	0.70	5.6	36.3	62.4	-44.7	2401
17	Mahmoud Hussein	6.22	3760	7.34	20.0	635	304	200	1872	581	0.0	148	0.27	0.70	6.6	43.7	71.4	-32.6	1751
18	Abu Elgolod	4.75	3329	7.67	12.5	865	80	212	1566	473	0.0	121	1.06	0.70	12.8	68.2	38.3	-15.2	860
19	Farm beside lifting station 46,750 km	4.92	3614	7.27	17.9	802	134	301	1702	457	0.0	201	0.14	0.70	9.7	56.8	42.2	-22.7	1301
20	Mohamed Abdallah	9.20	3152	7.26	31.6	319	105	557	1361	644	0.0	134	1.30	0.70	3.2	27.1	23.6	-34.2	1821

Table (2): Cont.

Ser. No.	Locality	EC	TDS	pH	K⁺	Na⁺	Mg⁺⁺	Ca⁺⁺	Cl⁻	SO₄⁻⁻	CO₃⁻⁻	HCO₃⁻	NO₂⁻ &NO₃⁻	PO₄⁻³	SAR	Na⁺ %	MH	RSC	TH
					Cations				Anions							Water parameters			
21	Sabry Ali	1.95	1437	7.59	11.4	160	182	100	681	168	0.0	134	0.29	0.70	2.2	25.5	75.0	-17.8	1000
22	Mohamed Salah	15.10	6495	6.84	61.3	1613	95	541	3063	987	0.0	134	0.71	2.80	16.8	65.8	22.4	-32.7	1741
23	Abdalla Khalifa	2.05	2072	7.40	15.4	432	80	160	817	159	52.8	356	0.93	4.20	6.9	55.6	45.1	-7.0	730
24	Salama Abdel -Allah	7.17	5280	7.45	12.3	1451	85	381	2723	454	0.0	175	0.93	1.05	17.5	70.5	26.9	-23.2	1301
25	Seliman Maqbol	5.46	4467	7.10	20.7	511	571	261	2424	444	0.0	235	16.22	1.75	4.1	26.9	78.3	-56.2	3001
26	Farm in Taia village	5.00	5692	7.50	15.3	1795	90	224	3063	343	0.0	161	0.15	0.35	25.6	80.4	39.7	-16.0	930
27	Farm to the south of Taia village	11.04	7165	7.12	13.4	1915	97	581	3744	613	0.0	201	1.03	1.05	19.3	69.0	21.6	-33.8	1851
28	Abdel-Baset Elsaid	10.06	7707	7.18	23.6	1923	523	40	4084	771	0.0	342	0.29	1.75	17.6	64.7	95.6	-39.4	2250
29	Um Oqba	5.66	6862	7.40	28.7	2165	122	257	3725	444	0.0	121	0.61	1.05	27.9	80.0	43.8	-20.8	1141
30	Seliman Hassan	0.67	692	7.59	13.1	95	29	100	78	108	66.0	268	0.57	1.05	2.1	34.8	32.4	-0.8	370
31	El-Shahat Ebrahem	11.63	7752	7.05	19.3	1279	365	1002	4186	733	0.0	168	0.88	1.05	8.8	40.8	37.4	-77.3	4002
32	Farm to the north of Taia village	11.45	4777	7.14	26.8	513	231	761	2174	883	0.0	188	1.01	1.40	4.2	27.9	33.3	-54.0	2852
33	Abu Kharab	12.73	7759	6.67	24.3	2131	450	100	4131	735	0.0	188	0.78	1.75	20.2	68.5	88.1	-38.9	2100
34	Mashrqy	11.24	5315	7.07	13.8	749	352	661	2772	619	0.0	148	0.64	5.60	5.8	34.3	46.7	-59.6	3101
35	Abd Alkarim	6.03	4200	7.36	22.9	535	219	641	2178	416	0.0	188	0.59	1.40	4.6	31.5	35.9	-47.0	2501
36	El-Masoody	4.28	2531	7.41	12.3	463	109	252	1021	378	0.0	295	1.08	5.95	6.1	47.9	41.6	-16.8	1081
37	El-Naga	16.90	8600	6.91	40.8	1620	462	782	4171	1337	0.0	188	0.52	5.25	11.3	47.4	49.3	-74.0	3852
38	Hassan Hammad	6.18	4609	7.20	15.0	695	182	701	2314	533	0.0	168	1.13	0.70	6.0	37.5	29.9	-47.3	2501
39	El-Tophaha	5.51	2337	7.39	8.5	297	149	313	956	479	0.0	134	0.32	3.85	3.5	31.5	43.9	-25.7	1393
40	El-Zarawita	6.99	5477	7.46	15.4	1094	238	489	2723	784	0.0	134	0.34	4.55	10.1	51.7	44.4	-41.8	2199
41	Salem Ali	6.63	5697	7.43	8.5	1400	171	393	2927	664	0.0	134	0.24	4.55	14.8	64.2	41.7	-31.5	1683
42	El-Fater	6.78	6144	7.33	11.5	1609	236	329	3403	394	0.0	161	0.27	3.50	16.5	65.9	54.2	-33.2	1793
43	Abu Mahmoud	1.86	1800	7.78	8.6	281	135	140	608	298	0.0	329	3.07	3.15	4.1	40.0	61.2	-12.7	904
44	Salem Soliman	2.03	2837	7.76	3.1	869	46	76	1361	213	6.0	268	0.68	0.70	19.4	83.1	49.9	-3.0	380
45	Ahmed Ebrahem	9.12	6408	7.29	19.3	1745	122	501	3335	559	0.0	128	0.86	0.70	18.1	68.1	28.5	-33.0	1751
46	El-Himida	9.79	7703	6.89	16.2	2064	277	473	4084	654	105.6	134	0.52	3.50	18.6	65.7	49.1	-40.7	2321
47	Wael Ebrahem	10.97	7948	7.15	13.1	1959	331	493	4325	625	0.0	201	0.29	4.90	16.7	62.0	52.5	-48.6	2593
48	Ragy El-Magfra (2)	1.20	954	7.82	7.9	264	4	20	102	140	0.0	416	0.96	0.70	14.0	88.2	25.4	5.5	67
49	Farm in Bir El-Abd city	2.08	1569	7.44	15.7	301	49	140	613	162	0.0	289	21.62	1.05	5.6	53.4	36.3	-6.3	550
50	Aisha El-Heed	15.93	8257	7.28	17.7	1821	397	573	3948	1340	0.0	161	1.42	5.25	14.3	56.2	53.2	-58.6	3063

Units in mg/l except pH, Electrical Conductivity (EC) in mmhos/cm at 25°C, Sodium Absorption Ratio (SAR) and Residual sodium carbonate (RSC) in epm, Total Hardness (TH) in mg/l, 1 mg/l of nitrate-N is equivalent to 4.5 mg/l of nitrate-NO₃ (Bauder *et al.,* 2004). Magnesium hazard (MH) % = (Mg²⁺×100)/ (Ca²⁺+Mg²⁺), where all ionic concentration expressed in equivalent per million (epm), according to Szabolcs and Darab (1964). Na % = (Na⁺) x 100/ (Ca²⁺ + Mg²⁺ + Na⁺ + K⁺), where the concentrations of ions are expressed in epm (Wilcox, 1955). TDS are the summation of anions and cations.
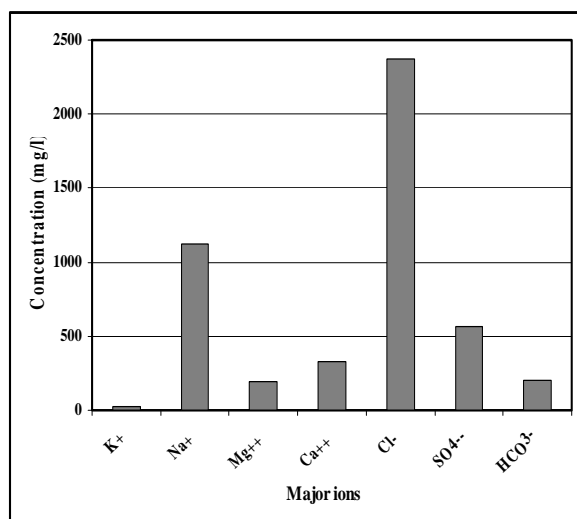
Fig. (4): Average values of major ions (mg/l) for the groundwater samples of the Quaternary aquifer, northwest Sinai, Egypt.
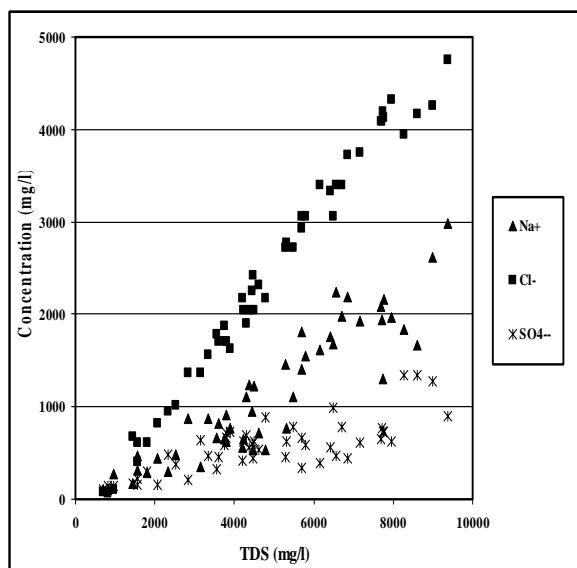
Fig. (5): Relationship between TDS and chloride, sodium, and sulfate concentrations (mg/l) for the groundwater samples of the Quaternary aquifer in the area northwest Sinai, Egypt.

Table (3): Concentration ranges for the measured constituents, water parameters and hydrochemical ratios in the groundwater samples of the Quaternary aquifer in the area northwest Sinai, Egypt.

		Minimum	Maximum	Average
pH values		6.67	8.19	7.36
E.C. (mmohs/cm at 25ºC)		0.67	19.75	7.42
TDS (mg/l)		692	9384	4817
Ions (mg/l)	**K$^+$**	3.1	80.4	21.2
	Na$^+$	86	2902	1121
	Mg^{+2}	4	571	196
	Ca^{+2}	20	1002	333
	Cl$^-$	68	4756	2374
	SO$_4^{-2}$	108	1340	570
	HCO$_3^-$	121	416	201
	PO$_4^{-3}$	0.35	5.95	1.90
	NO$_2^-$&NO$_3^-$	0.0	21.6	1.75
Water parameters	**SAR**	2	35	12.8
	Na$^+$ %	25.5	88.2	56.5
	MH%	14.7	97.1	46.8
	RSC	-77.3	5.5	-29.3
	TH	67	4002	1638
Hydrochemical coefficients (ratios)	**rNa/rCl**	0.33	3.99	0.83
	rMg/rCl	0.04	1.09	0.29
	rCa/rCl	0.01	3.03	0.38
	rCa/rMg	0.03	5.81	1.57
	rSO$_4$/rCl	0.08	1.55	0.26
	r(Cl-Na)/rCl	-2.99	0.67	0.17
	rCl/r(HCO$_3$+CO$_3$)	0.3	57.3	23.5

SAR: Sodium absorption ratio in epm, RSC: Residual sodium carbonate in epm, MH: Magnesium hazard in %, TH: Total hardness in mg/l.

The comparison of the average values of the calculated hydrochemical ratios of the Quaternary groundwater with those of sea water indicates that all ratios are higher than sea water except r(Cl-Na)/rCl ratio (Fig. 6 and Table 3).

Ion exchange between intruding sea water and fresh water aquifer matrix results in an increase in the rCa/rMg ratio when compared to the sea water ratio (Daniele *et al.*, 2010). Thus, the values of rCa/rMg ratio in 32% of the groundwater samples approach the value of the Mediterranean Sea sample (0.19) reflecting the effect of sea water intrusion. The calculated values of rSO$_4$/rCl of the groundwater samples 14, 26, 29, 42 and 47 are around the sea water value (0.11). This may also reflect the effect of sea water encroachment. Meanwhile, ninety percent of the samples are more than the sea water value, which may be due to the effect of evaporation and agricultural activities (Fig. 6).

In addition, the majority of Quaternary groundwater samples (70%) has values of rCl/r(HCO$_3$+CO$_3$) ratio more than 15.5, which indicates highly contaminated groundwater. The rest of samples (30%) vary between moderately contaminated and injuriously contaminated groundwater (according to Simpson, 1946). Thus, the Quaternary groundwater aquifer is highly contaminated with salt water intrusion.

The analyzed Quaternary groundwater samples comprise two main water types; CaCl$_2$, MgCl$_2$, reflecting the marine affinity (Fig. 7). The CaCl$_2$ type (48% of the groundwater samples) represents the hydrochemical composition of old marine water genesis. The MgCl$_2$ type (represents 42% of groundwater samples) reflects normal sea water composition. El-Salam Canal sample (2) belongs to this type. The NaHCO$_3$ water type (samples 8 & 48) suggests a meteoric origin and corresponds to surface running water or shallow water conditions. The Na$_2$SO$_4$ water type (samples 9, 30 & 44) corresponds to the deep meteoric water percolation (Fig. 7).
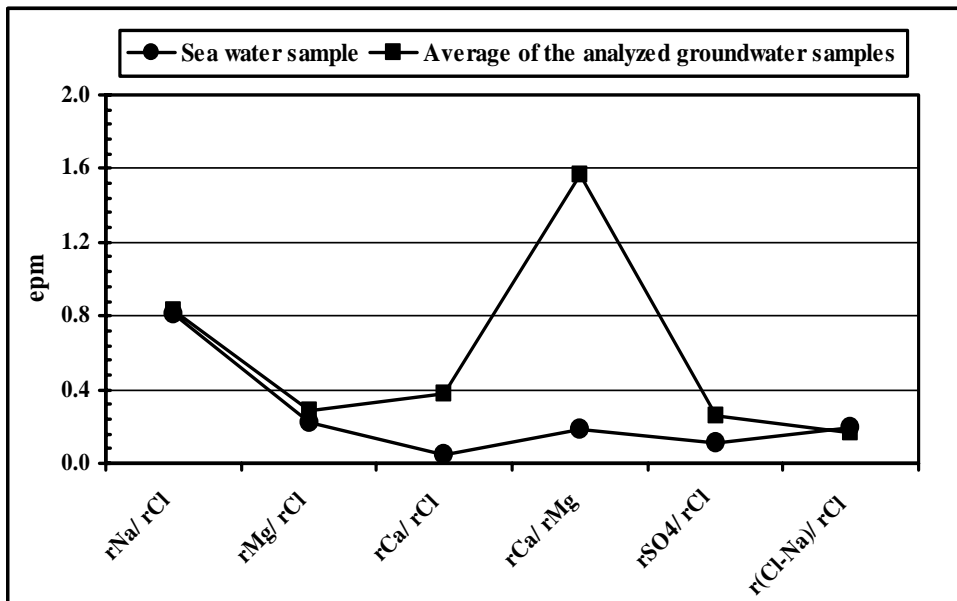
Fig. (6): The hydrochemical ratios in the Quaternary groundwater in the study area compared with sea water.
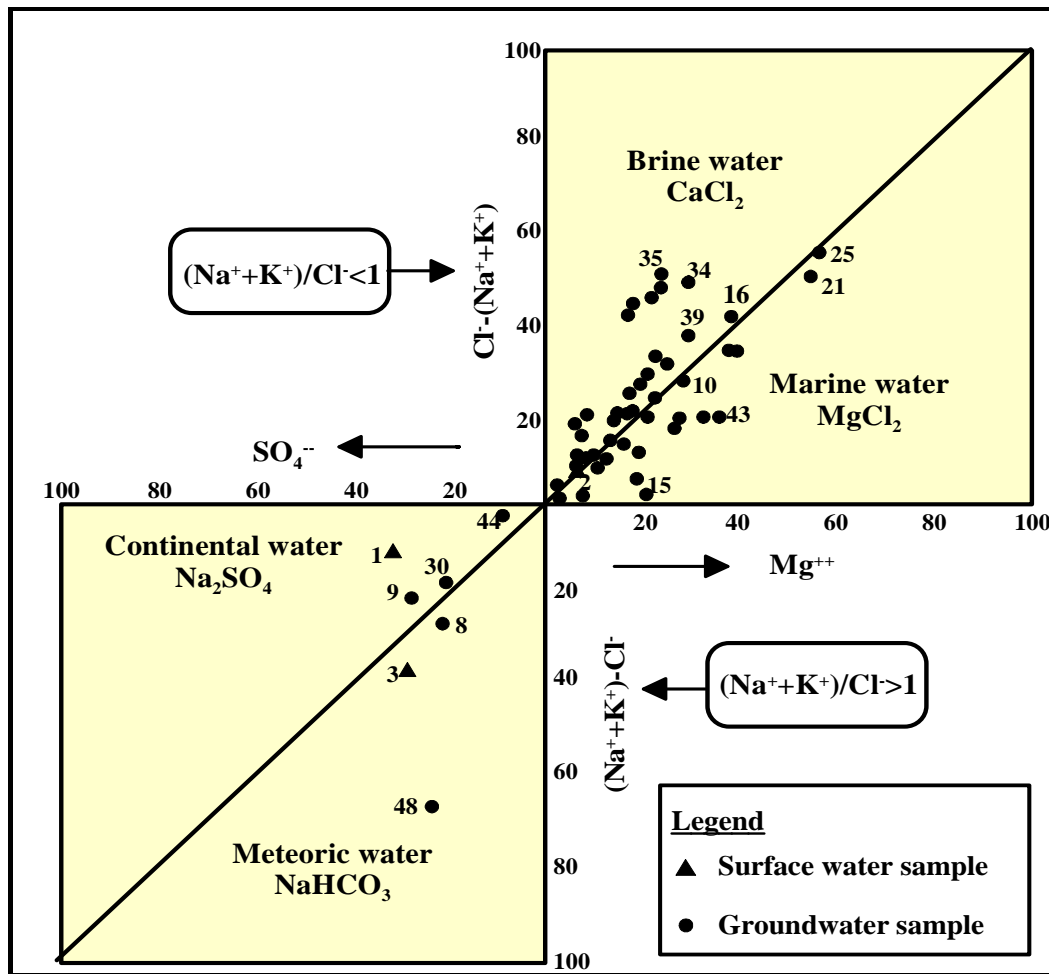
Fig. (7): Hydrochemical classification of water samples using Sulin's (1946) diagram in the area northwest Sinai, Egypt.

Evaluation of Groundwater Quality for Irrigation Purposes:

Several parameters, which affect both the plant and the soil, are used to assess the suitability of groundwater of the Quaternary aquifer of northwest Sinai for irrigation purposes.

Salinity Hazard:

Water with high salinity can limit growth of plants physically, by restricting the taking up of water through modification of osmotic processes. Most of groundwater samples (84%) have very high salinity water (Table 4). They are unsuitable and require high leaching before usage.

Regarding the relative tolerance of crop plants to groundwater salinity (Table 5), only 20% of the examined groundwater samples are suitable for irrigation of sensitive and moderately salt tolerant crops. Forty four percent of the samples can be used to irrigate salt tolerant crops such as sunflower, oats, soy bean, zucchini, broccoli, olive and peach**.** Twenty four percent of the samples can be used to irrigate very salt tolerant crops such as cotton, sugar beet, sorghum and wheat. Only 12% of the water samples are recommended to irrigate saline tolerant crops such as barley (grains) and tall wheat grass (Table 5 and Fig. 8).

Table (4): Classification of collected samples based on salinity hazard (Fipps, 1996).

Classes of water	Samples	%
Class 1, Excellent (TDS < 175 mg/l)	-	-
Class 2, Good (TDS= 175-525 mg/l)	-	-
Class 3, Permissible (TDS= 525-1400 mg/l)	9, 30 & 48	6
Class 4, Doubtful (TDS= 1400-2100 mg/l)	8, 21, 23, 43 & 49	10
Class 5, Unsuitable (TDS > 2100 mg/l)	1, 2, 3, 4, 5, 6, 7, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 24, 25, 26, 27, 28, 29, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 44, 45, 46, 47 & 50	84
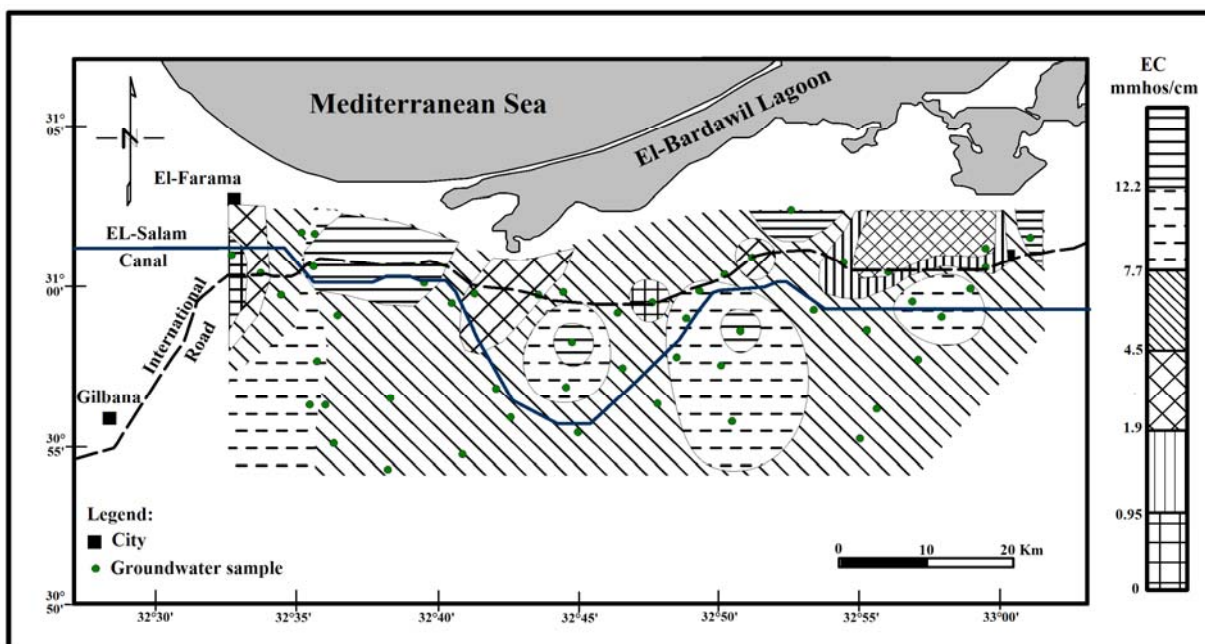
Fig. (8): Salinity zonation map of the Quaternary groundwater samples, northwest Sinai, for irrigation uses (categories classified according to Ayers and Westcot, 1976; and NWQMS, 2000).

Sodium hazard:

While a high salt content (high EC) in water leads to formation of saline soil, high sodium content (SAR) leads to development of an alkaline soil. Irrigation with Na-rich water results in ion exchange reactions: uptake of Na^+ and release of Ca^{++} and Mg^{++} (Khodapanah *et al*., 2009). This causes soil aggregates to disperse, reducing its permeability (Tijani, 1994). SAR of the groundwater samples ranges from 2 to 35 (Table 3). Moderate to high SAR values; causing alkali hazard, are recorded in 48% of the analyzed samples (Table 6 and Fig. 9). The relation between SAR and salinity (Fig. 10) reveals the following:

1- Water of very high salinity and very high SAR (C4S4); this class includes the majority of groundwater samples (50%). These water samples are unsuitable for irrigation in most soils; special soil management and high leaching are required.

2- Water of very high salinity and medium SAR (C4S2); this class contains 18% of the total groundwater samples (samples 6, 16, 20, 25, 32, 33, 35, 36 & 39). This water category is satisfactory for salt tolerant crops and soils of good permeability with special leaching.

3- Water of very high salinity and high SAR (C4S3); this class represents 14 % of the total groundwater samples (samples 10, 11, 17, 18, 19, 34 & 40) and El-Salam Canal water sample (2). These groundwater samples are generally unsuitable for continuous use in irrigation of most soils and require special soil management and high leaching.

Table (5): Relative tolerance of crop plants to groundwater salinity, northwest Sinai, Egypt (adapted from Ayers and Westcot, 1976; and NWQMS, 2000).

Classes of crops	Samples	%	Remarks
Class 1, Sensitive crops (EC> 0.95 mmhos/cm)	9 & 30.	4	**Field crops**: Bean (field), cowpea **Vegetables**: Beans, lettuce, onion, radish. **Fruits**: Avocado, strawberry.
Class 2, Moderately sensitive crops (EC= 0.95-1.9 mmhos/cm)	43 & 48.	4	**Field crops**: Broad bean, corn, flax **Vegetables**: Cabbage, pepper, potato, spinach, sweet corn, tomato. **Forages:** Alfalfa, clover, corn (forage), orchard grass. **Fruits**: Almond, apple, apricot, fig, grape, grapefruit, lemon, orange.
Class 3, Moderately salt tolerant crops (EC= 1.9-4.5 mmhos/cm)	8, 21, 23, 36, 44 & 49.	12	**Field crops**: Groundnut, rice, safflower. **Vegetables**: Beet. **Forages:** Tall fescue, barley hay, trefoil (small), harding grass. **Fruits**: Date palm.
Class 4, Salt tolerant crops (EC= 4.5-7.7 mmhos/cm)	1, 2, 4, 6, 7, 10, 11, 13, 16, 17, 18, 19, 24, 25, 26, 29, 35, 38, 39, 40, 41 & 42.	44	**Field crops**: Sunflower, oats, soy bean. **Vegetables**: Zucchini, broccoli. **Forages:** Bermuda grass, wheat grass**. Fruits:** Olive, peach**.**
Class 5, Very salt tolerant crops (EC= 7.7-12.2 mmhos/cm)	3, 5, 14, 20, 27, 28, 31, 32, 34, 45, 46 & 47.	24	**Field crops**: Cotton, sugar beet, sorghum, wheat.
Class 6, Generally too saline crops (EC> 12.2 mmhos/cm)	12, 15, 22, 33, 37 & 50.	12	**Field crops**: Barley (grains). **Forages:** Tall wheat grass.

Table (6): The sodium hazard of groundwater based on SAR Values (Fipps, 1996).

| Water class	SAR	Remarks	Groundwater samples	%
Low	1-10	Use on sodium sensitive crops such as avocados must be cautioned.	6, 9, 10, 11, 13, 16, 17, 19, 20, 21, 23, 25, 30, 31, 32, 34, 35, 36, 38, 39, 43 & 49	44
Medium	10-18	Amendments and leaching needed.	1, 2, 3, 4, 7, 8, 18, 22, 24, 28, 37, 40, 41, 42, 47, 48 & 50	34
High	18-26	Generally unsuitable for continuous use.	5, 26, 27, 33, 44, 45 & 46	14
Very high	>26	Generally unsuitable for use.	12, 14, 15 & 29	8

Fig. (9): Sodium adsorption ratio (SAR) zonation map of the Quaternary groundwater samples, northwest Sinai, for irrigation uses (categories classified according to Fipps, 1996)

Groundwater may be classified according to Na% (Wilcox, 1955). Regarding the examined Quaternary groundwater samples, Na% ranges from about 26% to 88% (Table 7); good to doubtful for irrigation uses. Ten percent of the Quaternary groundwater samples are unsuitable for irrigation due to high Na% (greater than 80%). Forty percent of the samples belong to the doubtful class of water. Fifty percent of the samples fall within the permissible and good water classes. The Na% zonation of the Quaternary groundwater samples is illustrated in Fig. (11).

Magnesium hazard (MH):

Although calcium and magnesium ions are essential for plant growth but they may associated with soil aggregation and friability (Khodapanah *et al.,* 2009). Magnesium hazard (MH) must be less than 50 to ensure safe and suitable water for irrigation (Khodapanah *et al.,* 2009). In the study area, the MH values range between about 15% and 97% (Table 3). Seventy percentage of the Quaternary groundwater samples have MH< 50% and are considered suitable for irrigation use. The remaining 30% of the samples are unsuitable for irrigation (Table 8 and Fig. 12).

Table (7): Water classes for irrigation purposes according to sodium percent (Wilcox, 1955).

Water class	Na%	Samples	%
Excellent	<20	-	-
Good	20-40	6, 9, 16, 20, 21, 25, 30, 32, 34, 35, 38 & 39	24
Permissible	40-60	10, 11, 13, 17, 19, 23, 31, 36, 37, 40, 43, 49 & 50	26
Doubtful	60-80	1, 2, 3, 4, 5, 7, 8, 15, 18, 22, 24, 27, 28, 29, 33, 41, 42, 45, 46 & 47	40
Unsuitable	>80	12, 14, 26, 44 & 48	10
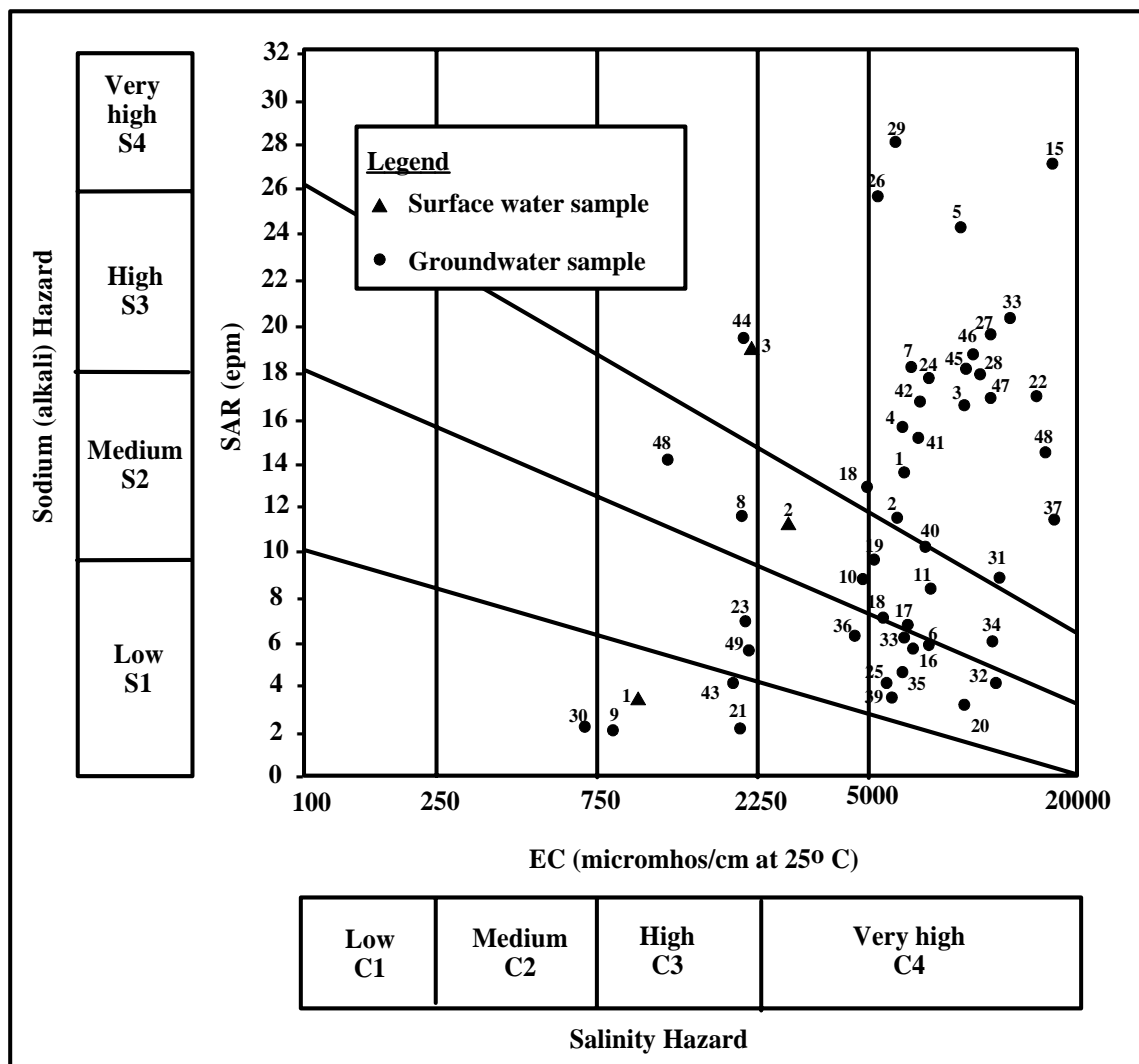
Fig. (10): Classification of irrigation waters (U.S. Salinity Laboratory Staff, 1954) for the analyzed groundwater samples.

Table (8): Classification of the Quaternary groundwater samples, northwest Sinai, according to magnesium hazard (MH), according to Khodapanah *et al.* (2009).

Classes of water	MH range (%)	Samples	%
Excellent	<50	1, 2, 4, 7, 8, 9, 11, 12, 13, 14, 18, 19, 20, 22, 23, 24, 26, 27, 29, 30, 31, 32, 34, 35, 36, 37, 38, 39, 40, 41, 44, 45, 46, 48 & 49	70
Unsuitable	>50	3, 5, 6, 10, 15, 16, 17, 21, 25, 28, 33, 42, 43, 47 & 50	30

Fig. (11): Sodium percent (Na %) zonation map of the Quaternary groundwater samples, northwest Sinai, for irrigation uses (categories classified according to Wilcox, 1955)
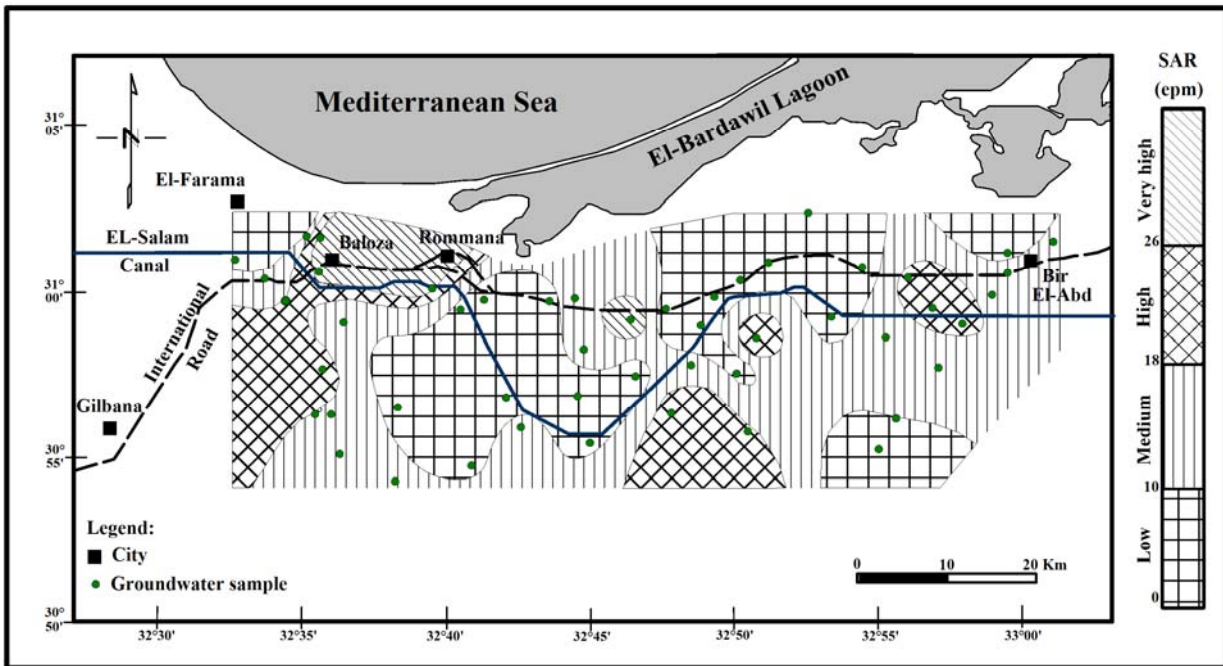

Fig. (12): Magnesium hazard (MH %) zonation map of the Quaternary groundwater samples, northwest Sinai, for irrigation uses (categories classified according to Khodapanah *et al.*, 2009)

Residual sodium carbonate (RSC):

Residual sodium carbonate (RSC) has been calculated to determine the hazardous effect of carbonate and bicarbonate on the quality of water for agricultural purposes. RSC<1.25 is suitable for irrigation purposes (Eaton, 1950). In waters having

high concentration of bicarbonates, there is a tendency for calcium and magnesium to precipitate as the water in the soil becomes more concentrated. As a result, the relative proportion of sodium in the water is increased in the form of sodium bicarbonate (Sadashivaiah *et al.*, 2008). All the analyzed water samples (surface- and ground-water) fall in the suitable class (RSC< 1.25), except groundwater sample 48 (RSC= 5.48 epm) belongs to the unsuitable class and poses bicarbonate hazard (Tables 1 & 2 and Fig. 13).

Total hardness (TH):

Hardness is an indication of the amount of calcium and magnesium in the water; expressed as mg $CaCO_3$/l. Water with hardness less than 150 mg/l (considered moderately hard based on Todd's classification, 1980), is considered desirable for plant growth. All the analyzed surface- and ground-water samples exceed the permissible limit (150 mg/l) and range from hard (150-300 mg/l) to very hard (> 300 mg/l) waters, except surface water sample (3) and groundwater sample (48), (Tables 1 & 2 and Fig. 14).

Nitrogen (NO_3^- & NO_2^-):

Nitrate ion (NO_3^-) is the common form of combined nitrogen in natural water. Nitrogen in irrigation water especially nitrate-nitrogen (NO_3-N), which often occurs at higher concentrations than ammonia in irrigation water and causes quality problems in crops such as barley and sugar beets and excessive vegetative growth in some vegetables (Bauder *et al.*, 2004). However, these problems can usually be overcome by good fertilizer and irrigation management. Regardless of the crop, nitrate should be credited toward the fertilizer rate especially when the concentration exceeds 10 mg/l NO_3-N (Bauder *et al.,* 2004). In the study area, all the analyzed surface and groundwater samples are within the safe limit, less than 45 mg/l, (Tables 1 & 2).

Phosphate (PO_4^{---}):

Phosphate (and nitrate) in surface and groundwater are generally associated with usage of nitrogen and phosphorus fertilizers. The acceptable limit for phosphate concentrations in irrigation water is between 0-2 mg/l (Shahinasi and Kashuta, 2008). All surface water samples and the majority of the analyzed groundwater samples (72%) fall in the permissible class and the rest of samples exceed 2 mg/l, indicating pollution in 28% of the total samples (Tables 1 & 2 and Fig. 15).
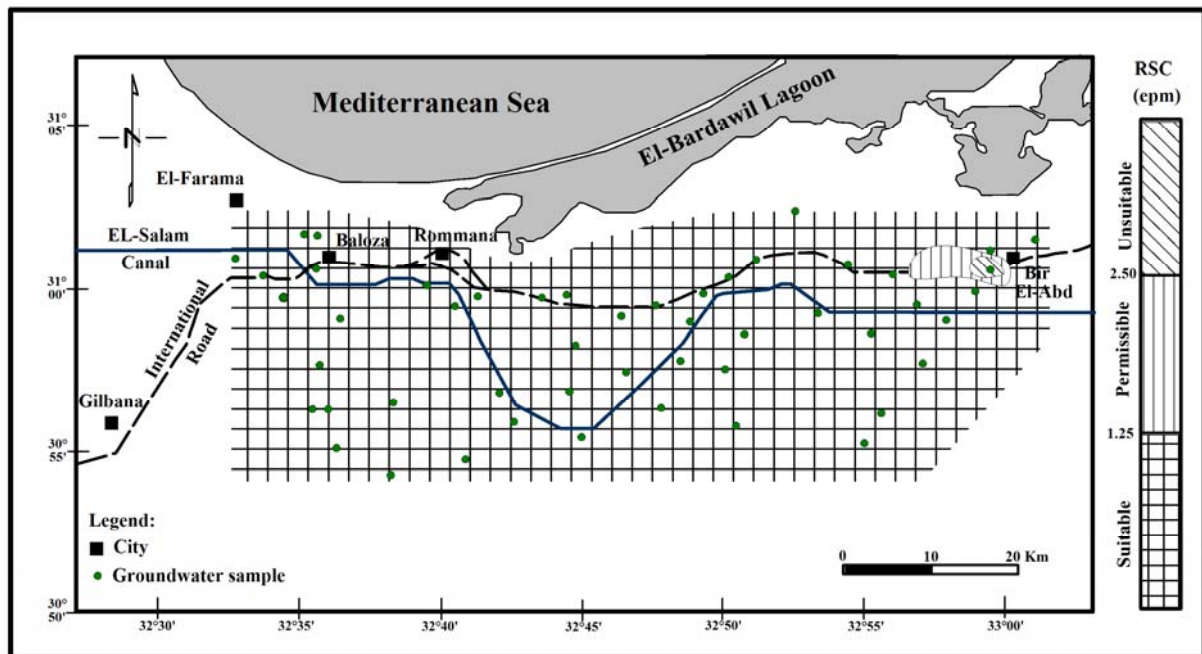
Fig. (13) Residual sodium carbonate (RSC) zonation map of the Quaternary groundwater samples, northwest Sinai, for irrigation uses (categories classified according to Eaton, 1950)

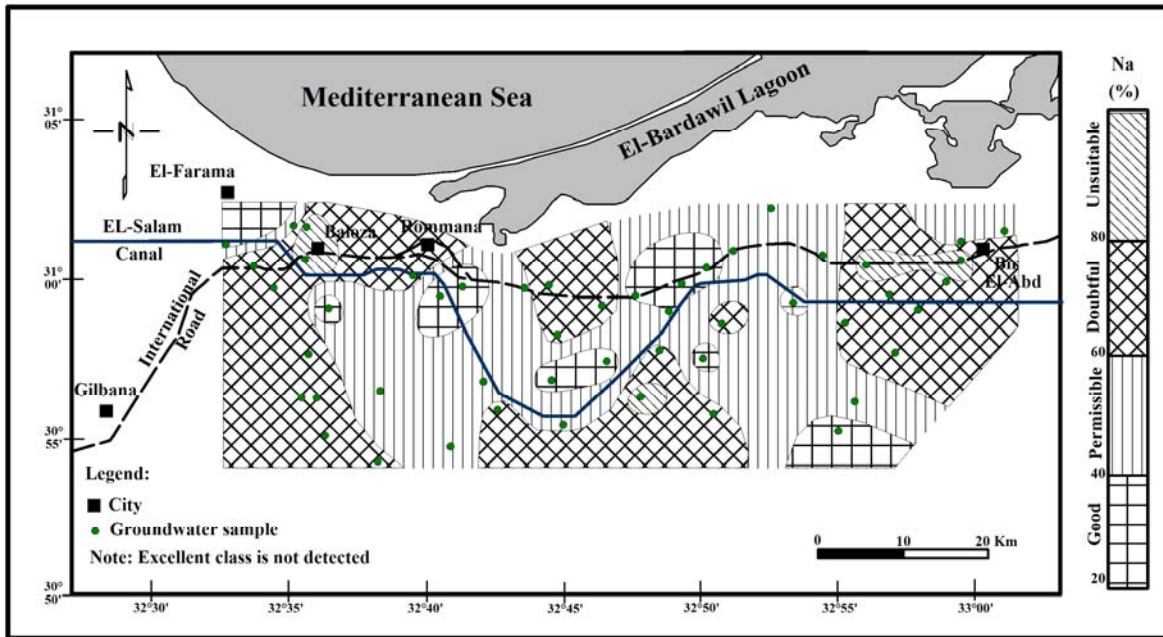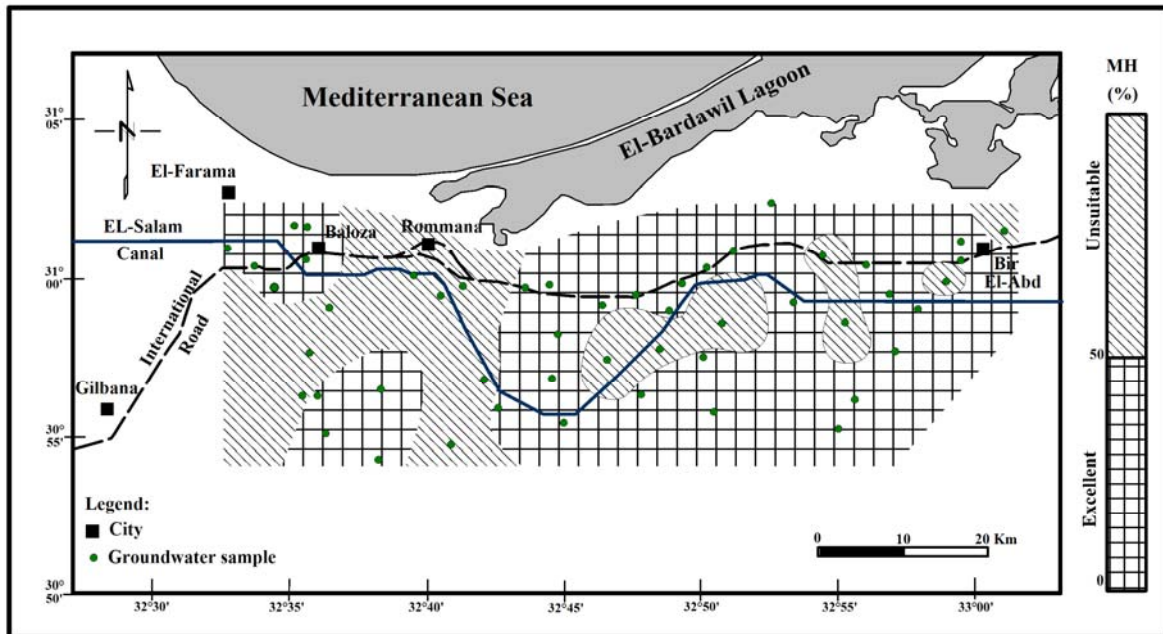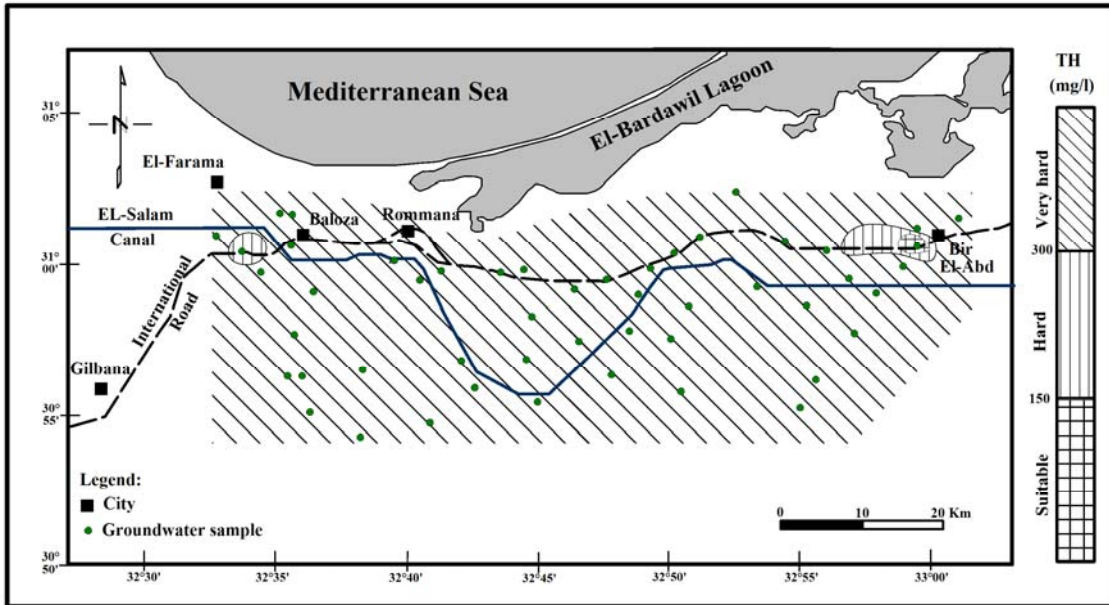Fig. (14): Total hardness (TH) zonation map of the Quaternary groundwater samples, northwest Sinai, for irrigation uses (categories classified according to Todd, 1980).
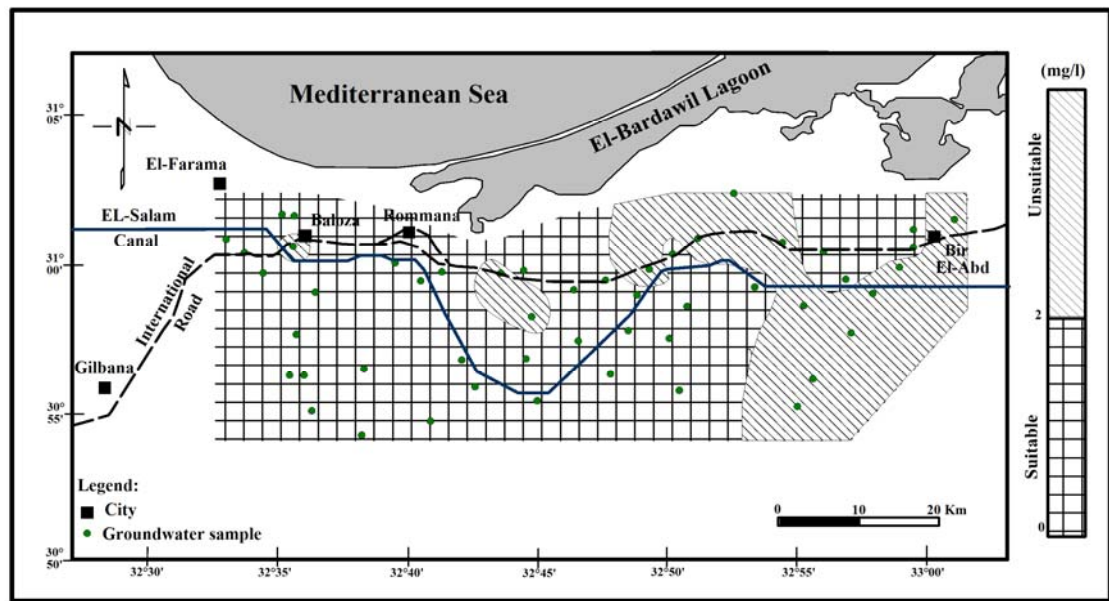
Fig. (15): Phosphate zonation map of the Quaternary groundwater samples, northwest Sinai, for irrigation uses (categories classified according to Shahinasi and Kashuta, 2008)

Excess chloride:

Excess chloride deposited on leaves cause foliar burn (Hopkins *et al.,* 2007). Based on Ayers (1975), chloride concentrations less than 142 mg/l show no chloride toxicity problems which increase if chloride concentration ranges between 142-335 mg/l. Irrigation water with chloride concentration exceeds 335 mg/l causes severe problem of chloride toxicity in most plants. All the analyzed samples of El-Salam Canal and the groundwater of the Quaternary aquifer, northwest Sinai suffer from chloride toxicity as chloride concentrations exceed 355 mg/l, except groundwater samples 9, 30 and 48 (Cl⁻ < 142 mg/l) which show no chloride toxicity problem (Tables 1 & 2).

Damage caused by high-chloride irrigation water can be minimized by planting less sensitive crops; avoiding foliar injury by using furrow, flood, or drip irrigation; and rinsing the plants at the end of each irrigation event if a source of high-quality water is available (Hopkins *et al.,* 2007).

Summary and conclusions:

The Quaternary aquifer is the most important source of water in northwest Sinai for agricultural purposes. It exists under unconfined conditions. The depth to water varies from 0.5 m in the north to 9.1 m below the ground surface and water level ranges between -3.7 m to 10 m in the northeast corner. The Quaternary groundwater is mainly brackish to saline in character with TDS range from 692 to 9384 mg/l. Chloride, sodium and sulphate increase with salinity increasing. The groundwater belongs to two main genetic water types; $CaCl_2$ (48%) and $MgCl_2$ (42%) of the samples, reflecting the marine origin.

Irrigation water quality was determined based on salinity, sodium, magnesium, and bicarbonate hazards, in addition to total hardness, nitrate, phosphate and excess chloride. Accordingly, more than fifty percent of the analyzed samples are unsuitable for irrigation under normal condition and requires special soil management and high leaching. Thus, for agricultural development special management of salinity control and certain kind of plants with good salt tolerance should be considered.

Only twenty percent of the examined groundwater samples are suitable for irrigation of sensitive and moderately salt tolerant crops. Forty four percent of the water samples can be used to irrigate salt tolerant crops such as sunflower, oats, soy bean, zucchini, broccoli, olive and peach**.** Twenty four percent of the samples can be used to irrigate very salt tolerant crops such as cotton, sugar beet, sorghum and wheat. The rest of groundwater samples percent (12%) are only recommended to irrigate too saline tolerant crops such as barley (grains) and tall wheat grass

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A Review of some Ecto-and Endo Protozoan Parasites Infecting *Sarotherodon Galilaeus* and *Tilapia Zillii* from Damietta Branch of River Nile, Egypt

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Abstract: The present study was carried out as a general survey searching for the possible protozoan parasites that can infect the Nile fishes *S. galilaeus* and *T. zillii*. A total of 125 live fish specimens were obtained from Damietta branch of River Nile and El-Sahel canal, Nile tributary. Examination of the investigated fish species revealed that, fishes were infected with eleven parasitic protozoan species belonging to eight genera. These species were: *Apiosoma piscicolum*, *A. conica*, *Scopulata epibranchialis*, *Vorticella* sp., *Ambiphrya ameiuri*, *Amphileptus* sp., *Chilodonella hexasticha*, *Tetrahymena corlissi*, *Trypanosoma mansouri*, *T. syanophilum* and *Trypanosoma* sp. Among the obtained parasites, the following were recovered for the first time in Egypt. *Apiosoma conica*, *Vorticella* sp., *Ambiphrya ameiuri*, *Amphileptus* sp., *Tetrahymena corlissi* and *Trypanosoma* sp. While *S. galilaeus* represent a new host for *Chilodonella hexasticha*. The recorded numerous parasites have pathological effects on the host fish with subsequent economic losses were discussed.
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1. Introduction:

In general protozoa are one of the major sectors of fish parasites that have been long neglected because of its inherent difficulty in studying compared to other larger parasites. Among protozoa, ecto and endo-parasitic protozoa occupy a very important sector as one of the hazardous threats to fish health. These parasites attack the fish causing massive destruction of skin and gill epithelium. Even moderate infection of these organisms on small fish may prove a fatal disease, since the infection may cause the fish to stop feeding (Meyer, 1968; Hoffman, 1970). Parasitic ciliates, particularly sessilines protozoa genera as *Apiosoma*, *Scopulata*, *Ambiphrya* and *Epistylis*, which infect skin and gills of fish. They are obligate parasites, which utilize gills and skin merely as a substrate for attachment. Thus, their pathogenicity is attributed to the mechanical interference with gas exchange activity (Paperna, 1980; Lom and Dykova 1992).

On the other hand endoparastic, trypanosomes are the most dangerous group that probably cause more diseases to fish than any other group of parasites, anemia and edema (Lom and Dykova 1992). In Egypt, fish parasitc protozoa gain a lot of attention (Ali, 1992; Ali et al., 2003-2007; Abdel-Meguid, 1995; Abdel-Ghaffar et al., 1998, 2008).

The present study aims to report on the occurrence of eleven protozoans found infecting two different host fish species. In addition, it will offer a review of some new species to egyptian protozoic fauna as well as add more detailed description of morphological characteristics of these parasites and its pathological effects.

2. Material and Methods

A total of 125 fresh water fish were collected from the River Nile at two locations, which are Demiatta branch near El-Mansoura and El-Sahel canal near Sherbeen city. The collected fish were transported to the laboratory in tank with good aeration. They were kept alive until required in aerated glass aquaria. The collected fish represent two species namely *Sarotherodon galilaeus* and *Tilapia zilliie*.

Fish skin, fins and gills were firstly examined by the naked eye for detection of any macroscopically visible lesions. Samples of mucus were scraped gently from the skin, fins and gills, then spread on a clean slide and freshly examined under phase-contrast microscope for the presence of ectoparasitic protozoans. Some of the positive slides were air-dried and stained according to Klein's dry silver impregnation method. Other positive slides were also air-dried, fixed with absolute methanol and stained with 10% Giemsa stain.

Blood samples were collected from the arteria caudalis using heparinized syringes. Thin blood films were made, air-dried, fixed with absolute methanol and then stained with 10% Giemsa stain for (20-30) minutes (Ali, 1992)

Detected protozoa were examined freshly, stained and identified according to (Shulman, 1984; Kazubski and Migala ,1974; Viljoen and Van As ,1985; Lom and Dykova ,1992). All measurements were taken in micrometers (µm) mean ± SD (range). Figures were drawn with aid of camera lucida.

3. Results

Among 125 examined fishes, 118 fishes were infected with different protozoan parasites. The detected protozoan parasites were classified into two main phyla; Ciliophora (Ciliates) and Mastigophora (Flagellates) that are summarized in (Table 1).

I- Genus: *Apiosoma*
A. piscicolum (Figs. 1A-C &4)

This peritrich is a solitary parasite, its body is goblet- like shaped, tapering towards its scopula by narrow stalk (Figs. 1B &4). The body is divided externally by a groove without cilia, to an oral body part measures 13.2 ±3.3 (9.9-16.5) µm and a basal body part measures 17.1±7.2 (9.9-24.2) µm. This groove is found at nearly one third of the body length from the peristome (Fig. 4). The peristomial lip is narrow and the peristomial disc (adoral spiral) is flat, winding counterclockwise and plunges into the buccal infudibulum. Infundibulum is slightly curved and extends to the non-ciliated groove. Contractile vacuole is large and the food vacuoles are distributed in the oral part only. The compact triangular macronucleus is observed at the level of or just below the groove and measures 13.8±2.8 (11-16.5) µm in length X 12.7±2.8 (9.9-15.4) µm in width (Fig. 1B). The micronucleus is oval and is situated above or alongside the macronucleus and measures 2.2X11 µm. Scopula is broader than stalk, sucker-like disc with undulant margin and measures 4.4(3.3-5.5) µm. Body free of cilia except for peristomial disc (three rows) and measures 33 ±1.1 (31.9-34.1) µm in length X 20.9±1.1 (19.8-22) µm in width. Transverse striations of pellicle are conspicuous and ranged from 33-42 in number (mean 37). Fig. 1C.

2- *A. conica* (Figs. 1D,E & 5)

This is a solitary, stalkless parasite with peculiar, conical body shape, gradually tapering to the scopula. The body measures 32.5±8.9 (21.8-43.2) µm in length X23.6±6.0 (13.8-38.1) µm in width. The non-ciliated groove found more than one third of the body length from the peristome and divided it to an oral body part measures 11.5±4.0 (7-16) µm and a basal part measures 12.1±2.0 (6.8-2.2) µm. The contractile vacuole is large (Fig. 1D). The peristomial lip is narrow and peristomial disc is flat and slanted. The epistomial disc is linguiform and elevated over

cilia of peristomial disc (Fig. 5). Infundibulum is short, curved and extends to the non-ciliated groove (Fig .1E). The compact rounded macronucleus is situated below the groove and measures 14.4±3.2 (11.2-17.6) µm in diameter. The micronucleus is rounded, situated below the macronucleus and measures 3.3 µm in diameter. Scopula broad, sucker-like disc with undulant margin and measures 15.6 µm.

II- genus: *Scopulata*
Scopulata epibranchialis (Figs. 1F, G & 6)

This sessile peritrich is solitary and stalkless. The body is barrel-shaped and measures 38.5±4.4 (34.1-42.9) µm in length X 26.4±3.3 (23.1-29.7) µm in width. The body is divided externally by a non-ciliated groove into nearly equal halves, an oral part measures 17.6±1.1(16.5-18.7) µm length and a basal one measures 19.2±7.2(12.1-26.4) µm length. The peristomial disc is broad and flat. The infundibulum is strongly curved and extends to the groove (Fig .1G). Macronucleus is frequently transverse-ellipsoidal, its transverse axis is longer and measures 14.6±3.6(11-17.6) µm in length X 17.1±2.8 (14.3-18.7) µm in width. It situated just below the groove (Fig .1F). Micronucleus is rounded, situated just below the groove and alongside of the macronucleus and measures 3.9±0.6(3.3-5.5) µm in length X 4.0±0.8(3.3-4.7) µm in width. Scopula is broad and flat (Fig.1G) but usually slightly narrower than the body, sometime bilobed (Fig.1F) and measures 4.4 µm in length (Fig. 6).

III- genus: *Vorticella*
Vorticella sp. (Figs. 1H & 7)

This parasite consists of two main parts, solitary zooid and scopula. The zooid is spherical-shaped and measures 72±6.0(66-78)µm in diameter. The peristomial disc is broad. The epistomial disc is vaulted, slightly elevated above the peristomial lips and slanted (Figs. 1H & 7). The peristomial lip is more or less outwardly and encircles the epistomial disc. The infundibulum is curved and lead to narrow cytopharynx. Large number of different sizes of food vacuoles are observed in fresh specimens. The nuclear apparatus consists of ribbon-shaped macronucleus, often sinuous, situated in the zooid center and measures 33 µm. The micronucleus is very small and far away the macronucleus and measures 1.1-2.2 µm. Scopula secretes contractile stalk provided with myonemes used for shortening and coiling the stalk. Stalk measures 20 µm in length and 6 µm in width. Transverse striations of pellicle are conspicuous and ranged from 70-82 (mean 76) (Fig. 1H). Numerous contractile and food vacuoles are present.

IV- Genus: *Ambiphrya*
A. ameiuri (Figs. 2A,B & 8)

Solitary sessil ciliates with large vase-shaped body. It measures 75.1±14.6(60.5-84.7) µm in length X 45.7±3.9(41.8-50.6) µm in width. The body is divided externally by a permanent equatorial ciliary girdle motionless into an oral part measures 29.7±2.2(27.5-33) µm in length and a basal part measures 17.9±6.9(11-27.8) µm in length (Fig. 8). The peristomial disc represent one turn around the slightly elevated epistomial disc. Infundibulum is triangular in shape (Fig. 2A). Macronucleus is ribbon-like forming an orally situated U-shape sinuous; its limbs descend parallel to each other and ends at the level of the ciliary girdle by hook-like shape. It measures 126.5X4.4 µm. Micronudeus is rounded situated adjacent to one end of macronucleus and measures 4.4X2.2 µm. Food vacuoles are distributed in the oral part. Scopula is in the form of a broad undulate disc but never exceeds the body width (38.6 µm in width). Reproduction in *A. ameiuri* is usually accomplished by binary fission, in which a new organism is pinched off the adult (Fig. 2B).

V- Genus: *Amphileptus*
Amphileptus **sp.** (Figs. 2C & 9)

This ciliate is compressed, long and lanceolate in outline. It measures 53.9±13.8(39.1-70.4) µm in length X 19.5±5.3 (14.2-24.7) µm in width. Longitudinal kineties (7-9) were observed on the right side while the left side bears longitudinal ciliary rows. Along the anterior edge of the body, there is a cytostomial slit which does not exceed one third of the body length. Nuclear apparatus consists of two oval macronuclei which are closely adjacent to each other being separated by only 1.3-1.9 µm distance (Fig. 2C). The macronuclei measure 8.5(8.1-9.0) µm in length X 6.4(5.3-7.5) µm in width. The micronucleus measures 2.7(2.1-3.4) µm in length X 2.2(2.1-3.2) µm in width, is often found in close contact with one of the macronuclei, in the area separating the macronuclei. Large contractile vacuole is always found in the posterior region of the body (Figs. 2C & 9). There are many food vacuoles with various sizes.

VI- Genus: *Chilodonella*
Chilodonella hexastcha (Figs. 2D,E, &10a,b)

The body is typical oval to foliate-shape dorsoventrally compressed and characterized by the presence of a notch at the anterior body margin. It measures 39±6.4(27.9-50.1) µm in length X 28.4±7.2(22.1-40.5) µm in width. The cytoplasm is coarsely granulated ventrally the ciliature of the body composed of right ventral ciliary band, three circumoral kineties and left ventral ciliary band (Fig. 2E). The right ciliary band is arched, long, number of ciliary kineties range from 6-8(mean7) and is meeting with the left one. The three oral kineties; two short circumoral ones in front of the oral opening and a long preoral one extending along the anterior line of contact of the two ciliary bands (Fig. 10a). The left ciliary band is straight, short and number of its kineties ranges from 7-9 (mean 8). There is a non-ciliated zone (naked zone). The cytostome occurs at the anterior part of the naked zone. It leads into a conspicuous cytopharynx (Fig. 2E). Cytopharynx is prominent and reinforced by 8-10 conspicuous nematodesmata (cuticular bands), forming a funnel-shaped tube with curved inner end (Fig. 10b). The cytopharynx may be slightly extruded to serve for boring into and disrupting the epithelial cells. Two contractile vacuoles are present. The macronucleus is rounded and measures 15.4 (13.1-17-8) µm in length X 13.2(12.2-14.3) µm in width. The micronucleus lies closely adjacent to the macronucleus and measures 4.4(3.2-5.1) µm in length X 3.1(2.3-4.1) µm in width.

VII- Genus: *Tetrahymena*
T. corlissi (Figs. 2F,3A &11)

Body is pyriform and measures 38.1±6.3(25.4-46.4) µm in length X 28.6±4.8(21.1-34.7) µm in width. The body is wholly covered with cilia. The number of meridional kineties ranges from 18-32(mean 25). All these kineties converge anteriorly around an apical loop (Fig. 3A). There are many contractile vacuoles with different sizes (Fig. 2F). Macronucleus measures 22.1(20-24.3) µm in length X 14.6(13.2-15.4) µm in width. Micronucleus is far from the macronucleus and measures 4.8(4.4-6.5) µm in length X 3.4(2.2-3.3) µm in width (Fig. 2F). Cytostome is small, oval and is situated at the anterior end (Fig.11).

VIII- Genus: *Trypanosoma*
1- *T. Mansouri* (Figs. 3B-D &12)

This trypanosome is polymorphic, showing three forms: small, intermediate and large (Figs. 3B-D). All morphometric data are shown in Table (2). The intermediate forms were the most abundant.
The general body of three forms is thin elongated and slender in shape and in many times they are curved in S-shape (Fig.12). The anterior end is more acute than the posterior one. The cytoplasm is finely granular and stained light red with Giemsa stain. The nucleus is situated mostly in the anterior half of the body or at least in front of the middle of the body. It is reniform-shaped and occupies the entire width of the body. Kinetoplast is oval. The flagellum originates from the kinetoplast and extends along the border of

undulating membrane. The undulating membrane bends into deep fold in close contact with the body cell and rise above body margin forming 8 to 15 festoons in plicate-shape. Then the flagellum extends beyond the anterior end of the body as a free flagellum. In the small forms, the undulating membrane is narrow, hyaline and produces not more seven folds (Fig. 3D).

2- *T. cyanophilum* (Figs. 3E & 13)

The body is elongated cylindrical, sinuously curved in horseshoe shape (Fig.3E). The anterior end is more acute than the blunt posterior one and both of them are folded back on themselves. The maximum width is at the nucleus level. This species is characterized by its deeply blue stained cytoplasm. The cytoplasm is also finely granular and has scattered vacuoles with different sizes. The nucleus is situated in the posterior half of the body. It is oval, occupies the entire width of the body and stained pink with Giemsa stain. The kinetoplast is oval and close to the posterior end of the body. Highly

conspicuous, sinuous undulating membrane forms strong 8 to 9 folds with about 33-35 festoons (Fig.13). The free flagellum is short. All morphmetric data are shown in Table (3).

3- *Trypanosome* sp. (Figs. 3F & 14)

Body of this species is stout with pointed anterior end and a short snout represent the posterior end. The cytoplasm is finely granular and stained light pink with Giemsa-stain. The nucleus is situated in the anterior half of the body or at least in front of the middle of the body (Fig. 3F). It is rounded and occupies the entire width of the body. The kinetoplast is oval shape lying at some distance from the posterior end. A distinct vacuole is often found in front of the kinetoplast. The undulating membrane is broader, less folded and end with hook-like shape at the level of the nucleus, then it becomes narrow and weakly folded (Fig. 14). The free flagellum is relatively long. Morphometric data are show in Table (3).

Table 1. Protozoan parasites reported in the present study.

Parasites spp.	Host fish species	Site of infection	References
Ph: Ciliophora			
1- Apiosoma piscicolum	Tilapia zillii	gills	Shulman (1984) ; present study
2- A. conica	T. zillii	gills	Shulman (1984) ; present study
3- Scopulata epibranchiails	Sarotherodon galilaeus	skin & gills	(Viljoen and Van As, 1983); present study
4- Vorticella sp.	S. galilaeus	skin	Present study
5- Ambiphrya ameiuri	S. galil	gills	(Lom and Dykova ,1992) ; the present study
6- Amphileptus sp.	S. galilaeus	gills & skin	Shulman (1984); (Lom and Dykova ,1992) ; present study
7- Chilodonella hexasticha	T. zillii S. galilaeus	gills skin	Shulman (1984); (Lom and Dykova ,1992); Ahmed et al., (2000) ; present study
8- Tetrahymena corlissi	S. galilaeus	skin	Shulman (1984); (Lom and Dykova ,1992) ; present study
Phylum: Mastigophora			
9- Trypanosoma mansouri	T. zillii	blood	Mohammed (1978) ; present study
10- T. syanophilum	T. zillii	blood	Mohammed (1978); Ahmed et al., (2000) ; present study
11- Trypanosoma sp.	T. zillii	blood	Present study

Table 2. Measurements (in µm) of various parts for the three forms of *Trypanosoma mansouri* from blood smears of *Tilapia zillii*.

Parameters	Small form	Intermediate form	Large form
Total length of the parasite including free flagellum	40.7(37.4-44)	48.4(46.2-50.6)	61.1(57.2-64.9)
Length of cell body	38(31.9-44)	35.8(33-38.5)	52.3(49.5-55)
Breadth of cell body	3.3(2.2-4.4)	3.3(2.2-4.4)	4.4(3.3-5.5)
Length of free flagellum	5.5(4.4-6.6)	12.7(12.1-13.2)	7.7(6.6-8.8)
Length of nucleus	4.4(3.3-5.5)	3.9(3.3-4.4)	5.0(4.4-5.5)
Breadth of nucleus	2.8(2.2-3.3)	2.8(2.2-3.3)	4.4(3.3-5.5)
Distance from anterior margin of nucleus to anterior end of body.	14.3(12.1-16.5)	12.1(9.9-14.3)	20.9(19.8-22)
Distance from posterior margin of nucleus to kinetoplast	22(18.7-25.3)	20.9(19.8-22)	25.3(24.2-26.4)
Distance from kinetoplast to posterior tip	2.8(1.1-4.4)	2.2(1.1-3.3)	1.1(0.8-1.3)
Length of kinetoplast	1.1(0.5-1.5)	1.7(1.1-2.2)	1.1(0.6-1.5)
Breadth of kinetoplast	0.9(0.8-0.9)	1.0(0.9-1.1)	0.9(0.9-1.1)
Width of undulating membrane	1.1(0.7-1.4)	2.2(1.0-3.4)	1.7(1.1-2.2)

Table 3. Measurements (in µm) of various parts for *Trypanosoma cyanophilum* and *Trypanosoma* sp. from blood smears of *Tilapia zillii*.

Parameters	T. cyanophilum	Trypanosoma sp.
Total length of the parasite including free flagellum	42.5(40.1-44.9)	43.3(41.1-45.2)
Length of cell body	38.2(36.7-39.7)	36.1(34.6-37.5)
Breadth of cell body	2.8(2.1-3.4)	4.2(3.2-5.2)
Length of free flagellum	6.3(5.4-7.2)	7.5(6.6-7.8)
Length of nucleus	4.2(3.5-4.9)	2.2(1.9-2.4)
Breadth of nucleus	2.4(2.2-2.6)	2.1(1.8-2.3)
Distance from anterior margin of nucleus to anterior end of body.	21.2(19.8-22.4)	15.2(14.1-16.2)
Distance from posterior margin of nucleus to kinetoplast	11.8(9.8-13.7)	19.1(18.2-20.1)
Distance from kinetoplast to posterior tip	1.4(0.9-1.9)	2.9(2.3-3.5)
Length of kinetoplast	1.3(1.2-1.4)	1.2(1.0-1.4)
Breadth of kinetoplast	0.7(0.3-1.0)	1.0(0.8-1.2)
Width of undulating membrane	1.2(0.9-1.4)	1.4(1.1-1.7)

Figure 1

Fig. 1. Morphology of Giemsa-stained *Apiosoma piscicolum* (A and B), silver-impreganated (C), Giemsa-stained of *A. conica* (D and E), *Scopulata epibranchialis* (F and G). Note broad and flat scopula arrow heads. Phase-contrast microscope of fresh *Vorticella* sp (H). cs, contractile stalk; cv, contractile vacuole; epd epistomial disc; fv, food vacuoles; in, infundubulum; ma, macronucleus, mi, micronucleus; ncg, non-ciliated groove; prestomial disc; pl, presomial lip; pts, pellicle transverse striations; sc, scopula; st, stalk; z, zooid.

Figure 2

Fig. 2. Morphology of Giemsa-stained *Ambiphrya ameiuri* and two small individuals (A and B), *Amphileptus* sp. (C), silver-impreganated of *Chilodonella hexastcha* (D); Giemsa-stained of *C. hexastcha* and *Tetrahymena corlissi* (E and F) al, apical loop; cb, cuticlar bands; ci, cilia; cp, cytopharynx, ct, curved tube; cys, cytostomal slit; L, left ciliary band; ma1 and ma2, macronucleus 1 and 2; mk, meridional kineties; no, notch; nz, naked zone; pk, preoral kinety; R, right ciliary band.

Figure 3

Fig. 3. Morphology of silver-impreganated *Tetrahymena* corlissi (A); Giemsa-stained *Trypanosoma mansouri* forms (B - D), *T. cyanophilum* (E); *Trypanosoma* sp. (F). fg, flagellum; hum, hyaline undulating membrane; k, kinety; n, nucleus; um, undulating membrane; v, vacuole.

Figs. 4, 5 &6. Line diagram of *Apiosoma piscicolun*
(**4**); *A. conica* (**5**); Scopulata epibranchialis
(**6**). Abbreviations as in Fig. (**1**).

Figs. 9, 10a, b & 11 Line diagram of *Amphileptus*
sp. (**9**); *Chilodonella hexasticha* (**10a**); apical
view of cytopharynx (**10b**); *Tetrahymena
corlissi* (**11**). c, circum oral kineties; co,
cytostomal opening; cy: cytostome; lk:
longitudinal kineties. Other abbreviations
as in Fig. (**2**).

Figs. 7 & 8. Line diagram of *Vorticella* sp. (**7**);
Ambiphrya ameiuri (**8**). ecg, equatorial
ciliary girdle; my, myonemes. Other
abbreviations as in Figs. 1 and 2.

Figs. 12, 13 & 14. Line diagram of *Trypanosoma
mansouri* (**12**); *T. cyanophilum* (**13**); *Trypanosoma*
sp. (**14**); bc, body cell; fo, fold; fs, festoones; ki,
kinetiy; sn, snout. Others abbreviations as in Fig.
(**3**).

4. Discussion:

Surveying of some ectoparasitic protozoa and blood parasites from *Sarotherodon galilaeus* and *Tilapia zillii* revealed the parasitism of these host fishes by 11 species representing 8 genera as follows:

Apiosoma piscicolum

This species was characterized by its goblet-like shaped body, narrow stalk, conspicuous transverse striations of pellicle and triangular compact macro-nucleus. The present parasite showed quite similarity to previously reported apiosomes by (Viljoen and Van As ,1985; Lom and Dykova ,1992).

A. conica

The investigated parasite was characterized by its conical body shape, stalkless and rounded compact macronucleus. This species closely resembles in the general shape and the size of body to *A. conica* reported by Shulman (1984), but the latter differs in site of infection (skin), host (9-spined sticklebacks) and locality (Neva Gulf). So the present parasite represent the first record in Egypt.

Scopulata epibranchialis

(Viljoen and Van As ,1985) created the genus *Scopulata*. Members of this genus are solitary, sessile, stalkless and the body is cylindrical with broad scopula. There are three species in the genus *Scopulata*; *S. constrica*, *S. epibranchialis* and *S. dermata*. However, some distinct features could be revealed between the present *Scopulata* and other three forms. *S. constrica* has a markedly constricted body at the groove. In *S. dermata* has triangular macronucleus. Accordingly, the parasite recorded herein is clearly not *S. constrica* or *S. dermata*. On the other hand the present recorded *Scopulata* conform well to the type specimens of *S. epibranchialis* in the shape of the body, shape and position of macronucleus and micronucleus. Thus the present specimens are comfortably identified as *S. epibranchialis*.

Vorticella sp.

According to (Lom and Dykova ,1992), the members belonging to this genus are free-living organisms and have contractile stalk, single zooid, ribbon macronucleus. The present recorded species conform well to genus characters. As far as our knowledge is concerned, this is the first record of *Vorticella* sp. from skin of *S. galilaeus*. The problem faced the identification of this organism is that it is free-living ciliates and colonize the fishes skin as facultative parasites. Therefore, (Migala and Kazubski ,1972), suggested that a great number of free-living ciliates teem on the skin of debilited,

moribund fish which lacking any defence reaction under adverse environmental conditions. The ciliates prey on the body surface of the fishes and feed on the tissues. The stalk coiling is produced by the contraction of myonems that resides in a helical form.

Ambiphrya ameiuri

Members of the genus *Ambiphrya* are characterized by a cylindrical and permanent equatorial ciliary fringe and macronucleus is in the shape of a long, thin and sinuous ribbon. Within this genus there exists two closely related species, *A. ameiuri* and *A. neobolae*. However, according to (Vijoen and Van As ,1985), *A. neobolae* have a deep constriction above scopula and the macronucleus ribbon shaped extends throughout the body. On the other hand, *A. ameiuri*, according to (Lom and Dykova 1992), has a ribbon-like macronucleus forming an orally situated horseshoe, the tips of which descend into the basal part with no deep constriction. Accordingly, *Ambiphrya* recorded in the present study identified as *A. ameiuri*. *A. ameiuri* described by (Thompson et al., 1947) for the first time from the gills of *Ameiurus melas melas* in North America then introduced in Russia and then in Europe and lastly the present study in Africa. This is the first recorded parasite from *S. galilaeus* gills.

Pathogenicity

All the previously mentioned sessilines utilize gills and skin merely as a substrate for attachment with their scopula. The scopula adheres directly to the substrate often being cemented to it with a thin layer of sticky substance. Heavy infection of these parasites can cause ulcers and may cause the fish to be more vulnerable to bacterial infections and lead to "red sore disease" Durborow (2003).

Amphileptus sp.

According to (Shulman, 1962; Lom and Dykova, 1992), members of this genus have lancet-like bodies bearing longitudinal arched ciliary rows on one side only and two oval macronuclei and single micronucleus. The present amphileptid showed some resemblance to *A. branchiarum* in the shape of the body and nuclear apparatus. However, *A. branchiaum* has large dimensions (56-120X35-70) μm and a larger number of kineties (20-25). The present Amphileptus sp. showed close resemblance to *A. piger* described by (Sonntag and Foissner, 2004) where the latter has body dimensions (55X13) μm and a single contractile vacuole. Due to the scarce literature about *Amphileptus* and the above mentioned differences, it was found to allocate the present parasite under the generic name only.

Chilodonella hexasticha

Shulman (1966) reported that all the members of genus *Chilodonella* are mostly free-living and two serious pathogenic species infecting freshwater fish. Until the 1970 the two parasitic *Chilodonella* species were usually confused, and it was mostly only *C. piscicola* that was recorded. (Kazubski and Migala, 1974) had redescribed the *Chilodonella* species and confirmed the occurrence of the two parasitic species on fish, based on a morphological analysis of the two ciliates. According to (Lom and Dyková, 1992), *C. hexasticha* rarly speices differs from *C. piscicola* the most dominant species in that it lacks a notch at the posterior body margin, has less numerous and more loosely arranged spaced kineties and smaller body size. The present species satisfy the characters mentioned above, and it is identified as *C. hexasticha*. *S. galilaeus* is recorded as a new host for *C. hexasticha.* The present result agrees with that of (Paperna, 1980; Lom and Dykova, 1992; Ahmed et al., 2000).

Pathogenicity

Since these ciliates are morphologically well adapted to adhesion to body surface and gills of fishes and have a rigid projected cytostomal opening, they will be obligate parasites of fishes and directly injure the fishes by boring and disrupting the epithelial cells. (Paperna and Van As, 1983) reported that the parasitium with *C. hexasticha* produced severe gill damage in the form of epithelial hyperplasia, which shrouded the fine respiratory epithelium and led to the death of the fish. Langdon et al. (1985) reported that heavy *C. hexasticha* infestation causes mass mortality among farmed and wild fish, the cases of death involving gill damage and fusion of adjacent filaments.

Tetrahymena corlissi

According to Jerome et al. (1996), the ciliates of genus Tetrahymena comprise at least 33 species. Most of these ciliates are free living, few are infecting various invertebrates. However, in literature we find tetrahymenids infesting fishes is very scarce. Based on the form of the body and the number of kineties, (Lom and Dykova, 1992) identified three species of genus *Tetrahumena* infected freshwater fishes; *T. pyriformis* has 17-21 kineties, while *T. corrlissi* has 25-31 kineties and *T. rostrata*, has 32-35 kineties. The present tetrahymenid is identified as *T. corrlissi*

Trypanosoma mansouri

Polymorphic trypanosome with three forms (small, intermediate and large) and there is no doubt that these forms belong to one species. The three forms resemble each other in general characters, such as position of the nucleus, structure and staining reaction of the cytoplasm and presence of free flagellum. (Tandon and Joshi, 1973) mentioned polymorphism in *T. maguri* from the blood of *Clarias batrachus* from India. Qadri (1962) reported dimorphism of *T. batrachi* from the blood of the same host *C. batrachus*. In Egypt, Mohamed (1978) described *T. mansouri* from the blood of *Chrysichthys auratus* and *Ch. reuppelli* as a polymorphic trypanosome. Comparing the general features of the present trypanosome with those of other previously described from freshwater fishes, it appears that these features resemble those of *T. mansouri* which was originally described by Mohamed (1978) from *Chrysichthys auratus* and *Ch. reuppelli*. The present trypanosome has greater body measurements where, the body length ranges from 57.2-64.9 μm while in *T. mansouri* Mohamed (1978), it ranges from 34.7-50.8 μm.

T. cyanophilum

Monomorphic trypanosome is characterized by its deeply blue stained cytoplasm, nucleus lies posteriorly and well festooned undulating membrane. This species originally described by Mohamed (1978) as dimorphic trypanosome from *Chrysichthys auratus* and *Ch. reuppelli*. Abu El- Wafa (1988) identified this species as *T. tilapiae* from different species of fishes. Later Negm El-Din (1991) synonymized this species with *T. cyanophilum*. The present investigated trypanosoma was in accordance with trypanosome described by Ahmed et al. (2000).

Trypanosoma sp.

Monotrophic parasite, characterized by stout body, nucleus is situated interiorly and presence of a distinct vacuole in front of the kinetoplast. Comparing the morphological description and morphometric data of this species with *T. mansouri* and T. *syanophilum* described in the present study from the blood of the same host *T. zillii* showed significant differences. Therefore the present parasite is tentatively identified as *Trypanosoma* sp.

Pathogenicity

The pathogenic potential of fishes trypanosomes depends on the intensity of infection. The heavy infection induces series of changes as anemia that induced by hemolysins secreted by live trypanosomes which lyse the RBCs and lead to mortality (Lom and Dykova ,1992).

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Political Parties: Extent and Nature

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Abstract: The political party is a means of connecting the political process with society and it helps to improve the political system. In addition, a central feature of any democracy is political party which serve as a vehicle through which citizens can come together freely to define their political and policy aspirations and campaign for public office. In fact political parties have developed alongside democracy, and it is commonly assumed that democracy cannot survive without them. This article is an overview from the extent and nature of the political party in any society.

1. Introduction

The idea of party development was born and developed in the early stages of political sociology. Lipset and Rokkan (1967), Lapalombara and Weiner, (1966) and Duverger (1964) were among those who pioneered sociological models of party formation, and they theorized the idea that parties are formed as part of a social and political maturation process which culminated in the mature, democratic party systems we know today. furthermore, a core feature of any democracy is the political party which serves as a vehicle through which citizens can assemble freely to define their political and policy aspirations, and campaign for public office (National Democratic Institute for International Affairs, 2001). The existence of at least one party occurs even in dictatorial and, indeed, totalitarian systems (Lapalombara and Wiener, 1966). Besides, if we accept the suggestion by Duverger (1964) and Michels (2001) that political parties are often little more than a small group of oligarchs, we cannot disregard their role as a connector between political systems and the community(Almond & Powell, 1966) and as "instruments of civil society" (Biezen, 2004, p. 18).

Consequently, the present paper focuses on the extent and nature of political party in any society and attempts to study the important of existence the political party to improve the democracy and political development. This research serves as a guide for government generally to implement plans which lead to the qualitative and quantitative growth of political party and specifically party organizations to be better prepared to meet and overcome obstacles that prohibit them in their development process. The outcome of this article also assist researchers in the field of development studies and lastly, this study can be as a guideline and reference for other researchers who wish to contribute to the effectiveness of political parties in the future.

Our argument is developed in four sections. First, we explain the method that is applied in this study. In our second section, we describe concept of political party. In third section, we study extent and nature of political parties. Last, we state the conclusion the study.

2. Methodology

The data of this study is gathered through secondary data. Secondary data is research based on secondary resources that already exist (Jennings, 2001). Secondary data also refer to previously published information that may conclude information, historical records and government reports (Yin, 2003).

Documentation is relevant to all case studies as it provides evidence of the phenomenon being studied (Yin, 2003).The secondary data method in current research included, journal articles, books and the internet.

3. Concept of Political Party

According to Lawson (1976) "no definition of party is ever entirely satisfactory but perhaps most useful as well as most common are those that focus on the political acts likely to be performed by party" (p. 2). On the other hand, the concept of a political party is multifaceted and can be defined in a number of ways. To start with, a party is a social organization. It means a party serves as a bridge between society and the government/state and represents the masses.

Anthony Downs (1957) points the "political party is a rational actor, which exists in order to fulfil its ideological goals" (p.28). In sum, he states an organization is considered a political party if it seeks to obtain voter support and power in decision-making. Fred Riggs (1968), for example, sees party as "any organization which nominates candidates for election to an elected assembly" (p.51). Joseph Lapalombara and Myron Weiner (1966) also say an organization deserve to be called the party that set up local units, seek electoral support from the general public, play a part in political recruitment, and be "committed to the capture or maintenance of power, either alone or in coalition with others" (p. 29). In addition, Joseph Schlesinger (1968) notes political party is "the political organization which actively and effectively engages in the competition for elective office" (p. 428).Moreover, other political scientists (Duverger, 1972; Epstein, 1980; J. Lapalombara, 1974; Miners, 1991; Sartori, 1976; Schattschneider, 1942) have critically discussed their understanding in the interpretation on political parties. These scholars point out that the electoral characteristic is the defining feature of a political party. Sartori (1976) defines a party as "any political group identified by an official label that presents at elections, and is capable of placing through elections (free and non-free), candidates for public office" (p. 63). Another author adopts a rather loose definition, and defines a party as "... any group, however loosely organized, seeking to elect governmental office holders under a given label" (Epstein, 1980, p. 9).

Janda (1980) also, supposes parties as "organizations that pursue a goal of placing their avowed representatives in government positions" (p. 5). Moreover, Lapalombara (1974, p. 509) states "a political party is a formal organization whose self-conscious, primary purpose is to place and maintain in public office persons who will control, alone or in coalition, the machinery of government". On the other hand, Duverger (1972) says "... political parties have as their primary goal the conquest of power" (p. 1). Miners (1991) also, gave a concrete picture of definition for this topic "political parties are normally defined as groups which seek to acquire political power through the capture of political office by winning an election" (p. 196).

Since the focus of this article will be extent and nature of political party, the usage of political parties in this study, therefore, adopts a wider role of parties is to act as a link between the state and society.

4. Extent and Nature of Political Parties

The wide literature on the nature and extent of political parties work done by various scholars such as; Lapalombara and Weiner (1966), Samuel Huntington (1993) Duverger (1964) Almond and Powell (1966), Lucian W. Pye (1966), Beyme (1995), Alan Ware (1996), Eldersveld (1964), Lawson (1976), Richard Gunther and Larry Diamond (2003), Mark Dickerson and Thomas Flanagan (1990), and Hein-Anton van der Heijden (2002). All these scholars agree that political parties are importance and necessary to any political system in the world.

Lapalombara and Weiner (1966) suppose that the political party, as a political institution is presented in all forms of the state and in all manner of political systems and governments even dictatorial and, indeed, he emphasizes that totalitarian systems seem unable to do without at least one party.   In other study, Beyme (1995) also demonstrates nature and extent of political parties in liberal democracies and points out to some duties of parties. From point of view, it is articulating and aggregating interests, political communication or the identification of goals; mobilizing and socializing the general public into the political system, elite recruitment and government formation are amongst of parties' duties.

Furthermore, according to Alan Ware (1996) the essence of a party lies in the coordination of individual resources towards the common goal of

exercising power within the state. Samuel Huntington (1993) also, identifies political parties as a key to political stabilization. He stresses that the party as the only modern source which can become a source of authority. He argues that the party creates the state.

Almond and Powell's (1966) classic book on the *Comparative politics: a developmental approach* furthermore, is a very good example of nature and extent of political parties. According to them the extent of political parties lies in political sociability, recruitment, nominate of efficient members for governmental positions; so they conclude, in this way, political parties become influential in stabilization of political structure and its compatibility with the present social context. The Authors also point out to extent of political parties' function and say political parties play a vital role in political structure of countries, because they categorize the plural and various demands into certain general subjects transferring them to the decision-making centre. Such a function makes political parties a connector between the society and the political system. This facilitates the decision-making duty of government because if policymakers are faced with many conflicting demands, which in turn makes the decision very difficult. However, they emphases that in the absence of political parties, rulers rely on strange methods such as scattering rumors which naturally cannot reflect the real demands of people.

Lucian W. Pye (1966) in his book *Party Systems and National Development in Asia* also, highlights nature and extent of political parties in this continent. He points to party systems in Asia and says that Asian politics are caught in a deep dilemma: they cannot get along without political parties, or work well with them. Historically, the introduction of political parties has apparently created as many problems as it has solved; and although by now Asians now have considerable experience in the announcement and living outside political parties, Asia has had pathetically little experience with working party systems.

The extent and nature of political party discussed by Bahar (1942) and Nozari (2001)'s work. They deepen our understanding of extent and nature of political parties in Iran. According of them, Iranian people in order to establish and maintain of civil society created first political parties in 1908.These studies show that in Iran after the formation of the parties, there were great attempts to make this western pattern play an important role in the traditional society of Iran, but due to the opposition of traditional

institutions and lack of people's knowledge about its necessity these attempts were not that successful.

According to Eldersveld (1964) the nature of the party structure is governed by three main factors: it is strongly influenced by environmental pressures, socio-economic conditions and political history through the sub-political culture, namely normative and operational codes adhered to in the power process, and by the time factor to the structural characteristics of the party usually evolve gradually overtime with room allowed to change or shift at critical points along the way.

There are some dissertations that also, done in the context of nature and extent of political parties. For example, on thesis of *The Role of Political Parties for Political System Support in Established and New Democracies* Paskeviciute (2005) collects data from the Comparative Study of Electoral Systems (CSES) 1996-2000 from eight established democracies. This dissertation develops and tests whether and how political parties influence the opinions of citizens in their political system. This research also examines the role of party identification for the support of citizen of the political system and therefore shows citizens identifying with political parties that take positions about the status quo of a political system. The author also states political parties influence system legitimacy by partisanship that facilitates party persuasion of their supporters, political representation, and party competition for government offices. In sum, according to this thesis parties organize the political world because they are the key actors in the operation of governments and parliaments. In addition, parties build the political sphere for many voters because they provide citizens with useful guidelines for dealing with the complexity of the political environment.

In other academic work that conducted by Lawson (1976, p. 1) the author displays that "parties have not been around very long; they are still not fully accepted everywhere as legitimate agencies of political action." He also suggests that,

> Parties are often a nation's most important personnel service. They recruit, process, and send forth a continuous stream of applicants for the top jobs in government. Often, parties themselves accompany victorious candidates into the realm of official decision making; in such cases party headquarters may become the central legislative bodies, and whole party

organizations may become in effect the nation's executive branch, responsible for the faithful execution of policy (p. 2).

In short, Lawson (1976) assumes that the domain of parties can be equal to the domain of politics itself, and all the functions that political systems can be made complete by the party. Furthermore, nature and extent of political parties is related to classifying of party organizations. For example, Duverger's (1964) classic book on *Political parties* is one the important research that have done already in classifying type of political parties. According to him the party system has shown in three main types: the single party, two-party system and a multi party structure.

He explains that "single party system has usually been regarded as a new political structure that developed in the twentieth century, exemplified by the former regimes in Germany, Italy and the Soviet government" (p. 225). According to him the party in a single party system aims to create new elites while creating and fashioning political leaders capable of governing with tight control since the masses themselves do not have the potential for governing themselves. The main difficulty inherent in such a system is the fact that the country's leaders are isolated from the masses.

Duverger also highlights that the two-party is closely associated with the Anglo-Saxon world, although it is neither universal among such countries nor exclusive to them. Two-party systems have exited also in Turkey and some Latin American countries, and gradual evolution towards such a system is becoming apparent in parts of continental Europe. According to him, the two-party system is not monolithic in itself, a fact clearly illustrated in a comparison between British and American models. In Britain, the party structure is highly centralized, particularly in the Labour party. In the United State on the other hand, there is the little organization beyond that of the state, and the power of national leaders and committees is strictly regulated and controlled (Duverger, 1964). Duverger (1964) shows that the type of the multi-party system is a bit difficult to establish, then he points out to the multi-party system in France and Belgium and says that; "the tripartite systems of France or Belgium, for example, show no common features, and there is little similarity between the quadric-partite systems of Scandinavia and Switzerland" (p. 229).

Richard Gunther and Larry Diamond's (2003) study on *Species of Political Parties: A New Typology,* likewise, is an effort to set many of the commonly used conceptions of parties into a coherent framework, and to define new party types whenever the existing models are incapable of capturing important aspects of contemporary parties. They suggest that although for nearly a century, political scientists (e.g. Duverger, 1964; Kirchheimer, 1966; Neumann, 1956) have developed typologies and models of political parties in an effort to capture the essential features of the partisan organizations that were the objects of their analysis for decades but the existing models of political parties are not adequately to capture the full range of variation in party types found in the world today.

Indeed, the researchers classified 15 types of party on the basis of three criteria: (1) the nature of the party's organization (thick/thin, elite-based or mass-based, etc.); (2) the programmatic orientation of the party (ideological, particularistic-clientele-oriented, etc.); and (3) tolerant and pluralistic (or democratic) versus proto-hegemonic (or anti-system). Nevertheless, this typology lacks parsimony; they believe that it captures more accurately the diversity of the parties as they exist in the contemporary democratic world. These can be seen in the figure below, which shows these party types in a two-dimensional array with "organizationally thin" parties towards the left and "organizationally thick" parties towards the right side of the diagram, and with party types that emerged in earlier historical periods towards the top of the diagram, and more recent entrants on the scene appearing towards the bottom.

Mark Dickerson and Thomas Flanagan (1990) also offer classifications for political parties. They separate political parties into five distinct categories: pragmatic, ideological, interest, personal and movement parties. Similarly, H. V. Wiseman (1966) reveals differences among political parties in terms of the style of their performance. He describes parties according to three typologies: a) secular or pragmatic bargaining parties; b) absolute value-oriented ideological parties; and c) traditional parties.

4.1 If extent of parties is limited in the world?

Nevertheless above literatures confirm the scope of functions of political parties in earlier decades but some studies show that extent of parties is limited in the world today. For example, Joseph Lapalombara (2007, p. 149) stresses that "the political party is everywhere in decline. Party identification is weakened. Party legitimacy is problematical. The professionals

have replaced old-line party leaders, and the once-critical party activists or cadres have largely disappeared".

The study of Hein-Anton van der Heijden (2002) shows that political parties in many countries around the world have lost many of their original functions (articulation of demand, political socialization, etc.) and are now part of the state and increasingly are aimed at efficient and effective management, rather than to transform society. According to him from the late 1960s forward, many countries in the world experienced the emergence of so-called new social movements like; women's movement, peace movement, and environmental movement as the emergence of power rivals such as NGOs, mass media and even European Union and Trans European Networks. Therefore, these new players have taken over some functions originally belonging to political parties.

5. Conclusion

The purpose of this overview was to study the extent and nature of political parties. It appears from this overview that political systems cannot be understood without understanding the political parties. In fact, it can be said that all of these researches attempted to demonstrate the nature and extent of political parties to bring the necessity and importance of parties to stability of political regimes in all forms of the state and in all manner of political systems and governments, even dictatorial.

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Pollution assessment of the aquatic resources in the Lagos lagoon system.

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Abstract: Sediment, water samples and fish (*Oreochromis niloticus* and *Chrysichtys nigrodigitatus)from each of Unilag,Ikorodu and Iddo Lagoons in Lagos State, Nigeria were analyzed for the presence* Zinc (Zn), Lead (Pb), Cadmium (Cd), Copper (Cu), Iron (Fe) and Chromium (Cr) using Buck Scientific 200A model, Atomic Absorption Spectrophotometer (AAS).Sediment contain highest concentration of Fe with a value of 113.02mg/kg against 0.96mg/L in water and 3.92mg/kg in fish, fish contain higher concentration of Zn 7.236mg/kg against 3.740mg/kg in sediment and 3.96mg/L in water. Cu is higher in fish 3.7mg/kg followed by water 2.96mg/L and sediment sample 1.163mg/kg.Cd,Cr and Pb were found not to be present in the water sample while these metals were found to be higher in the Sediment sample than fish tissue. Bioaccumulation was observed in tissues of *Oreochromis niloticus* and *Chrysichtys nigrodigitatus* as higher concentrations of metals were observed in fish tissues than in the water in which they live. The concentration of Zn in the water is above the limits permitted by the Lagos State Environmental Protection Agency (LASEPA) of 1.0 mg/L Zn set for water, there should be need for continuous monitoring of these Lagoons for heavy metals/pollution status.

Introduction

Fish, water, and bottom sediment are some of the major aquatic resources of marine environment, and they find their use and applications in all phase of life. The Minamata and Itai-Itai disasters in Japan in the 1960s, caused by poisoning and death of several people through the ingestion of fish contaminated by mercury and cadmium-contaminated rice respectively are still fresh in our memories. Ever since the tragic deaths, environmental pollution by heavy metals has received considerable attention in industrialized countries.

The ecological consequences of discharges of effluents from the Nigerian oil industry and domestic sewage and particularly, the environmental effects of heavy metals released from textile and mining industries have been widespread in recent years. Factors such as high population growth accompanied by intensive urbanization, increase in industrial activities and higher exploitation of natural resources including cultivable land have caused pollution increase. There has been a steady increase in discharges that reaches the aquatic environment from industries. There is an increasing concern regarding the roles and fates of trace metals in the Nigerian environment. Much of this concern arises from dearth of information on the concentration of these metals within the environment. The contamination of seafood by trace metals is a potential problem to man, aquatic organisms accumulate metals to concentrations many times higher than present in water.

Fish is a valuable and cheap food item, and also a source of protein to man. Concern about heavy-metal contamination of fish has been motivated largely by adverse effects on humans, given that consumption of fish is the primary route of heavy metal exposure (Nsikak, *et al*, 2007). In order to effectively control and manage water pollution due to heavy metals, it is imperative to have a clear understanding of their distribution pathways, fate and effect on biota. Some research findings have shown that heavy metals in aquatic environment could accumulate in biota especially fish as they are the most common aquatic organisms at higher tropic level (Olaifa *et al,* 2004).

This study was aimed at determining the concentrations of Zn, Pb, Cd, Cu, Fe and Cr in the sediment, water and fish of Lagos lagoon obtained from Unilag, Ikorodu and Iddo with a view to assessing the pollution status of the aquatic resources of Lagos lagoon.

Methodology
Sample Collection

Surface water, fish and sediment samples were collected from three (3) sample stations Unilag, Ikorodu, and Iddo, with the aids of water sampler, cast nets and van-veen grab respectively. The choice of sampling stations was influenced by coastal activities. Samples were collected from January to March 2009. Water and sediment samples were kept in plastic bottles and black polythene bags respectively. Water samples for heavy metal determination were acidified in pre-cleaned plastic containers on the field. Each sample was collected in an acid-cleaned polypropylene bottle, which was rinsed three times with the sample water prior collection.

The fish samples were kept in the ice pack right from sample station to the laboratory. The fish samples were identified in the laboratory, washed and frozen at -18^0C as soon as possible to avoid loss of sample integrity. Two species - *Oreochromis niloticus* and *Chrysichtys nigrodigitatus* were identified and reported for the fish samples at the study locations.

Sample Treatment and Analysis

The fish samples were allowed to defrost and then dried to constant weight in an oven at 105°C. Sediment samples were air dried and then ground using mortar and pestle and sieved through 2mm mesh sieve to remove coarse materials. A quantity 0.2g each of sediment and fish samples was digested using 0.02M HNO3 and HCl in the ratio 1:3 (aqua regia) in a fume cupboard at 80^0C (Obasoha, *et al,* 2007).

Analysis of all the samples was carried out using a Buck Scientific 200A model, Atomic Absorption Spectrophotometer (AAS) and the values expressed in milligram per litre (mg/l) for water samples and milligram per kilogram (mg/kg) for both sediment and fish samples, (Obasoha, *et al,* 2007).

Results
Heavy metals in Sediment

The mean concentrations of heavy metals in sediment samples are shown in figure 1 below. The highest value for Cu (11.63mg/kg) was observed at Ikorodu; and the least value (7.00mg/kg) was at Iddo location.

Figure 1:   Heavy Metals in Sediment Samples

	Cu x 100	Zn x 100	Cd x 100	Pb x 100	Fe	Cr x 100
Unilag	945	2045	45	611	7615	1363
Ikorodu	1163	3740	152	945	11302	1392
Iddo	700	1833	6	552	9135	1162
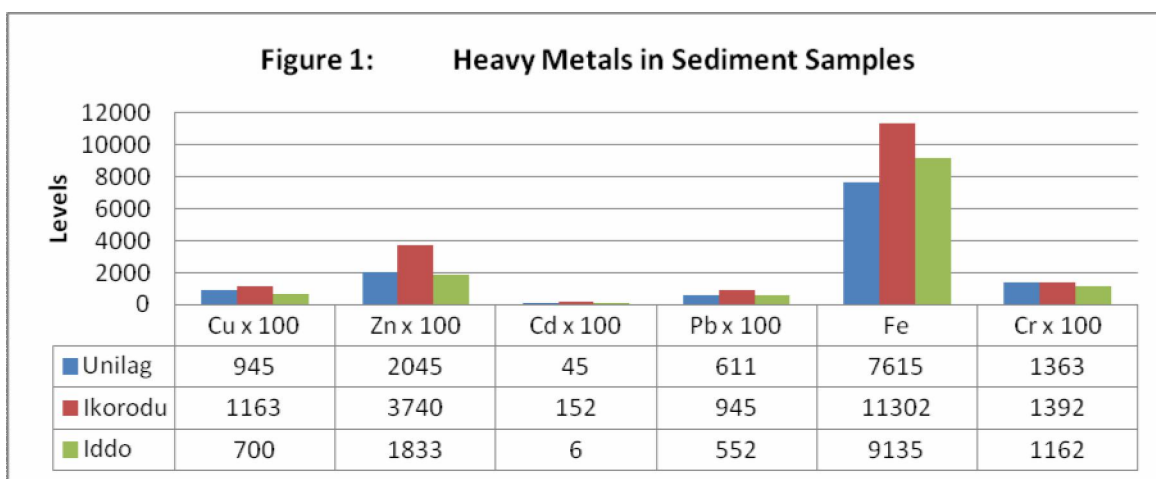
The highest value for Zn (37.40mg/kg) was recorded at Ikorodu, while the lowest (18.33mg/kg) was at Iddo. The highest mean value for Cd (1.52mg/kg) was recorded at Ikorodu, and the lowest (0.06mg/kg) was at Iddo. Lead has the highest value (9.45mg/kg) recorded at Ikorodu and the least (5.52mg/kg) at Iddo station while Fe and Cr has their highest values at Ikorodu, 113.02mg/kg and 13.92mg/kg respectively.

Heavy metals in Water.

The mean values for heavy metals in water samples are presented in figure 2 below. The highest mean value for Cu (1.59mg/l) was recorded at Ikorodu, while the least (0.11mg/l) was recorded at Iddo station. The mean concentration of Zn (2.96mg/l) obtained at Ikorodu was higher than Unilag and Iddo with mean values of (0.28mg/l) and (0.19mg/l) respectively.

Figure 2: Heavy Metals in Water Samples

	Cu	Zn	Cd	Pb	Fe	Cr
Unilag	0.26	0.28	0	0	0.3	0
Ikorodu	1.59	2.96	0	0	0.96	0
Iddo	0.11	0.19	0	0	0.3	0
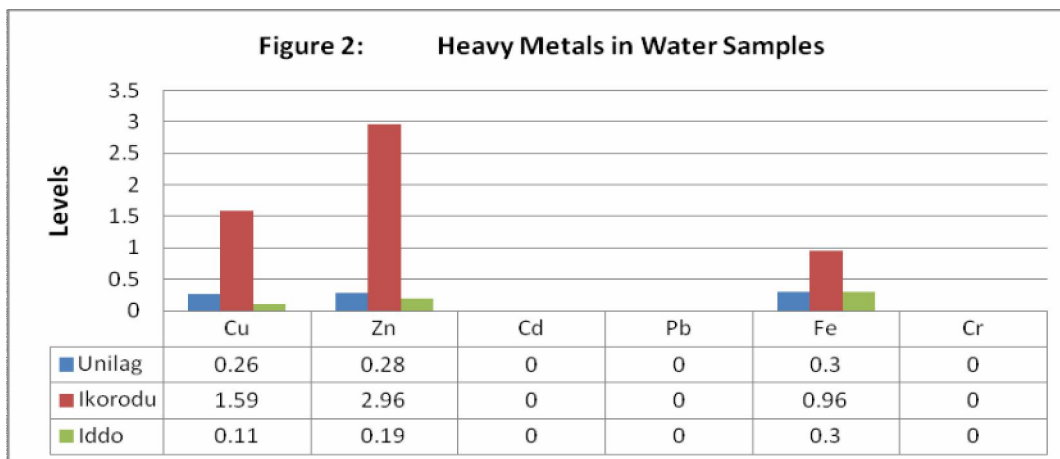
Cd, Pb and Cr were not detected in all the three stations while Fe has the highest mean value (0.96mg/l) recorded at Ikorodu and lowest in Unilag and Iddo with (0.3mg/l).

Heavy metals in Fish.

The highest value of Cu (3.7mg/kg) was recorded in Iddo and the least (2.59mg/kg) in Unilag. Zn has the highest value (7.23mg/kg) in Ikorodu and the least in (6.67mg/kg) in Iddo. Cd has the highest value (0.21mg/kg) in Iddo, and it was not detected in Unilag and Ikorodu. Pb was not detected in Unilag, but has its highest value (1.21mg/kg) recorded in Ikorodu and the lowest (0.71mg/kg) in Iddo. The maximum for Fe (3.91mg/kg) was observed in Unilag and the minimum value (2.82mg/kg) in Ikorodu while Cr has highest value (0.78mg/kg) recorded in Unilag, and the lowest value (0.65mg/kg) in Ikorodu.

Fig 3: Heavy Metals in Fish Samples

	Cu	Zn	Cd	Pb	Fe	Cr
Unilag	2.59	7.12	0	0	3.91	0.78
Ikorodu	3.04	7.23	0	1.21	2.82	0.65
Iddo	3.7	6.67	0.07	0.71	3.3	0.77
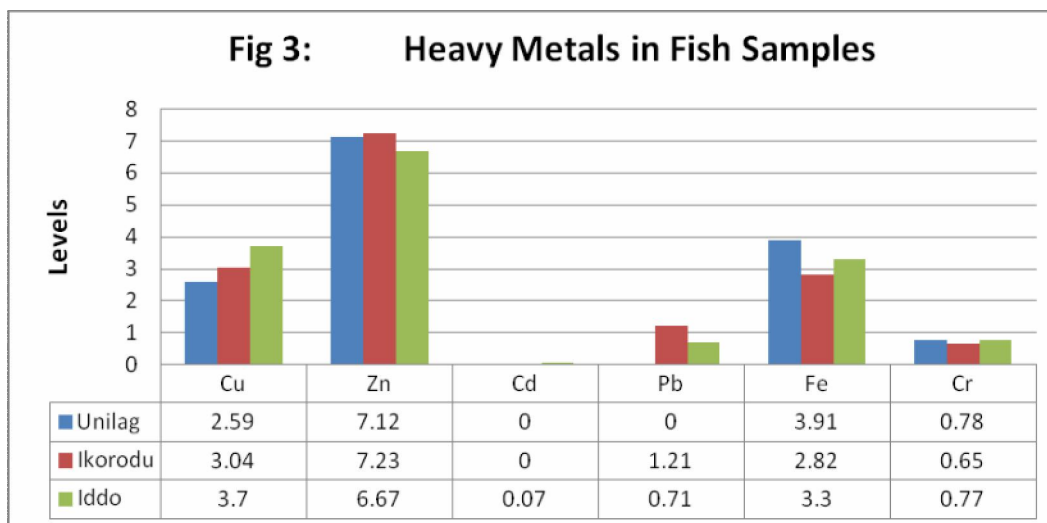
Discussion

The highest metal values in sediment recorded at Ikorodu, may be due to the fact that when metal pollutants are discharged into aquatic environment, they do not remain in aqueous phase but instead adsorbed onto the sediment. Thus, sediment serves as a sink for pollutants, hence the reason for its higher concentration of these metals. This is similar to the findings of Amoo et al (2005) where higher levels of these metals in sediment were obtained than in water of Lake Kanji in Nigeria.

It could also be as a result of many activities in the area which include the metal depot at Owode-Onirin, where metal scraps are deposited for sales. Other activities include the use of metal gears for fishing, dumping of metal containers of domestic sources, abandoned fishing canoes / boats with metal linings, etc. Leachates from these activities gained ingress into the Lagos lagoon through its tributaries. The lowest levels of heavy metals recorded at Unilag station is expected, as less human activity involving the use of heavy metals was not observed at this station.

The ranges of all the metals in the sediment samples from the three stations were on "a" pollutional status i.e. non-polluted environment as quoted in the pollutional status of sediments according to metals concentration by Prater and Anderson, 1977.

All the heavy metals analyzed in this study were detected in water, sediment and fish, except for Cd, Pb and Cr that were not detected in the water samples for all the three stations. The mean values of all the metals in sediment collected at the three sampling stations did not exceed the recommended threshold limits of 124 mg/kg, 0.678mg/kg and 30.2mg/kg set for Zn, Cd and Pb respectively by the United States Environmental Protection Agency (USEPA, 2007).

The concentration of Zn in the water is above the limits permitted by the Lagos State Environmental Protection Agency (LASEPA) of 1.0 mg/L Zn set for water while the mean values of Zn in water samples obtained from the three sampling stations are below the limit of 15.000mg/l recommended by Federal Environmental Protection Agency (FEPA, 1999).

Copper is one of the metals classified as essential to life due to its involvement in certain physiological processes and metabolic activities in organisms. However, elevated levels of Cu have been found to be toxic (Spear, 1981). Fe is found in natural fresh waters and has no health-based guideline value, although high concentrations in water give rise to consumer complaints (WHO, 2004).

The data obtained from this study also revealed that **Oreochromis niloticus** and **Chrysichtys nigrodigitatus** possess the requisite feature for use as bioindicator for heavy metal pollution monitoring.

Conclusion and Recommendations

The tendency of these metals to bioaccumulate in tissue of *Oreochromis niloticus* and *Chrysichtys nigrodigitatus* underscores the need for continuous monitoring of these stations to ascertain its suitability for use as a source of commercial fisheries. Zinc, iron, copper and chromium are essential in human diet. They all play significant roles in metabolic processes. In view of the importance of fish diet to man, it is necessary that heavy metal monitoring of the water and fish meant for consumption should be done regularly to ensure continuous safety of food. Safe disposal of domestic sewage and industrial effluents should be practiced and where possible recycled to avoid these metals and other contaminants from going into the environment.

1/19/2011

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Seasonal Variation Of Heavy Metals In Sediment And Water Of Lagos Lagoon

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ABSTRACT: The concentration of Chromium (Cr), Lead (Pb), Zinc (Zn), Cadmium (Cd) and physicochemical parameters were determined in Surface water and Sediment at different stations in Lagos lagoon during the wet season. The result showed that the concentration of Cr 0.039mg/l, Pb 0.043mg/l, Zn 0.107mg/l, Cd 0.17mg/l in surface water were generally low when compared to WHO standard "2004. The concentration of Zn in the water is within the limits permitted by the Lagos State Environmental Protection Agency (LASEPA) of 1.0 mg/L Zn set for water. The mean levels of heavy metals in the sediment of Lagos lagoon were generally low and fell within the acceptable limits described by WHO,2004 and FEPA.The average concentration for the heavy metals were Cr 0.046mg/g, Pb 0.054mg/g, Zn 0.730mg/g, Cd 0.523mg/g respectively. Comparism of these values with that of the surface water indicates that most metals were adsorbed to the sediment. None of the trace metals investigated were above the maximum permissible level set by world health organization (WHO). For the physicochemistry the salinity ranged from 0.0 ‰ to 3.1 ‰ indicating a typical freshwater condition, higher dissolved oxygen were also recorded this may be due to run-off during the wet season.
[Oshisanya, K.I., Unyimadu J.P., Shelle R.O.D., Nubi A.O, Ladigbolu, I.A., Oguguah N.M., Olumodeji,O.O., Adeleye A.O., Fashade, A.O. **Seasonal Variation Of Heavy Metals In Sediment And Water   Of Lagos Lagoon.** Journal of American Science 2011; 7(3):384-387]. (ISSN: 1545-1003). http://www.americanscience.org.

Key words: Sediment, heavy metals, Surface Water, Lagos Lagoon.

INTRODUCTION

The Lagos Lagoon complex is the largest lagoon systems of the Gulf of Guinea coast in West Africa (Hill and Webb, 1958).In Nigeria over 85% of all industries are situated in Lagos metropolitan area and their effluents ultimately get into the Lagos Lagoon complex directly or indirectly via drainages or streams and pollute the nursery grounds of both fishes and shrimps (Oyewo, 1998).

There is an increasing concern regarding the roles and fates of heavy metals in Nigerian environment. Much of this concern arises from the low level of available information on the concentration of these metals within the environment. The contamination of seafood by heavy metals is a potential problem to man. Human may be contaminated by organic and inorganic

Pollutants associated to aquatic systems by consumption of contaminated fish and other aquatic foods from this environment, aquatic organisms accumulate metals to concentrations many times higher

than present in water. The rate of heavy metal pollutants into natural waters in Nigeria is still largely unknown (Oyewo, 1998).

Heavy metals are vital source of pollution not just because they are toxic above a relatively low concentration but also because they are persistent, remaining in the environment long after the source of pollution has been removed (Voutsinou-Taliadouri, 1981).Contamination with heavy metals may have devastating effects on the ecological balance of the aquatic environment and the diversity of aquatic organisms becomes limited.

Basics of samples of water, sediment and biota from Lagos lagoon is due to many renown scientist such as Ajao , Okoye *et al*. had researched this lagoon and came up with the fact that the lagoon is continuously under the effect of pollution by industries around the city.

The aim of this study is to determine the Level of heavy metals in water and sediment in different stations of Lagos lagoon. This study becomes

imperative, not only because of the threat of heavy metals to public water supplies, but also the damage caused to the aquatic life (Canli et al, 1998).

MATERIALS AND METHOD

Study Area

Lagos Lagoon lies between longitudes 30 22'E and 30 40'E and latitude 60 17'N and 60 28'N.The lagoon is generally shallow with a depth of between 0.3 and 3.2m in most parts with the exception of some dredged parts, notably in the Lagos Harbour,where depth is greater than 10m.

Collection of water and Sediment sample

Surface water samples from the selected locations were collected by dipping plastic containers of 1.5 ml to about 6-10 cm below the surface film.

Some analysis were done in-situ while the plastic containers were well corked, labeled and carried to the laboratory where it was stored in a refrigerator before the commencement of further analysis.
The sediment was collected with the aid of a grab and stored in a polythene bag.

Chemical Analysis of Samples.

Temperature was determined using mercury in glass thermometer calibrated in degree centigrade (^{o}C). The surface water temperature was measured on site. Ph, Salinity, Turbidity and Conductivity were measured in-situ using Horiba water checker model U-10.

The Dissolved oxygen was determined by Winkler's titrimetric method and Alkalinity was estimated titrimetrically using 0.02 Hcl with methyl orange as indicator.

Chromium (Cr), Lead (Pb), Zinc (Zn) and Cadmium (Cd) in the samples was assayed using Atomic Absorption Spectrophotometer (AAS) model standards were prepared from 1000mg/l stock solution of the metals of interest.

Serial dilutions were made to obtain 1.0, 2.0 and 3.0 mg/l.The equipment was calibrated using deionized water as blank.

RESULT AND DISCUSSION

The Concentration of the heavy metals determined in the surface water and sediment is indicated in Figure 1 and 2, while the physicochemical parameters are in figure 3.

The mean levels of heavy metals in the sediment of Lagos lagoon results showed that the concentration of the metals were generally low and fell within the acceptable limits described by FEPA 1998 and WHO, the average concentrations are Cr 0.046mg/kg, Pb 0.054mg/kg, Zn 0.730mg/g, Cd 0.523mg/kg, the mean levels in surface water is generally low when compared to WHO standard 2004. The metal levels in sediments were higher than those in the lagoon surface water and animals are known to take up and accumulate heavy metals from contaminated soils Madejon,et al, 2003.

Although levels of Cd (0.173), Cr (0.039), Pb (0.043) mg/g, were within the normal range in minimum allowable in diet of man however, continual consumption could lead to accumulation to with adverse health implications since Cd has been linked to renal diseases and cancer Kjellstroem, T., 1986. Zn showed the highest mean relative to other metals and this result can be deduced to conform to the expected since Zn forms part of trace metal required by the body for healthy functioning.

The highest salinity recorded was 3.1% in Iddo, most stations recorded zero salinity indicating a fresh water condition, Ajayi, S.O, Osibanjo, O (1981). The DO recorded at all stations were high and above the WHO standard (Table 3) this could be attributed to flood water dilution. The highest PH was 9.1 and the water samples were Turbid during the period of study, the particulate matters brought into the lagoon by surface run-off and flood must have been responsible for the high turbidity recorded in the wet season.
Lagos lagoon has been subjected to contaminating materials, capable of initiating the impairment of the water quality.

CONCLUSION

The metal levels in sediment were higher than those in the Lagoon surface water and this confirms that sediments are important hosts for toxic metals. The occurrence of enhanced concentrations of heavy metals in coastal sediment such as that obtained in Lagos lagoon can be a good indication of man induced pollution rather than by natural enrichment through geological weathering.

Metals from Lagoon surface water could be a contributing source to the levels in animal's hence continual assessment is highly essential.
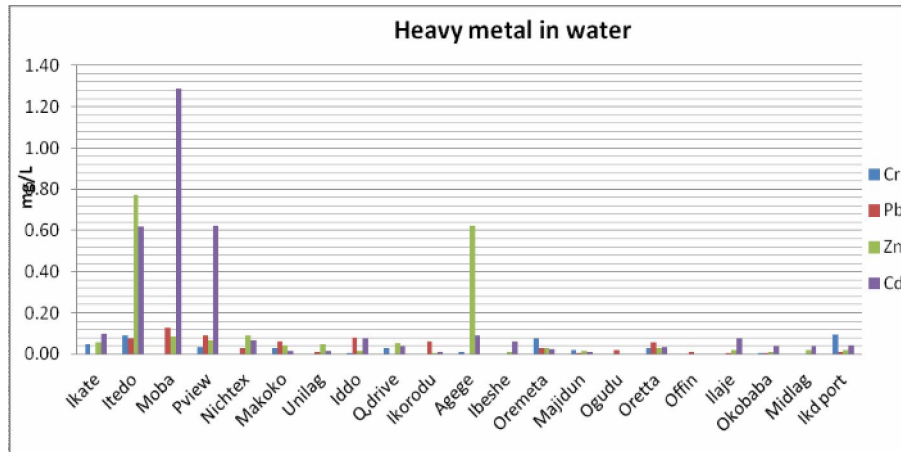
Figure 1. Distribution of heavy metals in Surface water.
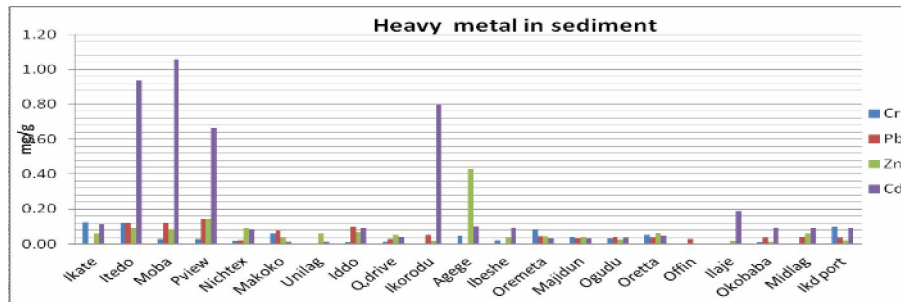
Figure 2. Distributions of heavy metals in the Sediment.
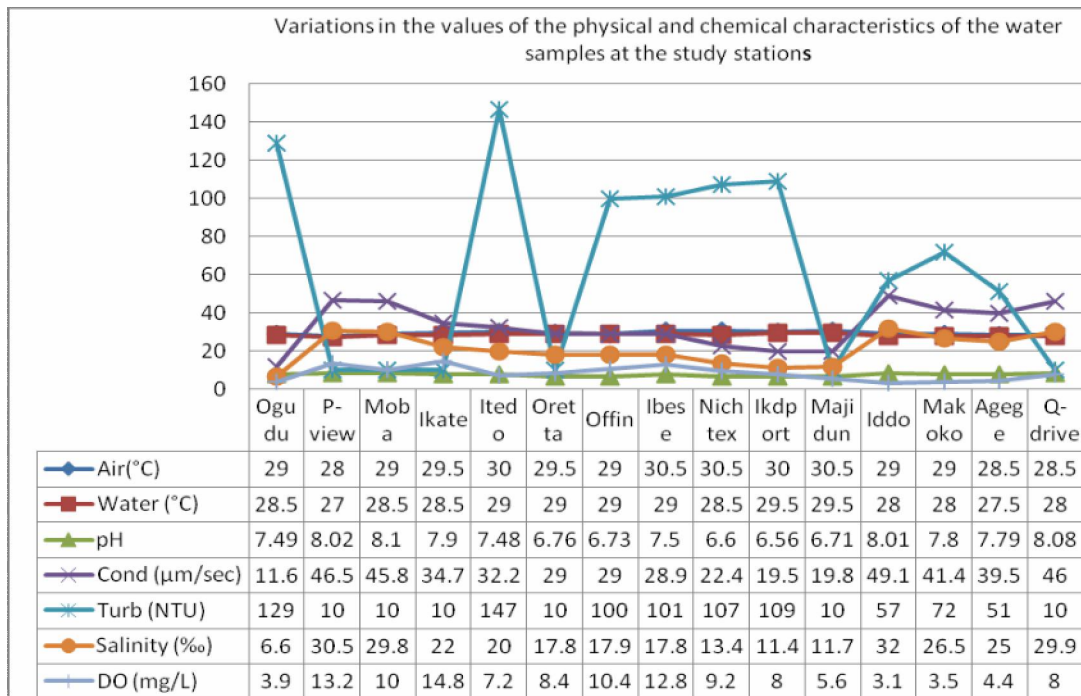
Figure 3. Distribution of the physicochemical parameters in the station

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The Response of *Galega officinalis* Plant to Different Nitrogen Sources and their Effect on Active Ingredients and Biological Activity

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Abstract: *Galega officinalis,* goat′s rue, a plant native to Euro-Asia and North Africa was introducing to Egypt as an objective for introducing important medicinal plants. The plant claimed to be useful in diabetic treatment, reduce weight and as anti platelet aggregation. Plant density and different nitrogen sources (organic, bio and chemical fertilizers) were tried to evaluate their effect on growth, yield, active ingredient, and its biological activity as anti diabetic. The results revealed that wide propagation distance produced higher growth and yield than the narrower one. The mineral nitrogen proved to be the effective source followed by compost then cattle manure on growth and yield of leaves, whole plant, flower, and sucker number. Total alkaloid was determined in leaves and it fluctuated with no clear trend, however was higher in sample taken at June. The fertilization had no effect on alkaloid accumulation.
Goat′s rue total alkaloid showed the highest reduction percentage in blood glucose level after 2 hours in diabetic rats.

1. Introduction:

Galega officinalis, commonly known as goat`s rue, French lilac, Italian flitch or professor weed, is an herbaceous plant in the Faboideae subfamily. It is native to the Middle East, but it has been neutralized in Europe, western Asia and western Pakistan. The plant has been extensively cultivated as a forage crops, an ornamental, a bee plant and as green manure (lasseigne, 2003). Its name drives from gale "milk" and ega "to bring on" as Galega used as a galactogogue in small domestic animals.

Galega officinalis has been known since the middle ages for relieving the symptoms of diabetes mellitus, upon analysis it turned out to contain guanidine a substance, that decrease blood sugar by decreasing insulin resistance, Pundarikakshudu *et al.* (2001) reported the anti- bacterial activity of *Galega*. ethanolic extract both gram negative and positive bacteria. Peiretti and Gai (2006) reported the nutritive value, fatty and amino acids content of Galega herbage during growth cycle and in regrowth in samples collected at progressive morphological stages and during regrowth.

Other uses of Galega were indicated as its usefulness in weight reduction (Palit *et al.*, 1999), inhibition of platelet aggregation (Atanasov and Spasov, 2000), against plaque (Bunney, 1986) in foot bath and ointment to hasten skin healing after surgery (Pandarikakshudu *et al.*, 2001). It increases and stimulates milk lactic secretion and milk production in animals (Gonzalez-Andres *et al.*, 2004)

In a project deals with plants have antidiabetic effects, this plant was introduced to Egypt, where different agricultural treatments will be tried to adapt this plant to Egyptian conditions.

This investigation deals with using different fertilization rates and kinds and their effect on bio-mass production, alkaloid content and its biological activity as anti– diabetic.

2. Material and Methods

Seeds of *Galega officinalis* were directly sown in the field on 20 March 2008 and germinated on 28 March. Seven treatments beside control were performed as follows;

1. control,
2. cattle manure 20 m^3/ acre,
3. Rhyzobacterin + 15 m^3 compost/ acre
4. Phosphorin + 15 m^3 compost / acre
5. Compost 20 m^3/ acre
6. Ammonium nitrate 100kg/acre(33.5%N)
7. Ammonium nitrate 200kg/acre (67%N).

The propagation was done on rows 60cm apart at a distance of 20 and 40cm between plants. The treatments were arranged in complete randomized blocks, each one replicated 3 times in an area of $3m^2$ (3 x1 m).

Different fertilizers of varied sources were added four times on May 16, June 4, October 17 and November 27. the compost and cattle manure added

before cultivation through land preparation.The bacterial extract was added at a rate of 10 ml diluted to 1 liter water where each ml of the bacterial extract contains 10^{-6} bacterial cells.

The compost obtained from sekem company (Cairo – Belbeis Desert Road, El-Horreya, Cairo) was added and analyzed; its values were presented in Table (1)

Table (1) chemical Analyses of Compost.

Constituent	values
Bulk Density kg/m^3	510
Moisture Content %	18.2
Electrical conductivity dS/m	9.65
pH	7.6
Total Organic Carbon %	24.6
Total Organic Matter %	42.41
C/N Ratio	18.22
NH_4-N, mg/kg	880
NO_3-N, mg/kg	450
Total Phosphorus %	1.6
Av. Phosphorus mg/kg	410
Total Potassium %	2.3
Av. Potassium mg/kg	620
Trace Element (ppm)	
Fe	960
Zn	280
Mn	210
Cu	140

Nematodes	nil
Weeds Germination	nil
Parasites	nil
Pathogenic	nil

Cattle manure was also analyzed and the data was compiled in Table (2)

Table (2): Analyses of cattle manure.

Weight of m^3 /kg	685
Humidity	6.19%
Total nitrogen	3.45%
Ammonia mg/kg	922.1
Nitrate mg/kg	74.3
Total phosphorus %	0.53
Total potassium %	2.06
Organic matter	63.14
Organic carbon %	61.11
Iron mg/kg	-
Mn mg/kg	188.6
Cu mg/kg	55.3
Zn mg/kg	875.6

The first record for vegetative growth was obtained on 15 th June 2008 and the same growth

parameters were recorded in different dates; 24th July, 6th September, 27th November, 31th December 2008 and 12th March 2009.

The following measurements were recorded: plant height over ground in cm, sucker's number, whole plant weight fresh and dried, and leaves weight fresh and dried in grams per plant, and flowers fresh and dry weight.

The plants were cut off 10cm over ground at the end of September, growth was continued, where 3 samples were taken on; November, December 2008 and on March 2009.

Growth parameters

The growth aspects measured were compiled in Tables 3, 4, 5, 6 and Fig.1 for the samples taken through the growth season and regrowth.

Extraction of crude alkaloid

About 200g dried powdered leaves of *Galega officinalis* was extracted with 5% Hcl in ethanol different times till exhaustion. The combined extract was evaporated till 10 ml and then pH was adjusted to 11 by adding NH_4OH.

The total extract is partitioned between chloroform and water. The chloroform layer was concentrated till dryness (total alkaloids), and then weighed and total alkaloid as percentage was calculated according to the method of Edeoga *et al.* (2005).

Ethanol extract

About 500g dried powdered leaves of *Galega* was percolated with ethanol 70% (3L, five times). The combined ethanol extract was evaporated till dryness.

The crude alkaloid, standard galegine (metformin) as wells as ethanol extract were applied on TLC aluminium sheet with the following solvent systems:
1- Chloroform- MeOH (9:1).   2- Benzene: EtOAc (8:2). 3- Benzene: EtOAc (9:1).

The plates were sprayed with Dragendorff reagent to visualize the color of alkaloid; the different R_f values were presented in Table (7).

Acute toxicity of crude alkaloid
Animals and diet:

Male Albino mice weighing from 20-25g were obtained from Animal House of National Research Centre, Egypt. The mices were fed on a basal pellet diet. All animals were maintained in plastic cages with free access to tap water.
Experimental design

Acute toxicity assay of total alkaloid studied on eight groups of mices, each group contained 6

mices. The test was carried out among equal sized groups of lethality in mice receiving progressively increasing oral dose levels of the total alkaloid (250, 500, 750, 1000, 1250, 1500, 1750, 2000 mg/kg b.wt).

After administration of alkaloid, animals were observed closely during first 48h and then every 12h for one week.

The experimental work on mices was performed with approval of the animal care experimental committee, National Research Centre, Egypt, according to the guidance for care and use of laboratory animals.

Oral glucose tolerance test (OGTT)

Male Albino rats were fasted overnight (at least 12h). They were divided into three groups containing six animals each. Control rats (group 1) were given 1 ml distilled water. Metformin (as a reference drug) and galega alkaloids at concentration of 200 mg/kg (b.wt.) were administered orally using a syringe to the second and third groups. After half an hour of extract administration, the rats of all groups were orally administrated with 2g/kg b.wt of glucose. Blood samples were collected from the retro-orbital plexus just prior to glucose administration and 30, 60 and 120 min, after glucose administration.

Loading-Plasma was separated and blood glucose levels were measured immediately by the glucose oxidase method (Trinder, 1969).

Chromatographic investigation:

Chromatographic investigation was performed to check the occurrence of alkaloid either in ethanol extract or precipitated as crude alkaloid in comparison with metformin which is considered as reference. Different solvent systems were tried, where Rf values on TLC were present in Table (7).

3. Results

Six samples were taken from the experiment including the different growth parameters beginning 15th June and then with intervals of 45 days.

The first sample taken on 15th June 2008 illustrated in Fig. (1), the data obtained indicate that all treatments increased growth parameters studied. The most potent treatment was ammonium nitrate in its high rate. Whole plant weight was 100.68 against 23.96 gm in control. These finding was true at the two spacing distance 20 and 40cm. As to compare between the two spacing distance, 40cm gave more biomass specially with treatments of compost (98.95) and the two rates of ammonium nitrate (67.91 and 119.18 gm) which produce more vegetative growth than plants propagated at 20cm space.

The data of the second sample (Table, 3) reveals that all the parameters increased either in

plant height or weight as the plant progressed in age. When the different treatments were considered, it was found that cattle manure (105cm) and the high rate of ammonium nitrate (108.76cm) produced taller plants. On the other hand the vegetative growth was vigored by cattle manure (379.40g) and compost (320g) treatments, with plants cultivated at 20cm space distant.

With regard to plants cultivated at 40cm, cattle manure produced the tallest plants (106.77), overall the treatments, but the vegetative growth was stimulated by compost treatments and ammonium nitrate only.470.0 and 405.67g/plant respectively.

The third sample taken represent the progress occurred through growth season Table (4), meaning, increase in all parameters studied over two previous samples. Maximum length of plants was produced from plants fertilized with compost and ammonium nitrate 200 kg/acre, (100.43 and 100cm) respectively.

Suckers number was the highest in treatment of cattle manure followed by ammonium nitrate 100 kg/acra., 54 and 39 suckers / plant respectively.

Cattle manure and ammonium nitrate in its high rates produced the highest fresh weight or biomass (whole plant). The same finding was reported with leaves fresh and dry weights. As to consider the wider distance of cultivation all the fertilization treatments vigorates the whole plant and leaves fresh weight over the control, due to treatments of ammonium nitrate, compost and rhizobacterin which produced the highest yield.

The fourth sample represents the regrowth obtained after cutting 10cm over ground and fertilized as previously performed then left to grow. With this condition which can be described as the re-growth of another cut, treatments of compost, and cattle manure increased plant length to nearly the same length (56cm) but lower than the previous two samples taken on July and Septemper. Compost highly increased whole plant fresh weight to reach 1011.20 gram/plant followed by the two rates of mineral nitrogen, 700.67 and 885.10g/plant, respectively.

Wider distance and ammonium nitrate (the highest rate) synergize the production of whole plant fresh weight to double fold the plants propagated at the narrower distance 2252.0gm/plant and consequently the leaves fresh weight 1716.87 gm and dry weight437.8 gm., Table (5)

The fifth sample was taken on 31-12-2008 75 days old from cutting .Fig. (2) clearly represents the variation in vegetative parameter due to fertilization treatments and propagation distances. All the growth parameters increased than the sample taken at the end of November regardless the low temperature prevail at this time. Treatments of compost and mineral nitrogen in the lower dose

greatly increased plant length, whole plant fresh weight, leaves fresh and dry weights with plants cultivated at the narrower distances however wider distance produced lighter yield, and shorter plants.

The last sample taken from plants (75days old, Table (6) showed taller plants than that of the previous samples. No effect of the different treatments was revealed on plant height as control plant produced the tallest ones except with ammonium nitrate in its lower dose treatments. Cattle manure affects sucker no. greatly in wider distance of cultivation while compost treatment increases the no. of suckers in the narrower cultivation distance.

With respect to the effect of fertilization treatments on whole plant fresh weight, only compost stimulates this aspect on plants propagated at 20 cm. In plants propagated at 40 cm distance, all fertilization treatments increase whole plant fresh weight and leaves fresh and dry weights, the maximum growth yield was produced as an effect of ammonium nitrate in its lower rate.

A glance on all the samples taken, it was found that ammonium nitrate in its two rates added was the treatment of choice to increase the vegetative parameters studied on goats rue plants. Compost and or cattle manure came in the next order. In this respect compost produces higher yield or biomass especially in sample taken at low temperature (on December) as it is hydrolysable during the hottest dates and then releases its content in winter. The wider distance is the most suitable for propagation than that of 20cm irrespective of the high yield obtained with narrower one due to the high density of plants per acre.

Chromatographic analysis:

The chloroform and alcohol extract beside the standard metformin were applied on TLC plates in a trial to separate the alkaloids. In all the solvents applied one red spot representing the galegine or metformin was detected and has the Rf values represented in the following Table (7).

It was found that solvent I was the best to separate and detect the galegine alkaloid from all other plant components.

Determination of alkaloids:

Alkaloids were determined as total in the leaves of the different treatments according to the method of Edeoga *et al.* (2005), where it was determined gravimetrically.

The total alkaloids as galegine were estimated as percentage in leaves and were compiled in Fig (3) where it could be concluded that alkaloids are higher in June, in younger organs (the plant is about 3 months old) then decreased towards maturation till November where the plant was cut

over the ground. Fertilization was added at the end of November and new growth was progressed. The alkaloid percentage again increased gradually but not reached the higher percentage observed in June.

From Table (8a) and Fig.(3) it was reveled that the maximum alkaloid content was accumulated through November, at sample taken after cutting and contain higher biomass and higher alkaloid content.

Biological studies: Acute toxicity

To use this plant as hypoglycemic remedy, toxicity must be estimated. In the present investigation, no death in animals in any groups administered crude alkaloid except the group administered 2000mg/kg body weight, in which one rat died. So this plant is safe till 2 gram/kg b.wt.

Glucose tolerance in rats

Administration of 2g/kg b.wt. glucose to normal rats increased serum glucose to 106.6 mg/dl after 60 min. in group I. The administration of metformin as a reference and extract of Goat's rue suppressed the elevation of serum glucose at 60 and 120 min.

Goats rue extract induced the highest reduction in blood glucose after 2 hours as illustrated in Fig. (4)

4. Discussion:

The demand for medicinal plants is related to the great culture significance attached to medicinal plants. The growing demand has not only resulted in increased hazard for over exploitation of wild population but also on increased interest of cultivation. Major interest of different factors affecting yield components and active ingredients is fertilization. Interest which is concentrated on nitrogen fertilization and the usage of different sources especially organic one to minimize using mineral sources, enormous researches are dealing with this factor on different medicinal plants. In August 2010 through the ICHS at Lisbon, (the International Conference of Horticultural Science). different sources of nitrogen and different levels (urea, ammonium nitrate ammonium sulphate), at 0, 180, 240, 300, 360 kg N/Ha were applied as treatments for two medicinal plants *leonotis* and *Artemisia*. The results proved that the plants treated reacted positively with addition of the three nitrogen sources with most of the treatment showing a significant increase in the fresh mass yield specially with the lowest level of fertilizer applied, in South Africa presented by Prinsloo *et al.*(2010). With an Iranian experiment dealing with chamomile using organic cultivation and bio fertilizer and their effects on growth, yield and essential oil content. Salehi *et*

al. (2010) concluded that it seems that organic cultivation of German chamomile can consider as an alternative system for conventionally system in production of yield and essential oils. Ferreria *et al.* (2010) in Brazil observed a linear increase in biomass production according days after transplantation and the highest production occurred at 180 kg/ ha nitrogen in an experiment using different nitrogen rates of 0, 45, 90, 135, and 180 kg N / ha and different harvest ages of 152, 242, 332 and 428 days after transplanting of *piper aduncum* plants. Biesiada *et al.* (2007) on their work on *Calendula* proved that form and rate of nitrogen had a significant effect on chemical composition. Plants fertilized with urea had high level of phenolic compound than other mineral nitrogen sources. Rosemary plants treated with bio-fertilizer (*Azotobacter vinelandii*) through its different cuts, cause slight improvement in growth characters and its content of essential oil amounted to 0.75 % as revealed by Leithy *et al.* (2006). Combination of mineral and chemical fertilizers with Barvar phosphate biofertilizer caused the highest seed yield and mucilage content of *Plantago ovata* as described by Majid *et al.* (2007)

All the results reviewed revealed the beneficial effect of either mineral nitrogen or organic or biofertilization which coincided with the result obtained with this investigation. Organic fertilization provides nutrients to plants, improve the soil physical structure, increase water retention, reduces the erosion losses and favors the biological control and even are beneficial than mineral ones in reducing the residual effect of heavy metals and pollution

Hendawy and El- Gengaihi (2010) in comparative studies on the role of organic, bio and mineral fertilization of nitrogen and phosphorus found that mineral nitrogen gave quick and significant effect on Borage and Echium plants. Organic and bio fertilization however increase both growth characters and fixed oil but were in second rank after mineral ones which release quickly than organic ones like compost.

Dealing with distance of cultivation, Chaves *et al.* (2010) from Brazil concluded that biomass production was inversely proportional to the special arrangement, with the greater biomass production (1034.93 kg / ha) in narrower spacing, although no statistical difference was verified between distance (1m×1m) and (1.0 m ×1.5 m). The same response was observed for the production of essential oil of *Piper callosum.*

Table (3)   **Mean values of some growth parameters of *Galega officinalis* under two different spacing as influenced by different fertilizer treatments (24/07/2008)**

Treatment	20 cm						40 cm					
	Plant height (cm)	Sucker No.	Fresh weight whole plant (g)	Leaves fresh weight (g)	Leaves dry weight (g)	Flower fresh weight (g)	Plant height (cm)	Sucker No.	Fresh weight whole plant (g)	Leaves fresh weight (g)	Leaves dry weight (g)	Flower fresh weight (g)
Control	74.00	11.00	233.52	156.73	33.38	2.58	85.33	10.67	209.98	127.86	27.23	2.45
Cattle manure	105.00	14.10	379.40	236.33	50.34	3.31	106.77	11.00	209.79	114.58	24.41	9.23
Rhizobacterin	98.00	12.33	290.67	240.00	51.12	19.30	104.33	15.00	181.03	143.74	30.62	4.35
Phosphorine	78.67	13.33	262.17	181.87	38.74	2.20	85.00	11.33	198.13	102.19	21.77	5.40
Compost	93.33	12.00	320.00	219.67	46.79	5.47	95.33	17.00	470.00	332.21	70.76	8.82
Ammonium nitrate 100kg/fed	86.33	21.33	264.67	257.33	54.81	11.00	92.17	17.33	405.67	165.80	35.32	8.98
Ammonium nitrate 200 kg/fed	108.67	11.00	265.00	164.07	34.95	12.37	96.00	23.00	433.00	187.00	39.83	4.30
L.S.D. (5%)	4.41	2.35	10.42	12.22	3.85	1.92	5.36	2.01	10.74	10.61	3.52	1.55
L.S.D. (5%) (20*40 cm)	4.11	2.15	9.6	11.03	2.93	1.41						

Table (4)   **Mean values of some growth parameters of *Galega officinalis* under two different spacing as influenced by different fertilizer treatments (06/09/2008)**

Treatment	20 cm						40 cm					
	Plant height (cm)	Sucker No.	Fresh weight whole plant (g)	Leaves fresh weight (g)	Leaves dry weight (g)	Pods weight (g)	Plant height (cm)	Sucker No.	Fresh weight whole plant (g)	Leaves fresh weight (g)	Leaves dry weight (g)	Pods weight (g)
Control	77.67	23.00	195.00	195.00	44.46	31.51	84.67	18.00	252.67	126.70	28.89	10.91
Cattle manure	91.67	54.43	234.77	234.77	53.53	18.07	77.33	27.10	278.87	186.80	42.59	16.57
Rhizobacterin	84.00	30.67	204.77	204.77	46.69	35.24	73.00	26.20	384.77	193.87	44.20	16.25
Phosphorine	90.20	15.87	148.27	148.27	33.80	13.33	72.87	27.33	321.67	244.00	55.63	20.45
Compost	100.43	27.67	185.43	185.43	42.28	17.53	80.67	42.67	369.33	207.87	47.39	27.53
Ammonium nitrate 100kg/fed	99.67	39.53	224.93	224.93	51.28	27.17	69.53	19.00	292.53	165.27	37.68	10.81
Ammonium nitrate 200 kg/fed	100.00	22.67	259.67	259.67	59.20	19.99	115.67	35.87	626.43	321.67	73.34	37.93
L.S.D. (5%)	3.08	2.74	8.72	13.54	7.9	2.05	4.31	2.6	7,66	13.61	5.49	2.11
L.S.D. (5%) (20*40 cm)	3.22	2.68	7.41	10.08	5.86	1.93						

Table (5)   **Mean values of some growth parameters of *Galega officinalis* under two different spacing as influenced by different fertilizer treatments (26/11/2008)**

Treatment	20 cm						40 cm					
	Plant height (cm)	Sucker No.	Fresh weight whole plant (g)	Leaves fresh weight (g)	Leaves dry weight (g)	Fruit wt. (g)	Plant height (cm)	Sucker No.	Fresh weight whole plant (g)	Leaves fresh weight (g)	Leaves dry weight (g)	Fruit wt. (g)
Control	50.43	46.87	599.33	399.77	101.94		43.10	23.77	292.50	201.00	51.26	
Cattle manure	56.10	30.43	630.00	554.33	141.36		55.20	31.43	502.53	308.33	78.63	
Rhizobacterin	53.43	24.33	540.87	330.10	84.18		42.87	22.10	557.33	385.43	98.29	
Phosphorine	42.53	24.00	646.77	502.43	128.12		62.20	43.67	1260.67	925.67	236.05	
Compost	56.00	43.67	1011.20	668.67	170.51		50.43	37.33	753.53	535.33	136.51	
Ammonium nitrate 100kg/fed	50.53	42.10	700.67	520.67	132.77		61.43	25.00	1190.10	838.00	213.69	
Ammonium nitrate 200 kg/fed	48.10	37.43	885.10	622.67	158.78		69.33	57.43	2252.00	1716.87	437.80	
L.S.D. (5%)	3.92	2.94	38.06	37.11	8.75		4.6	3.92	42.61	45.01	8.22	
L.S.D. (5%) (20*40 cm)	2.84	2.19	35.62	32.19	7.04							

Table (6)   **Mean values of some growth parameters of *Galega officinalis* under two different spacing as influenced by different fertilizer treatments(12-03-2009)**

Treatment	20 cm					40 cm				
	Plant height (cm)	Sucker No.	Fresh weight whole plant (g)	Leaves fresh weight (g)	Leaves dry weight (g)	Plant height (cm)	Sucker No.	Fresh weight whole plant (g.)	Leaves fresh weight (g.)	Leaves dry weight (g.)
Control	92.20	28.10	447.20	346.20	83.09	74.43	25.77	274.77	130.43	31.30
Cattle manure	77.10	53.00	441.53	347.33	83.36	74.67	32.00	343.10	258.87	62.13
Rhizobacterin	73.10	28.67	412.00	282.87	67.89	62.00	35.10	359.20	248.00	59.52
Phosphorine	93.87	26.53	294.67	202.67	48.64	63.53	34.10	309.67	186.77	44.82
Compost	86.10	49.33	477.33	341.87	82.05	75.67	54.87	368.43	258.33	62.00
Ammonium nitrate 100kg/fed	101.67	44.10	409.20	349.33	83.84	76.67	33.32	416.37	316.53	75.97
Ammonium nitrate 200 kg/fed	78.33	24.43	364.33	249.87	59.97	95.33	45.00	389.43	264.10	63.38
L.S.D. (5%)	4.23	1.62	12.13	14.3	7.42	5.23	1.84	16.7	12.6	6.88
L.S.D. (5%) (20*40 cm)	4.01	1.24	9.7	10.22	4.18					
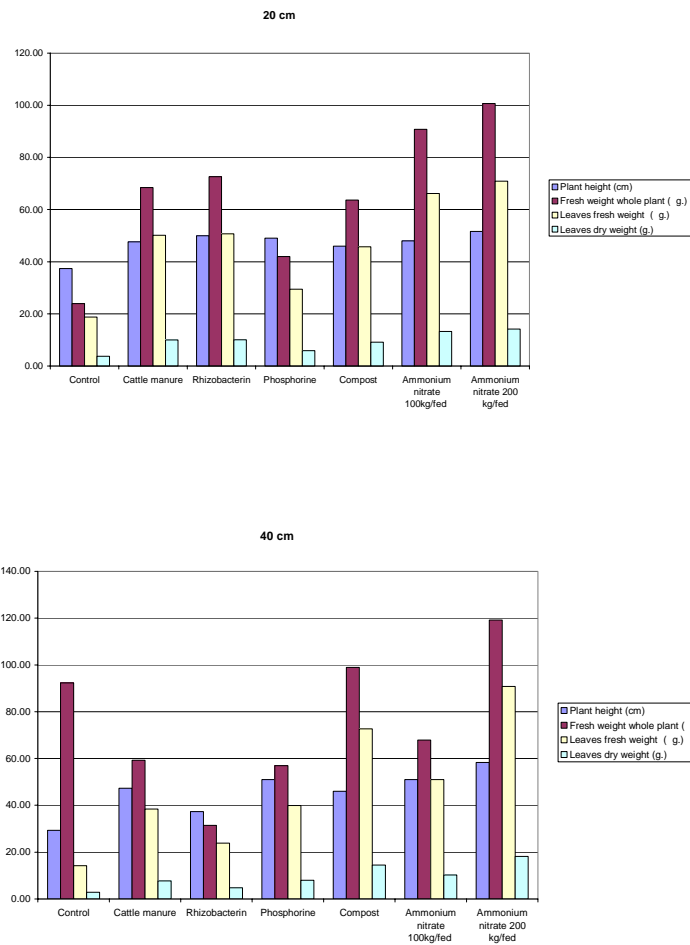
Fig.(1) mean value of some growth parameters of goats rue under two different spacing as influenced by different fertilizer treatments at first sample June 2008.
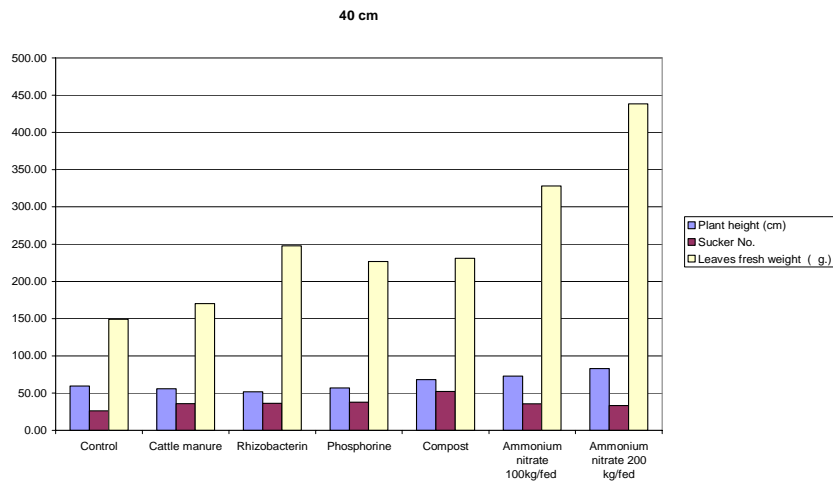
Fig.(2) Mean values of some growth parameters of *Galega officinalis* under two different spacing as influenced by different fertilizer treatments (31-12-2008)

Table (7): R$_f$ values of alkaloid in different extracts and with different solvent systems

	Solvent I	Solvent II	Solvent III	Corresponding to galegine
	R$_f$ values			
Alcohol extract	0.184	0.897	0.50	
Crude alkaloid	0.184	0.890	0.55	
Metformin	0.186	0.893	0.55	

Table (8): Percentage of alkaloid through different plant stages

	Date of cut / Treatments	June	July	Sept.	Nov.	Dec.2008	March 2009
20 cm	Control	7.6	3.0	1.4	1.2	2.2	1.6
	Cattle manure	5.8	3.6	3.2	1.4	3.2	3.6
	Rhizobacterin	5.2	6.8	3.2	1.0	2.6	2.4
	Phosphorine	4.8	0.0	2.8	2.0	2.4	1.6
	Compost	6.2	2.6	2.6	1.8	2.0	2.0
	Amm.Nitrate 100	7.8	3.0	3.0	1.6	2.2	2.4
	Amm.Nitrate 200	7.2	3.6	3.6	1.6	1.0	2.8
	Mean	**6.37**	**3.23**	**2.83**	**1.51**	**2.23**	**2.34**
40 cm	Control	8.0	2.8	2.0	1.2	1.8	3.6
	Cattle manure	3.2	4.0	3.6	2.4	2.6	3.8
	Rhizobacterin	7.0	3.0	4.8	1.0	2.8	2.4
	Phosphorine	3.8	3.4	4.2	2.4	2.4	1.6
	Compost	5.0	3.0	3.2	2.4	2.2	2.2
	Amm.Nitrate 100	8.4	3.4	4.2	2.2	3.6	2.6
	Amm.Nitrate 200	6.6	3.2	4.8	1.6	2.8	2.8
	Mean	**6.00**	**3.26**	**3.83**	**1.89**	**2.60**	**2.71**

Table (8a): Mean alkaloids content as galegine in leaves of *Galega* plant under different fertilization treatments. (g/ plant)

	Date of cut / Treatments	June	July	Sept.	Nov.	Dec.2008	March 2009
20 cm	Control	0.29	1.00	0.62	1.22	1.77	1.33
	Cattle manure	0.58	1.81	1.71	1.98	2.76	3.00
	Rhizobacterin	0.53	3.48	1.49	0.84	1.74	1.63
	Phosphorine	0.28	0.00	1.95	2.56	1.97	0.78
	Compost	0.57	1.22	1.10	3.07	2.37	1.64
	Amm.Nitrate 100	1.03	1.64	1.54	2.12	2.37	2.01
	Amm.Nitrate 200	1.02	1.26	2.13	2.54	0.56	1.68
	Total	٤.٣٠	١٠.٤١	10.54	١٤.٣٤	١٣.٥٣	١٢.٠٧
40 cm	Control	0.23	0.76	0.58	0.62	0.75	1.13
	Cattle manure	0.25	0.98	1.53	1.89	1.24	2.36
	Rhizobacterin	0.33	0.92	2.12	0.98	1.94	1.43
	Phosphorine	0.30	0.74	2.34	5.67	1.52	0.72
	Compost	0.73	2.12	1.52	3.28	1.42	1.36
	Amm.Nitrate 100	0.86	1.20	1.58	4.70	3.31	1.98
	Amm.Nitrate 200	1.20	1.27	3.52	7.00	3.44	1.77
	Total	٣.٨٩	٨.٠٠	١٣.١٩	٢٤.١٣	١٣.٦٢	١٠.٧٥

Fig.(3) Mean alkaloids content as galegine in leaves of Galega plant under dfferent distance
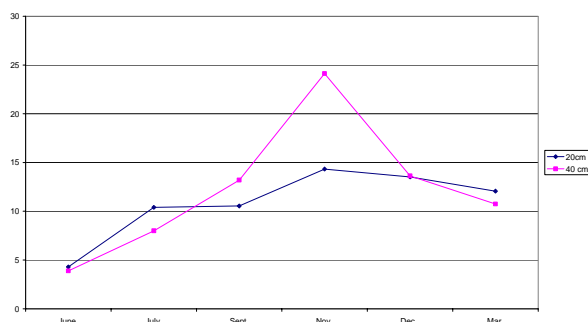
Fig. (4) Effect of metformin and galega total alkaloid on the oral glucose tolerance in rats.
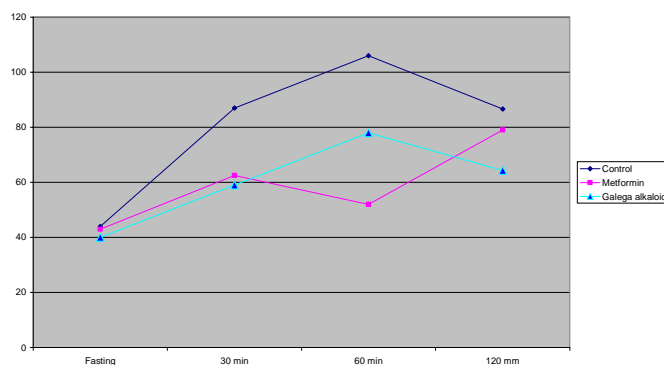
Dealing with the effect of fertilization on the percentage of alkaloid no clear effect was observed due to fertilization. Control plant accumulates more alkaloid than all the treatments. So, it can be concluded that alkaloid are plant stage dependent and not influenced by fertilization. On the other hand plant growth was affected by different fertilization applied which reflects on the production of alkaloid per plant, represented in Table (8) and Fig (3).

5. Conclusion

Ammonium nitrate in its two rates was the treatment of choice to increase the vegetative parameter studied. Compost or cattle manure came in the next rank. Wider distance of propagation (40 cm) is the most suitable cultivation distance. One major alkloidal spot was detected in crude alkaloid extract and in alchoholic one. Total alkaloid is higher in young leaves then decreased through maturation, when this alkaloid administered to rats it proved to have anti-diabetic activity.

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Graphical Abstract
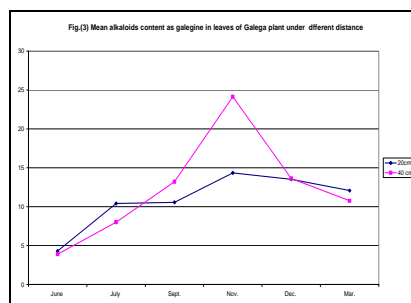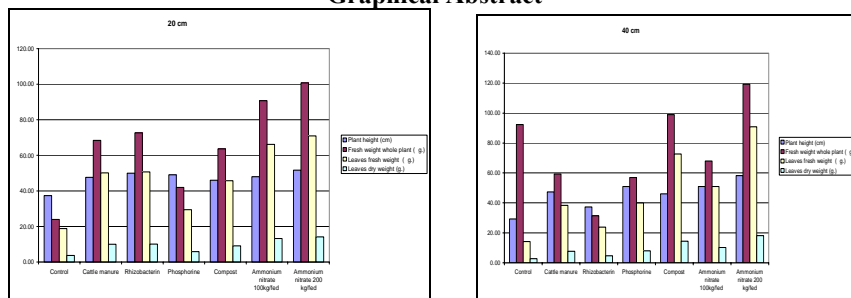
Fig.(3) Mean alkaloids content as galegine in leaves of Galega plant under different distance

The mineral nitrogen proved to be the effective source followed by compost then cattle manure on growth and yield of leaves, whole plant, flower, and sucker number. Total alkaloid was determined in leaves and it fluctuated with no clear trend, however was higher in sample taken at June.

2/1/2011

Effect of Putrescine and Uniconazole Treatments on Flower Characters and Photosynthetic Pigments of *Chrysanthemum indicum* L. Plant

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Abstract: The effect of Putrescine at the concentration of 100,200 and 300 ppm and Uniconazole at 20, 40 and 60 ppm in addition to control (distilled water) on flower characters, total carbohydrates and photosynthetic pigments in flowers of Chrysanthemum plant during 2004/2005 and 2005/2006 had been evaluated studied. The obtained data indicated that all flower characters and chemical composition were significantly increased by foliar application of Putrescine at the three concentrations. Uniconazole treatments delayed start of flowering after spraying, decreased pedicle length and length of flower stalk, while it increased yield of flowers, diameter of inflorescence, vase life, total carbohydrates in the flowers and photosynthetic pigments chl. (a),(b) and carotonoids . The highest values were found when plants were treated with 200 ppm Putrescine and 20 ppm Uniconazole.

1. Introduction:

Chrysanthemum indicum L. plant is one of the leading cut flowers and potted plants in the international market. It is a perennial herb grown in Egypt as one of the most important cut flowers and pot plants, the inflorescence consists of several ray and disc flowers (florets) and such, is called a (flower head),which is in markets because of its beautiful shape and longevity in vases. Chrysanthemum is a short day plant because of its habit of flowering only under short day conditions. A major reason being that the time of flowering can be modified throughout the year. The development of Chrysanthemum industry as a major ornamental cut flower and pot plant enterprises supports the major thrusts of the government to develop the non –traditional export products that will boost the industry to earn foreign currency.The immediate benefits from Chrysanthemum production will substitute the imported flowers with locally produced ones.

The Polyamines; namely Putrescine (Put), Spermine and Spermidine are well known as affecting different plant developmental processes (Kumar *et al*., 1997) and(Martin-Tanguy,2001). They modulate several growth and developmental processes Viz., cell division, differentiation, flowering, fruit ripening, embryogenesis, senescence and rhizogenesis. In all these plant growth processes, polyamines have been described showing various roles such as a new class of plant growth regulators, Hormonal Second Messengers (HSM) and one of the reserves of carbon and nitrogen at least in cultured tissues. At cellular pH values, these compounds behave as cations and can interact with anionic macromolecules such as DNA, RNA phospholipids and certain proteins (Hepy and Persson, 1990). PAs may act as anti-senescence agents (Galston and Kaur-Sawhey, 1995) and this property is largely based on a decrease in PA content with leaf aging and senescence in excised leaf or leaf segments. The mechanism of senescence inhibition by PAs may also be related to their possible inhibition of ethylene synthesis (Lee *et al*., 1997).This property may account for PAs acting as effective scavengers of free radical generated in a number of chemical and in vitro enzymes.

Uniconazole is one of plant growth regulators having important role in crop production, toward manipulation of plant growth and yield. Triazole plant growth regulators induce a variety of morphological and biochemical responses in plants (Fletcher and Hofstra, 1988).

The aim of this study is to investigate the effect of both Putrescine and Uniconazole on flower characters and photosynthetic pigments of Chrysanthemum *plant.*

2. Materials and Methods:

Putrescine is (tetramethylene Diamine $C_4H_2N_2$, molecular weight 88.15), (Wettable powder with 98% activity) obtained from Sigma Chemical Company and Uniconazole Sumitomo Chemical Japanese Company Limited plant growth retardant kindly, supplied by their scientific Bureau in Cairo.

The substance (Wettable powder with 10%activity) [(E)-1-(P-Chlorophenyl) 4.4-Dimethyl-2-(1,2,-4 Triazole1-1l)1-Penten-3-ol],with molecular formulae $C_{15} H_{18} Cl N_{3o}$, molecular weight 291.78, water solubility 8.4 ppm at 25°C, solubility in methanol 20-10 (W/W% at 21°C) were used.

The plants were sprayed with bioregulators three times (1st November, 1st December and 1st January 2004/2005 and 2005/2006) with freshly prepared solutions of Putrescine (100, 200 and 300 ppm) and Uniconazole (20, 40 and 60 ppm), while the control plants were sprayed with distilled water. The experiment was a complete randomized blocks design, with six treatments plus control. Each treatment has three replicates (each replicate contain five plants). The volume of the spraying solution was maintained to cover the whole plant foliage till run off point.

The flowers were weighed, dried in an electric oven at 70 °C[o] till a constant weight, ground to fine powder by an electric mill and stored for chemical analysis to estimate total carbohydrates.

Additional samples were taken after ten days from each spray to estimate flower characters (flowering start after spraying, flowers yield, inflorescence diameter, pedicle length of inflorescence, length of inflorescence stalk, vase life (days), and fresh and dry weight of inflorescence.

Total carbohydrates were determined in the dry powder of chrysanthemum flowers using colorimetric method at wave length 490 nm as described by (Herbert et al., 1971).

Chlorophyll (a), (b) as well as carotenoids were determined in the fresh material 10 days after each spray as described by (Wattestein,1957).

Statistical analysis**:** Data obtained were subjected to standard analysis of variance procedure. The values of L.S.D were obtained whenever F values were significant at 5% level, according to (Snedecor and Cochran, 1980).

3. Results and Discussion:
Flowering start after spraying

Data in Table (1) indicated that spraying Chrysanthemum plants with Putrescine at 100, 200 and 300 ppm lead to early blooming than control plants. The longest period, which was obtained in Jan. and Feb. from application of 200 ppm Putrescine, and 300 ppm in Dec. and Jan. then 100 Putrescine in Dec. in both seasons, respectively. On the other hand, spraying the plants with Uniconazole at 20, 40 and 60 ppm delayed flowering in January, February and March for the 1st and 2nd season, respectively as compared with control plants, while in December all used concentrations did not produce any flowers. It may be due to the effect of Putrescine

on the IAA synthesis, which increases the synthesis of IAA enzyme and increasing the tryptophane levels, which are precursors of IAA hormone.

The results are in agreement with those obtained by(Koriesh et al ,1989), (Dutta et al.,1993) and (Kang et al.,1995) on Chrysanthemum. They found that growth regulators enhanced earlier flowering than untreated plants.

Yield of inflorescence

Data presented in Table (2) indicate that foliar spraying of Chrysanthemum plants with Putrescine at the rate of 100,200 and 300 ppm caused a significant increase in flower yield in both seasons than control plants.The highest values were found at 200 ppm Putrescine, giving 147and 161 for the 1st and 2nd season, respectively. Application of Uniconazole treatments at 20 ppm gave the highest values 103and 104 of inflorescence plants in the 1st and 2nd season, respectively. These results are in agreement with obtained by(Mahgoub et al.,2006 b) on *Dianthus caryophyllus* plants they found that Putrescine at 200 and 400 ppm caused a significant increase in flower yield as compared with untreated plants.

With regard to the effect of Uniconazole; our results are in agreement with those obtained by(Bekheta ,1992), (Wu et al.,1996), (Bekheta,2000) and (El-Kadey,2002) they found that using Uniconazole increased yield over than untreated plants.

Diameter of inflorescence

Data presented in Table (2) indicate that spraying Putrescine at 200 ppm significantly increased diameter of flower head, giving 8.0 cm and 8.6 cm/ inflorescence compared with control plans that gave 5.6 cm and 5.9 cm/inflorescence in the 1st and 2nd season, respectively. Other concentrations increased also the diameter of flowering heads as compared with the untreated plants, in both seasons

In case of Uniconazole application, it was found that low concentrations 2o and 40 ppm significantly increased the inflorescence diameter by 21.4 and 23.2% over than that of unsprayed plants in the 1st season. While spraying the plants with 20 ppm Uniconazole in the 2nd season gave 7.2 cm showing its significant effect, while the other rates were insignificantly effective.

Similar finding was obtained by (Haggag, 1997) on Chrysanthemum. (Dutta et al., 1993) on some Chrysanthemum cultivars, found that Uniconazole increased flower diameter than control plants.

Pedicle length of inflorescence

Data in Table (2) showed that foliar application of Chrysanthemum plants with Putrescine increased insignificantly pedicle length of flowers as compared with the control plants in the 1[st]season. In case of the

2nd season, the application of 200 and 300 ppm significantly increased the pedicle length of flowers than application of 100 ppm Putrescine 9.8cm as compared with control plants 9.0cm in both seasons.

On the other hand, using concentrations of 40 and 60 ppm Uniconazole significantly decreased pedicle length of flowers than the control plants 8.9 and 9.0 cm for the 1st and 2nd season.

Length of inflorescence stalk

Data in Table (2) revealed that foliar spraying of Chrysanthemum plants with Putrescine significantly increased the length of flowering stalk in the two seasons. The highest value 30.6 and 32.3cm had been recorded for plants treated with 200 ppm as compared with control plants 20.7 and 22.0 cm in the 1st and 2nd season, respectively.

In case of Uniconazole; data showed that as the concentration was raised the recorded mean value for this character decreased to reach its minimum value 16.6 and 17.3 cm for the 1st and 2nd respectively, as compared with control plants 20.7 and 22.0 cm. These results were in harmony with those obtained by (Mao *et al*., 1991) on *Salvia splendens* and (Haggag, 1997) on Chrysanthemum, who stated that PP-333 application reduced flower stalk length.

Vase life/days

Data in Table (2) indicated that the control treatment recorded 11.3 and 14.3 days for flowers vase life, while using 200 ppm Putrescine gave the longest period of flower vase life 26.0 and 27.0 days in 1st and 2nd seasons, respectively. In 1st season treated plants with 100 and 300 ppm Putrescine gave 20.7 and 24.3 days. The same trend was obtained in the 2nd season.

Application of Uniconazole increased significantly the flowers vase life. The longest vase life 19.0 and 20.0 days had been recorded for plants treated with 20 ppm in the 1st and 2nd seasons, respectively.

These results are in agreement with that obtained by(Mahgoub *et al*. ,2006 b) on *Dianthus caryophyllus* plants.

The increment of vase life of flowers due to growth regulators may be due to increasing protein content in petals and ovaries (Lukaszewska,1988), or due to cytokinins, which are able to reduce and delay the production of endogenous ethylene.

Also polyamines may retard senescence by inhibiting ethylene production (Suttle, 1981). The increment of polyamines was accompanied by inhibition of lipid peroxidation and the inhibition of lipid peroxidation may be one of the mechanisms responsible for the anti-senescence effects of polyamines. (Borrell *et al*., 1997)

Fresh weight of inflorescence

Data in Table (2) indicated that spraying the plants with Putrescine had significantly effect on the fresh weight of flowers. The highest value had been obtained from spraying the plants with 200 ppm.

Using Putrescine at the concentration of 100 ppm and 300 ppm increased fresh weight having 6.3and 7.0 g for the 1st season and 6.6 and 7.3 g for the 2ndseason, respectively.

Application of 20 ppm Uniconazole increased significantly fresh weight of treated plants. The main estimated values were 5.3 and 6.3 g while the control plants, produced 4.5 and 4.6 g in both seasons, respectively. Raising the application rate to 40 ppm and 60 ppm decreased as the concentration was raised.

Putrescine was more effective on increasing fresh weight of Chrysanthemum plants, as compared with application of Uniconazole. This may be explained that Putrescine enhanced the accumulation of the photosynthetic products in the plant tissues, i.e. flowers.

Dry weight of inflorescence

Using Putrescine at 100, 200 and 300 ppm gave the values of 2.4, 3 .0 and 2.7 g as compared with the control 1.58 g in the 1st season. Similar trend was found in the 2nd season.

Application of Uniconazole at 20, 40, and 60 ppm recorded the values 1.94, 1.91 and 1.76 g for the 1st season. The same trend was found in the 2nd season. From these results it was noticed that using Putrescine at 200 ppm and Uniconazole at 20 ppm resulted in the highest increase in the dry weight of chrysanthemum flowers. These results are in agreement with those obtained by (Singh and Bist ,2003) on rose, (Mahgoub *et al.,* 2006 b) on *Dianthus caryophyllus* and (Mahgoub *et al*.,2006 a) on *Calendula officinalis*, (El –Quesni *et al*. ,2007) on *Bougainvillea glabra* plants, they found that paclobutrazole or Putrescine increased dry weight of flowers.

These results may be due to the promotive effect of Putrescine, which is essential for plant growth and differentiation and thus involved in various physiological processes (Flores and Galston, 1982 and Friedman *et al*., 1989).

Chemical constituents
a- Carbohydrate content

Data in Table (3) showed that all concentrations of the two bioregulators increased the total carbohydrates content in flowers of chrysanthemum and the highest values were found when the plants were treated with Putrescine at the

rate of 200 ppm and Uniconazole at the rate of 20 ppm. These increment in total carbohydrates content may be attributed to the increase in photosynthetic process efficiency, which led to increase assimilation of leaf CO_2.These results are in agreement with those obtained by, (Mahgoub *et al*.2006 b) on *Dianthus caryophyllus* and (El-Quesni *et al*., 2007) on *Bougainvillea glabra* plants as they obtained increases in the total carbohydrates content in the plants treated with different concentrations of Putrescine.

Concerning the effect of Uniconazole on the carbohydrates content in plants, these results are in agreement with those obtained by ,(Mahgoub *et al*., 2006 a) on *Calendula officinalis* , (Bekheta *et al*., 2003) on *Thymus serpyllum* plants and (El-Quesni *et al.*, 2007) on *Bougainvillea glabra* plants.

b. Photosynthetic pigments
Chlorophyll (a)

Data in Fig. (1) Illustrated that spraying Chrysanthemum plants with Putrescine significantly increased the content of Chl. (a) in recent leaves of plants.

In the 1st spray data showed that Treating plants with 200 ppm Putrescine caused the highest values of the content Chl. (a) for the 1st and 2nd seasons, respectively, followed by 300 ppm Putrescine, whereas the lowest concentration resulted in the lowest values of Chl. (a) for the control plants in the three sprays compared with the other treatments. The highest values were showed with plants treated with 200 ppm in the st spray, followed by the same concentration and in the 3rd spray at the two seasons, respectively.

Spraying the plants with 20 ppm significantly increased chl.(a) over the control in the 1st and 2nd season,respectively, followed by 40 and 60 ppm Uniconazole, which gave the lowest value of chl.(a) within the treatments but higher than the control in the two seasons (Fig.1).

In case of 2nd spray, in the 1st season, the data indicated that all the values of chl.(a) content were over control plants, when the plants were treated with 20, 40 and 60 ppm Uniconazole. The highest content was recorded in the plants treated with 20 ppm Uniconazole, which was higher than the untreated plants, whereas there was no different ratio, when the plants were treated with 40 and 60 ppm. In general chlorophyll (a) content clearly differed from control in the 1st spray of the two seasons,

this means that the used bioregulators showed only their effect early at the beginning of their application, while after that the application was less effective.

Chlorophyll (b)

Spraying the plants with 200 ppm Putrescine produced the highest content of Chl. (b) in all sprays than that for control plants, while decreasing the concentration to 100 ppm reduced the content of Chl. (b) in both seasons (Fig. 2).

Regarding to Uniconazole treatments the data showed that spraying the plants with Uniconazole at the rates of 20, 40 and 60 ppm significantly increased the Chlorophyll (b) content; giving the highest content at the lowest concentration (20 ppm), while raising the concentration resulted in reduction of chlorophyll (b) content. The highest values were found in the 1st spray in 1st season.

The application of three sprays showed that their greatest effect had been obtained at the 1st spray.

Total carotenoids content

Data in Fig. (3) Revealed that, spraying Chrysanthemum plants with Putrescine and Uniconazole at the different concentrations affected significantly on increasing total carotenoids content in the two seasons. The highest values of carotenoids were obtained in plants treated with 200 ppm. Concerning Uniconazole treatment it was found that spraying the plants with 20 ppm resulted insignificant increase in the $1^{st}$and 2nd season at the 1st spray, followed by the $2^{nd}$and finally by the 3rd spray.

These results are in agreement with those obtained by (Talaat *et al*., 2005) on *Catharanthus roseus* and (Youssef *et al*., 2004) on *Matthiola incana*. Polyamines stimulated some physiological responses including vegetative growth and photosynthetic activity (Yanghua *et al*., 1996 and Chattopadayay *et al*., 2002). They found that different concentrations of Putrescine increased chl (a), (b) and total carotenoids, respectively.

With regard to the effect of Uniconazole on photosynthetic pigments, the obtained results are in harmony with those obtained by(Bekheta *et al* .,2003) on *Thymus serpyllum* plants and(El-Quesni *et al*., 2007) on *Bouganivillea glabra* plants, they stated that treating the plants with triazole compound at 100 ppm concentrations increased chlorophylls contet.

Table 1. Flowering start after spraying of *Chrysanthemum indicum* L. plant as affected by Putrescine and Uniconazole during 2004/2005 and 2005/2006 seasons

Treatments	2004/2005				2005/2006			
	Dec.	Jan.	Feb.	Mar.	Dec.	Jan.	Feb.	Mar.
Control	4	11	18	15	8	14	17	15
Put., 100 ppm	15	22	28	20	17	24	26	20
Put., 200 ppm	25	28	35	26	24	29	33	28
Put., 300 ppm	27	31	22	25	29	33	24	22
Uni., 20 ppm	——	5	10	7	——	6	10	9
Uni., 40 ppm	——	7	6	9	——	9	5	9
Uni., 60 ppm	——	5	7	5	——	4	6	6

Table 2. Effect of Putrescine and Uniconazole on inflorescences characters of *Chrysanthemum indicum* L. plant during 2004/2005 and 2005/2006 seasons

Treatment	flower Yield /plant (No)		Flower diameter (cm)		Pedicle length (cm)		Length of inflorescence stalk (cm)		Vase life (days)		Fresh weight (g)		Dry weight (g)	
	Season													
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control	72	078	5.6	5.9	8.9	9.0	20.7	22.0	11.3	14.3	4.5	4.6	1.58	1.60
Put., 100 ppm	104	108	7.5	7.7	9.1	9.8	27.3	28.6	20.7	22.0	6.3	6.6	2.40	2.45
Put., 200 ppm	147	161	8.0	8.6	10.0	11.5	30.6	32.3	26.0	27.0	7.1	8.2	2.99	3.12
Put., 300 ppm	108	120	7.8	8.1	9.8	10.1	28.2	29.8	24.3	24.0	7.0	7.3	2.66	2.59
Uni ., 20 ppm	103	104	6.8	7.2	7.5	08.7	18.1	19.2	19.0	20.0	5.3	6.3	1.94	2.30
Uni ., 40 ppm	097	100	6.9	6.5	6.8	07.7	17.8	18.7	18.0	18.3	5.3	6.0	1.91	2.18
Uni ., 60 ppm	089	090	6.6	6.4	6.0	07.0	16.6	17.3	17.0	18.0	4.9	5.0	1.76	1.78
L.S.D 0.05	4	5	1.1	0.9	1.4	0.9	2.2	2.4	2.0	2.4	0.2	1.1	0.15	0.54

Table 3. Carbohydrates contents (D.W %) in flowers of *Chrysanthemum indicum* L. plant as affected by Putrescine and Uniconazole treatments during 2004/2005 and 2005/2006 seasons.

Treatment	Flowers	
	2004/2005	2005/2006
Control	10.8	14.3
Put., 100 ppm	15.0	14.3
Put., 200 ppm	17.4	21.7
Put., 300 ppm	15.0	19.6
Uni., 20 ppm	19.7	21.5
Uni., 40 ppm	16.8	16.0
Uni., 60 ppm	14.0	15.8
L.S.D 0.05	3.7	2.1

Put= Putrescine   Uni= Uniconazole
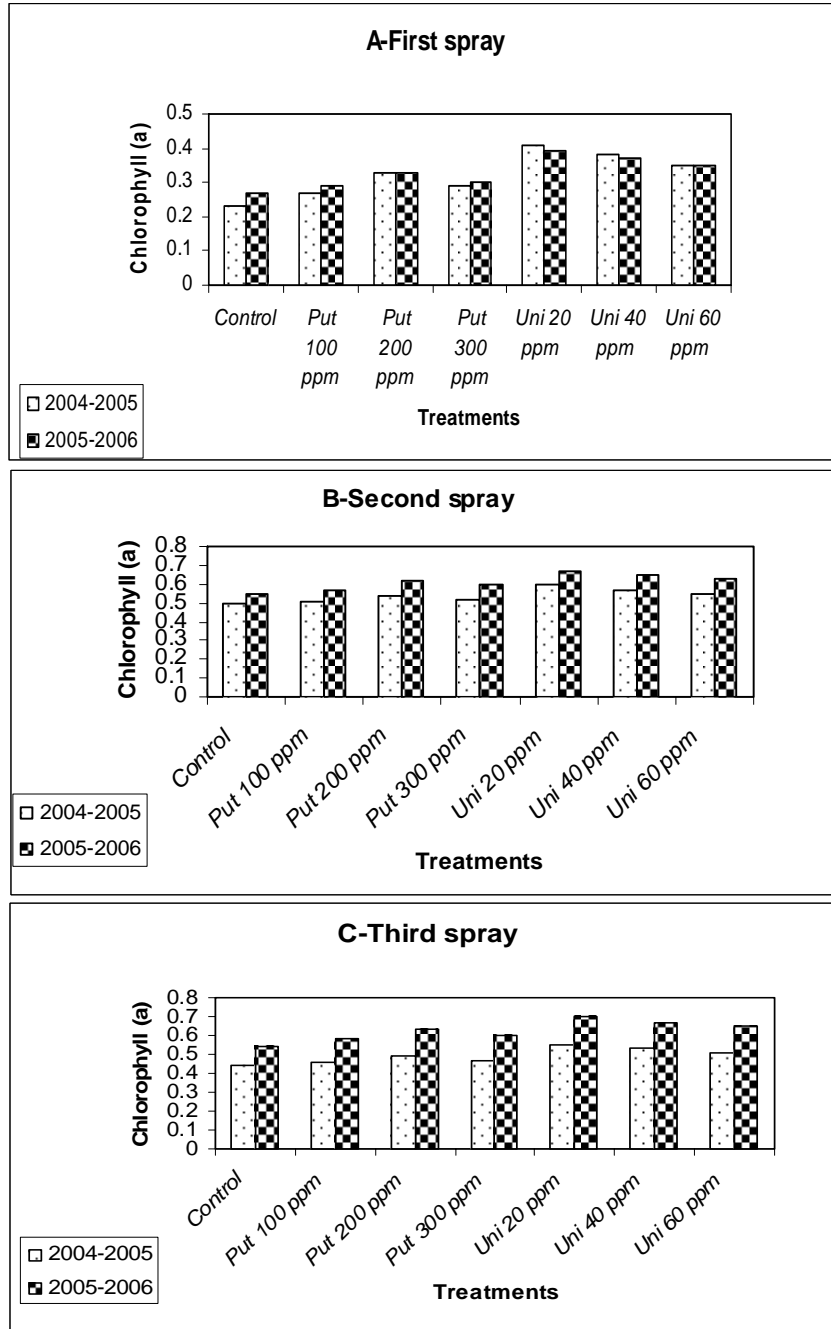
Put= Putrescine   Uni= Uniconazole

Fig.1. Chlorophyll (a) content (mg/g F.W) of *Chrysanthemum indicum* L.plant as affected by Putrescine and Uniconazole treatments during 2004/2005 and 2005/2006 seasons.
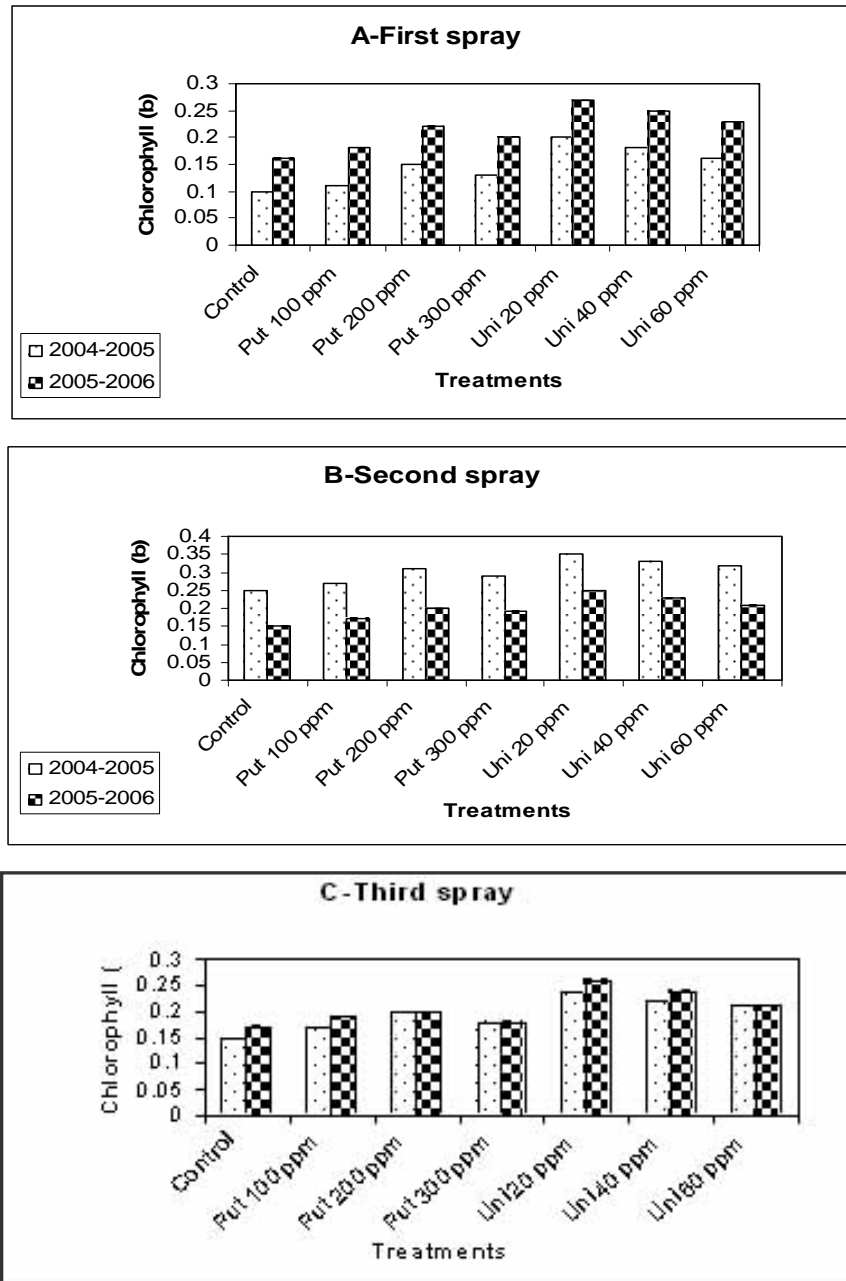
Put= Putrescine   Uni= Uniconazole

Fig. 2. Chlorophyll (b) content (mg/g F.W) of *Chrysanthemum   indicum* L. plant as affected by Putrescine and Uniconazole treatments 2004/2005 and 2005/2006 seasons.
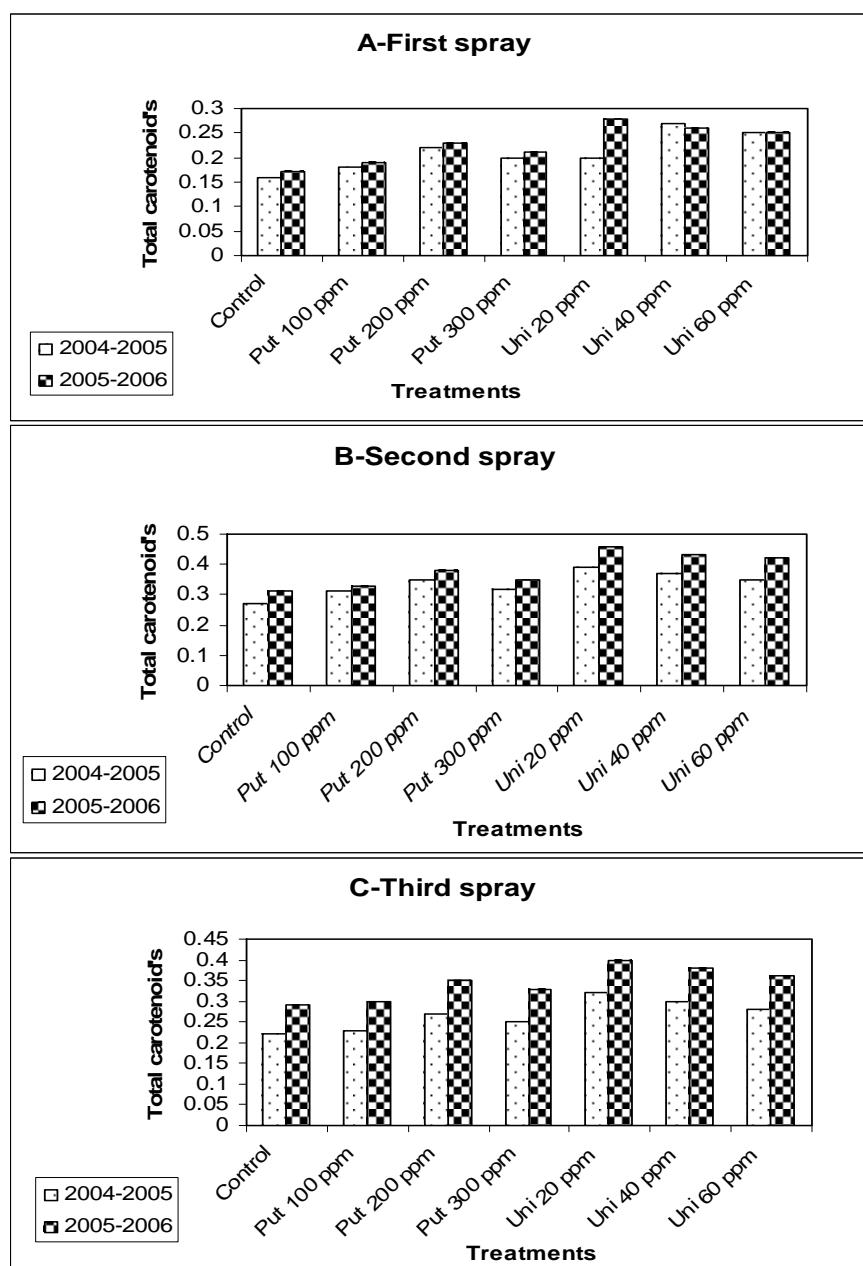
Put= Putrescine   Uni= Uniconazole

Fig . 3.   Total carotenoids content (mg/g F.W) of *Chrysanthemum indicum* L. plant as affected by Putrescine and Uniconazole treatments 2004/2005 and 2005/ 2006 seasons.

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The empowerment of rural women through Information and Communication Technologies (ICT)

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Abstract: Rural women are among those major groups at society who previously were considered less by planners, due to specific reasons in the past. And this problem is more observable at developing countries. While, by looking at women's history of economic and social life, we can find that this great group, continuously have played basic role in forming economic condition of country. This great group consistent with men have had active role at areas of social-economic activities and always have had major part on economic production of society. ICT is now recognized as a technological tool which can serve as a catalytic intervention in respect of transforming the lives and livelihoods of rural families.The economic and income divides between urban and rural areas can be overcome only by the technological upgradation of rural professions.

Introduction:
Information and Communication Technologies or ICTs as they are collectively called have made enormous strides. In a relatively short period of time of our planet's history, its impact has been felt across the globe and through various strata of society. It would not be bold to say that no one has been left untouched in some way or the other. However, the benefits have not been spread evenly. In fact, this has given rise to a new form of divide often called the "digital divide". Due to the lack of resources and particularly the economic might, the poor never attract the attention and thus never get to draw the full benefit from these emerging developments.

As this book will show, what is ironic is that these very same developments hold the key to massive transformative potential in their lives. There is a window of opportunity for the rural masses and the poor to make a game-change switch. So who would play that catalytic role?

According to some predictions, we are moving into an 'information society' by which few aspects of our lives will remain untouched. It is claimed we will have access to unlimited quantities of information; we will be able to watch any number of TV channels from all over the world; we will do our shopping and banking from home. Still deeper changes will take place in society. For example, we are told patterns of employment will change - we will be able to work from home, choosing how and when we work. We are told that the cause of these unprecedented transformations in society is the 'communications revolution'. Are these scenarios credible, even feasible, or are they merely examples of authors' imaginations running wild? This course gives an insight into the developments in communications technology which give rise to these predictions. It includes an overview of the technology and examines some of the social implications which are claimed for the new developments. Broadcast media are covered including delivery systems such as cable and satellite transmission.

Technologies such as videoconferencing and the Internet, the so-called 'convergent technologies', are also included; digital coding techniques now enable computers to store, process and deliver motion video as well as audio and text; modems can connect these same computers to others anywhere around the world. Thus it is no longer meaningful to discuss the broadcast media without consideration of computer technologies. This is not a technical course; there are no scientific theories or mathematical equations at all.

The global economy requires the kind of necessity and purpose of educational institutions. Since the current trend towards reducing incomplete information and access to accurate information is growing, other schools can not control time to transfer a set of prescribed information from teacher to student during a fixed time point are, but schools must to promote Culture of "Teaching for Learning For example, acquisition of knowledge and continuous learning skills which make possible during the individual's life. According to Alvin Toffler, illiterate in 21st century, who was not read and write but those who do not know which fail to learn or remember are illiterate. (Jauhari, 2004).

Concerns about educational quality and educational opportunities with the necessity of developing those

most vulnerable are the accumulation of globalization is symbiotic. Generally, "the changes of globalization in developing countries, on low-income groups, especially women and girls and" low skill workers, as well as all groups applying for and obtaining new skills to press. (Bellamy and Taylor, 1998).

In the rural context, development involves use of physical, financial and human resources for economic growth and social development of the rural economies (Burkey, 2000). The term rural development also represents improvement in quality of life of rural people in villages. As per Chambers (1983) "Rural Development is a strategy to enable a specific group of people, poor rural women and men, to gain for themselves and their children more of what they want and need." Singh (1999) defines Rural Development as "A process leading to sustainable improvement in the quality of life of rural people, especially the poor". The fact of the matter is that three quarters of the world's poor, about 900 million people are in rural areas, and the Millennium poverty target set by Millennium Development Goals (MDG), cannot be met unless the world addresses rural poverty. "Sustainable Rural Development can make a powerful contribution to four critical goals of: Poverty Reduction, Wider shared growth, Household, national, and global food security and Sustainable natural resource management" (World Bank, 1997). Hence worldwide there is a growing emphasis on development of rural economy of the countries. Any improvement, in the social or economic status of rural areas would not just directly benefit rural poor but would also bring down the migration-pressures on cities and contribute by positive ripple effect in global stride towards development.

The process of development in a country is to be aided by its governance. The goal of governance "should be to develop capacities that are needed to realize development that gives priority to the poor, and creates needed opportunities for employment and other livelihoods" (The World Bank, 1992, UNDP, 1994). Increased number of poor, hungry or marginalized people in a country represents decrease in its quality of governance. To promote development, various studies have proposed governance in the contextual realities of each country, including veritable participation of citizens in the governmental decision-making process (Grindle, 2004; Evans and David, 2006).

Communications and Information Technologies continue to undergo rapid change and will continue to impact all aspects of the university. Some of these impacts are obvious to both the technical and the casual user but many take place in the background. They include: new approaches to old activities, new activities that were previously not possible, more personalization of communication and applications, and increasing efficiency and effectiveness in everyone's work environment.

Women form great part of total workforce that needed for agriculture part at universe, as one of the intangible factors at agriculture economy. So, statistics that was represented in relation to extent of women's activity is very lower than real extent. Because in this statistics, mostly, seasonal jobs, part time job, no wage job and their housekeeping activities, aren't considered. rural women, have different roles and duties such as husband, mother, crops producer , participate at ranching activities , planting ,maintaining, harvesting, processing, marketing and preparing food. Rural women maybe venturing to culture cash products, while cultivating subsistence products and if they have no farm land, they have to work for others instead receiving wage. We can consider such women as agriculture propagator, production expert and even in some case as policy maker. Other than activity at agriculture field, women's participation at rural development is critical and is considered in order to supply adequate and needed food (Lahsaeizadeh, 2000). According to women's role at family, they can be considered as base of development and progress and unfortunately according to universal tangible realities, they possess unfavorable position at international level (Changizi Ashtiani, 2003).

For example, difference at levels of policy making, investing and receiving salary for equal activity, are universal phenomena. extent of women's participation at economic activities, extent of women's activity at economic activities, is confirmation on lack of adequate attention to women's affair and their added value, because rural women work alongside men, at all levels of producing agriculture crops and livestock products and generally all affairs, and also spend their little leisure time for handicrafts such as rugs and carpets and etc. so it is necessary to establish self acknowledgement fields, directing women's economic and social ability and programming to attract their participation at different activities. At rural area, women have more significant role on family economy and inside activities and cause economic prosperity of society. yet, women couldn't gain their real position as active citizens who have talent for participation at economic, politic, social and cultural arena at most countries, especially developing country, and still their activities in economic calculations aren't considered, and they be considered as intangible workforce. Disappointing estimation about number of active rural women and underestimate about extent of their participation at economic activities is confirmation on lack of

adequate attention to women's affairs and their added value. they are major force to create revolution and potential sources to progress rural economy and increasing extent of growth rate of producing food productions, although traditionally, farming and ranching, has been male profession, but women's role was never restricted to house and family, so they are active outside (farming, ranching, forestry and …) other than inside activity (Balali, 2005).

Empowering rural women:
Empowerment is capacity that woman can obtain in cultural and social environment, for economic independency and self reliance, by controlling over emotional decision making and far from violation. Empowering means, evolution and developing activities through non governmental organizations (NGOS) that lead empowerment to improve economic dimensions. (Amiri, 2000)

Enabling is process that, during it, people of society do activities to overcome barriers of advancement that finally cause their domination to determine their own density. The term "enabling" means overcoming fundamental inequalities. So it is different from self-reliance. (UNICEF, 1997)   2-
Enabling, enables individual to overcome any problematic condition and consider barriers and problems as part of life and positive campaign. Finally, enabling provides energy to overcome most intellectual barriers and external problems at private life.

Thus, among all what have been said, it is possible to present suitable definition of enabling women, as follows:
"Process of explaining women about themselves (and also men about them) for instances that they must or want to do, and growth of their willingness and courage until they reach to needed competency "(management of rural and tribal women).

it should be noted here , that major factor which should be considered about women's ability , is eliminating individual and social barriers , and finally preparing field of economic and social participation for women at all fields . purpose of women's participation , is because of their dominance on all affairs of village including decision making process , organizations , forums , enterprising posts and … that involve , participation at all social and economic dimensions .

Criteria of empowering women:
 Enabling as a theory of policy making for women, in it present five criteria:
Welfare, access, Concientisation, participation and control.

1-   **welfare criteria :** In this criteria, men and women as human resources of development should enjoy of desirable welfare conditions and equality (Paknazar, 2000).

Most of timing developmental programs, have worked on base of women's welfare. They have considered and provided some services for women who were passive recipient of these services. But these services were limited to physical needs and mostly were considered to revive their role of productivity, again. sometimes , it has been said that this approach has begun at colonial era and has considered women from poor country and intended services for them that dose not exceed from that poverty level . Agricultural and industrial projects were designed for men and social programs for women and children. Most of welfare programs were inadequate or its success was limited. Considerable point in this criteria is that men and women as human resources of development should enjoy equality and desirable welfare conditions. At this stage, women's material welfare and their enjoyment of welfare programs, compared to men (nutrition, death rate and …) were considered. And women's role as producer to supply their own needs isn't very important.

access criteria : Lack of access or limited access for women to sources including (fields, job, capital and training) cause that their functions at production is less than men (Paknazar 2000). Access to facilities, sources, designed program and projects for women and access to schools and … are in this part. Just whenever most of other legal, cultural and social issues being solved, men and women would equally access to sources and facilities. Concept of enabling at this stage is that women have equal right to access to sources at family and greater society.

3- Concientisation criteria: Women should know that their problems aren't due to their individual inefficiency and shortage but it has emerged by social system in which discriminations has become formal and acceptable issue. (Araghzadeh, 2002). This stage is more critical and important than other stages. Because women can participate at development activities not just be passive users. Women have real equality at development, just when be aware. Concientisation will help to increase women's ability to equality at participation at society. At this stage, women face with critical analysis with society and will find that what has been considered natural and unchangeable reality, is changeable. (Bakhshoodeh, 2005).

4- Participation criteria: One the most important items that this criteria has considered , is men and women's equal participation at decision making process of affairs of family at society (Paknazar 2000) . Men and women both should participate at process of assessment needs, designing, performing and evaluation of projects and development programs

(UNICEF, 1998). In summary, this criterion means women's participation at all stages of surveying needs, detecting problems, planning, management, performing and valuation.

5- Control criteria: This criterion emphasize on this point that in addition to equal access of men and women to development sources , they must have adequate control on these sources that this issue is balance criterion , between men and women so that no one exceed other one (Paknazar 2000) . Women should have opportunities for decision making at workplace and home. If woman is producer, should be shared with part of her interest and wage. Women like men, should be able to choose her individual and social field and able to make decision and also development activities should be facilitator of these processes.

FAO (food and agricultural organization) addresses these three purposes as strategic goals while enabling women:

1- equality between men and women to access production sources
2- women's participation at policy and decision making
3- decreasing rural women's workload and increasing job opportunity and income for them (Paknazar 2000)

within theoretical framework of enabling women , having control on sources is presented as highest stage at women's participation process on development , but existing data at most developing countries , indicates that not only rural women haven't any control on financial resources of family but even they were deprived to access to sources and credits , specially through formal credits system (Shaditalab, 2002) .

The question that arises here is that what relation is there between enabling women and micro-credits programs? Nowadays, micro-credits are considered as effective mechanism to eradicate poverty for women. Interests of micro-credits further increasing women's income, include:

- improving women's role in family
- Increasing women's confidence, not only through obtain financial success through business activity, but through increasing women's access to social services and communication with other women.
- Changing at social level (social class) at perspective of women's role.

Conclusion:
Regardless of the wide differences in ICT access between rich and poor countries and between different groups in the country, there are concerns that challenge the application of ICT in education with the existing differences among the lines of

economic, social, cultural, geographic and gender will be broader. Everyone equal opportunities in terms of suitability for participation are necessary, but access to various factors, either as users or as producers through their sources is difficult and heavy. Therefore, the primary differences enhance and even grow. Consequently, programmers' international education is faced with a difficult challenge and how to help solve the problem and its development.

Rural women at agriculture activities have key role as producer. Rural women are most efficient among society women and are such individuals that work in productive occupations, thus it is obvious that attention to rural women as powerful force at rural development can have very positive impacts at this regard.

One of the issues that government should pay attention to is rural development issue especially at undeveloped countries. in this countries due to lack of proper policy making to improve quality of people life level of these areas, villagers migration to cities has increased considerably and led to urbanization growth and emergence of problems and also psychological, social, cultural and economical abnormalities especially at agriculture and ranching part. Also method for growth and rural development growth, require research at this field which can help government in order to economic, social and cultural programming and policy making. Creating local organizations and regional institutions with affective women's attendance and villager participation to solve problems are among important and affective substances that should be considered in regional programming. at developing and changing process of developed economy system of agriculture, value of women's activity changed as form of money which previously was as no wage workforce at family , and was given to her. Other than agriculture part (i.e. industry and public services) which are main field of women's work, rural women's participation is very important. The most important issues about women's social and political participation are participating at programming, decision making, performing decisions and valuing results.

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The importance of Information and Communication Technologies (ICT) in agriculture development in developing countries

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Abstract—In recent years, assistance from developed countries to developing countries has intensified. Information and Communication Technologies (ICTs) have also been widely deployed in developmental programmes, leading to the creation of a new field – ICT for development. This paper reviews a number of projects that introduce technically innovative ICTs that are intended for the development of marginalised rural areas.
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Keywords: Information and Communication Technologies (ICT), agriculture development, developing countries

Introduction:

In the rural context, development involves use of physical, financial and human resources for economic growth and social development of the rural economies (Burkey, 2000). The term rural development also represents improvement in quality of life of rural people in villages. As per Chambers (1983) " Rural Development is a strategy to enable a specific group of people, poor rural women and men, to gain for themselves and their children more of what they want and need." Singh (1999) defines Rural Development as "A process leading to sustainable improvement in the quality of life of rural people, especially the poor". The fact of the matter is that three quarters of the world's poor, about 900 million people are in rural areas, and the Millennium poverty target set by Millenium Development Goals (MDG), cannot be met unless the world addresses rural poverty. "Sustainable Rural Development can make a powerful contribution to four critical goals of: Poverty Reduction, Wider shared growth, Household, national, and global food security and Sustainable natural resource management" (World Bank, 1997). Hence worldwide there is a growing emphasis on development of rural economy of the countries. Any improvement, in the social or economic status of rural areas would not just directly benefit rural poor but would also bring down the migration-pressures on cities and contribute by positive ripple effect in global stride towards development.

The process of development in a country is to be aided by its governance. The goal of governance "should be to develop capacities that are needed to realise development that gives priority to the poor, … and creates needed opportunities for employment and other livelihoods" (The World Bank, 1992, UNDP, 1994). Increased number of poor, hungry or marginalised people in a country represents decrease in its quality of governance. To promote development, various studies have proposed governance in the contextual realities of each country, including veritable participation of citizens in the governmental decision-making process (Grindle, 2004; Evans and David, 2006). Several institutions and experts accept Governance as a reflexive process, wherein policies, institutions, outcomes and analysis interact, to maximise the process of participatory development (UNDP, 1997; Ludden, 2005; Mehta, 2006).

There are two opposite perspectives on the role of ICT in society. One part of scholars views computers and the Internet as magic entities with the power to transform society. They consider the Internet as a new medium of communication, helping to cope with issues of social exclusion, social inequality. According to Manuel Castells (2002: xxxi), this is one of the reasons "why, after three decades of existence, it emerged from specialized communities in the world of researchers, techies, hackers, and countercultural communities, to catch fire in business and in society at large". But there are others who consider the new ICT as a tool to strengthen social inequality and widen the information gap, when one part of the population (haves of information) uses digital devices, while the

other part of the population (non-haves) is in a digital divide.

The last decade has seen a number of exciting changes in access to and use of information and communication technologies in rural areas. And not just in the comparatively wealthy countrysides of Western Europe or North America –even rural areas with some of the lowest levels of income in the World, from Kerala in India to Lindi in Tanzania, have seen an explosion in access to communications technologies, and these technologies are proving increasingly powerful as new applications spread. It is no exaggeration to say that this extended access has made a real difference to the quality of life of billions worldwide, including perhaps more than a billion people in rural areas.

It is no surprise that this 'ICT revolution' has proven a powerful source for creative vision by utopian thinkers the world over. Not least, Tom Friedman's best-selling works suggested that ICT has shrunk the world "from a size medium to a size small," offering new opportunities to poor people and regions to compete. He suggested that the Internet, along with globalization "are acting like nutcrackers to open societies," so that the Middle East is on the edge of a democratic revolution –and so on. Internet visionary George Gilder went as far as to suggest that the Internet presages "the overthrow of matter… that will make the new millennium a time of awakening to the oceanic grandeur and goodness of the universe" (see Kenny, 2006, for sources).

he Millennium Development Goals of the United Nations address a series of global social development issues, including halving extreme poverty by 2015 [1]. Since the publication of the millennium goals in 2000, much attention in the field of ICT has been focused on bringing ICT to poverty-stricken areas. Such projects have often not been evaluated scientifically, so there was until recently little evidence of the efficacy of ICT for development (ICT4D) in these environments [2]. However, most evidence supports deployment of ICTs in such areas and by some reports, ICTs can assist with several of the MDGs simultaneously [3]. Mostly off-the-shelf technologies have been used in innovative ways, but with the advent of funding by large international development organizations and also private companies, the field of ICT4D has increasingly become an area of technical innovation.

Information and communication technologies (ICT), including radio and television and the newer digital technologies like computers and the Internet as potentially are introduced powerful tools and activators of educational reform and changes.different ICT, when properly applied can be developed to help access to education and the

relationship between training and workshops to strengthen the increasingly digital, the quality of education also helped to create teaching and learning in an active process connected to real life high take. However, the experience of being raised by ICT in the classroom and other educational sites around the world during the last few decades proves that is not automatic fully realize the potential benefits of ICT training.(Guptaand et al, 2004).

In the past few years, the power of Internet as a communication medium has captured the imagination of developmental organizations around the world. A number of projects have been undertaken in various parts of the world attempting to provide sustainable digital access to rural communities.

Wider Access of ICT:

The reach of information and communications technologies around the world has been expanding for decades. There is one television set for each four people on the planet (World Bank, 2005). In India, over 112 million households have a TV. As early as 1995, television exposure in China was estimated at one billion people (Jensen and Oster, 2006). The reach of radio is probably even greater. But the recent past has seen particularly rapid rollout of access to telephones and the Internet, as technology advance has driven down costs. Fixed phone connection and rental charges have more than halved worldwide over the past decade. Meanwhile, mobile handset costs have dropped to as low as $50 and companies in LDCs claim that they can profitably provide service at an average revenues per user of just $60 a year. As a result, the proportion of households worldwide that had a fixed telephone almost certainly surpassed 50 percent in 2003. And even more rapid growth in access has been driven by mobile telephony. The number of mobile subscribers worldwide increased from 11 million in 1990 to 1.7 billion in 2004.

This access has extended to people previously far from any phone. In Burkina Faso, for example, there were fewer than 7,000 telephones outside the capital city in 1990, serving a population of 8.3 million people spread across an area of over 100,000 square miles. There was no mobile phone service. In 2002, the mobile footprint (the area of the country where a mobile phone signal is available) covered 5.4 million people outside of the capital—far more than half of the population living outside of Ouagadougou. More widely, perhaps 83 percent of rural people in South Asia had access to a telephone in their village in 2002. In Africa, a 2001 survey of Ghana, Uganda and Botswana found that, even in rural areas, between 75 and 80 percent of respondents had made a phone call in the last three months. Across the globe, an estimated 86 percent of the World's

population, including a considerable majority of rural populations, were under the mobile footprint in 2004 –and it appears quite likely that total telecoms access rates are even higher than that (Keremane and Kenny, 2006).

While the recent spread of the Internet has been somewhat less dramatic than that of the telephone, its speed would be unprecedented for a communications technology were it not for mobiles. Both Internet and mobiles reached ten percent of the world's population within fifteen years of invention, and there are already far more Internet users in the developing world than in the developed. The number of users tripled over the 2001-2005 period in the developing world, reaching over 440 million (UNCTAD, 2006). Of course, rural areas in developing countries in particular do still see very low Internet usage. A few years ago, but ten percent of Thailand's Internet users were rural, despite the fact that rural people made up nearly 70 percent of the country's population (Kenny, 2006). Many rural areas of even comparatively rich developing countries still see Internet usage rates at below one percent of the population. Nonetheless, the opportunity to use the Internet has spread far and wide, even if usage itself has not.

More Uses of ICT:

Again, there is a long record of ICT use having an impact on development outcomes in rural areas. Over 700,000 secondary-school students in remote villages in Mexico watch the Telesecundaria program of televised classes. While students enter the program with lower mathematics and language test scores than the average, by graduation they have caught up in math and halved the language-score deficit (de Moura et al, 1999). Survey evidence from within developing countries has long suggested that rural areas with access to telephones see lower prices for inputs, higher prices for outputs, larger non-farm incomes, a greater number of small and medium enterprises and better delivery of public services (Forestier et al., 2002).

As technologies have spread, so have their impact. Robert Jensen and Emily Oster of the National Bureau of Economic Research study the rollout of cable television access in rural India and conclude that the introduction of cable in a village is associated with higher female school enrollment, declines in fertility and increased female autonomy (Jensen and Oster, 2007). The size of these effects is large: within two years of introduction, between 45 and 70 percent of the difference between urban and rural areas on these measures disappears, and the impact of cable TV on fertility decisions is as large as increasing the length of time girls stay in school by around five years.

Regarding the spread of mobile technologies, Grameen Phone has leased cell phones to poor rural women who set up local village pay phone shops. In a review of the early experience of the Grameen Phone project, this service was found to be of considerable benefit both to the provider and the users. Not least, the average operator was earning between 24 and 40 per cent of household income from providing phone services and the estimated consumer surplus from phone usage ranged as high as $2.70-$10 per call (Richardson et al. 2000).

This consumer surplus derives in part from the significant power of communications to improve market outcomes. In Kerala in India, mobile phone service was introduced over the period 1997-2001. One result was a dramatic improvement in the efficiency and profitability of the fishing industry. As mobile phone service spread, it allowed fishermen to land their catches where there were wholesalers ready to purchase them. This reduced waste from between 5-8 per cent of total catch to close to zero and increased average profitability by around 8 per cent. At the same time, consumer prices fell by 4 per cent (Jensen, 2007).

ICTs for development

The idea that the Internet and related technologies might have an important role in aiding developmental efforts has captured a central place in international policy debates. Over the course of the last few years, statements affirming the need to close the so-called 'digital divide' between social groups with and without access to the internet have been made through several UN agencies, and at meetings of developmental organizations around the world. The idea of digitally oriented development is as powerful and seductive as the technology upon which it is based. No single technological revolution has changed the lives of current generations in the way that the Internet has. For example, it took at least a century before the printing press touched 50 million individuals. It took 38 years for radio to reach the same number, and thirteen years for television. But the World Wide Web, in only four years, exceeded the 50,000,000 mark[13]. Never before has a communications revolution spread so rapidly.

The promise of digital development is that it might have the same reach as the original Internet boom of the mid 1990s – only this time the most disprivileged communities, those who had missed out on earlier waves of technology, might be able to 'leapfrog' over their more developed competitors. The greatest obstacles to rural development in developing economies – large distances and

inadequate infrastructure, might be obviated by instant access to virtual institutions that provide banking, education, health care, neonatal information, agricultural advice, and so forth.

At the same time, questions are being asked if ICTs are the way to go in developing countries, such as those in South Asia, where most rural populations lack running water and sanitation systems, electricity is still a scarce and intermittent resource, roads are poor and education a luxury. The value of IT for rural development is accompanied by this dilemma for decision makers and multilateral funding agencies: should the very limited resources for rural development be applied to developing IT capacities, or are they best used for other high priorities such as schools, hospitals, and dispensaries?

The concept of "Digital Divide":

One of the most hyped phrases in the context of ICTs for development is "Digital Divide". Kenneth Keniston[13] of MIT introduces the concept of, not one, but four digital divides. The first divide is that which exists within every nation, industrialized or developing, between those who are rich, educated, and powerful, and those who are not.

A second digital divide, less often noted, is linguistic and cultural. In many nations this divide separates those who speak English or another West European language from those who do not. This is quite notable in India, and is further compounded by linguistic issues. An estimated 60-80% of all Web sites in the world are in English while almost all the rest are in one of the major 'Northern' languages like Japanese, German, French, Spanish, Portuguese, and increasingly Chinese. But in India, like the rest of South Asia, only an estimated 2-10% of the population speaks fluent English while the rest (more than 900 million Indians and about 1.2 billion South Asians) speak other languages.

The third digital divide follows inevitably from the first two -- it is the growing digital gap between the rich and the poor nations. The fourth digital divide is the difference between the lifestyles of those who are in the IT or similar sunrise industries like Biotechnology and those who are in the other professions.

Bridging the Digital Divide:

With the understanding of digital divide, several agencies are also talking about bridging the digital divide. Interestingly, ICTs have been touted as the solution to development. It has been suggested that digital access could well be linked to wealth accumulation. Victor, Philip et al[15] suggest a positive correlation between teledensity and GDP per capita for low and middle-income countries. As for Internet access, Thomas Schauer[18] feels that Internet has not existed for sufficiently long, and it is not possible to examine whether poor countries which have put a focus on overall development (wealth first!) subsequently have better opportunities to create an information society or whether the strategy should be to invest massively into the IT infrastructure in order to create subsequent wealth.

So then what is the promise of ICTs towards reducing the "digital divide" and the "income divide" associated with it? It would be interesting to look at the case of ITC initiated e-Choupal project in India. This is an excellent example of how Internet access has created wealth for farmers from about 6000 villages in MP in India[10]. The project initiated in September 2001 by ITC was intended to gain control over Soya procurement supply chain. ITC harnessed the power of information technology to fill institutional voids in the Soya procurement chain by avoiding middlemen and directly buying Soya from the farmers. According to ITC "The intermediary has information and, thus, extracts a greater margin. So we said to ourselves if you bring this information to the farmer and use go- betweens where they are adding value directly, you have a business model." To do that, ITC first leased three Soya processing and collection centers. These centers were created in the mid-90s and had, since then, gone under. Then it started scouting villages around these centers for lead farmers (sanchalaks) to head each choupal. The computer was placed at the sanchalak's house and he was trained to use it. Having put them in place, ITC started to pump information on daily mandi prices through the Internet into the sanchalak's homes. Farmers would gather as they did at choupals, check the prices and head out to the collection centers to sell their produce. The idea of heading out to the collection centers struck the right kind of chords among the farming community. Here, because systems were efficient, the transactions are completed in a few hours rather than days as they used to. So they came to the collection centers in droves. The sanchalaks, for their part in directing farmers to these collection centers, were paid a commission of 0.5% for each tonne of soyabean that originated from their choupal. On ITC's part, the procurement costs of Soya came down from Rs 700 per tonne to approx. Rs 300 per tonne. The math looked roughly like this. On an average, it cost Rs 40,000 to set up a basic Choupal. In places where connectivity was terribly

poor, and telephone lines to connect to the Internet still a pipe dream, ITC invested in V-SATs. These investments jacked up costs by as much as Rs 1 lakh. According to ITC, it has been able to recoup the investment in three sowing seasons (18 months). At the time of going to print (January 2003), MP had 1,045 e-choupals spread over 6,000 villages that covered six lakh farmers. ITC is now planning to apply the model to wheat procurement, which is a market, multiple times that of Soya market.

Rural Information Needs:

But what relevance do ICTs have to rural consumers? Can ICTs be the solution to poor infrastructure for health, telecom and education in rural India? What are the information needs of the rural consumer? Many of these questions are answered by an NIC, Government of India study[6]. Based upon a survey in a rural area in the state of Bihar, following information categories were arrived at:

1. **Health**

2. **Agriculture**

   - Rainfall (forecasting)

   - Cropping Pattern

   - Modern Techniques of Cultivation/Farming

   - Irrigation (Sources)

   - Information on Market and Market Prices

3. **Education**

   - Distance Education/Learning

   - Information on Schools & Virtual Schooling

4. **Government Information**

   - Information on Soft loans & Financial Institutions

   - Information on Government Go downs

5. **Land Records**

Conclusion:

A common strategy in higher education ministries in developing countries is public and private sector partnership in strategy or pursue rapid ICT projects is based. This partnership has different forms such as grant aid private sector interaction with public assistance, donated educational equipment and components by companies to public schools, providing technical assistance for planning, management and consolidation tools and human resources at the local level. But after financial aid, testing programs based on ICT is critical.

Many of the ICT training programs based on the charitable agencies aid have been unable to have high durability. Because the government has failed in its financial assistance in this situation none of the local communities to provide resources do not needed to continue these programs. Two strategies in here "to support government and local communities to move" are important. Since the 21st century, is century of education support about youth in Asia, to find sustainable ways to bridge the digital age in Asian countries is a real priority. And work through partnership that local leaders and guides are experts it can be lasting forever.

Several recommendations that emerged from the discussions emphasized on the need to think of ICT in education beyond computer aided learning and investigate the potential other technologies like community radio and other medium. These mediums could not only be cost effective but also has a greater outreach potential. It was also pointed out that low cost software solutions for e-learning that have scopes for innovation, should be incorporated in large scale projects. With an indication to open source solutions, the sessions recommended that such solutions should become a part of the overall policy for implementating technology supported education interventions.

Sustainability and scalability of project are also issues that needed serious considerations. While moving beyond the pilot and experimental phase, projects especially those that needs a considerable financial contribution should have a viable sustainability model for up scaling. It was also recommended that implementers needs to be cautious when selecting areas for implementing ICT in education projects.

Projects should also not lose priority of the education objectives. In some cases ensuring school accountability system and teachers attendance may be more important that investing time and resources in ICT integration in schools. One fact that emerged in the sessions was that ICTs effectively computers, initiated in government department and schools were being used as decision support in education. Essentially, clear criteria, norms and standards needs to be developed for the information that was being used for decision-making.

This paper is a multidisciplinary study of ICT initiatives for rural development. It emphasizes adoption of a more systematic approach for

integrating Traditional Knowledge Systems (TKS) and ICT inputs to ensure sustainability of rural e-governance projects. The study of literature related to rural development and e-governance has indicated various issues impeding success of such initiatives. The main issues are lack of localization of content for rural communities and inadequate participation of rural communities in design of rural ICT initiatives. The study therefore suggests the use the systems-approach to integrate the relevant TKS along with ICT initiatives in the design of e-governance systems for rural development. This participatory approach can lead to creation of more acceptable and sustainable e-governance projects.

Regardless of the wide differences in ICT access between rich and poor countries and between different groups in the country, there are concerns that challenge the application of ICT in education with the existing differences among the lines of economic, social, cultural, geographic and gender will be broader. Everyone equal opportunities in terms of suitability for participation are necessary, but access to various factors, either as users or as producers through their sources is difficult and heavy. Therefore, the primary differences enhance and even grow. Consequently, programmers' international education is faced with a difficult challenge and how to help solve the problem and its development.

Promoting ICT in education, when done without careful study, can lead to the marginalization of those with more favorable conditions are unknown. For example, "women compared with men, because of illiteracy, lack of higher education, lack of time and mobility and poverty, controlling access to ICT and fewer opportunities for training are relevant. Also, more boys than girls' access to computers at home and school are not strange to say that if more boys than girls are willing to work with computers. The report of the University Association of American Women is that "Although some girls have an important gender gap have been limited, but today's technology, technology club, and boys in public schools while its own problems and programs are settled girls use computers for word processing the brand". In an assessment in four African countries, the activities organized by World links remote international cooperation on projects between teachers and students in developing countries will promote, despite creating programs without regard to sex contacts, sexual inequalities remain Uganda and Ghana. In addition, while more girls than boys in relation to academic performance and advanced communication skills program will enjoy more than boys, but they were unable to perform their technological skills were. A set of economic factors,

organizational and cultural differences involved in the social.

"The high ratio of students to computers and politics, whoever came first, the first is used in accordance with the girls wanted it." Girls travel restrictions in the early hours of daily work and home responsibilities are that this will limit their access. Also because local patriarchal beliefs dominate the boys are in the computer lab environment. Including proposed measures to address this discrimination, strategies to encourage schools to create "fair use" in the computer labs and the holding of meetings and sexual sensibilities conductivity decreased defense duties after school girls. ICT provides access to only a small part of the action is created equal. Equal attention should also be applied to ensure the technology really "is used by learners and ways of how well their needs will cure.

An educational program that reinforced this approach shows the overall program is bilingual. The program seeks to establish technology learning centers for bilingual teachers, students, teachers, parents and community members. Technical teams from each center three students, two teachers and the director of the Center with at least one female student and a teacher are female.

Another example of a general approach to the application of ICT in education, radio education project Gobi Women of Mongolia, which seeks to provide professional and educational structure of women's favorite courses around the nomads and their opportunities for income generation.

It contains topics such as livestock rearing, family support (family planning, health, nutrition and health) to create income in the application of local raw materials and basic skills for the job is a new market.

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Necessary of attention to indigenous knowledge toward improving agriculture

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Abstract: society. Indigenous knowledge is different with scientific knowledge that was established by universities and scientific communities. This knowledge is basis for decision making at field of agriculture, health, education, food and natural sources Indigenous knowledge is set of all knowledge and skills that people enjoy in one geographical area (in one environmental conditions) that most of their skills and knowledge be transmitted to next generation , and new generation would be adapted with them and add to it Since, each knowledge is consequent of individual interaction with environment, so indigenous knowledge is consequent of indigenous people interaction with their environment. Chambers with emphasis on people's role at development process believes that "rural people's knowledge" term is more eloquent than other terms for indigenous knowledge. Our purpose of rural people are producer farmers , input buyers , agriculture production sellers and etc. "people" in above phrase emphasis that this knowledge is more verbal and less has been written . This word also referred to whole knowledge system which contains concepts, beliefs, and attitudes and also contains gain, store and transmitting knowledge process.
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Introduction:

Experience shows that indigenous knowledge not only has no contradiction with formal knowledge but different indigenous knowledge features, put it as well complementary for formal knowledge. Indigenous knowledge is accessible, useful and cheap. Its perspective is holistic and its transmission is verbal. Knowledge is dynamic and time-tested, and while it has grown within local natural and social environment, so it is very sustainable with indigenous condition. Indigenous knowledge refers to both component and whole part of culture of each nation and this component and whole integration is so that stop to change traditional society of life without indigenous knowledge out of its cultural origin and therefore would lose it concept and effectiveness.

in recent decades following issues had been recognized very essential : programming and performing development plans , indigenous knowledge at farming , pest control , ranching , veterinary , nutrition , medicine , watershed management , foresting , architecture , urban planning , social associations and decision making method as sustainable technology . At on hand, reason of this great evolution can be found due to wrong policy and at the other hand in undesirable environmental consequences of these policies.

Studies have given new dimension to agriculture research. Now, in many countries the managers of agriculture resources are the people who are trained in western countries. So if the manager become familiar with the culture and environment roots of indigenous system of resource management, they won't do mistake. Indigenous agriculture is based on cooperation of farmer with nature. Recently researchers of ecological agriculture have more attention to these systems. The result of these studies is important from two sides:

1- At the first, in the process of agriculture renovation in the third world that is indeed unavoidable, the indigenous agriculture knowledge and local methods in management of agriculture resources is to be destroyed and simultaneity environmental regions are on the verge of destruction. Modern agriculture prefers huge profit from resources and didn't pay attention to environmental, cultural, social and economic varieties of traditional agriculture. So incongruities of agriculture development plans are not compatible with rural needs and talents and also rural conditions. By recognizing indigenous agriculture features such as traditional classification for identifying plant and animal species and using of indigenous practices

like simultaneous cultivation of compatible crops, we can get useful information about suitable ways for agriculture. Surely these guidelines will be more compatible with rural needs and agriculture and environmental features of each region and won't be reckless to social, economic and environmental complex issues (Appleton and Jeans, 1995).

2- Second, with studying indigenous agriculture we can get points that will help us to design the same systems in industrial countries. Sustainable agriculture which is taken from indigenous systems will remedy the shortcoming of modern agriculture. In a single-product of modern farm, life circles of nature has changed by using chemical poison that give no chance for using principles of ecological agriculture. But completeness (evolution) of culture and environment is the result of local agricultural systems (Ahmed, 2000). In indigenous agriculture, variety and alternation of cultivation make minimize the possibility of farming products destruction. Although these systems have resources limitation, but they use of learning advantage and intellectual ways for use of animals, soil and compatible farm species. For this reason, researchers of ecological agriculture know these systems as unexampled kinds to specify constant static scales for agriculture activities. In industrial countries they use of these scales for designing and managing ecological production systems (Emadi and Amiri Ardekani, 2004). Indigenous knowledge of each nation has enabled them to supply their needs from natural sources without reducing these sources. So, indigenous knowledge collection of world is valuable source of practices and time-tested tool that would be useful for sustainable development of all societies. At third world countries, unconsidered triumph of world development policies has led to various social, economic, cultural and environmental issues (Agrawal, 2002).

Imbalance population growth, non-sustainable efficiency of natural sources and unequal distribution of resources, goods and services made involved societies in confusing issues and impasses. In these countries , inappropriate sampling of abroad countries and inordinate imports (e.g. heterogeneous and non-indigenous technology) devastated independent collection of micro local systems , and instead has established heterogeneous and dependent system to global economy system , that obviously couldn't supply people's needs. Since , this development process is formed without considering social , cultural and environmental consequents so isn't continuing and human have to find strategies which can make development sustainable and humane(Popzan, 2002) .

Indigenous knowledge owners of world in current age (which known as information age) have valuable experiences from industry age and from inappropriate exploitation of their natural sources. These countries have learned that exporting produced goods is better than selling petroleum. enforcing indigenous productive system at villages and also encouraging youths and teens to learn indigenous knowledge at on hand , and preparing suitable research condition for applied-sciences scholars in order to identify better and increasing applied aptitude of indigenous knowledge at the other hand , is equal to protection and sustainable use of natural resources(Zare, H and Yaghoubi, 2003).

From Robert Chambers' view, power and wealth are at industry and at cities, and poverty and deprivation are part of villager's life. Power and wealth of cities of world has absorbed experts, sources and needed research facilities for producing and disseminating knowledge. Knowledge of these modern centers is considered scientific, advanced, and valid and enjoys premium technology. He labeled this group as "first" and in contrast "last" for deprived villagers. Because, preferences and values of these two groups are different.

Their knowledge and attitudes are also different. he believes that since "first" development remedies and their attitudes have led to fault , irregular and deprivation , so deprived villager's attitudes and knowledge should be considered serious in order to reach to improve conditions for this part of human society as they need and demand(Azkia, M and Imani, 2008) .

Features of indigenous knowledge
Some of these features are as follow:

Indigenous knowledge is holistic: indigenous knowledge is gained by sense and inspiration force and leads information unity. In spite of formal knowledge that is aural, visual and analytic. Indigenous knowledge is verbal: writing and documenting indigenous knowledge would make it out of reach of villagers who can add to it, if it would not follow applied activities.

Indigenous knowledge is practical: it is possible to write about indigenous knowledge but it is impossible to educate and learn it through books

and articles. Only way to learn it is close view and follow professor.

Indigenous knowledge isn't explanatory: it isn't possible to expect one master (e.g. mason, apothecary, farmer) to explain his method efficiency in a way that is apprehensible to us (literate people)

Indigenous knowledge is local: villager's knowledge has formed in itself environmental and climate framework. Effective indigenous knowledge at one geographical area isn't necessarily effective at other area (Nowroozi, A and Alagha, 2000).

Indigenous knowledge is general : while, formal knowledge emphasis is on saving time and removing ideas and also monopoly of knowledge at universities and research institutes , but indigenous knowledge is , receptive , incentive and needs to more people's participation at learning , developing and add to it. Furthermore, in verbal cultures, it is impossible to separate science from world and even include it to computer and book. Every human are important in indigenous knowledge.

Indigenous knowledge is deteriorating quickly: by every death of old indigenous people, great knowledge resources would be lost also, so every action toward gathering indigenous knowledge is necessary.

Learning by doing: repeating action in order to sustain and enforce indigenous knowledge through "learning by doing" is one of features of indigenous knowledge in real operation environment (Emadi and Abbasi, 2001)

Villager's knowledge and especially indigenous knowledge systems have various dimensions that is include linguistic knowledge, zoology, ecology, climate, agriculture, ranching and professional skills. Range and value of this knowledge hasn't been considered. Four aspects of various dimensions of rural knowledge were selected and were analyzed, In order to change attitudes and reformer's behavior of rural development. These dimensions are: agriculture operations, rural knowledge about nature, rural people's aptitudes and abilities and their experiences (Razavi, 2002).

In Chambers' opinion, indigenous knowledge or rural knowledge has various dimensions that he classified them to four parts in order to explain more and better about diversity of indigenous knowledge that are as follow: A: farming activity; B: knowledge in relation to nature; C : indigenous people's aptitude and ability; D: indigenous people's test . indigenous people's knowledge originated from exact viewing of environment; since indigenous villagers have direct contact with phenomenon and also see all different processes at nature so have especial aptitude and ability compared to outside people. Maybe least known aspect of indigenous villager's knowledge is

essence of tests that they do which maybe these tests are available to choose "bests" and some other for "minimizing risks" (Dewes, 1998).

Sustainable agriculture

Sustainable agriculture is kind of agriculture that is toward human's interests and has more efficiency of using resources, and also is in balance with environment. This definition is in harmony with changing social and politic factors at agriculture development .and also it referred to kind of agriculture that is enable to produce enough foods without destroying world sources or polluting environment. It is also kind of agriculture that is follow with social values, agriculture family's welfare and supplying needed foods.

Generally sustainable agriculture is every kind of production system which follows theses goals:

More complete mixing of natural processes such as food cycles, nitrogen fixation, and relation of pests and natural disasters with agriculture productions processes.

Decreasing use of that non-farming, outside and non-renewable inputs in order to reduce damage to environment or less damage to farmers and consumer's health.

More fair access to interests and productions opportunities and progress in order to access to forms of agriculture that is fairer, and also increasing self reliance between farmers and villagers (Chambers, 2000).

Using more potential biologic and genetic aptitude of plant and animal species.

Using more local knowledge including innovative approaches that scholars didn't understand it completely or farmers didn't accept it extensively.

Combined agriculture would prepare this opportunity for common systems to apply needed reforms without creating inclusive changes in it toward organic systems. Therefore, aforementioned systems are considered as medium between common intensive agriculture and organic agriculture methods.

Two principles have especial importance at sustainable agriculture that is:

at early 1980's , with the emergence of new concepts , renewable agriculture and sustainable agriculture evolved and indeed it was based on "ecological interplay affect" .now, this concept forms alter indigenous agriculture philosophy.

Sustainable agriculture presented from 1987 at global scale. In this principle, "agricultural interplay affects with society" is presented. Three issues are important about sustainability: first is enough income especially between poor people. Second is increasing access opportunity to food and its consumption. This

means that more food should be prepared through increasing production and improving marketing. Third issue contains protecting and improving natural resources (Louise, 2000).

Conclusion and discussion:

Necessity of considering indigenous knowledge at developing extension programs is emanated from where that is considered as principal components and sustainable human development items is emanated from same sources. At sustainable human development, people are considered as "goal" of social and economic policies that their range of their selections would be extended in order to actively participate at decision making. Therefore, people's participation is one of tools of sustainable agriculture development. But active rural people's participation at extension programs as a form of sustainable would not be possible unless by believing role of rural people's knowledge, vision and skills (Brouwer 1998).

At one research as a name of "analyzing position of indigenous knowledge at sustainable rural development" that was done by Buzarjomhore (2005) it was signified that although there are some differences between indigenous and formal knowledge, but they should not be compared, because they are complementary of each other and it is possible to gain successes by synthesizing them that is impossible lonely. Base on new paradigms of rural development in order to solve rural problems, we should first refer to indigenous solutions and if it was working, then we should reinforce it; if not we should test and use outside solutions. Findings of one research done by Emadi and Amiri (2004), as "Synthesizing indigenous knowledge and formal knowledge as necessity for accessing to sustainable rural development", has shown that dominated belief among educated groups toward natives and their knowledge is precondition of every interaction, synthesis and relation. Creating revolution in formal education systems in order to attending empirical knowledge area is considered as one of main necessity of this synthesis that is outcome of years of researches. Researchers attention to "exploiter's accumulated experimental and historical wisdom" is one of other necessities of this revolution by using cooperative, qualitative and filed methods. Also, applying mutual extension ways and creating revolution at communication system between governmental, education-extension centers and farmers and rural people so that they be interacting, was considered as precondition and necessities. At researches as "indigenous knowledge at development process" done by Karimi (2003) , findings show that

indigenous knowledge is principal factor and main source at the field of research of sustainable development , decreasing poverty , enabling local men and attracting their participation at activities and rural development programs, developing and producing appropriate technology, self-reliance of rural societies and country.

So, effort and national commitment and multi-dimensional support is very critical for recording, valuing, extending and exchanging this rich source and also preparing mechanism and practical strategy for synthesizing this knowledge with new knowledge and agricultural development programs.

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Magnetic resonance imaging of the brain in the diagnostic evaluation of microcephaly

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Abstract: Microcephaly is defined as small head size characterized by occipito-frontal circumference (OFC) at least 2 standard deviations (SD) below the mean for age and sex. Microcephaly is associated with numerous disorders of diverse etiology. Radiology plays a fundamental role in determining the etiology. MRI is often the imaging modality of choice. **Aim of the work:** To assess the contribution of brain magnetic resonance imaging (MRI) in establishing an etiological diagnosis in children presenting with microcephaly in the first two years of life. **Methods**: Nine hundred Egyptian patients attending the general pediatric and neurology clinic of Benha University Hospital (BUH) and clinic of inherited metabolic disorder at the centre of social and preventive medicine of Cairo University Children Hospital (CUCH) were screened for microcephaly. This was done by measuring the (OFC), and then MRI was performed to all microcephalic patients. Other investigations done according to the condition. **Results:** Fifty five patients out of 900 cases were microcephalic, below the 3rd percentile of Egyption charts. Male and female distribution was 31 (56.4%) and 24 (43.6%) with ratio of 3:2.The ages of presentation ranged from 2 months to 84 months with mean age of 20.6 ± 15.6 months. All patients were symptomatizing before 24 months with mean age of (6.5 ± 4.2 months). The patients were classified according to the final diagnosis into 3 groups: primary microcephaly 11cases (20%), secondary microcephaly 29 cases (52.72%) and undiagnosed cases 15 cases (27.28%). The most frequent MRI finding is brain   atrophy in 11(20%) cases followed by demylination in 10(18.18%) cases, leukomalecia &atrophy in 7(12.7%) cases, demylination &atrophy in 6(10.9%) cases, basal ganglia lesion in 5(9%) cases, congenital brain malformations in 4(7.3%) cases, microcephalic changes in 3(5.5%) cases and leukomalecia only in 2(3.6%) cases. **Conclusion:** MRI is considered as a golden standard in the evaluation of brain abnormalities in patients with microcephaly. It is diagnostic in congenital brain malformations and in combination with history & clinical findings, it can suspect the diagnosis, as in ARM, Leigh syndrome & HIE cases or point to specific test for diagnosis as in MLD & PKU.

1. Introduction

Microcephaly is an important neurologic sign and is usually defined as a head circumference (HC) more than 2 SDs below the mean for age and gender (1). Microcephaly may be described as primary and secondary. Primary microcephaly in which the brain fails to grow to the correct size during pregnancy (2). Secondary (also known as acquired) microcephaly is a condition in which a child's head circumference is within the normal range at birth and for an undefined period thereafter, but then does not increase as fast as normal and, as a result, crosses percentiles to below the second percentile (3). It is important to measure a patient's head circumference at birth. Serial head circumference measurements are more meaningful than a single determination, particularly when the abnormality is minimal (4). The assessment of microcephaly is both clinical and radiological (5). Microcephaly has been associated with numerous genetic etiologies and because the genetics of microcephaly is a rapidly evolving field, currently available data likely underestimate the importance and

relevance of genetic testing as part of the diagnostic evaluation of children with microcephaly (6). Many of the microcephaly genes identified to date have been associated with specific phenotypes, allowing more targeted clinical testing. Available screening tests for chromosomal deletions and duplications include karyotyping, subtelomeric fluorescent in situ hybridization, and bacterial artificial chromosome or oligo-based comparative genomic hybridization (7).

Investigations of microcephaly requires a systematic consideration of each of the etiologic causes, search for risk factors in the mother , details of pregnancy, birth , developmental progress and a detailed family history (8). MRI is considered as a golden standard in the diagnosis of microcephaly as it often reveals findings that are more difficult to visualize on CT, such as migrational disorders, callosal malformations, structural abnormalities and disorders of myelination (9). Advances in neuroimaging and genetics have improved understanding of the causes of microcephaly, suggesting new approaches to classification and testing(2). Once the cause of

microcephaly has been established, the physician must provide accurate and supportive genetic and family counseling. Because many children with microcephaly are also mentally retarded, the physician must assist with placement in an appropriate program that will provide for maximum development of the child (10). The present study aims to assess the contribution of brain magnetic resonance imaging (MRI) in establishing an etiological diagnosis in children presenting with microcephaly in the first two years of life.

2. Subjects and Methods:

Subject selection

Among 900 patients referred from the general pediatric and neurology clinic of Benha University Hospital (BUH) and clinic of inherited metabolic disorders at the centre of social and preventive medicine of Cairo University Children Hospital (CUCH). Fifty five patients were found to be microcephalic according to Egyption charts of *Ghaly et al., (2002)(11)*. This study was conducted in the period of March 2009 to September 2009.

Inclusion criteria:

Our subjects were patients presenting with microcephaly before the age of 2 years.

Methods

- **History:** Full history taking was obtained from all cases including: Personal history , Present history ,Developmental history , Perinatal history and Family history including family pedigree analysis

§ Examination:
General Examination:

All cases were subjected to general examination including anthropometric measures (head circumference, height and weight) using Egyptian growth charts 2002.

Local Examination:

Our patients were subjected to neurological examination including motor power, tone and reflexes (deep &superficial), any abnormal movement and cranial nerves examination.

§ Investigations:

Radiological Investigations: MRI was used for all cases of the study: It was performed on 1.0 Tesla unit (Signa, GE, Milwaukee) using a head coil. Some MR examinations were performed on 1.0 Tesla (Gyroscan NT 10; Philips Medical systems). MR examinations, using head coil, included; Axial and coronal T2 FSE using 2000-5000/98-120 TR msce/TE msec, one to four signals acquired, 16-22 cm FOV with or without a rectangular Field, 192-256 x 256 matrix, 3-5 mm thick sections with 0.3-0.5 mm intersection gap. Axial TI

wighted images were obtained using 400-650/20-30 TR msec/TE msec, 2-4 signals acquired, 128-192 x 256 matrix, 16-22 cm FOV, and 3-5 mm thick contiguous sections. Sagital TI WSE MR imaging (400-700/11-20. 1-2 signals aquired, 128-192 x 256 matrix, 20-40 cm FOV, 5 mm thick sections with 0-2.5 mm intersection gap. Additional coronal TI WES was obtained using the same parameters used for axial TI. Axial T2 FLAIR (Fluid attenuated inversion recovery) images were done using 10,000/140/2,200 TR msec/echo time msec/inversion time msec, one signal acquired, 20-22 cm FOV, 192x256 matrix, 4-5 mm thick sections with 1.0-2.5 mm intersection gap.

Laboratory investigations:

Basic metabolic investigations were done to all patients including:

§ Complete blood picture, random blood sugar, liver function tests, kidney function tests, arterial blood gases, and anion gap calculation in cases of metabolic acidosis.

§ Serum ammonia & lactate, urine ketones and reducing substances.

Specific metabolic investigations:

§ TMS: tandem mass spectrometry, using MS/MS laboratory method, which is a technique that allows the screening of multiple disorders in the same blood spot collected on the standard card. More than 20 preventable genetic disorders can be screened by blood drop**.**

§ Organic acids profile in urine using Gas chromatography mass spectrometry (GC/MS) was performed for patients with abnormal acylcarnitine profile or those with unexplained mental retardation or compensated metabolic acidosis. This qualitative urinary organic acid method is capable of detecting over 200 organic acids for a large numbre of metabolic conditions.

§ Definitive diagnosis by enzymatic analysis was done in some cases such as Aryl sulphatase An in MLD (Metachromatic Leuko Dystrophy).

§ Other metabolic investigations: Pyruvate, VLCFA (very long chain fatty acid): for suspected perioxosomal disorders.

I.Q (Intelligence Quotient), TORCH screening & karyotyping needed in some cases accordingly. Other studies as (FISH for William) which done in cases suspected to be syndromatic.

Neurophysiologic study

ERG, VEP, Fundus examination, ABR, EEG, EMG and Echocardiography were carried out.

3. Results

Our results revealed that, out of 900 patients attending the neuropediatric clinic, 55 (6.11%) patients were found to be microcephalic**.** Male and female distribution among the study group was 31 (56.4%) and 24 (43.6%) with ratio of 3:2.

The ages of presentation ranged from (2 months to 84 months) with mean age of 20.6 ± 15.6 months. However, all patients were symptomatizing before 24 months with mean age of 6.5 ± 4.2 months .The 55 patients of the study were classified according to their final diagnosis into 3 groups; Group1: primary microcephaly 11 patients (20%), Group secondary microcephaly 29 patients (52.72%) and Group undiagnosed group 15 patients (27.28%) . Group1 subclassified into: genetic; (9.1%), syndromatic; (3.6%) and congenital brain malformations (7.2%). Group sub-classified into: perinatal causes (HIE) or cerebral palsy group; 12 cases (21.81%), neuro-metabolic; 10 cases (18.18%) including aminoacidopathy (PKU and homocystinuria) and organic aciduria (MMA **2**cases and 3-MCG **1**case), neurodegenerative; 3 cases (5.45%) including 2 cases MLD and 1 case of Rett syndrome and mitochondrial disorders (Leigh syndrome) 4 cases (7.27%).

Table (1) demonstrates the clinical manifestations of the studied groups. The most prevalent clinical manifestation was developmental delay in all cases 55 (100%), followed by tone abnormality in 46/55cases (83.63%). The dysmorphic features were detected in 22/55 (40%). Abnormal movements in 15/55 (27.27%). Eye affection was found in 6 cases (10.91%) followed by hearing affection in 3 cases (5.45%). Associated anomalies and speech affection (dysartheria) were detected in 2 (3.64%) cases each. The most frequent history findings were positive consanguinity in 35 cases (63.63%) and seizures in 27cases (49.09%). Table 3 shows analysis of clinical presentation of 40 diagnosed cases. MRI findings in all the cases (table 3)reveals that, the most frequent MRI finding is brain atrophy in 11(20%) cases followed by demylination in 10(18.18%) cases, leukomalecia & atrophy in 7(12.7%) cases, demylination &atrophy in 6(10.9%) cases, basal ganglia lesion in 5(9%) cases, congenital brain malformations in 4(7.3%) cases, microcephalic changes in 3(5.5%) cases and leukomalecia only in 2(3.6%) cases.

MRI of Autosomal Recessive Microcephaly is shown in figure (1). MRI of Congenital Brain Malformation (Cortical dysplasia and incomplete lissencephaly) is shown in figure (2). MRI of Hypoxic ischemic Encephalopathy is shown in figure (3). MRI of Aminoacidopathy (PKU) is shown in figure (4). MRI of Methylmalonic academia (MMA) shown in figure (5). MRI of Metachromatic leukodystrophy is shown in figure (6). MRI of Rett syndrome is shown in figure (7). MRI of Leigh syndrome is shown in figure (8).

Table (1): Clinical manifestations in the study groups.

Clinical manifestation	number	%
Positive Consanguinity	35	63.63%
Abortion	17	30.90%
Sibs death	12	21.82%
Other sibs affection	17	30.90%
Family history of Similar condition, other neurological disorder or mental retardation.	15	27.27%
Maternal illness	9	16.36%
History of DCL(disturbed conscious level)	2	3.64%
Seizure	27	49.09%
Abnormal movements	15	27.27%
Hypertonia **Hypotonia** **Normotonia**	27 19 9	49.09% 34.54% 16.36%
**Developmental delay** **Motor delay** **Mental delay** **GDD(Global developmental delay)** **Regression**	_**55**_ 9 3 36 7	_**100%**_ 16.36% 5.45% 65.45% 12.73%
Dysmorphic feature	22	40%
Associated anomalies	2	3.64%
Eye affection **albinotic fundus (3cases) 5.45%** **pallor of optic disc (2cases) 3.64%** **high myopic (1case) 1.81%**	6	10.91%
Hearing affection	3	5.45%
Speech affection (dysartheria)	2	3.64%

Table (2): Distribution of patients of the study groups according to their final diagnosis.

	Final diagnosis	Number (%)
Primary microcephaly 11/55 (20%)	**1-Autosomal Recessive microcephaly**	**5/55(9.09%)**
	2- Syndromatic	**2/55(3.63 %)**
	-Cornelia de lange syndrome	1/55(1.81%)
	-Smith lemli opitz syndrome	1/55(1.81%)
	3-Congenital brain malformations	**4/55(7.27%)**
	-Pachygyria	2/55(3.63%)
	-Lissencephaly	1/55(1.81%)
	-Cortical dysplasia and incomplete lissencephaly	1/55(1.81%)
Secondary microcephaly 29/55 (52.72%)	**1-(HIE)hypoxic ischemic encephalopathy**	**12/55(21.81%)**
	2- Neurometabolic	**10/55(18.18%)**
	a)Aminoacidopathy	**7/55(12.72)**
	-PKU(phenyl ketonuria)	6/55(10.9%)
	-Homocystinuria	1/55(1.81%)
	b)- Organic aciduria	**3/55(5.45%)**
	-MMA(methyl malonic acidemia)	2/55(3.63%)
	-MCG(Methyl crotonyl glycinuria)	1/55(1.81%)
	3-Neurodegenerative	**3/55(5.45%)**
	- MLD (metachromatic leukodystrophy)	2/55(3.63%)
	- Rett syndrome	1/55(1.81%)
	4-Leigh syndrome	**4/55(7.27%)**
Undiagnosed cases 15/55 (27.28%)		**15/55(27.28%)**
Total number (%)		**55(100%)**

Table (3): Analysis of clinical presentations of diagnosed cases (40cases)

clasification	sub clasification	Final Diagnosis	Consanguinity	Abortion	Sibs death	Other sibs affection	1-disturbed conscious level 2-seizure(s)	Dysmorphic features	Tone: spasticity	Tone: Hypotonia	Tone: Normotonia	Dev. Delay: Motor	Dev. Delay: Mental	Regression	Global developmental delay(GDD)	Vision affection	Hearing affection	Speech affection
Primary Microcephaly	1- Genetic	ARM* 5 (9.09%)	+ve (3)			+ve		+ve			+ve	+ve (3)			+ve (2)			+ve (1)
	2- Syndromatic	CDS*1 1 (1.81%)	+ve					+ve							+ve			
		SLOs*2 1 (1.81%)	+ve	+ve			s+ve	+ve							+ve			
	3- Congenital brain malformations	Pachygyria 2 (3063%)	+ve (1)	+ve (2)			s+ve (2)		+ve (2)						+ve		+ve (1)	
		Lissencephaly 1(1.81%)	+ve	+ve			s+ve		+ve						+ve			
		Corticaldysplesia+Lissencephay1 (1.81%)		+ve			s+ve	+ve	+ve						+ve			
	1 – P	HIE*3 12 (21.8%)	+ve (6)	+ve (3)	+ve (2)		s+ve (11)	+ve	+ve (11)	+ve (1)		+ve (9)			+ve (3)	+ve (2)		
	2-Neuro-metabolic — a-Amino acidopathy	P KU*4 6(10.9%)	+ve (4)	+ve (2)	+ve (1)	+ve (6)	s+ve	+ve	+ve (4)		+ve (2)				+ve			
		Homc*5 1(1.81%)					s+ve			+ve					+ve			
	2-Neuro-metabolic — b-Organic aciduria	MMA*6 2 (3.63%)	+ve (2)			+ve (1)	D C L (2)		+ve (1)		+ve (1)				+ve			
		3MCG*7 1(1.8%)	+ve				D C L (1)		+ve						+ve			
Secondary Microcephaly	3- neuron-degenerative	MLD*8 2(3.63%)	+ve (1)	+ve	+ve (1)				+ve				+ve	+ve				
		Rett syndrome 1(1.81%)	+ve	+ve				+ve	+ve				+ve	+ve				+ve (1)
	4- Mitochondrial	Leigh syndrome 4(7.27%)	+ve (3)	+ve			s+ve (2)		+ve (1)	+ve (3)			+ve	+ve				

*Autosomal Recessive microcephaly,*1 Cornelia de lange syndrome, *2 Smith lemli opitz syndrome , *3 hypoxic ischemic encephalopathy , *4 phenyl ketonuria ,*5 Homocystinuria ,*6 methyl malonic acidemia , *7 Methyl crotonyl glycinuria , *8 metachromatic leukodystrophy.

Table (4): MRI findings of all cases of the study group

CLASSIFICATION	sub classifications	Final Diagnosis	Microcephalic changes	Brain atrophy	Leukomalecia	Leukomalecia/Atrophy	Demylination	Demylination/Atrophy	Congenital brain Mal formation	Basal Ganglia lesion	Others
Primary microcephaly	1-Genetic	Autosomal recessive microcephaly(ARM)5 (9.09%)	3	1							Colpocephaly&Interh emispheric cyst.
	2-Syndrometic matic	Cornelia de lange Syndrome (CDLS)1(1.81%)		1							
		Smith lemli Opitze Syndrome (SLOS)1(1.81%)									Incomplete agenesis of corpus callosum.
	3-Congenital brain Mal formations	Pachygyria2(3.63%)							2		
		Lissencephaly1(1.81%)							1		
		Cortical dysplesia & incomplete Lissencephaly1(1.81%)							1		
Secondary Microcephaly	1-Perinatal	Hypoxic ischemic encephalopathy (HIE)12 (21.81%)		3	2	7					
	2-Neuro metabolic / a- Amino acidopathy	(PKU) Phenyl Ketonuria6 (10.9%)					2	4			
		Homcystinura 1(1.81%)					1				
	2-Neuro metabolic / b- Organic aciduria	(MMA) Meth-yl Malonic Acidemia 2 (3.63%)					1			1	
		(3-MCG) 3-Methyl crotonyl glycinuria1(1.81%)		1							
	3- neurodegenerative	Metachromatic leukodyst –rophy (MLD) 2(3.63%)					2				
		Rett syndrome 1(1.81%)		1							
	4- Mitochondrial	Leigh syndrome 4(7.27%)								4	
undiagnosd	? Mitochondrial			3				2			2 Normal
	? Neurodegenerative						4				
	? Syndromatic			1							3 Normal

Autosomal Recessive Microcephaly

Fig (1) MRI axial section showing; Microcephaly, Trigonocephaly,colpocephaly and inter- hemispheric cyst and abnormal gyral pattern.

Congenital Brain Malformation (Cortical dysplasia and incomplete lissencephaly)

Figure 2 (a)	Figure 2 (b)

Figure 2 (a,b) : MRI brain T1WI Axial (a) and Sagittal (b) sections of female patient aged 6 month showing cortical dysplasia(thickening) with lissencephaly (incomplete type) , due to the presence of a smooth brain surface with shallow Sylvain fissure and some gyral formation

HIE (Hypoxic ischemic Encephalopathy)

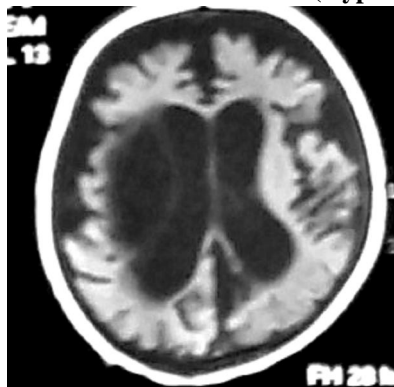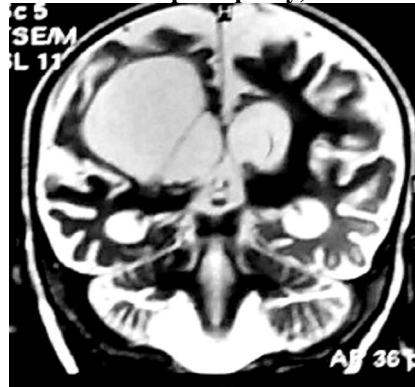
Figure 3 (a)	Figure 3 (b)

Figure 3 (a,b): MRI brain Axial T1WI (4a) and Coronal T2WI (4b) , sections of HIE male patient aged 1 year showing oblong shape cystic encephalomalecia (6x3 cm) with in the right deep partial periventricular region, brain atrophy appears as (dilated ventricles) central atrophy and (deep sylvian fissure and prominent sulci) peripheral atrophy.

Aminoacidopathy, Phenyl Ketonuria(PKU)
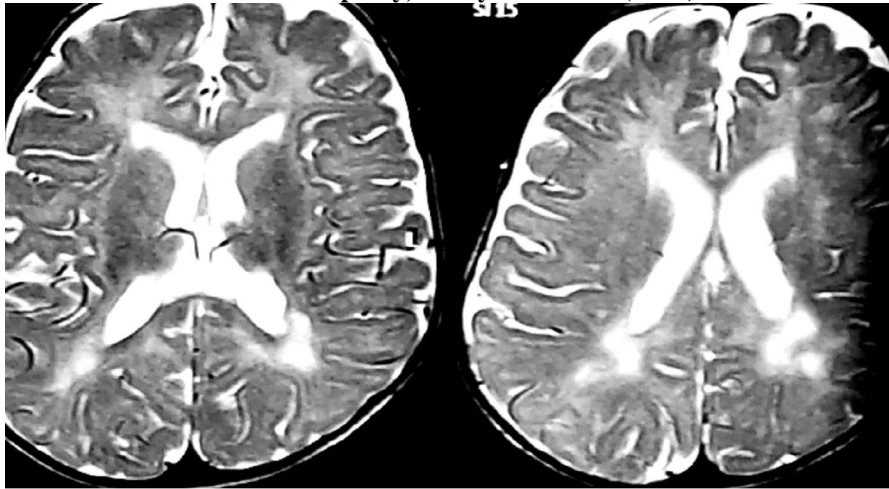
Figure 4 (a)
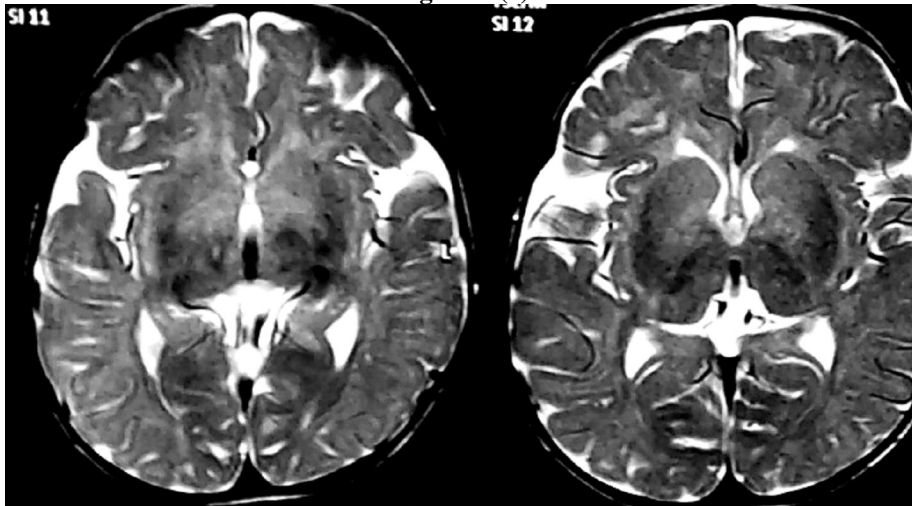
Figure 4 (b)

Figure 4 (a,b) : MRI brain Axial sections T2WI of PKU female patient aged 16 month. (a) Showing defective mylination of the white matter appears mainly priventricular (b) showing cerebral atrophy appears as (dilated ventricles) central atrophy and (deep sylvian fissure and prominent sulci) peripheral atrophy .

MMA (Methyl Malonic Academia)

Figure 5: MRI brain Axial section FLAIR of male patient aged 18 month, proved to be MMA, showing diffuse high signal intensity lesion involving the white matter bilaterally with central and cortical atrophy.

Metachromatic Leukodystrophy (MLD)
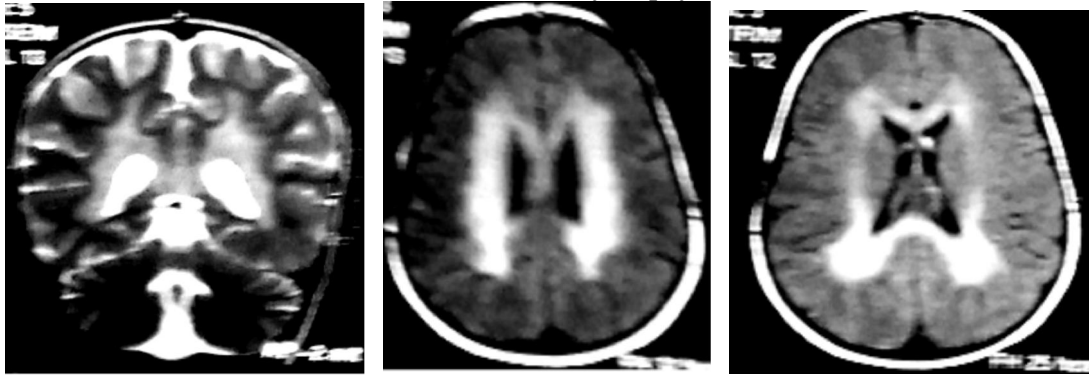
Figure 6(a)   **Figure 6(b)**   **Figure 6(c)**

Figure 6 (a,b,c) : MRI brain coronal (a) , axial (b,c) flair sections of female patient aged 2 years diagnosed as MLD showing diffuse high signal intensity lesion involving the periventricular white matter mostly peritrigonal , forceps major and minor sparing the sub cortical u fibers , basal ganglia , thalamus and the posterior fossa.

(Rett syndrome) Cerebral and cerebellar atrophy

Metachromatic Leukodystrophy (MLD)
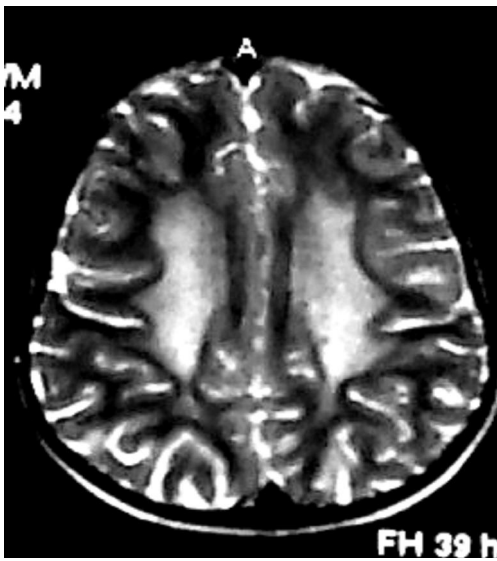
Figure 6 (d): MRI brain axial (d) T2 WI of the same patient showing centrum semi oval with leopard appearance**.**
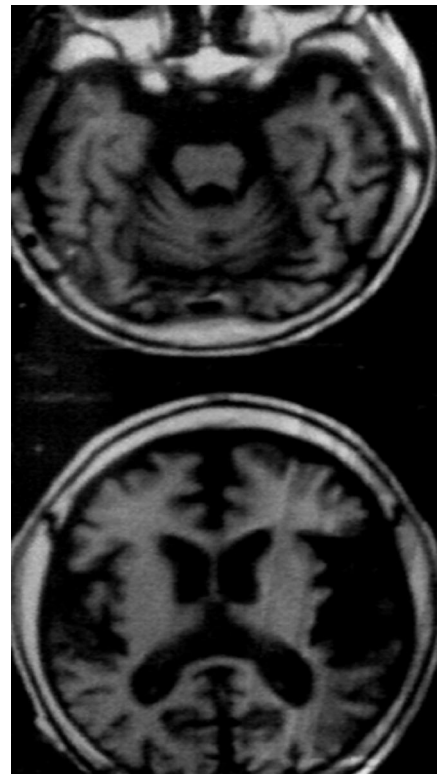
Figure(7) : MRI brain Axial sections T1WI of female patient aged 3 years diagnosed as Rett syndrome showing cerebellar atrophy and cerebral atrophy appears as (dilated ventricles)central atrophy and (deep sylvian fissure and prominent sulci)   peripheral atrophy

Leigh syndrome
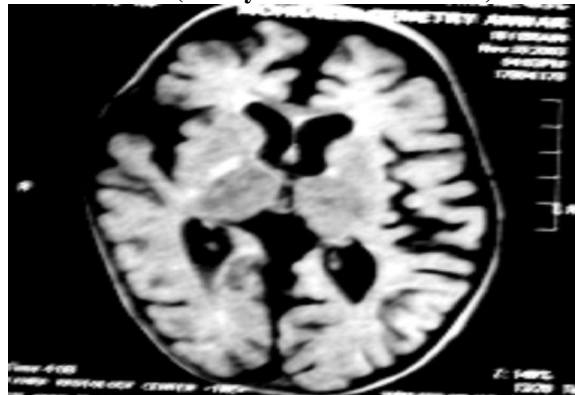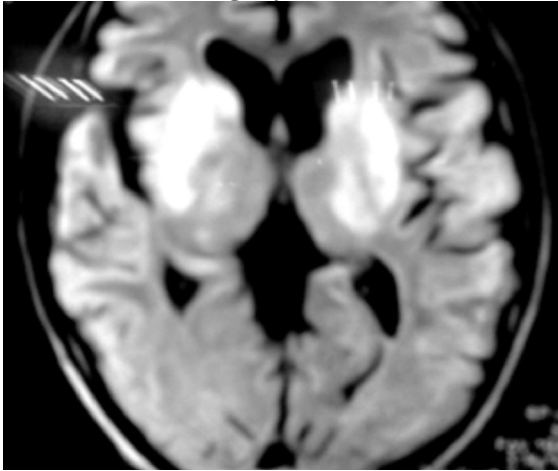

Fig (8) : Brain MRI axial section FLAIR of patient aged 1 year diagnosed as Leigh syndrome showing bilateral affection of basal ganglia which shown as high signal intensity in lentiform , caudate and thalamus.

4. Discussions

The yield of neuro imaging in microcephaly ranges from 43% to 80% in many studies *(2).* The yield of neuroimaging in the current study is 72.72%.We found that secondary microcephaly is the most frequent type, the same was reported by Peter et al., (3). Coexistent conditions with microcephaly include epilepsy (40%), cerebral palsy (20%), mental retardation (50%), and ophthalmologic disorders (20% to 50%) (*2).*These disorders are comparable to our results as we found epilepsy in(50%), cerebral palsy in(21%),GDD in (65%) and ophthalmologic disorders in (11%). The usefulness of MRI is apparent as certain malformations (e.g., lissencephaly, schizencephaly) are well known to be associated with severe neurologic impairment and specific gene abnormalities have been found in several of these disorders(2). The current study showed that all cases with congenital brain malformations had microcephaly since birth, hypertonia, hyperreflexia, seizures and GDD. This is in accordance with *Herman & siegel (12) and Poirier et al., (13).* Our patients with pachygyria had MRI findings of large gyri, few sulci and thick cortex on both cerebral hemispheres. Those with lissencephally had MRI findings of agyri, thickened cortex, straight grey white matter and shallow sylvian fissure. This is in accordance with *Barkovich &Raybaud (14)).* Cortical dysplasia and incomplete lissencephally was diagnosed when MRI showed abnormal cortical thickening, incomplete lissencephally & periventricular abnormal signals and this is in agreement with *Abdel Razek et al., (15).*

Microcephaly has been associated with numerous genetic etiologies, including syndromes *as*

Angelman*,* Cornelia de lange and Smith lemli opitz *(6).* Cornelia de lange , Smith lemli opitz ,Leigh and Rett syndromes were diagnosed among our cases. The current study revealed (ARM) in (9%) of cases, while other studies by *Barkovich et al., (16)* and *Wycliffe et al., (9)* reported higher incidence of (ARM) 15.5% to 53.3%, and this may be explained by, the unfeasibility of molecular genetics to diagnose more cases. The diagnosis of (ARM) in our patients was based mainly on the presence of dysmorphic facial features, mental retardation coupled by MRI findings of microcephalic changes (small cerebral hemispheres, shallow sylvain fissure and simplified gyral pattern), this is in agreement with *Rajab et al.,* (17). Despite advances in medical and technological possibilities, perinatal asphyxia is still a matter of concern due to its considerably high rate of mortality and morbidity *(18).* Twelve cases (21.81%) were diagnosed as HIE or cerebral palsy , they presented with microcephaly, motor delay mainly, history of perinatal problem especially during delivery which required NICU admission after delivery(7 cases) and a static course since birth, these clinical criteria are in agreement with *Adcock& Papile (19).*In this group , seizures appeared to be the most common feature (11/12) cases, the same found by *Hahn &Olsan(20).*The main MRI findings in this group were atrophy (central & cortical) in 3/12cases, Peri ventricular leukomalecia in 2/12 cases and both in 7/12cases, so 9/12 cases (75%)of HIE cases demonstrated PVL on MRI. Valeo & Tom (21), reported that MRI findings of 351 of children with CP showed that more than 42 percent had damage to white matter and nearly 90 percent of the children scanned displayed cerebral pathology, including basal ganglia, cortical and subcortical areas, as well as malformations, and infarcts, they concluded that MRI is an important diagnostic tool in cerebral palsy. Metabolic disorders are more likely to cause postnatal onset microcephaly and are typically associated with global developmental delay (GDD). The prevalence of metabolic disorders among children with microcephaly is 1% to 5% *(22).*The current study revealed metabolic disorders in 10 (18.18%) patients, this large number in relation to other studies, could be attributed to the large percent (63.63%)of consanguineous marriages found in our study. The yield of metabolic testing in microcephaly is higher when a parental history of consanguinity is present *(23).* In the current study, patients diagnosed as PKU presented with microcephaly, MR, positive history of consanguinity, fair complexion, agitated behavior, GDD and convulsion in some cases. MRI findings of PKU cases showed demylination in the periventricular area with central and cortical atrophy, this is in agreement with *Harald et al., (23) and Hahnel (24).* Two of our cases were diagnosed as (MMA), they presented with history of coma and admission to ICU,

microcephaly, myoclonic seizures and GDD. Laboratory findings in MMA were metabolic acidosis, increased C3 by (Ms/Ms), methylmalonic, methylcitrate and hydroxy propionic acid in organic acid profile,this is in agreement with *Radmanesh et al. (25)*.MRI findings of MMA cases showed bilateral diffuse abnormal signals intensity in the white matter in one case and bilateral affection of both globus pallidus(GP) in the other one, and this is in agreement with *Girgis et al.,(26).*

MRI findings in cases of MLD show diffuse high signals involving periventricular white matter bilaterally and sparing the sub cortical U fibers, basal ganglia and thalami .The posterior fossa and the centrum semiovale show leopard skin appearance, the same reported by *Kim et al., (27).*

Leigh syndrome diagnosed in 7.27% of our cases (4/55). The main clinical manifestations were microcephaly, history of developmental regression, hypotonia, dystonia, metabolic acidosis and high lactate level. MRI findings in this group revealed basal ganglia affection. AL Kartikasalwah &LH Ngu (28) in their study on Leigh syndrome ,reported that, the symmetrical necrotic lesions in the basal ganglia and/or brainstem which appear as hyperintense lesions on T2-weighted MRI is characteristic and one of the essential diagnostic criteria, together with neurological problems should prompt the clinician to investigate for Leigh syndrome. In our study, the case diagnosed as Rett syndrome presented with microcephaly, history of regression and GDD, non purposeful hand movements and autistic behavior. This is in agreement with Moog et al., (29). MRI findings of this case demonstrated, cerebral and cerebellar atrophy and this is in agreement with Carter (30).

5. Conclusion

MRI is considered as a golden standard in the evaluation of brain abnormaities in patients with microcephaly**.** It is diagnostic in congenital brain malformations and in combination with history & clinical findings, it can suspect the diagnosis, as in ARM, Leigh syndrome & HIE cases or point to specific test for diagnosis as in MLD & PKU.

Abbreviation: HIE (hypoxic ischemic encephalopathy)- HC (head circumference)- TMS (tandem mass spectrometry) –PKU (phenylketonuria) - MLD (Metachromatic leukodystrophy) –ERG (electroretinogram) –VEP (Visual evoked potentials) – ABR (auditory brain stem)

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2/2/2011

Pay-As-Bid versus Uniform Pricing Mechanism in Restructured Power Systems

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Abstract: Energy markets have specifically different mechanism in quite varying countries. Even in one country, it might be that mechanism of electrical market is different from each other. What is similar in this markets is satisfying load or demand as a main target. In the worldwide electricity markets, ordinary mechanism of market clearing implied as a uniform price, while mechanism of payment in Iran's electricity market based on the model of pay-as-bid by energy generation companies. This paper is surveying these two mechanisms and introducing its weak and strong points.
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1. Introduction

Before restructuring in electricity industry, the structure of generation, transmission, distribution and operation from network was known as a vertically integrated chain. In this situation, government with public service provider had duty to supply energy in the cheapest way for the generation cost from end users and would be paid to all producers (R. Green, 1999). In supply side, this received expenses will spend on crew cost, fuel cost, Variable Operation and Maintenance (VOM) cost, loss, Infrastructure expansion planning. Therefore, total of the receipts and payments manage integrated in these systems (Shrestha et al., 2001). This management and operation of power systems, named as vertically integrated too, because this chain of generation, transmission and distribution manage entirely. In this area, practically there is not any difference between units with high efficient and the low one. Successful privatization experiences in various industries such as communication and transportation and airlines in many countries due to put the energy supplying chain into prospective in order to enter in a competitive market. In result, the process of privatization of electricity Industry has begun. With intention to the topic competition and accounting arguments and their focus on these chains, in each of these countries demonstrated specific regulation for considering this issue (Kirschen and Strbac, 2004).

The main goal in restructuring in restructure in electricity industry is decentralization from government sector into private one, developing competition, and increasing long term efficiency and

most important of all long term social welfare. It is to note that regulation approved with this goal (Shahidehpour, et al. 2002). Due to lack of motivation for generation companies with low efficiency for improving their generation and their technologies and increasing the unit efficiency, operation of conventional generation units is very least efficient. In this case, cost based model obtained from generation level and their supplementary imposed costs. In fact, cost function is proposed by generation level. This cost function shows that the cost of energy production corresponding with generating level. Finally, this cost will be aggregate and segregate correspondingly with the demands (Bhattacharya et al., 2001). In restructured competitive power market, the condition is vice-versa of this case. In the other words, the conditions are similar to other economic systems based on offer and demand, the amount of tendency of Genco's for each level of generating level determined and offered to market. Consequently, the high efficiency unit has more tendencies to generation for a specific price than a low efficiency unit does. So electricity market will manage similar to an economic-management system. In this case, units should offer their capability for supplying the energy (Javadi and Monsef, 2008).

2. Operation of Generation Units in Vertically Integrated Power Systems

In conventional power system, at first, load forecasting for generation scheduling and the amount of energy demand estimated for next 24 hours (It means the day-ahead load forecasting). In

the second order of merit, economic dispatch of supplying the load is carried out. In this case, demands have a price elasticity of zero and considered as a solely constraint. In this case decision-making attributes is marginal cost of generation units. In other words, the units that selected for generation should have lesser cost of generation than marginal cost or the generation cost of them should be equal with marginal cost. From point of view of economy, marginal cost has profound concepts that can search these concepts in economical documents. In this model, the marginal cost will defined via the amount of load.
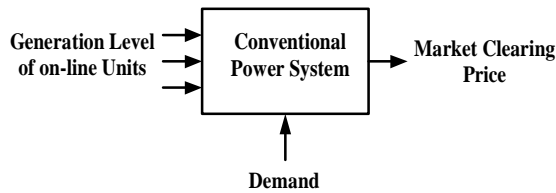
Figure .1 Operation model of conventional power system

3. Operation of Generation Units in Restructured Power Systems

In modern power system, the condition is vice-versa of above case. In the other words, generation would offer their marginal cost of generation units under operation in the first step. Optimum generation level will be distinct by these offers. In this regard, customer desire about maintaining the demands will be receive. Market Clearing Price (MCP) will be reached by crossing of aggregated supply curve and aggregated demand curve. Because of particular properties of electricity commodity, the price elasticity is extremely low (Javadi et al., 2009). In this paper, due to have simple analysis, loads are non-elastic. In the following section different PAB mechanisms will present.
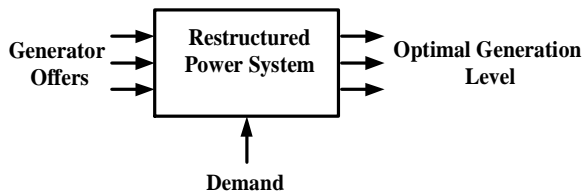
Figure.2 operation model of modern power system

3.1. Pay-As Bid versus uniform pricing Mechanism

There are two mechanisms after settling the market and defining the MCP, for pay to generation units. One of them is Pay-as-Bid (PAB) and another one is Uniform Pricing (UP). In PAB mechanism, each of the generation companies (Genco's) that are in generation scheduling will be paid based on its

offer bid. In UP mechanism, all of the Genco's that are in generation scheduling received the price of settling the market after defining the MCP and determining the most expensive generation unit (that will determine the final accepted price). Consequently, all of the Genco's will receive similar price. For this reason, this mechanism is uniform pricing (Bouffard et al., 2005). This article surveys the characteristics and differences between these tow mechanisms. The following simple example illustrates these characteristics and differences:

Suppose that we have two generation units (A and B). Offering their desire supply function as below:

Table.1 Generation steps of unit A

Unit A	A1	A2	A3	A4	A5
Declared(MW)	0-10	10-30	30-100	100-150	150-200
Declared($/MWh)	10	20	30	35	50

Table.2 Generation steps of unit B

Unit B	B1	B2	B3	B4	B5
Declared(MW)	0-20	20-40	40-60	60-90	90-120
Declared($/MWh)	15	22	25	40	45

If the hourly load demand is as figure 3, settling the market price and payment are obtained as follows:
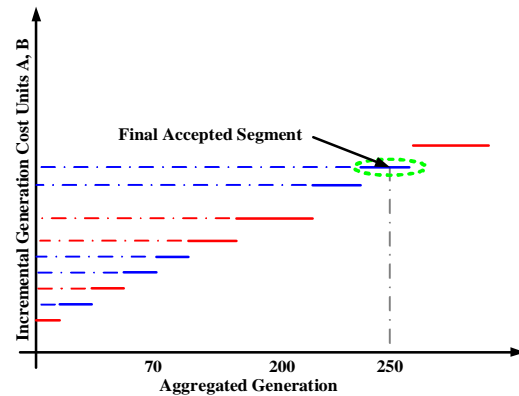
Figure 3. (a) Mechanism of market based on PAB
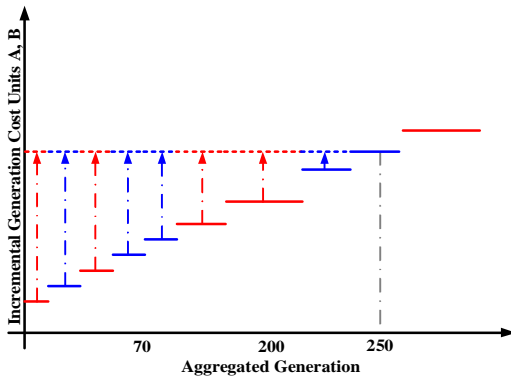
Figure 3. (b) Mechanism of market based on UP

Suppose that three generating technology A, B and C are available which should supply the load at any moment. A is a 400 MW unit, B is a 500 MW unit and the capacity of unit C is 120 MW. Figure 4 shows the load duration curve (LDC).
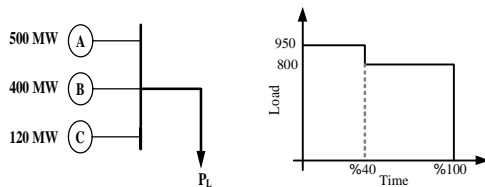
Figure 4. LDC of studied example and diagram of generation system and load

Figure 4 shows that in %40 of time, load demand is 950 MW and in the remaining %60 of time, load is 800 MW. LDC is obtained from the Daily Load Curve (DLC). Through arranging the load from ascending to descending and with knowing the information of load demand for 24 hours, DLC is obtained; and with knowing the information of load demand for 8760 hours, Annually Load Duration Curve (ALDA) is attained. From point of view of economic, marginal cost is the slope of cost function. Therefore marginal costs are achieved for three different generating technologies. See figure 5.
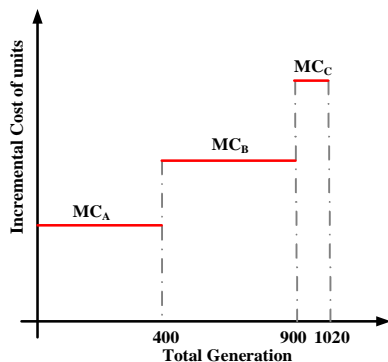
Figure 5(a). Increasing marginal cost curve
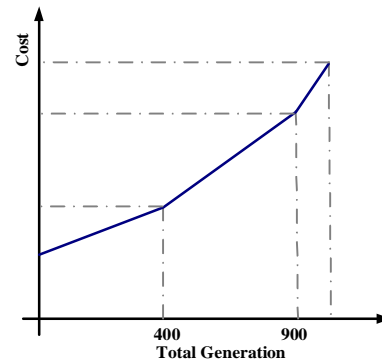
Figure 5. (b) Comparative cost curve
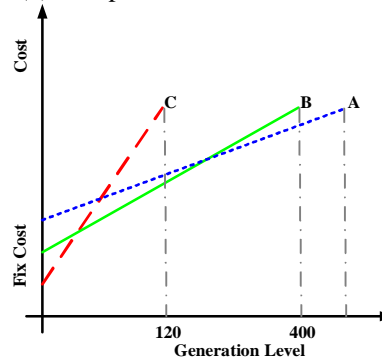
Figure 5(c). Cost model of generation units

3.2 Pay-as-Bid mechanism and profit calculation

Profit is calculated by taking the revenue and costs. The difference between cost and revenue is profit of entity. Accordingly, profit is computed as follows:

Assume that the generations cost for each unit is marginal cost. So for each unit, the cost of generation is consists of fix and variable cost proportional with amount of generation that its mathematical expression is a first-order linear function as follows:

$$Cost_i = Fix_i + Var_i \times P_i \qquad (1)$$

For each unit, revenue is calculated as follows:

Unit A:
This unit is cheapest so in on-line all of the time.

$$Rev_A = (0.4 \times 400 \times \pi_A + 0.6 \times 400 \times \pi_A)$$
$$-(Fix_A + 400 \times \pi_A) = -Fix_A \qquad (2)$$

Unit B:
This unit is a full load unit in %40 of the time, but in the remaining %60 of the time, the unit is on-line with 400 MW.

$$\mathrm{Rev}_B = (0.4 \times 500\pi_B + 0.6 \times 400\pi_B)$$
$$-(Fix_B + 0.4 \times 500 \times \pi_B + 0.6 \times 400 \times \pi_B) \qquad (3)$$
$$= -Fix_B$$

Unit C:

This unit is an expensive unit. So it is on-line with 50 MW in %40 of time and it's off-line in the remaining %60 of the time.

$$\mathrm{Rev}_C = (0.4 \times 50 \times \pi_C)$$
$$-(Fix_C + 0.4 \times 50 \times \pi_C) \qquad (4)$$
$$= -Fix_C$$

If all of the generation units offered their marginal costs to the market, they are lost the fix cost. The amount of their losses is equal to their fix costs. It can be shown that in this mechanism, Is not desirable for Genco's to offer their marginal cost to the market.. It is to note that Genco's presented a price that is more than the marginal cost. So it is a worry for end user. As mentioned previously, one aim of restructuring is to increase social welfare from both consumer and Genco's. In this case, there is not any tendency to energy generation in short term and there is not any positive signal for investing in generation sector in long term. Before presenting the other countries energy policy and approaches in their operation of their electricity markets, we would like to calculate the profit in uniform mechanism (Soheily, 2000).

3.3 Uniform mechanism and profit calculation

Consider the earlier example; Unit A is a full load unit; unit B is a full load unit in %40 of the time, but in the remaining %60 of time, the unit is on-line with 400 MW so this unit is a marginal unit and would be a price maker and unit C that supplies the load (50MW) in %40 of the time (at on-peak time) determines the price in this range. In this mechanism, profit calculated as;

$$\mathrm{Rev}_A = (0.4 \times 400\pi_C + 0.6 \times 400\pi_B)$$
$$-(Fix_A + 400\pi_A) \qquad (5)$$
$$= (0.4\pi_C + 0.6\pi_B - \pi_A)400 - Fix_A$$

$$\mathrm{Rev}_B = (0.4 \times 500\pi_C + 0.6 \times 400\pi_B)$$
$$-(Fix_B + 0.4 \times 500\pi_B + 0.6 \times 400\pi_B) \qquad (6)$$
$$= 0.4 \times 500(\pi_C - \pi_B) - Fix_B$$

$$\mathrm{Rev}_C = (0.4 \times 50 \times \pi_C)$$
$$-(Fix_C + 0.4 \times 50 \times \pi_A) \qquad (7)$$
$$= -Fix_C$$

According to the result, it is clear that only conditions for unit C are similar to the PAB mechanism. Note that, the amount of loss is less than for other units that have lesser cost or they have not any loss at all. So as a generally result and for profit, the amount of risk in bidding strategy of generation units in PAB mechanism is more than UP mechanism. In addition, rising costs seems quite reasonable in such circumstances. Suppose that there are some incentives for investing in private sector and Genco's, too.

4. Calculation of loss of load cost and market intervention

In both mechanisms, there are conditions that in them operation prices of generation units for covering fix costs are more than marginal costs of their generation. So prices will be more expensive. This case is more common in PAB mechanism.

Suppose that unit C is not generating energy. It is not delivering to network, too. Therefore, power system has e loss of load (50 MW) in %40 of the time. Ordinary the loss of load is 50-100 times of real cost of energy generation. In this case, system pays more prices because of no facing with loss of load. Assume that the system is using a virtual generator unit for making to equivalent with loss of load. So fix and quasi-fix costs for this unit are zero and variable costs are value of loss of load (VOLL using in reliability calculation of network).

Add a virtual generator unit (equal with loss of load) to previous example in UP mechanism. In this case, if the VOLL exists, there will be no more loss for each unit. For example, in unit C:

$$\mathrm{Rev}_C = VOLL \times T_D + \pi_C(T_C - T_D)$$
$$-(Fix_C + \pi_C \times T_C) \qquad (8)$$

Finally
$$VOLL \times T_D - \pi_C T_D = Fix_C \qquad (9)$$

$$\mathrm{Rev}_C = Fix_C - Fix_C = 0 \qquad (10)$$
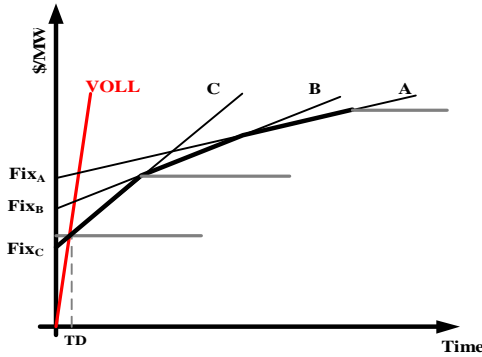
Figure 6. Comparative cost curve and the relation between VOLL and Fix_C

Consequently, there is not any loss for unit C because it accepted as last unit in market. Obviously, condition of profit is positive for other units. Capacity payment index is a product of loss of load probability (LOLP) and VOLL and defined as:

$$CP = LOLP(VOLL - SMP) \qquad (11)$$

Where,
CP: Capacity Payment of unit
LOLP: Loss of Load probability
VOLL: Value of Load Loss
SMP: System Marginal Price

Units of CP are $\dfrac{\$}{kWh}$ and $\dfrac{\$}{MWh}$.

Suppose that an investor wants to install a new generation unit in TD range for supplying the load. Construction cost of On-peak load unit is identified as;

$$CCOPU = LOLP(VOLL - SMP) \qquad (12)$$

In PAB mechanism; such as Iran Electricity Market, if there is tendency to CP, so there will be tendency to generation expansion planning (GEP) and augmenting the role of existing generation units. In this case, high efficiency generation companies have high profit. For that reason, there is a comparative mood for generating energy.
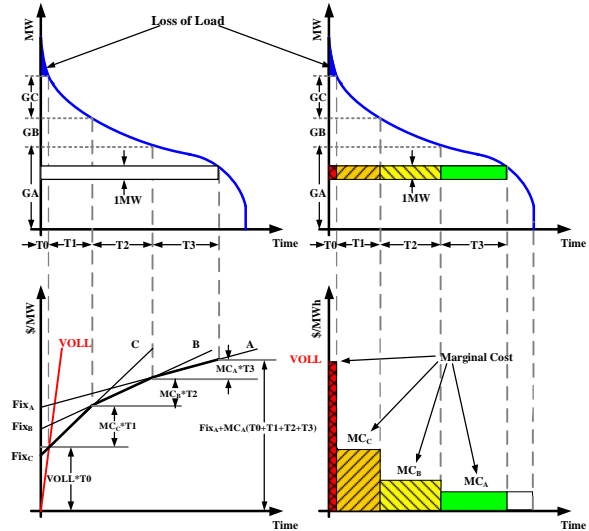
Figure 7. Cognitive model of Intermediary marketing interfering mechanism

As a unit is going to be more efficient, the possibility of being online and gaining CP in the hours of being on-line will increase. Therefore, this incentive factor creates a positive signal to improving efficiency of generation units in long-term and competition in being on-line in short-term. Genco's have tendency to present real price of generation (marginal cost of generation) in this market and comparative conditions. As a result, in this case, it can be said that CP can make a kind of balanced and rational competition between generation units because of in this condition, bidding strategy to be closer to the marginal cost.

5. Discussions

In this paper, market clearing mechanism in Electricity market and accepted mechanism in other countries in a comparative and analytical process have been carried out. Results shown that in PAB mechanism, there is increasing of offered prices by Genco's. In this area, investment analysis in GEP, investors should have some stimulus signals. This issue is very important for market operator and consumer, too. In this mechanism, payment as installed capacity should to pay similar to mechanism of other countries. It have been shown that, this case cause to increasing the tendency of Genco's to improving conditions of their generation units for best profit in short term and for investing in long term. It is clear that the amount of generation and consumption is not in idealistic conditions, so this approach is more efficacious than implementation of full rivalry mechanisms. In these conditions, end user demands and the amount of generation will manage and are going to a balanced comparative mood.

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3/8/2011

The role of rural women's self-reliance in improving Livelihood

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Abstract: Rural women are among those major groups at society who previously were considered less by planners, due to specific reasons in the past. And this problem is more observable at developing countries. If rural women can work through receiving credits, loan and others finance facilities at favorite jobs and live through earned income (as it called "self-reliance and independence"), so undoubtedly we would see changes in social, economic and cultural relations of village. Rural women's financial self-reliance has many social & economic influence as it made them self-sufficiency, it changes economic behavior and it makes women independent, it will be effective in economic development in family & society, it also improve the women's roles in society and it causes self-confidence in women, it builds family strength and it causes to respect the women rights more than before and women will become equal with men in all their rights, of course we won't have patriarchy in the family. The women's empowerment in the rural society will increase because of all the aspects of rural women's self-reliance and their position will be confirmed.

1- Introduction:

In the development countries, rural societies which are poverty for geographic reasons such as being far from urban societies or because of mountainous of zone and also as the roads are impassable and some other reason, they became deprived of many human development programs. Unfortunately these societies are suffering of mortality because of poverty but what is clear here is that we can't attribute such privation to geography and nature of the zone. Every country is tying to solve such critical conditions by applying depoverty policies. (Bakhshoodeh and Salami, 2005)

Poverty spreading in village is a global issue. According to the Fao finding about % 75 of world's poor people that are more than 1 milliard people are living in rural zone and more than % 70 of this poverty people are women. As the most of the people who are poor are living in village and are women is the reason for insufficiency of rural development programs.

One of the other basic barriers in development of rural women is their independent inaccessibility to get credits for investment in their job. Although their illiteracy is the big barrier to use of bank credits, but this view that women are dependent people that their husband should decide about their financial decisions

is the other reason that rural women couldn't access to official credits. Maybe these barriers are the reason

why rural women are happy about applying micro-credit thought in village. (Najafi, 2007).

Having investment (capital) independency enforce people to think about economic from different angles. He should study the ways for using capital, he must consult with authority and experienced people and he will investigate about relevant markets. Such things will help him to be authoritative & independent. But how rural women can get such independency? Are the women created inherently for housekeeping, parenting and working or is there any opportunity for rural women to show their skills in economic & social development?

From 1970, the waves of thought about micro-credits and run of small activity in villages was one of the suitable way get increased for invest improvement in rural occupations.

The said plan because of special grants such as giving loan with low wage and no interest and with long reimbursement could give farmers this opportunity to don't rely usurers and jobber intermediaries. Indeed giving micro-credits to rural women was more effective. Because along agriculture activities which need more investments,

the women with using micro-credits couldn't only show their talent in rural production, but also could improve their economic & social empowerments and they could also participate in social activities. (Chabokru et al, 2005).

Women's self-reliance and independency were the outcome of giving credits to women and in some cases were the obstacle of receiving credits by women which is necessary to explain about them shortly.

2- Financial self-reliance of rural women:

Although women in our society (Iran) have the main role in economic production but they are not aware of financial exchanges. In other word, we can say the family's father is responsible for financial issues. The women and other rural people should know that the aim of women's financial self-reliance is helping the families economic and it doesn't mean that when rural women became independent should forget their previous relation with the family. The financial self-reliance shouldn't make rural women proud and destroyed their relation with the family. Also, the rural men should be aware that women's self-reliance is their right and isn't in contrary with the family's economic principles. (Amiri, 2000).

3- Economic effects of rural women's financial self-reliance:

it is possible that rural women's financial self-reliance made some crudities (malformations) in the family for a short time, for example, rural women became proud after financial independency and find the independence & Excellency sense in themselves but such problems will be small and for a short time.

The rural women's self-reliance has positive effects which is useful for women and their family and also will help their economic improvement that we will mention some of them. (Chowdhury, 2005).

3-1- Self-reliance and financial independency:

The income of the rural women makes them financially independent. The financial independency will let them to spend their wage in the ways that they like. Of course their dependency to their family won't let them to spend their wage out of their family needs. Because of this, their financial independency will let them and their family to be self-reliance. (Ghaffari, 2000).

3-2- Change economic behavior:

Although we are familiar with the rural women's role in the village and family's economic, but they direct & indirectly start a new economic relation, with finding modern jobs & financial independency. Catching loan from financial organizations has forced

them to have economic schematization for loan reimbursement and to have intellectual economic behaviors. So after that rural women become active in economic activities. In rural traditional economic, women only have productive role and they don't have any role in economic planning, providence and they don't pay any attention to profits and losses. But in this new condition, for managing affairs in best way, the women have to be active in all of the affairs from production to dispense and also in others economic aspects. In other words, women will not be a productive only; they will contribute in managing of economic activities and will find various economic behaviors. (Araghzadeh, 2002).

3-3- Independency:

The rural women will not dependent economically to their father or husband because of financial independency, this independency is very important to women who have children or they have lost their husband, because the financial problems have forced the rural women to have marriage which is not suitable for their children & themselves. Although the women can solve their financial problems with this kind of marriage but they will have many cultural, social & mental problems. If these women could manage their life with having a job, they can improve their family & kinship's relation.

The rural men & women should notice that their financial independency is not the meaning of an independency in their family, social & cultural affairs and making consensus between financial & economic affairs is necessary for family's consistency. (Fiona Steele et al, 2008).

3-4- Help to economic growth

The rural women's financial self-reliance will increase their motivation for finding a good job. As a result our rural & urban society will develop by working of women. And it will help direct & indirectly to our society's economic development. As the women constitute about half of the rural & urban's population, so by increasing their production, our society will develop economically. (Jameela, 2010).

Conclusion & discussion:

If rural women could provide a job for them by getting credits, loan and other financial convenience, through their income they can get self-reliance or financial independency and we will see social, cultural & economic change in village. The question here is that if these changes have positive or negative aspects in the village? It's natural that every change in social phenomenon has both positive and negative aspect, but which is Important here is that which

aspect is more than the other and it depends to different condition in various societies. In our rural society there is an especial social & cultural kind that it's outcome maybe different and in some case inconsistent. With these actions rural women could be in idealistic economic condition and they could live with out dependency to their husband's income. In most of the villages in Iran there is patriarchy in the families which is not acceptable for the most of the rural people and groups. When rural women became financially independent, it's acceptable to see its cultural & social outcomes.

Hashemi and others (2004) found that joining to Gramin Bank, has meaningful positive effects on controlling women, and helps to family income.

Ellen and her colleagues (2009) used approach called it "credits and education at Bolivia, Ghana, Honduras, Mali and Thailand". This approach looks for empowering women through financial services with education. In this approach, women get familiar with importance of credits through education and extension and also familiar with ways to access it through establishing different groups.

Shahnaj and chaudhury(2009) in research as "credits and its role on empowering women " concluded that there is meaningful relation between attending in credits programs and empowering women, at economical dimensions.Ruhal amin and others (2010) found that those who joined credit funds had more ability rather than those who didn't. Jameela (2010) presented that credit programs has shown lot of affects on empowering women so that has increased their social, politic and economic ability. Thus it is obvious that credits programs and its educational and empowering programs can be affective on social, humane and economic development or rural society, if it be associated with proper and gradual practices and base on reciprocal communications principles and apply opinion of local society. Maybe the main challenges that threaten credits associations, is lack of necessary emphasizes on social dimensions and on reinforcing their basics, that practically cause that this social foundations lose its efficiency soon and practically changed to unsuccessful institution.

In order to overcoming dominant consideration, experts believe that we should consider following in protection process of these social institutions
-Relating public established institutions with each other and networking established institutions
-Emphasis on stability and self reliance of management system of credits institutions from financial and economic dimensions
-Efforts to gain local confidence and credibility among contacts

-Effectiveness of costs and economic and financial efficiency inside established institutions
Also following suggestions has been offered:
- providing extension educations for men in order to believe economic role of their women, and give them chance of corporation on all economic, credits fields
- Since that base of credit association, forms base on People Corporation, so it's good chance to use these communities to expand extension-education activities. so it is better to consider special programs on different extensional filed such as agriculture, ranching, family health, housekeeping economy and other fields accordance to condition of region and rural women's needs.

Giving the right that women make decision, independency to their family, increasing the cultural knowledge among them& making relation with new institutions, having independency in making decision about marriage, occupation, migration & something like this are the right that women have got it.

Women by getting these rights can make change in the rural cultural & social issues which make disfunction & crudity in their family's relation. However, rural women's self-reliance has caused improvement in the economic, social & cultural issues. For solving women's self-reliance problems we can do these activities:
- Giving promotional services for increasing rural women's skills in various fields.

- Giving promotional instructions to men for believing their women's economic role & their women opportunity to participate in all economic, authority & … aspects.

- Increasing rural women's knowledge in all social, political, cultural & economic fields.

- Making use of micro-credits programs to motivate & support women for doing economic affairs better & finally to make women self-reliance.

Its result is that, exploiter can't access to desirable condition of production efficiency at first. Secondly, he would incapable for loan repayment. Third, his activity doesn't contain consistency. Fourth, remarkable part of provided credits would exit from production cycle due to exploiter's incapability and lack of skill in exploiter. His technical and occupation skill would improve, if credit is being provided for exploiter as a credit

program. and he knows and can applies loan properly and well timed for production and activity, so condition of production and level of income, level of life and would improve.

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Decentralization in agricultural management in rural activities

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Abstract: Agricultural extension is a non-formal type of education that provides advisory services by the use of educational approach in acquiring knowledge and skills to deal with the growing needs of global world. Diverse agricultural extension funding and delivery arrangements have been undertaken since the mid-1980s by governments worldwide in the name of "privatization." When agricultural extension is discussed, privatization is used in the broadest sense – of introducing or increasing private sector participation, which does not necessarily imply a transfer of designated state-owned assets to the private sector. In fact, various cost-recovery, commercialization, and other so-called privatization alternatives have been adopted to improve agricultural extension. The form and content of decentralization has dominated development discourse and public sector reform agenda in Kenya in the last two decades. The case of agricultural extension service presents decentralization in a difficult context partly due to lack of information on its possible diverse impacts especially on resource poor farmers.

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Introduction:

Un-fortunately in developing as well as low income countries agricultural extension has failed in diffusing new technology to its ultimate users (Government of Malawi, 2000) and further deterioration witnessed with the passage of time (Eicher, 2001). The failure of agricultural extension services for last decades is under constant pressure to be responsive to ever-growing challenges of food production.

Over the past two decades many countries have undertaken to decentralize government functions and transfer authority and responsibilities from central to intermediate and local governments, and often to communities and the private sector. Decentralization is potentially important to agricultural knowledge and information systems, but decentralization is not an end in itself, and successful decentralization strategies must address three challenges—establishing a national framework for decentralization, developing subsector approaches, and enhancing capacities of various participants for coproduction of decentralized goods and services. Agricultural extension services are under increasing pressure to become more effective, more responsive to clients, and less costly to government. Decentralization is an increasingly common aspect of extension reforms. Field extension advisory services are well suited to decentralized approaches, but a comprehensive extension system requires a range of extension support services and programs, some of

which (strategy formulation, training, monitoring and evaluation, specialized technical support) are often best carried out at the central level.

The prime challenges in the traditional public extension systems enlisted as outdated, top-down, paternalistic, inflexible, subject to bureaucratic inefficiencies that results less ability to cope with the dynamic demands of modern day agriculture (World Bank, 2002; Obaa et al., 2005). In some countries the change is occurring with its natural pace but in many developing countries these have been accelerated by structural adjustment reforms (Chapman & Tripp, 2003).

Like other developing country Pakistan is also an agrarian country, whose economy is highly dependent on agriculture having 23% share to GDP (Government of Pakistan, 2005). But still the performance of agriculture sector at the farm level remains significantly below the potential and limited due to the weak institutional formwork in disseminating agricultural technology to the farmers (Farooq, 2005). Research scientists evolving new methods and technologies to meet the challenges of new era and the farming community also has a potential and courage to adopt but the third component i.e. agricultural extension, which serves as a technology transfer vehicle and play a significant role in increasing the productivity, farm incomes and ensure food security has been very much weak since independence (Luqman et al., 2004; Farooq, 2005). The extension services in the country have not been

able to achieve their goals effectively, because of a number of bottlenecks. These include weak research-extension linkages, lack of adequate resources for on-farm demonstrations, poor mobility, inadequate research and training in extension methodology and lack of an effective system of continuing education for extension personnel at various levels (Sandhu, 1993). Among major filed crops wheat, rice, cotton and sugarcane accounts for 90.4% of the value added in major crops and 37.1% of the value added in overall agriculture (Government of Pakistan, 2005). The low production of these crops depends upon a number of factors including ineffective and isolated agricultural extension system.

Decentralizing:

Decentralization as transfer of authority and responsibility for government functions from central government to intermediate and local governments, and often to communities and the private sector has become widespread over the 1980s and 1990s. Countries with diverse systems and traditions of government have pursued decentralization initiatives for many reasons, including especially the failure of government to meet expectations under centralized approaches to economic management and service approaches to organizing public administration. Though not yet widely applied to agricultural research and extension, decentralization strategies are potentially important to these agricultural knowledge and information systems. Decentralization is frequently viewed from one of two different perspectives (Johnson, 2000).

1. The democratic view emphasizes the aspect of empowering local people to control and direct their own public programs; and

2. The administrative view emphasizes the efficiency gains resulting from improved administration and effectiveness of public programs due to local control. Decentralization is generally expected to: encourage local financing and ownership of programs, result in more efficient and equitable allocation of government resources, provide incentives for production and service delivery, ensure lower-cost service delivery, build local capacity, and respond more effectively to local needs. (Khan, 2002).

For rural programs, decentralization offers hope for correcting the urban bias that results from the geographic dispersion of rural people, the difficulties for them to organize to promote their interests, and the discrimination against agriculture inherent in many country policy frameworks. Decentralization of agricultural extension and research seeks to increase user participation in

technology programs and make programs more accountable to users. (Eicher, 2001).

Enthusiasm for decentralization needs to be tempered with some caution. In small countries, decentralization may be unnecessary and in very large countries decentralization to the state or provincial level may still leave programs distant from user influence. Definitive evidence of the impact of decentralization is limited and not everyone benefits from any reform. Furthermore, decentralization does little to improve intraregional disparities, may bring oppressive elites into power, and can lead to greater inequalities in allocation of government resources.

Thus, decentralization has the potential to increase access to and cost of services, but specific targeting mechanisms and strong central oversight are needed to avoid inequities in service access and quality. (Farooq, 2005).

Decentralization of Public Sector Extension:

Pubic extension services are being forced to change. In the 1990s agricultural extension services were attacked for being inefficient, irrelevant, ineffective, and poorly targeted. The need for reform was obvious and national systems responded with three major strategies— privatization, decentralization, and program revitalization. Although cost reduction has been the force behind many changes, the principal objective of reforms should be an attempt to improve quality of services to clients Decentralizing extension services, when implemented effectively, can transform exten- sion and address a range of generic problems.

Decentralized extension brings decisionmaking processes closer to clients and makes programs more responsive to user needs. Service providers become more accountable to clients and better oversight increases efficiency of operations. Decentralization itself can introduce a new dynamism in programs and can promote diversity in service providers and program approaches, thus serving as a first step toward privatization. In addition, reforms to revitalize and privatize programs can accompany decentralization reforms, which generally involve: (World Bank, 2003).

- Administrative decentralization—moving responsibilities for extension to local levels of government;
- Political decentralization—expanding user influence on program priority setting, planning, and management; and
- Fiscal decentralization—giving financial management responsibility to local governments or requiring cofinancing from local governments and producer groups.

Extension services differ from research in two important ways that affect their potential for decentralization. First, extension advisory services (field extension services) come in direct contact with clients and provide services that have a high private-goods content. These characteristics make field extension services a much better candidate for decentralization than research, which typically has a longer-term payoff. Local producers are more willing to commit resources to pay for effective extension services from which they realize immediate direct benefits. Still, there remains a need for other extension services to address "externalities"—environmental problems, food quality or safety concerns, or social equity issues (that is, special needs of small farmers)—that are in the public interest, but are not a priority for individual producers or decentralized institutions. This requires continued central support for extension. A second difference between research and extension is the scope and scale of programs. (Williamson, 2002).

Research institutions are generally smaller and more concentrated. Extension programs typically operate across the country, provide information on a wide range of technologies from various sources, and draw on traditional knowledge and farmer innovation to improve producer organization, management, production, and marketing functions. The broad demands on extension require strategies that incorporate a variety of approaches to providing services.

Despite the apparent suitability of extension service provision to be decentralized, they are often highly centralized. A World Bank study of 19 countries found that in the early 1990s 13 countries or regions showed almost no evidence of decentralization of extension services. Colombia, Jiangxi (China), the Philippines, and Nusa-Tenggarra-Timor (Indonesia) were relatively highly decentralized, and Poland and Tunisia showed some decentralization. The study found that:
• When extension is decentralized there is a fairly good balance in fiscal, administrative, and political decentralization;
• Political decentralization (the role of elected officials) lags other elements of decentralization; and
• NGO involvement is moderate and farmer participation is significant in extension.
Underlying these conclusions was the fact that institutional development and civil society provide important support to decentralizing extension services. (FAO, 2001).

Administrative Decentralization:
Deconcentration is intrinsic to extension services that are provided in dispersed fields and communities throughout a country. Cropping systems, markets, agroecological zones, and eth9 nic and cultural characteristics of farmers can vary widely within a country, and moving administration closer to field services can substantially improve program management through better understanding of local conditions. Administrative decentralization goes further by making extension programs directly responsible to local authorities. The challenge in any successful decentralization reform is that of maintaining overall program quality and coherence. Decentralized extension programs are limited if the decentralized administration lacks awareness of new technologies, sources of assistance, and extension methodologies. Although decentralized administrations can effectively integrate local institutions, organizations, and technologies into an extension system, major benefits from formal extension often come from integrating external knowledge into the local system. Lack of coordination between local administrations can be a problem. If many localities promote a single commodity, the result might be overproduction and low prices. Similarly, separate localities might finance the same feasibility studies, training programs, or extension materials. Implementing an integrated watershed or regional development plan might prove impossible if programs in each administrative region are completely independent. Other potential problems include the lack of career opportunities for extension staff in decentralized programs, and difficulties with monitoring and evaluation when local administrative units lack ability to compare targets, results, and achievements with other areas. (Khan, 2002).

Extension program quality depends fundamentally on good linkages with other programs— specialized training for extension agents and farmers, technical backstopping by subject matter specialists and information services, other extension services (mass media, fairs), and other development programs (credit programs, market development programs, input supply).

Some of these linkages can be maintained at the local level, but many require higher level coordination to ensure efficiency and quality support.

Fiscal Decentralization of Extension Services:
Government inability to sustain financial support for large extension systems has been a motivation for the many reforms that attempt to reduce public sector funding, introduce private financing, or eliminate government programs that compete with the private sector. Typically, these strategies tend to decentralize extension financing. Although an objective of many decentralization

reforms has been to reduce government expenditures, local governments generally have limited resources and limited ability to raise funds. Central governments therefore must usually continue financing for extension services through intergovernmental financial transfers (IGFTs), and must also finance the considerable costs of reform and local capacity development. This increases total financing requirements for extension, at least over the short term. Over the longer term, decentralizing extension services might reduce government financing requirements by: (1) increasing efficiencies through better oversight and greater flexibility in funding decisions and (2) increasing cofinancing by being more responsive, and demonstrating greater benefits, to users. Cofinancing grants (IGFTs) to local governments or farmer groups are an important element of fiscal decentralization, but they present two significant problems: (Chapman & Tripp, 2003).

• Many local organizations lack capacity to plan, manage, and evaluate extension programs and lack the contacts and financial management capacity to procure needed services; and

• Resource-rich farmers are better able to cofinance services and capture program benefits, even if program objectives are to assist weaker elements of rural society. Still, many new initiatives are using subgrants of various types for local subprojects, and future program design can draw on this experience Decentralization programs must address these two problems. Training and orientation, program promotion, and support services are critical to enable target clients and local organizations to take over extension responsibilities under new decentralized systems. Later, as programs are implemented, a strong monitoring and evaluation system is needed to provide management with information necessary to understand who is benefiting from the program and what real impact it is having (Farooq, 2005).

Conclusion:
Decentralize extension services where possible, with emphasis on giving users control over program planning, implementation, and evaluation.
• Provide for adequate centralized support systems for decentralized extension services, especially support for training, subject matter specialists, and production of extension materials.
• Adapt strategies to local institutional environments to accommodate country legal frameworks, political traditions, administrative structures, and social and agroecological conditions. Extension strategies can emphasize decentralization when there is already a strong political decentralization in the country, but should proceed cautiously when decentralization is not yet well established.

• Determine on a case-by-case basis whether decentralized services should be managed by local governments, community/producer organizations, or local governments bin conjunction with producer/community organizations.
• Provide clear division of responsibilities between the different levels of government and other program participants.
• Develop procedures for policy formulation and priority setting in mixed systems to reconcile central government financing and policy objectives (poverty alleviation, food security, and environmental conservation)b with local peoples' priorities that emerge from the decentralized program governance.
• Provide for needed fiscal transfers from central government to decentralized implementing agencies to finance decentralized extension services, recognizing that over the short term decentralization rarely reduces requirements for central government financing.
• Structure fiscal transfers to give users maximum influence over programs and to promote institutional pluralism in service provision. This empowers users and develops capacities in a range of public and private providers, such that the most competent institutions are able to provide the services.
• Provide for extensive planning, promotion of the rationale and principles behind reforms, and training in new operational procedures before launching decentralization reforms.
• Provide for needed investments in development of local capacity (local governments, executing agencies, community or producer groups), as such implementation capacity is critical to success of decentralization reforms.
• Establish effective systems to monitor and bevaluate decentralized programs, and ensure that the data are available at all appropriate blevels. Central monitoring should be sensitive to equity issues and the possibility of local elites capture of programs, thus excluding services to the poor or women.

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Proprietary of Total Intensity Magnetic Data to Detect the Subsurface Structures and Tectonics of Southern Sinai Peninsula, Egypt

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Abstract: This study presents the analysis and interpretation of magnetic data to map the subsurface structural framework of the present area. Structural interpretation of the magnetic data was achieved through applying advanced processing techniques that provide automatic delineation and depth estimation of the magnetic structures. Some structural elements could be deduced from the qualitative interpretation of such magnetic anomalies. Phase-shifts of magnetic anomalies due to the local direction of the geomagnetic field vector can be corrected using a reduction-to-pole filtering operation. At the interpretation stage, the analysis of the RTP magnetic data, which included low-pass/high-pass filtering by power spectrum and separation of the magnetic causatives of shallow sources from those of deeper sources through the matching band-pass filtering. The horizontal gradient and local wave number tools were used for locating the magnetic sources and their properties. In this case, peaks in those methods can be used to locate sources representing the edges of thin horizontal sheets and estimate their strike directions which, used to delineate the tectonic framework of the investigated area. The shallow structural depths located between 1.5 to 2.5 km (red color) dominate the majority of the southern part, as well as some parts in the northern part. While the deep depths 2.5 to 5.5 km (blue color) dominate the northcentral and westcentral parts. The mapped structures reveal that, the area is affected by a set of faults trending mainly in the NE-SW, NW-SE and N-S directions. Moreover, the area is dissected by a set of deep basement swells and troughs, as well as shallow anticlinal and synclinal trends controlled mainly by the predominant faults.
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Keywords: Proprietary; Magnetic Data; Subsurface Structure; Tectonics; Southern Sinai Peninsula; Egypt

Introduction:

The study area occupies nearly the southern half of Sinai Peninsula, that located between the Gulf of Suez and Gulf of Aqaba. It lies between latitudes 27° 50' & 29° 30' N and longitudes 33° 00' & 34° 45' E (Fig.1) . It is noticed that, the concerned area is an active seismic area, the higher rate of seismic activities at the southern end of the Gulf of Suez is interpreted as a result of the triple junction between the African and Arabian plates and Sinai subplate (Ben-Menahem, 1979).This region is considered as a Tertiary cratonic rift between Northeastern Africa and the Arabian Peninsula , its rifting phase ceased when continental separation became more oblique, due to the predominant movements of the left-lateral transform fault, that extends north north-eastward through the Gulf of Aqaba to the Dead Sea (Patton et al.,1994, USGS 1998 and Reilinger et al., 2006).

Many geophysical researches have been applied on this area to delineate the subsurface structures and tectonism. It was indicated that, the structures of the study area are related to the Gulf of Suez and the Red Sea tectonics. These structures extend from the basement rocks upwards into the sedimentary sequences and divided the area into several major faulted blocks, that have great importance in sealing the oil traps (Rabeh, 2003).It was noticed that, the southern part of Sinai Peninsula tends to move towards the east (NNE) at an angle smaller than that of the northern part(Rabeh et al,2008).

In general, the geophysical magnetic method is mainly based on the measurements and analysis of small variations in the earth's magnetic field within any area. An aeromagnetic map is a reflection of the distinctions in the magnetic properties of the underlying rocks. So, these variations encountered in the measured magnetic field are attributed to the distribution of the subsurface magnetically-polarized rocks. The sedimentary rocks are of weaker magnetic properties than the underlying basement rocks, especially the mafic rocks. Therefore, the magnetic methods are used to delineate the structural and lithological configuration of the basement rocks.

The main available type of geophysical data for the current study is a magnetic map reflecting the distribution of the total intensity magnetic anomalies within the study area (Fig. 4). The current study deals

with the interpretation of land- magnetic data of South Sinai to map the subsurface structures and the tectonic setting of the area.
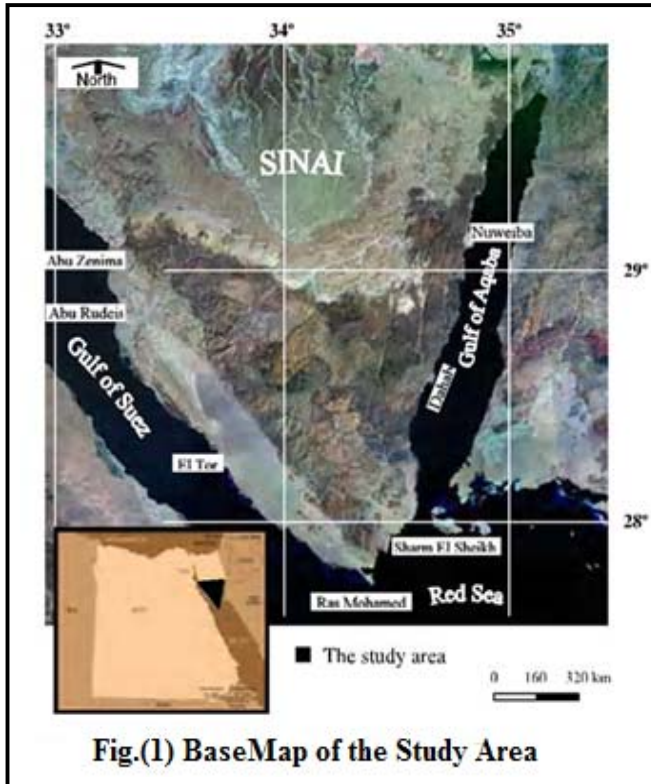
Fig.(1) BaseMap of the Study Area

Geological Outlines

Sinai Peninsula displays a variety of simple and complex structural forms, so it is considered the glamorous region from the geological stand point of view (Abu-Al-Izz,1971).

Geomorphologically, South Sinai is characterized by elevated mountains district assuming a triangular shape with its apex at Ras Mohamed to the south , and a central plateau in the form of two main questas , El Egma to the southwest, and El Tieh to the north (Abu –Al-Izz,1971).

The surface rock units of the study area range from Pre-Cambrian basement rocks to the Quaternary deposits (Fig.2). The basement rocks (Precambrian) are located in the southern region of the area, while the Paleozoic rocks are located in the middle area and the Mesozoic rocks occupy the eastern and western parts of the study area. The Quaternary deposits lie on either sides of the Precambrian rocks along the Gulf of Suez and Gulf of Aqaba. The basement rocks of South Sinai are gently dipping northward, with a consequent thickening of the overlying sediments.

Structurally, both the basement rocks and the thin sedimentary cover exhibit a large number of

surface tectonic elements of varying lengths and trends (Fig., 3), as shown by Neev, 1975, Agha,1981 and Said,1990. Ahmed and Hassaneen (1985) had addressed the predominant directions of the fault systems in Sinai, through the interpretation of the magnetic map of Egypt. They summarized the directions into the following categories:

1) The N-S direction is predominant.

2) The E-W direction (Mediterranean Sea trend).

3) The NE-SW direction is presented by five directions , which are 10°, 25°,45°,50°and 70°from the north (ENE is the Syrian Arc trend and NNE is the Gulf of Aqaba trend).

4)The NW-SE direction is represented by three directions, which are 15°,25° and 40° from the north (Gulf of Suez trend).

Tectonically, the wrenching along the Gulf of Aqaba is reflected in the form of strike-slip faults cutting through the basement rocks and the overlying sedimentary section, as well as en-echelon folds (Abdel Khalek et al., 1993). The northward extension of the Gulf of Aqaba leads to the Dead Sea –Jordan transform fault system, which links the Arabian Plate convergence in Southern Turkey with the active seafloor spreading in the Red Sea . The Aqaba – Levant structure is thought to have formed by left-lateral shear motion (Freund et al.,1970) with relative displacement ranges from tens of meters up to 9.8 km. The creation of the pull-apart basins is mainly related to strike-slip motion (Garfunkel,1981) with N-S to NNE-SSW fault trend (Lyberis,1988).
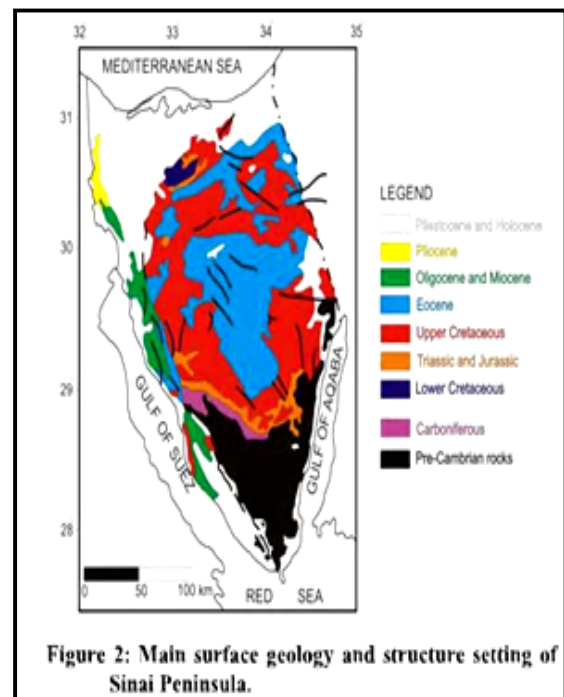
Figure 2: Main surface geology and structure setting of Sinai Peninsula.
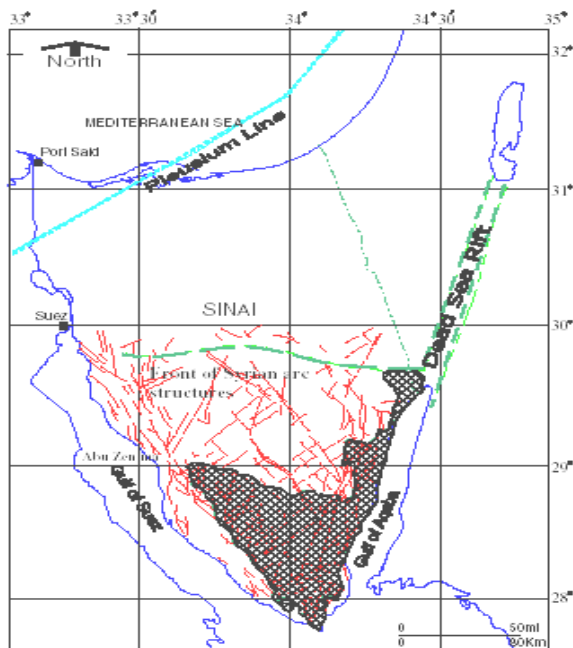
Fig.(3) Tectonic map of South Sinai (modified
after Neev 1975 and Agha 1981)

Main Concepts

There are some principles should be taken into consideration when working with the magnetic data. The interpretation of magnetic data is not unique, because it is controlled by many factors; for example, depth to top of the causative feature, its shape, its azimuth, its magnetic susceptibility, that is mainly related to its petrographical composition. Such factors are related to the subsurface anomalous features, that produce their magnetic signatures at the surface. The availability and use of some of these factors during the interpretation reduce the ambiguity in the magnetic interpretation. Such factors can be taken from the available well data and regional geology of the area. The available magnetic data for this study have been corrected for the diurnal variations, instrument drift, and for the errors in positioning and height keeping.

Insights On The Original Magnetic Data

The close study of the total intensity map with scale of 1:500000 and 30 nT contour interval (Ismail et al. 2001) (Fig. 4) indicates that, most of the observed anomalies show NE-SW , NW-SE and N-S trend patterns with some sharp gradients at varying locations. Since the magnetic maps are related directly to the basement rock features, so this indicates the presence of a basement relief change.

Close investigation of the total intensity land magnetic map (Fig. 4) revealed that, the western side of the map is occupied by three positive elongated

anomalies intervened by two negative ones, all are trending NW-SE. The northern part of the area seems to be affected by three high positive large extent anomalies, which are oval shaped trending E-W .The southern area of the map is characterized by a large E-W high amplitude anomaly, along with a small round NE-SW positive anomaly. The central and eastern parts of the area are characterized by the presence of large number of negative anomalies trending E-W, ENE-WSW and N-S directions. These negative anomalies may be due to lithologic variation of the basement rocks and or due to the faulting process in this part. There is a large oval shape positive anomaly occupies the central part of the mapped area trending NNE-SSW direction, which may be referred to shallow structure or high magnetic susceptibility feature.

Magnetic Data Processing

The processing of the magnetic anomalies is based on the analysis of the computer-digitized information using different processing techniques at different altitudinal levels from the compiled total magnetic data shown in Figure (4). These techniques involve; first the reduction to the north magnetic pole. The reduced to the north magnetic pole digitized data were used for additional investigative techniques, that helped integrative to deduce the structural set-up for the basement of considered area.
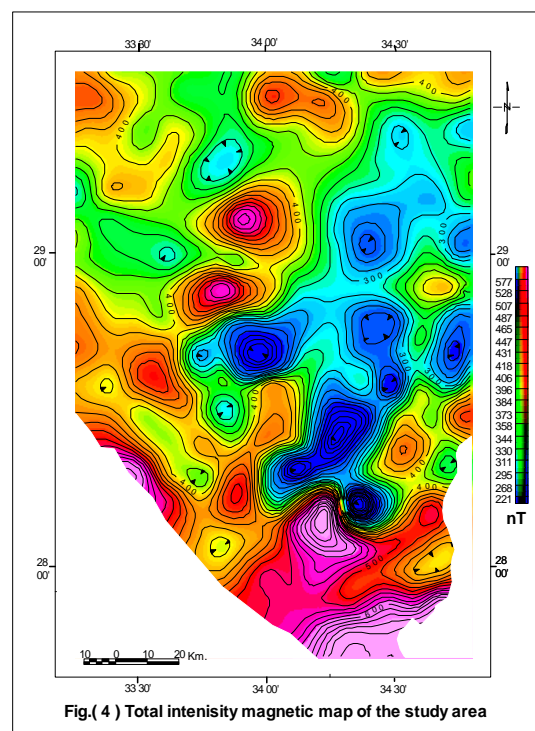
Fig.(4) Total intensity magnetic map of the study area

The Reducion to the North Magnetic Pole:

A reduction-to-pole (RTP) transformation is typically applied to the total magnetic data to minimize the polarity effects (Blakely, 1995). Reduction-to-the-pole is a filtering technique used to align the peaks and gradients of magnetic anomalies directly over their sources. These effects are manifested as a shift of the main anomaly from the center of the magnetic source and are due to the vector inclination and declination of the measured magnetic field. The RTP alteration usually involves an assumption that, the total magnetizations of most rocks align parallel or anti-parallel to the Earth's main field(declination=2.38^o, inclination=42.32^o and IGRF total intensity value=42803 nT, for the study area). In the present study, the total intensity land magnetic anomaly data are reduced to the magnetic pole (RTP), according to Geosoft Oasis Montaj (2007).

Inspection of the RTP map (Fig.5) shows that, the southern and southeastern parts of the area are characterized by the presence of a very high amplitude oval shape positive anomaly trending NNW-SSE, together with an elongated one having a big aerial extent trending E-W. This region represents the basement rocks lying between the two gulfs, expressing their structural directions. While the western area of the map shows a high positive amplitude anomaly, comprising three small oval shape ones trending NW-SE direction parallel to the Gulf of Suez with moderately steep gradient, indicating intermediate depth sources. The central and northeastern parts of the area are characterized by very low amplitude anomalies trending NNE-SSW and N-S directions, with almost oval shape.
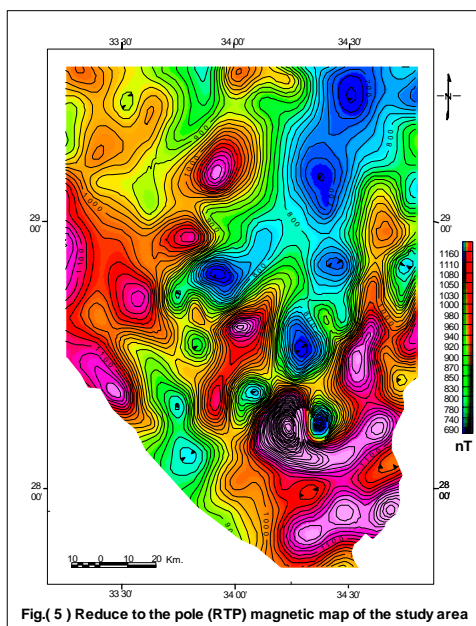
Fig.(5) Reduce to the pole (RTP) magnetic map of the study area

1-Low-pass and High-pass filtering of the RTP Magnetic Data

Filtering the magnetic data is an essential process prior to the analysis and interpretation. The objective of the filter is to recondition the data set and to render the resulting presentation in such a way as to make it easier to interpret the significance of anomalies in terms of their geological sources. Therefore, the most effective way to filter the data is through understanding the geologic control and the desired filtered results. Several filtering techniques can be performed in the frequency domain. However, one of the most traditional filters, used in the potential field, is the separation of long (deep) and short (shallow) wavelength anomalies. The success of this technique depends on the proper choice of the cut-off wavelength used in the filter design. The cut-off wavelengths and information about the contribution of the short and long wavelengths in the spectrum can be obtained from the calculated radially-averaged power spectrum of the data.

Two-dimensional power spectrum curve of the present RTP data, using Geosoft Oasis Montaj (2007), shows two linear segments related to the long and short wavelength components with frequency bands ranges from 0.0 to 0.3 and from 0.3 to 0.95 wavenumber, respectively. Following Spector and Grant (1970), the slope, of these two linear segments were used to estimate the average depths to the tops of the deep-seated and near-surface magnetic sources, respectively. These depths are average estimates for the entire area and do not reflect a resolved and detailed such topography of the basement surface. The frequency bands corresponding to these linear segments were used through the band pass filter technique to produce the low-pass and high-pass magnetic component, maps (Figs.6 & 7).

The regional (low-pass) magnetic anomaly map (Fig.6) is characterized by large, homogenous and high amplitude anomalies, which are caused by deep-seated causatives. Inspection of this map indicates that, broad and oval shape high intensity negative magnetic anomalies extend from the northeastern corner to the central part of the area. This belt trends in the NE-SW direction and degrades by steep gradients. The southeastern corner of the mapped area is characterized by a broad aerial extent and high intensity positive magnetic anomaly with elongated shape, trending in the NW-SE direction. It is surrounded by steep magnetic gradients from its northern and western sides, indicating the existence of major deep-seated faults around this anomaly from both sides. Broad positive magnetic anomalies with oval shape occupy the western part of the study area with gentle magnetic gradients trending NNE-SSW.

Residual (high-pass) magnetic anomalies map (Fig.7) are of primary importance in reflecting the structures presented on the basement surface or the magnetic sources occurred within the sedimentary cover. Examination of such map revealed that, the map is characterized by high frequencies, short wavelengths, small size, weak intensity, sharp low amplitude and nearly round-shaped anomalies. The

southern part of the study area is characterized by numerous positive and negative small elongated to round shaped, and high amplitude anomalies, trending in the NE-SW, N-S and E-W directions. The central and northern parts have large elongated and nearly rounded anomalies, trending in the NE-SW and N-S directions.

Fig.(6) Low-pass filtering magnetic map of the study area

Fig.(7) High-pass filtered magnetic map of the study area
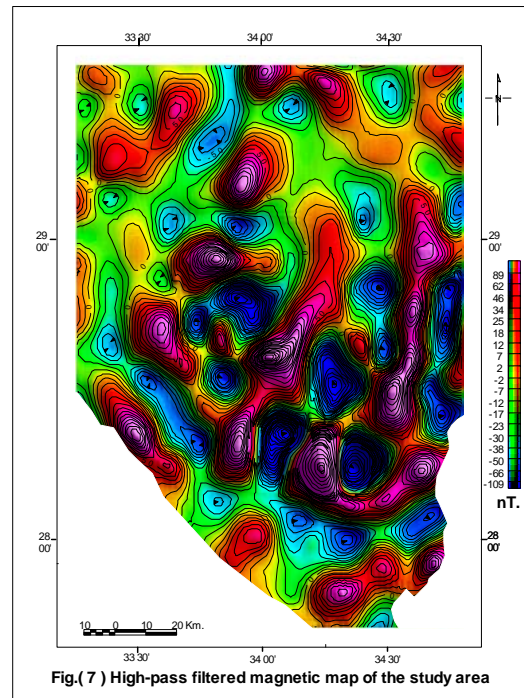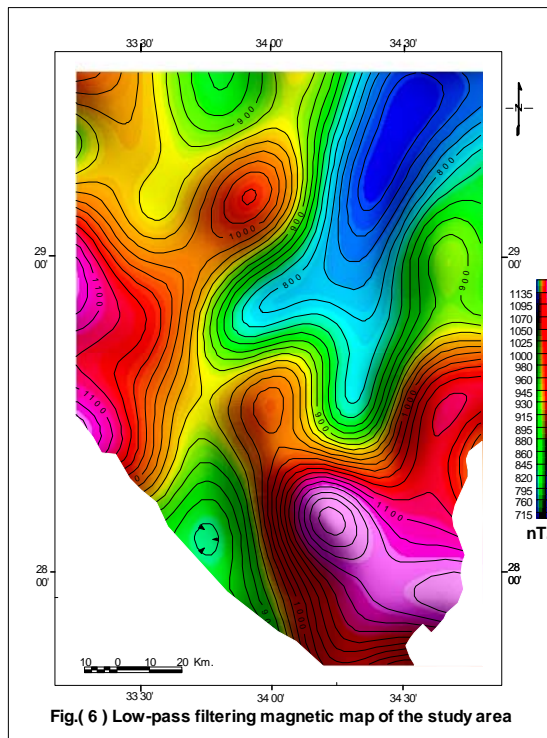
2-Extracting the magnetic sources using matched bandpass filtering

The idea of matched filtering is to fit the Fourier power spectrum of the workable aeromagnetic data with a series of equivalent power spectra corresponding to simple magnetic layers in the earth (Syberg, 1972).

Typically, there will be a noise layer containing low-amplitude, very short-wavelength magnetic noises largely unrelated to geologic sources, a layer corresponding to the near-surface magnetic sources and one or more layers corresponding to the deeper magnetic sources.

The matched bandpass filtering (Phillips, 2000) is a technique used to separate the magnetic anomalies produced at different source depths. In particular, anomalies produced by near-surface sources, such as shallow geologic units and cultural features can be separated from anomalies produced by deeper geologic units. Normally, the aeromagnetic signals produced from the near-surface sedimentary

geologic units and cultural (man-made) features will have much lower amplitudes and much shorter wavelengths than the aeromagnetic signals resulted from the deeply buried crystalline geologic units within the basement rock. In this situation, the Fourier band-pass filtering can be used to isolate and enhance the anomaly wavelengths associated with the shallow (or deep) sources. This is reflected by varying slopes in the Fourier power spectrum of the aeromagnetic data, which has been averaged for all azimuths.

The RTP magnetic map of the study area (Fig.5) can be used to illustrate this process (using MFDESIGN and MFFILTER programs of Phillips, 1997). Figure (8) contains the RTP anomalies produced by shallow geological sources with equivalent dipole layer for this band pass located at 0.487km. The intermediate wavelength map (Fig.9) involves the RTP anomalies resulted by geologic sources at depth nearly of 1.5 km. Moreover, the long wavelength RTP map (Fig.10) includes the anomalies

inferred from the deepest and broadest features of the section at depth of about 5.4 km.

Automated Interpretation of Source Parameters

Various methods are elaborated for estimating the source locations and other source parameters from total magnetic data, both in profile form and in map form. This section compares the results of two analogous interpretation methods, that work on map data: the horizontal gradient method and the local wavenumber method.

1- Horizontal Gradient Magnitude (HGM)

Horizontal gradient is a simple approach to locate linear structures, such as contacts and faults from potential field data. For magnetic field *M(x,y)*, the horizontal gradient magnitude *HG(x,y)* is given by Cordell and Grauch, (1982 & 1985):

$$HG(x, y) = \sqrt{\left(\frac{\partial M}{\partial x}\right)^2 + \left(\frac{\partial M}{\partial y}\right)^2} \qquad (1)$$

This function peaks over magnetic contacts under certain assumptions: (1) the magnetic field and source magnetization are vertical, (2) the contact is vertical and (3) the sources are thick (Phillips, 2000). Violation of the first two assumptions leads to shift of the peaks away from the contact location. Violation of the third assumption leads to secondary peaks parallel to that of the contact. When these assumptions are satisfied, the method is effective in detecting lineaments that may correspond to basement faults and contacts. Moreover, the method is less susceptible to noises in the data, because it only requires calculation of the two first-order horizontal derivatives of the magnetic field.

When applied to the reduced-to-pole magnetic data, the horizontal gradient method (Cordell and Grauch, 1985; Blakely and Simpson, 1986; and Roest and Pilkington, 1993) assumes that, the sources are isolated in vertical contacts separating thick geologic units. Peaks in the horizontal gradient magnitude of the reduced-to-pole magnetic field are used to locate the vertical contacts and estimate their strike directions.

The horizontal gradient magnitude (HGM) method is considered as the simplest approach to define the contact locations such as faults, where the maximum horizontal gradient (more properly the maxima of the total horizontal gradient) of the anomaly slope is located near or over the body edge. That is, the horizontal gradient operator in the map form produces maximum ridges over the edges of high dense basement blocks and faults or other dense

bodies. In addition, the horizontal gradient highlights linear features, related to contacts, in the data set.

2-Local Wavenumber Method

The local wavenumber method (also known as source parameter image method SPI) is a technique for calculation of source depths from magnetic data. Local wavenumber is a technique based on the extension of complex analytical signal to estimate magnetic depths. The original SPI method (Thurston and Smith, 1997 and Smith and others, 1998), as implemented by Phillips(2000) assumes that, the sources are isolated and linear, without a presumption of thickness and works for two models: a 2-D sloping contact or a 2-D dipping thin-sheet. For the magnetic field *M*, the local wavenumber (Thurston and Smith, 1997) is given by:

$$k = \frac{\dfrac{\partial^2 M}{\partial x \partial z}\dfrac{\partial M}{\partial x} - \dfrac{\partial^2 M}{\partial x^2}\dfrac{\partial M}{\partial z}}{\left(\dfrac{\partial M}{\partial x}\right)^2 + \left(\dfrac{\partial M}{\partial z}\right)^2} \qquad (2)$$

For the dipping contact, the maxima of *k* are located directly over the isolated contact edges and are independent of the magnetic inclination, declination, dip, strike and any remnant magnetization. The depth is estimated at the source edge from the reciprocal of the local wavenumber.

$$Depth_{(x=0)} = \frac{1}{k_{max}} \qquad (3)$$

One more advantage of this method is that, the interference of anomaly features is reducible, since the method uses the second-order derivatives. The SPI menu provides options to compute the two horizontal derivative grids in the space-domain, and the first vertical derivative grid in the frequency-domain, using the standard montaj filters and default settings. However, the user may compute these grids separately, if desired.

In practice, the method is used on gridded data by first estimating the direction at each grid point. The vertical gradient is computed in the frequency domain, and the horizontal derivatives are computed in the direction perpendicular to the strike using the least-squares method.

The fore-mentioned methods are illustrated for the investigated area. The derivative maps (horizontal gradient and local wavenumber) are shown in the figures (11&12) respectively. Crests of the derivative maps (in white) correspond to the estimated source locations.

On the other hand, the contact locations and depths resulting from the fore mentioned two methods are shown in figures (13&14). The local wavenumber method greatly overestimates the source depths relative to the other one. Otherwise, the two results contain nearly similar features. The inspection of the two maps elucidates the shallow depths at the southeastern and southwestern parts, as well as some separated parts from the central area and another shallow part in the northwestern corner. However, the deepest depths (from 3.5 to 5.5 km) are located at the central northern part of the area (blue) and at the west central part, as well as other intermediate depths (from 2.5 to 3.5 km) occupied the central and northeastern parts. The shallow depths (ranged from 1.5 to 2.5 km) are illustrated in the other parts of the map area. Generally, the local wavenumber method is quite sensitive to noises in the data to interference effects between nearby sources, which can result in over estimation of the source depths.

Fig.(8) The short-wavelength map contains magnetic anomalies produced by the shallowest geologic sources and noise in shaded relief

Fig.(9) The intermediate-wavelength map contains magnetic anomalies produced by geologic sources at intermediate depths in shaded relief

Fig.(10) The long-wavelength map contains magnetic anomalies from the deepest and broadest features of the geology in shaded relief

Fig.(11) Horizontal gradient magnitude of the RTP magnetic field with source locations

Fig.(12) Local Wavenumber of the RTP magnetic Field

Fig.(13) Sources depth as estimated by horizontal gradient method with contact locations

Fig.(14) Sources depths as estimated by local wavenumber method

Structural evolution

The main target of the magnetic data interpretation of an area is the establishment of the structural features complicating the continuity of the evaluated sedimentary section and the underlying basement complex. Accordingly, the deep-seated structural features and the shallow-seated structural elements are represented in the study area, belt-wise and zone-wise, as shown as follow:

A-Deep-seated structural features:

The deep-seated structural features of the study area (Fig.15) are integrated as basement swell and trough belts intervened by faults of varying trends. However, the northeastern border of the map

area is concordant with a trough belt of NE-SW trend, which is bifurcated into two splits at its lower end. This trough is formed from two negative features, the first one is trending N-S to the east and the second one trend NNE-SSW at the central part of the map area.

Such a trough belt is followed southeastwardly by, moderate swell through NE-SW and NNW-SSW faults of eastward and westward throws, respectively. Also, that trough belt is followed northwestwardly by a major swell through a NNE-SSW fault of eastward throw.

Added, the fore-mentioned southeastern swell orients mostly NNE-SSW parallel to the Gulf of Aqaba, with mostly NW-SE bifurcation at its southern part, which carved at its northern end. While, the second major northwestern swell is formed from high relief and NE-SW trend with NNW-SSE splitting at its central part toward the northwestern corner. This swell system is bounded by a major NE-SW normal fault to the west and intervened internally by NE-SW and N-S faults throwing inwardly toward the in-between minor trough.

B -Shallow-seated structural elements:

The shallow-seated structural elements of the study area (Fig.16) are cumulated as anticlinal (red colored) and synclinal (blue colored) zones dissected by faults of varying parameters. By this way, the considered area is characterized by the presence of N-S anticlinal zone at the eastern part of the map, followed southwardly by another anticlinal zone orients NE-SW with N-S splitting at the western end.

These anticlinal zones are followed eastwardly by a complex synclinal zone through a N-S fault. This synclinal zone is configured by two segments, northern NNW-SSE one with NNE-SSW split and southern N-S with mostly NE-SW split. The fore-mentioned segments are separated by a NNW-SSE strike-slip fault.

Added, the second NNW-SSE complex anticlinal zone is located to the west of the map area with mostly NE-SW bifurcation at its central part and another NE-SW bifurcation at its northern part. Such a northern split is formed from two segments separated by NE-SW strike-slip fault and followed southwardly and northwardly by two NE-SW low relief synclines through a NE-SW normal fault.

Moreover, other two separate synclinal trends are located at the southwestern corner parallel to the Gulf of Suez and at the southeastern corner parallel to the Gulf of Aqaba. A series of separated NW-SE and NE-SW low extended synclines are observed at the southwestern corner (parallel to the

Gulf of Suez) and southeastern part (Parallel to the Gulf of Aqaba). These synclines are followed outwardly by low relief anticlinal trends in the same directions through normal faults, beside a moderate NE-SW anticlinal zone observed at the northwestern corner, which is formed from two splits (NE-SW and NNW-SSE).

Fig.(15) Deep-Seated Structural Features of the Study Area

Fig.(16) Shallow- Seated Structural Features of the Study Area
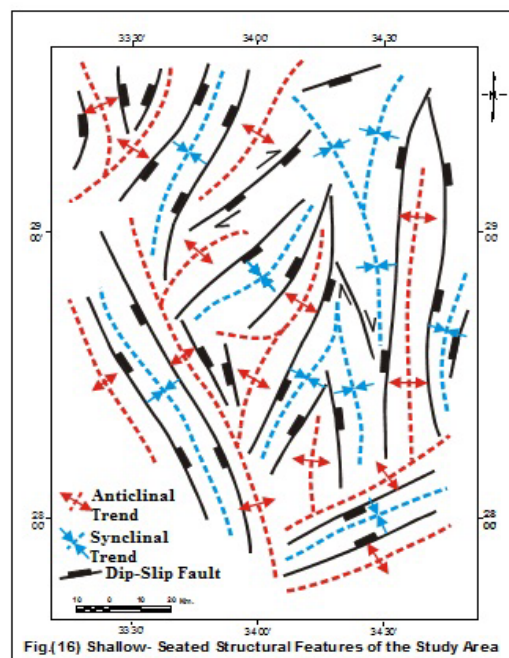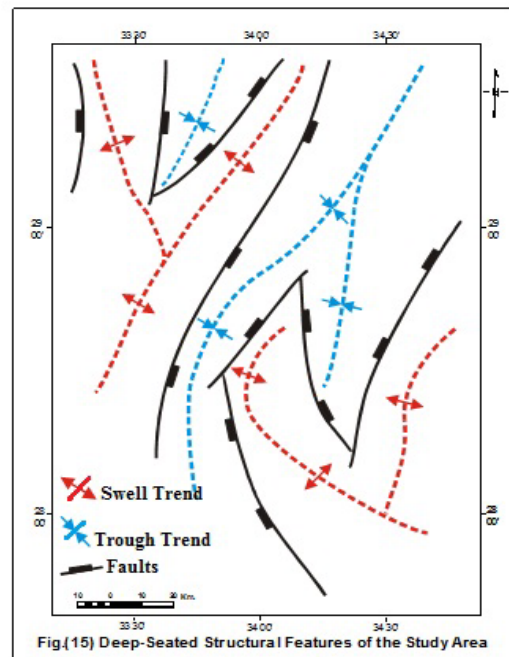
Results and Conclusions

This study was carried out using the available total intensity magnetic data. The data were analyzed using the most advanced and suitable techniques. These techniques includes the reduce to the pole filter, low-pass/high-pass filtering, matching band-pass filter, horizontal gradient magnitude and local wave number tool. The phase-shift effects in the total magnetic anomalies resulting from a non-vertical geomagnetic field vector can often be removed using the reduction-to-pole filtering, in which the intended effect is to move the anomaly peaks and gradients directly over their sources to aid in the interpretation.

The magnetic anomalies produced by deep geologic sources can be separated from anomalies produced by shallow geologic effects using power spectrum filtering, and depending on the anomalies wavelength using matched bandpass filtering. Also, the automated interpretation methods can be used to estimate the locations and depths of magnetic sources. Because each automated method makes different assumptions about the sources, the use of several methods is recommended in order to provide a reality check for the results. For that, we used the horizontal gradient and local-wavenumber methods for automatic revealing of the source locations and depths. The application of these tools discriminated the variable sources of specific depth ranges for the residual and regional anomalies. The average depth values to the causative sources are widely ranges between 1 to 5.5 km, as deduced from these derivative methods.

Tectonically, the magnetic methods were critical for detecting the geometry of the basement rocks and the structures related to tectonic forces. The results indicate that the principal tectonic trend is oriented in the NE-SW (N45^{o}-65°E) direction, which connected to Syrian Arc tectonics in this part of the peninsula. The NW-SE (N35^{o}-45° W) tectonic trend is a second order of predominance. This trend originated due to the opening of the Gulf of Suez, and is normal to the NE-SW tension axis (Said,1990). The NNE-SSW (N15^{o}-25°E) tectonic trend , related to Gulf of Aqaba tectonics, can be detected as a third order of predominance. Finally, we can conclude that the area is highly affected by the tectonic related to the Gulf of Suez, Red Sea and Gulf of Aqaba. It is affecting both basement and sedimentary rocks, dividing the study area into several faulted blocks.

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Clinical value of transforming growth factor beta as a marker of Fibrosis in adolescents with Chronic Liver Diseases

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Abstract: Background: Hepatic fibrosis is the final common path of liver injury in most chronic liver diseases and can lead to cirrhosis, which is responsible for the majority of clinical complications. Our aim is to asses the clinical value of serum transforming growth factor β (TGFβ)　as a fibrogenesis marker in adolescents with chronic Liver Diseases**. Methods:** We measured serum levels of TGF-β in 25 adolescents with chronic liver disease and 25 healthy controls, and determined their relationship to frequently used liver function tests and liver biopsy findings. **Results:** Serum Transforming growth factor β was significantly higher in patients than in controls (P < 0.001). Significant positive correlation between TGFβ and TSB (r = 0.4682 and p< 0.05). High significant positive correlation was noted between TGFβ and stage, grade of liver fibrosis, PT and duration of illness as p is <0.001and r is 0.9409, 0.7447, 0.5293 and 0.5952 respectively. Highly significant negative correlation was found between TGFβ and prothrombin concentration (PC) and serum albumin level as p is < 0.01 and r is -0.6460 and -0.5371 respectively. Sensitivity of TGFβ in diagnosis of fibrosis was 65%, specificity was 94% and area under curve (AUC) was 0.812. The cut-off value of TGFβ used to discriminate significant fibrosis was 22.6 ng/ml and it was a dependant predictor factor for diagnosis of fibrosis with positive predictive value 75.5% and negative predictive value 90.4 %. **Conclusions:** TGF-β had the ability to discriminate patients with significant fibrosis and may be useful in reducing but not replacing the need for liver biopsy.

1 Introduction :

Chronic liver diseases (CLDs) are defined as the continuity of clinical and biochemical evidence of hepatic dysfunction for longer than six months (1). Hepatitis B and C (HBV, HCV) are, and will remain for some time, major health problems in Egypt. Both infections can lead to an acute or silent course of liver disease (2). Chronic viral hepatitis, non-alcoholic steato-hepatitis (NASH) and AIH are the most common causes of CLD among adolescents (3). Hepatic fibrosis, which represents the wound healing response of the liver, is a common sequel of liver injury characterized by excess deposition and altered composition of extra-cellular matrix (ECM) (4). Hepatic stellate cells(HSCs)are the major source of ECM and regarded as the principle cell type in the development of hepatic fibrosis.They are activated by a variety of mechanisms, including cytokines, chemokines and others (5).Transforming growth factor-beta (TGF-β) is released by activated HSCs and appears to be the main fibrogenic mediator (6).Up till now, liver biopsy is essential in establishing the diagnosis of liver fibrosis. Beside invasiveness, liver biopsy has many complications like sampling error and cost (7). Moreover many patients are reluctant to over go repeated biopsies, which limit the ability to monitor disease progression (8). This situation strengthens the need for harmless, alternative and complementary non invasive serum biomarkers (9),that are safe, inexpensive and reliable (10). Non-invasive diagnosis of liver fibrosis has been extensively evaluated in adult populations

(11),(12). In contrast, in adolescents, data are lacking and liver biopsy is still the only reliable tool for diagnosing the histological features.

Our aim is to asses the clinical value of serum Transforming growth factor β (TGFβ) as a fibrogenesis marker in adolescents with chronic Liver disease.

2. Subjects and Methods:

A mixed retrospective and prospective study was conducted from October 2009 to June 2010.Twenty five patients with chronic liver disease (group 1) were chosen from hepatology clinic of Benha university hospital (Kalyobia Governate) and the Liver Institute (Menofia Governate). Twenty five healthy children matched for age, sex, locality and socioeconomic state served as control (group 2). Written consent was taken from parents before including their children in the study. Patients were included if their ages ranged from 10-16 years and with chronic liver disease. Patients with gastrointestinal bleeding (acute attack), chronic renal failure, and hepatic encephalopathy were excluded from the study. All cases were subjected to: full history taking, complete clinical examination including; Liver (surface, edge, consistency and span), spleen (surface, edge, consistency and size), presence or absence of ascites. Presence or absence of manifestation of liver cell failure (edema, bleeding tendency, jaundice and angiomas) was recorded. Laboratory investigations including: complete blood count,fasting blood sugar, blood urea and creatinine, liver function tests including(ALT, AST, serum bilirubin (total and direct), serum albumin, prothrombin time and concentration. TGFβ was measured, using DRG TGFβ ELISA kit.

Ultrasonography-guided liver biopsy was done for chronic hepatitis patients. Liver biopsies were performed using true cut needle. Biopsy specimens were fixed in formalin and embedded in paraffin. Liver fibrosis and necroinflammatory activity were evaluated according to Ishak staging and grading score where histological activity index (HAI) ranged from 0 to 12, while fibrosis score ranged from F0 to F6 (13).

3. Results

Demographic data among studied groups, including sex, residence and age (mean age 13.06±2.5) years in group 1 compared to (13.1±2.1) years in group 2 are shown in table (1).Chronic hepatitis C was the most common etiology of chronic liver disease among our cases (44%) followed by autoimmune hepatitis (24%), chronic hepatitis B (16%), glycogen storage disease (8%), congenital hepatic fibrosis and Alpha1 anti-trypsin deficiency (4%) each as shown in table (2). Table 3 shows clinical characteristics of studied cases. Table 4 shows laboratory data of studied groups and revealed highly significant difference between patients group and control group as regard serum transaminases level, albumin level, total & direct billirubin, prothrombin time and concentration, also serum level of TGFβ were highly significantly elevated in group (1) than in group (2) ($P < 0.001$). Grading of liver fibrosis revealed that; there were 8,9,5,2 and 1 patients in grade 2, 3, 4, 7and 8 respectively. Staging of liver fibrosis revealed that; there were 8, 7, 2, 5 and 3 patients in stage 1, 2, 3, 4 and 5 respectively. Figure (1) shows significant positive correlation between TGFβ and TSB as r is 0.4682 and p is <0.05. High significant positive correlation was found between TGFβ and (stage, grade of liver fibrosis, PT and duration of illness) as p is < 0.01 and r is 0.9409, 0.7447, 0.5293 and 0.5952 respectively are shown in figures 2, 3,4and 5. Highly significant negative correlation between TGFB and PC and serum albumin level was found as p is < 0.01 and r is -

0.6460 and -0.5371 respectively as shown in figures 6 and 7. Sensitivity of TGFβ was 65%, specificity 94% and area under curve (AUC) was 0.812 as shown in figure 8. The cut-off value of TGFβ used to discriminate

significant fibrosis was 22.6 ng/ml and it was a dependant predictor factor for diagnosis of fibrosis with positive predictive value 75.5% and negative predictive value 90.4 %.

Table (1) Demographic data among studied groups

	Cases(25)	Control(25)	Z / (t)	P
Age (years)				
Range	10-16	10-16		
Mean +S.D	13.06+2.5	13.1+2.1	0.45	> 0.05
Sex				
Male	13 (52%)	11 (44%)	0.56	
female	12 (48%)	14 (56%)		> 0.05
Locality				
Urban				
No	10	11		
%	40	44		
rural			0.28	
No	15	14		> 0.05
%	60	56		

Table (2) Distribution of studied cases regarding the etiology of liver disease

Etiology	Frequency	
	No	%
Chronic Hepatitis C	11	44
AIH (Autoimmune hepatitis)	6	24
Chronic Hepatitis B	4	16
GSD1(Glycogen storage disease type 1)	2	8
Congenital hepatic fibrosis	1	4
Alpha1 anti-trypsin deficiency	1	4
Total	25	100

Table (3) Clinical characteristics of studied cases

	Frequency	
	No	%
Hepatomegaly	16	64
Splenomegaly	16	64
Jaundice	10	40
Pallor	7	28
Portal hypertension	5	20
Ascites	3	12
Lower limb edema	1	4

Table (4): Laboratory data in studied groups

	Group 1 (25)	Group 2 (25)	(t)	P
AST (IU/L)				
▪ **Range**	12- 430	17- 40		
▪ **Mean+S.D.**	72.3+ 88	23.3+ 5.6	2.7	< 0.01**
ALT (IU/L)				
▪ **Range**	10- 625	15- 36		

▪ **Mean+ SD**	77.4± 120	22.8± 6.1	2.25	< 0.05*
Total billirubin mg/dl				
▪ **Range**	0.3- 6.6	0.2- 1		
▪ **Mean+SD**	2.6±1.8	0.66±0.24	5.4	< 0.001**
Direct billiruban (mg/dl)				
▪ **Range**	0.09-2.1	0.01-0.18		
▪ **Mean+SD**	0.81 + 0.71	0.056+0.05	5.3	< 0.001**
S albumin (gm/dl)				
▪ **Range**	2.1-4.8	3.8 – 5.1		
▪ **Mean+SD**	3.3 + 0.72	4.43 + 0.44	6.2	< 0.001**
Prothrombin time PT(sec)				
▪ **Range**	11.5- 18	11- 13		
▪ **Mean+SD**	13.4±1.8	12.2±0.44	3.2	< 0.01**
Prothrombin concentration PC %				
▪ **Range**	54- 100	95- 110		
▪ **Mean±SD**	84.8±16.9	99±3.1	4.2	< 0.01* *
TGFβ (ng/ml)				
Range	12.3- 45.1	9.8- 20.7		
Mean±SD	25±10.3	14.9±3	4.6	< 0.001**

* significant value
** high significant value
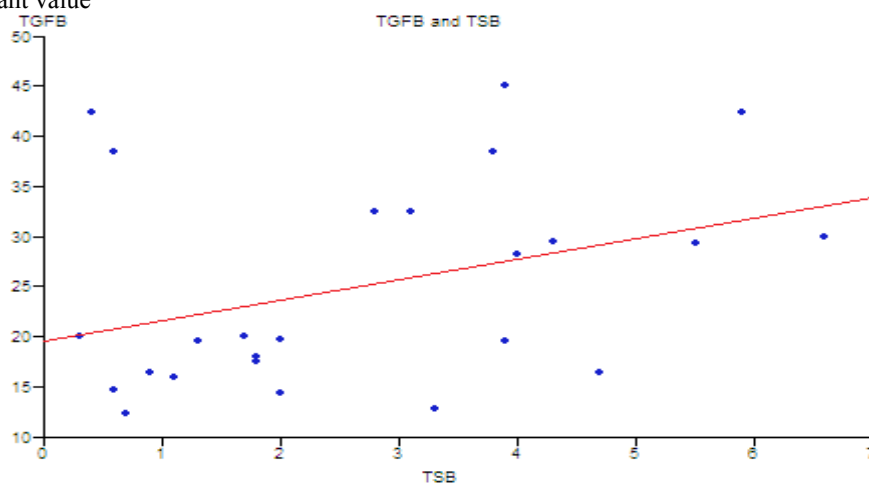
Figure 1: Correlation between TGFβ and TSB.
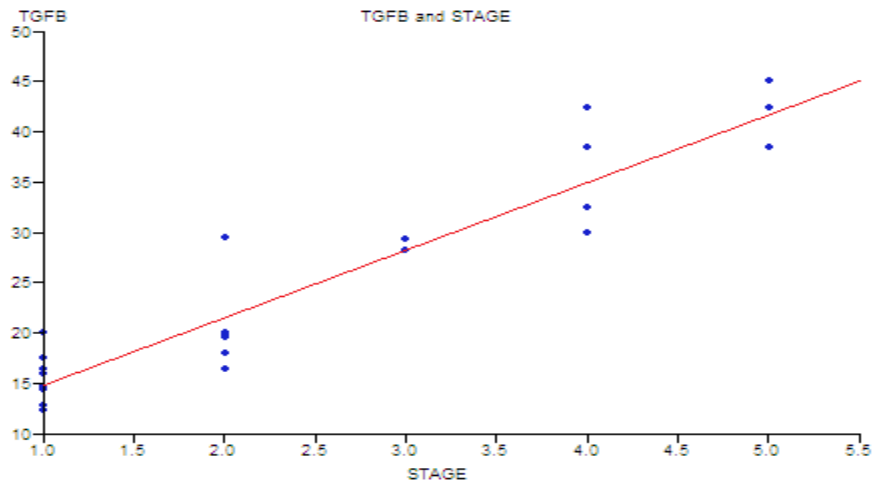
Figure 2: Correlation between TGFβ and stage of liver fibrosis
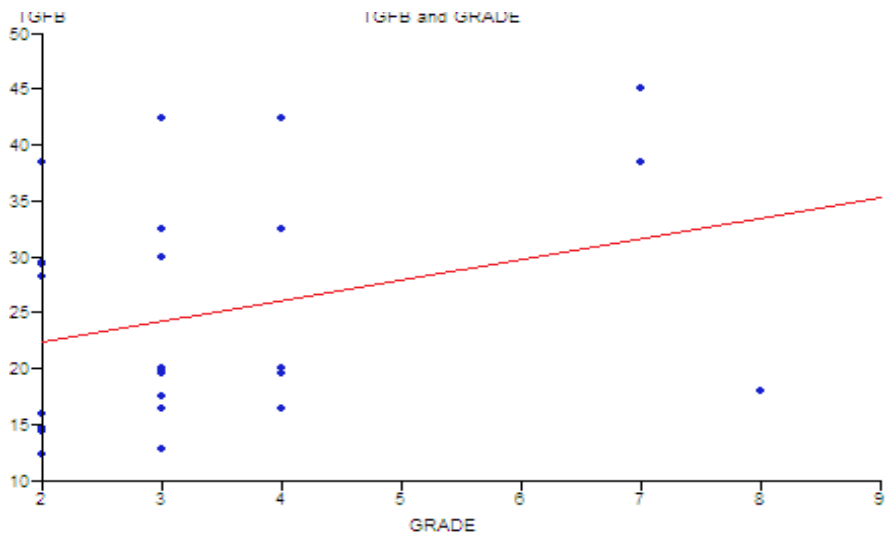
Figure 3: Correlation between TGFβ and grade of liver fibrosis
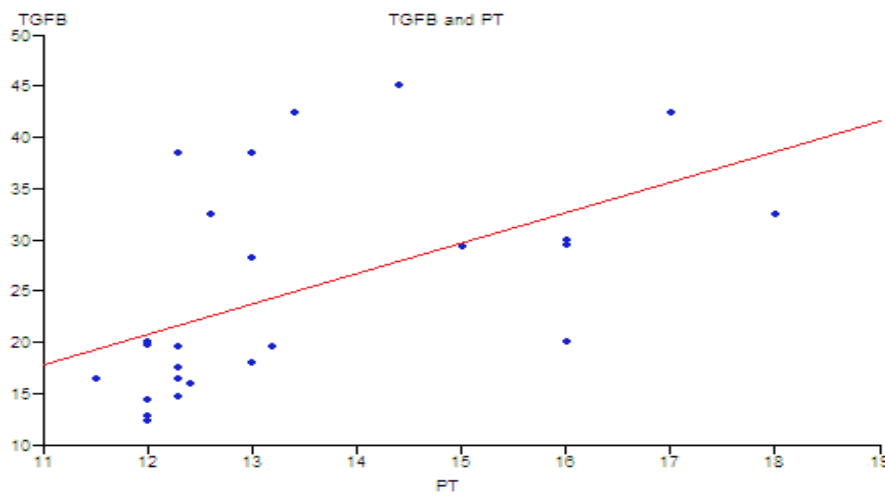
Figure 4: Correlation between TGFβ and PT.
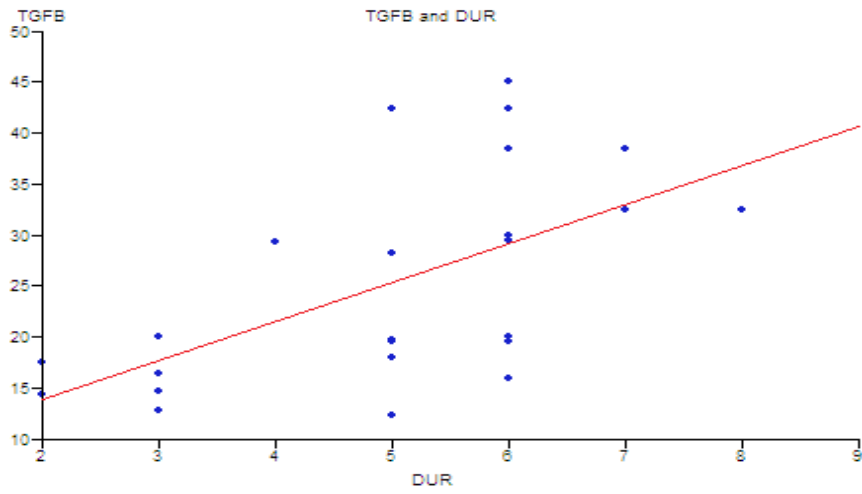
Figure 5: Correlation between TGFβ and duration of illness.
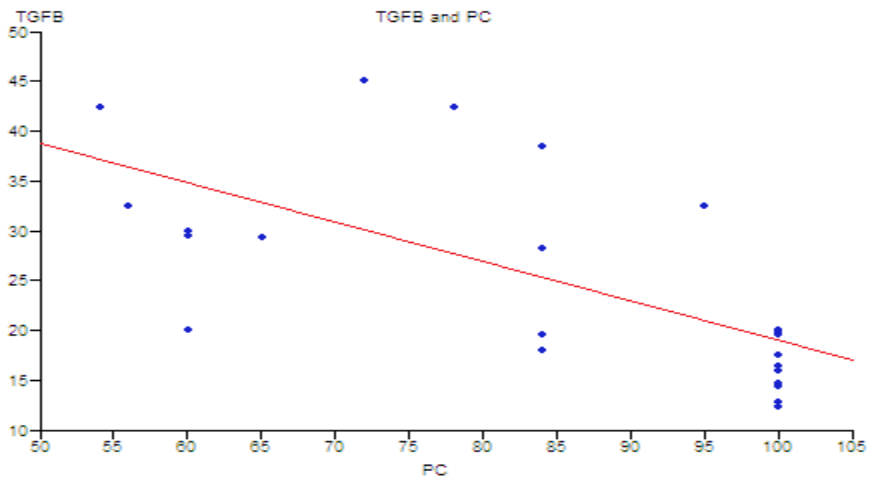
Figure 6: Correlation between TGFβ and PC.
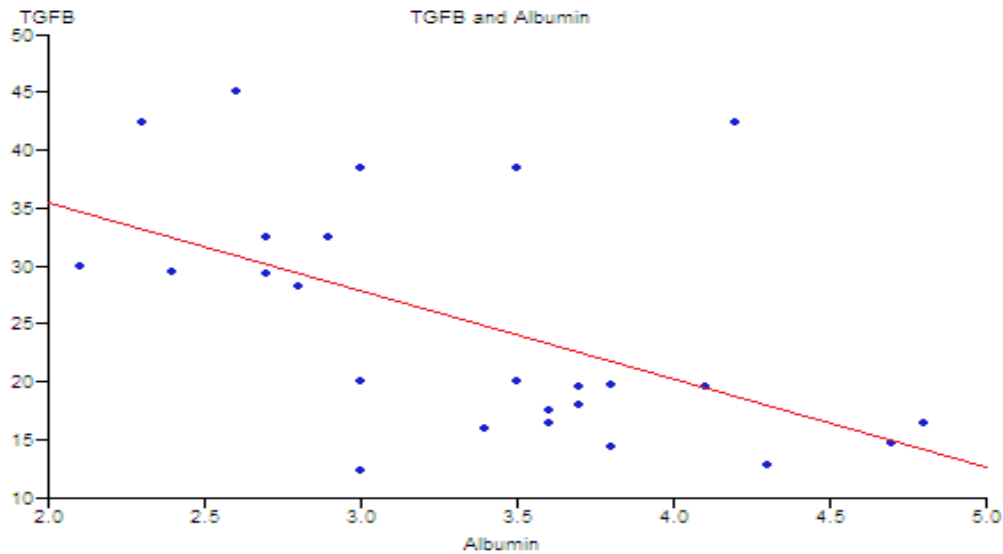
Figure 7: Correlation between TGFβ and serum albumin level.
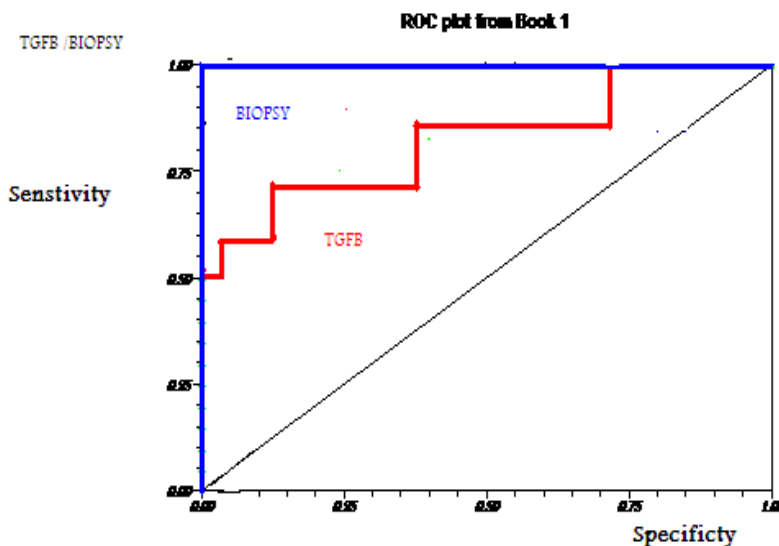
Figure 8: Nonparametric receiver operating charachteristic(ROC) curve for assessing the diagnostic value of TGFβ as an indicator of liver fibrosis.

4. Discussion

In the normal liver, HSCs express very little TGF-β and hepatocytes essentially none. When injury strikes, inflammatory cells are drawn to the site of injury and HSCs undergo activation and becoming fibrogenic (14), (15).

The current study revealed that, serum level of TGF-β was highly significantly elevated in patients with chronic liver disease than control. This significant elevation might reflect the fibrogenic process in the liver.

Luo et al., 2001(16) found a significant elevation of TGF-β1 in liver cirrhosis, yet its correlation with activity was moderate. In another Egyptian study done by Abdel-Ghaffar et al., 2010(17) they found that; TGF-β1 was significantly increased in children with chronic liver disease than control. There are conflicting results in literature as to which TGF-beta level increase or remain unchanged in patients with chronic hepatitis. Hong-Lei Weng et al., 2009(18) reported elevated TGF-β1 serum levels in patients with chronic hepatitis B virus (HBV)/hepatitis C virus (HCV) infections. On the other side Liberek et al., 2009(19) reported that ; in

chronic hepatitis group of patients the plasma TGF-beta level did not differ from the control group and did not correlate with grading and staging of the liver tissue fibrosis and they concluded that, this finding may be due to low level of fibrosis observed in the studied children.. These contradictory results may be explained by the finding of Wasmuth et al.,(20) that, progression of hepatic fibrosis have been attributed to age, sex, and exogenous factors, e. g., coinfections and that, host genetic factors play key roles in the modulation of hepatic fibrosis. Our results showed that TGF-β correlated positively with PT, TSB, stage and grade of liver fibrosis and negatively with PC and serum albumin levels. Our results are in agreement with Flisiak and Prokopowicz, 2000(21) as they found a correlation between elevated TGF-β and impairment of some synthetic liver functions, and *Filiask et al., (2002)*(22)who reported that TGFβ was correlated positively with liver fibrosis. Also in accordance with our results, Iagoda et al. , 2006(23) studied correlations between growth factors and histological changes in the liver in 48 patients with chronic viral hepatitis and hepatic cirrhosis, they found that the blood level of transforming growth factor-beta (TGF-beta) increases according to increase in histological activity and the degree of hepatic fibrosis and that there is a positive correlation between TGF-beta and the degree of hepatic inflammation and fibrosis. The stimulatory effect of TGF-beta on collagen synthesis by fat-storing cells is observed *in vitro* at a concentration of 10 ng/ml (21). In our study, the level of circulating TGF-beta was two fold increase and a level more than 22.6 ng/ml had a sensitivity of 65% and specificity of 94% in identifying significant fibrosis. In the study of Abdel-Ghaffar et al., 2010(17) they found that TGF-β1 more than 54.8 ng/ml had a sensitivity of 78.6% and specificity of 71.4% in identifying significant fibrosis. The difference in the results between our study and other studies could be explained by the difference in the mean age of the cases and accordingly the aetiology of chronic liver disease. Hong-Lei Weng, 2009(18) reported that TGF-β1/Smad2 signaling in liver fibrogenesis is not a generalized feature and detected in an etiology-dependent manner.

In conclusion, TGF-β may be used to predict significant fibrosis and/or cirrhosis in children with chronic hepatitis B & C and other causes of chronic liver disease. That is to say, non-invasive markers will likely reduce but not replace the need for liver biopsy, which may be useful in monitoring of disease development and treatment effectiveness and might be an inseparable part of assessment of chronic hepatopathies.

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Comparing of yield and yield components of rice hybrid in different irrigation regimes and nitrogen levels

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Abstract: In order to investigating the effect of different irrigation regimes and nitrogen fertilizer on yield and yield components of hybrid rice a biennial experiment was conducted at rice research institute of Iran during crop season 2008-9. experiment was arranged in split plot based on completely randomized block design with 3 replications in which water regimes were main factor included Continuous Submergence and Alternative Submergence conditions (irrigation intervals of 5, 8 and 11days) and nitrogen fertilizer levels were sub factor included 0, 90, 120 and 150 kg/ha. Grain yield in I1 to I4 were 7342, 7079, 7159 and 5168 kg/ha in 2008 and 4372, 4343, 4674 and 4208 kg/ha in 2009 respectively. Number of grains per panicle in I1 to I4 was 304,307,311 and 272 in 2008 and 183,180,181 and 179 in 2009 respectively. Weight of 1000 grain in I1 to I4 was 22.4, 22.2, 21.8 and 21.1 g in 2008 and was 22.8, 23, 23.1 and 23.2 g in 2009 respectively. Unfilled grain in I1 to I4 was 27.1, 32.9, 30.3 and 39.2 percentage in 2008 and was 55.2, 48.4, 50.4 and 46.8 percentage in 2009 respectively. Mean grain yield in irrigation regimes in 2009 compare to 2008 decreased 34% because mean number of grains per panicle in 2009 compare to 2008 decreased 40% and mean unfilled grain percentage increased 56%.

Keywords: irrigation, nitrogen, water use efficiency, rice, Iran

1. Introduction

Increased efficiency in the use of water is essential for future food security in Asia where rice production has to be increased by 70% of the present amount by the year 2025.decreasing water availability for agriculture threatens the productivity of the irrigated rice ecosystem and ways must be sought to save water and increase the water productivity of rice (Guerra et al., 1998). Conventional water management in lowland rice aims at keeping the fields continuously submerged. Water inputs can be reduced and water productivity increased by introducing periods of none submerged conditions of several days (Bouman and Tuong, 2001).

Panicles with a low percentage of sterile flowers permit the application of higher doses of nitrogen and produce better yields (Yoshida, 1981). Rice grain yield was recorded highest in case the N application ranged between 90-250 kg per ha (Bali et al., 1995).

Worldwide, freshwater availability for irrigation is decreasing because of increasing competition from urban and industrial development, degrading irrigation infra-structure, and degrading water quality (Molden, 2007).

Zhong and Huang (2002) report that grain yield and dry matter increased when application of nitrogen fertilizer increased.

Nitrogen is normally a key factor in achieving optimum lowland rice grain yields (Fageria et al., 1997). It is worth mentioning utilization especially usage of nitrogen fertilizer is very significant factor in growth of rice. When nitrogen fertilizer used in tillering, paddy yield increased (Bacon, 1989).

2. Material and Methods

In order to investigating the effect of different regimes of irrigation and nitrogen fertilizer on yield of hybrid rice an experiment was conducted at rice research institute of Iran during crop season 2008-9. experiment was arranged in split plot based on completely randomized block design with 3 replications in which water regimes were main factor included continuous submergence and alternately submergence (irrigation intervals of 5, 8 and 11days) and nitrogen fertilizer levels were sub factor included 0, 90, 120 and 150 kg/ha. For all treatments, drainage basins have been mounted from which waste water belonging to each replicate treatments were exited. Each experimental plot had 15 lines with five meter in length and the planting method was considered to be 25×25 cm. The nursery construction took place in April and transplanting to the field happened in early may. In order to use fertilizer, based on the soil test and instructions of the technicians the rice investigation organization the amount of P and K was calculated and

applied to every plot. The amount of irrigation water applied was monitored at each plot from transplanting till maturity, by using flow meters installed in the irrigation pipes. Yield was measured with $6m^2$ harvesting of every plot. The mean daily temperature during the growing season (May-Sep) was 26 and 23.6 in 2008 and 2009, respectively.

Total rainfall during the growing season (May-Sep) was 236mm (50 mm in flowering to harvest) and 157mm (108mm in flowering to harvest) in 2008 and 2009, respectively.

Total sunshine hours in flowering to harvest was 256 and 173 h in 2008 and 2009, respectively. The yield and yield components were analyzed by using MSTATC software. The Duncan's multiple range tests was used to compare the means at 5% of significant.

3. Results

The effect of irrigation regime on Grain yield, Biomass, Harvest index, Number of grains per panicle, Weight of 1000 grain, unfilled grain percentage was significant and no significant on Number of tiller, Length of panicle and Plant height in 2008 (table 1).

Irrigation regime had no significant effect on all of plant characteristics in 2009 (table 2).

The effect of nitrogen levels on Grain yield, Biomass, Number of tiller, Number of grains per panicle, Weight of 1000 grain, unfilled grain percentage, Length of panicle and Plant height was significant and no significant on Harvest index in 2008 and 2009 (table 1 and 2).

Grain yield in I1 to I4 were 7342, 7079, 7159 and 5168 kg/ha in 2008 and 4372, 4343, 4674 and 4208 kg/ha in 2009 respectively (Table 3, 4).

Grain yield in N1 to N4 were 3471, 4588, 4783 and 4755kg/ha in 2008 and 3471, 4588, 4783and 4755 kg/ha in 2009 respectively (Table 3, 4).

Biomass in I1 to I4 were 13245, 12647, 12698 and 11641 kg/ha in 2008 and 12155, 12919, 11939 and 12350 kg/ha in 2009 respectively (Table 3, 4).

Biomass in N1 to N4 were 9873, 12685, 13284 and 14389kg/ha in 2008 and 10095, 11455, 13386and 14427 kg/ha in 2009 respectively (Table 3, 4).

Harvest index in I1 to I4 were 51, 53, 53 and 42 percentage in 2008 and 48, 48, 49 and 49 percentages in 2009 respectively (Table 3, 4).

Harvest index in N1 to N4 were 50, 49, 52 and 48 percentage in 2008 and 48, 49, 49 and 49 percentages in 2009 respectively (Table 3, 4).

Number of tiller in I1 to I4 was 245,252,250 and 228 in 2008 and 276,278,276 and 272 in 2009 respectively (Table 3, 4).

Number of tiller in N1 to N4 was 209,240,249 and 277 in 2008 and 236,272,284and 307 in 2009 respectively (Table 3, 4).

Number of grains per panicle in I1 to I4 was 304,307,311 and 272 in 2008 and 183,180,181 and 179 in 2009 respectively (Table 3, 4).

Number of grains per panicle in N1 to N4 was 282,291,305 and 316 in 2008 and 165,174,190 and 196 in 2009 respectively (Table 3, 4).

Weight of 1000 grain in I1 to I4 was 22.4, 22.2, 21.8 and 21.1 g in 2008 and was 22.8, 23, 23.1 and 23.2 g in 2009 respectively(Table 3, 4).

Weight of 1000 grain in N1 to N4 was 21, 21.9, 22.4 and 22.2 g in 2008 and was 22.5, 23.1, 23.1and 23.3 g in 2009 respectively (Table 3, 4).

Unfilled grain in I1 to I4 was 27.1, 32.9, 30.3 and 39.2 percentage in 2008 and was 55.2, 48.4, 50.4 and 46.8 in 2009 respectively (Table 3, 4).

Unfilled grain in N1 to N4 was 37.5, 31.1, 32 and 29 percentage in 2008 and was 49.1, 44.4, 47.2 and 60.1 in 2009 respectively (Table 3, 4).

Length of panicle in I1 to I4 was 29.4, 31.1, 30.5 and 29.6 cm in 2008 and was 25.6, 25.8, 26.2 and 25.2 cm in 2009 respectively (Table 3, 4).

Length of panicle in N1 to N4 was 29.1, 30.3, 30.3 and 30.9 cm in 2008 and was 24, 26, 26 and 27 cm in 2009 respectively(Table 3, 4).

Plant height in I1 to I4 was 105.8, 107.6, 107 and 106.7 cm in 2008 and was 92, 94, 91 and 92 cm in 2009 respectively (Table 3, 4).

Plant height in N1 to N4 was 100.8, 106.7, 109.2 and 110.4 cm in 2008 and was 86, 94, 94 and 96 cm in 2009 respectively(Table 3, 4).

Table 1. Analysis of variance in plant parameters in 2008

S. O. V	df	Yield	biomass	Weight of 1000 grain	Length of panicle	unfilled grain	Plant Height	Number of tiller	number of grains per panicle	Harvest Index
I	3	12452331**	5364477.1*	3.8**	7.1 ns	316.8**	6.9 ns	1464.8 ns	3974.2*	333.7**
N	3	11838173**	44436179.4**	5.1**	6.6*	159.3**	218.7**	9429.3**	2746.9**	33.6 ns
I×N	9	1211276*	4554914.2*	.051**	4.1*	38.7*	16.2 ns	986 ns	322.1 ns	43.1 ns
Cv (%)		10.59	10.93	.47	4.3	10.67	3.74	17.47	6.55	9.48

Table 2. Analysis of variance in plant parameters in 2009

	Weight of 1000 grain (g)	Length of panicle(cm)	Unfilled grain (%)	Plant Height (cm)	Number of tiller	number of grains per panicle	biomass (kg/ha)	Yield (kg/ha)	Harvest Index (%)
Irrigation									
Continue submerge	22.4 a	29.4a	27.1c	105.8a	245a	304a	13245a	7342a	51a
5 day interval	22.2 b	31.1a	32.9b	107.6a	252a	307a	12647ab	7079a	53a
8 day interval	21.8c	30.5a	30.3bc	107a	250a	311a	12698ab	7159a	53a
11 day interval	21.1d	29.6a	39.2a	106.7a	228a	272b	11641b	5168b	42 b
Nitrogen									
N1	21d	29.1b	37.5a	100.8c	209b	282c	9873c	3471b	50a
N2	21.9c	30.3a	31.1b	106.7b	240ab	291bc	12685b	4588a	49a
N3	22.4 a	30.3a	32b	109.2ab	249a	305ab	13284ab	4783a	52a
N4	22.2 b	30.9a	29b	110.4a	277a	316a	14389a	4755a	48a

Table 3. Effects of irrigation management and nitrogen levels plant parameters of rice in 2008

S. O. V	df	Yield	biomass	Weight of 1000 grain	Length of panicle	unfilled grain	Plant Height	Number of tiller	number of grains per panicle	Harvest Index
I	3	ns	ns	ns	ns	ns	ns	ns	ns	ns
N	3	468361**	67839329**	1.37**	13.9**	566**	220**	45**	2465**	ns
I×N	9	ns	ns	ns	ns	ns	ns	ns	421*	ns
Cv (%)		11.5	16	1.02	2.7	21.5	8.57	8.6	6.45	2.5

Table 4. Effects of irrigation management and nitrogen levels plant parameters of rice in 2009

	Weight of 1000 grain (g)	Length of panicle (cm)	Unfilled grain (%)	Plant Height (cm)	Number of tiller	number of grains per panicle	biomass (kg/ha)	Yield (kg/ha)	Harvest Index (%)
Irrigation									
Continue submerge	22.8a	25.6a	55.2a	92a	276a	183a	12155a	4372a	48a
5 day interval	23a	25.8a	48.4a	94a	278a	180a	12919a	4343a	48a
8 day interval	23.1a	26.2a	50.4a	91a	276a	181a	11939a	4674a	49a
11 day interval	23.2a	25.2a	46.8a	92a	272a	179a	12350a	4208a	49 a
Nitrogen									
N1	22.5c	24c	49.1b	86c	236d	165b	10095c	3471b	48a
N2	23.1b	26b	44.4b	94b	272c	174b	11455b	4588a	49a
N3	23.1b	26b	47.2b	94b	284b	190a	13386a	4783a	49a
N4	23.3a	27a	60.1a	96a	307a	196a	14427a	4755a	49a

4. Discussions

Mean grain yield in irrigation regimes in 2009 compare to 2008 decreased 34% because mean number of grains per panicle in 2009 compare to 2008 decreased 40% and mean unfilled grain percentage increased 56%.

Numbers of grains per panicle and unfilled grain percentage were two important yield components and have direct effect on increasing of grain yield.

The mean daily temperature during the growing season (May-Sep) was 26 and 23.6 in 2008 and 2009, respectively.

Total rainfall during the growing season (May-Sep) was 236mm (50 mm in flowering to harvest) and 157mm (108mm in flowering to harvest) in 2008 and 2009, respectively.

Total sunshine hours in flowering to harvest was 256 and 173 h in 2008 and 2009, respectively.

Mean daily temperature during the growing season (May-Sep) and Total sunshine hours in flowering to harvest in 2009 were lower than 2008, therefore mean number of grains per panicle decreased and mean unfilled grain percentage increased.

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Expression of Maspin, KI-67 and CD105 as Predictors of Postoperative Recurrence in Laryngeal Carcinoma: perioperative planning and proposed reconstructive tools

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Abstract: Background: Maspin, a member of serpin super-family, has multifaceted biological functions and an unique tumor suppressing activity. Several studies showed that maspin suppresses tumor growth, angiogenesis, invasion and metastasis. The present study investigated the relationship between maspin expression, Ki-67 proliferative index (PI), CD105-assessed microvessel density (MVD) and postoperative recurrence in laryngeal squamous cell carcinoma (SCC). **Patients and methods:** Subcellular pattern of maspin expression was immunohistochemically evaluated in 28 cases of laryngeal SCC treated by total laryngectomy with reconstruction but without primary radiotherapy with a follow-up period from 10 to 36 months. The expression and interaction between Ki-67, CD105 and maspin were also studied. **Results:** Two patterns of positive maspin expression; cytoplasmic (n=9) and nuclear-cytoplasmic (n=16) were recognized. Significant inverse correlation between nuclear-cytoplasmic pattern and both Ki-67 PI (P=0.049) and CD105-assessed MVD (P=0.016) were disclosed. Comparing the two groups of patients with (pR+) and without (pR-) evidence of postoperative recurrence, none of the studied clinicopathological parameters (age, sex, pathological grade, tumor stage, and nodal stage) was significantly associated with recurrence (all p>0.05). The nuclear-cytoplasmic maspin expression was significantly higher in pR- patients (p=0.018), while higher Ki-67 PI and CD105-assesed MVD were significantly correlated with pR+ group (p= 0.007 & p = 0.004 respectively). **Conclusion:** The present results suggest that absence of nuclear localization of maspin and high Ki-67 PI and CD105-assessed MVD may predict a higher risk of recurrence in laryngeal SCC patients.
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Keywords: carcinoma, larynx, maspin, nuclear, Ki-67, CD105, recurrence.

1. Introduction

Laryngeal cancer is the second most common cancer of the respiratory tract with an estimated incidence rate of 5.1/ 100,000 cases in males worldwide in the year 2008 and the European incidence rate of 10/100,000. The age-standardized mortality (world standard) in laryngeal cancer for males is 2.2/100,000 [1]

Tumor site (glottic, supraglottic and subglottic) and staging determine the treatment modality and influence survival rates. For example, for patients with T1 or T2 laryngeal cancer, the American Society of Clinical Oncology (ASCO) recommends in its clinical practice guidelines an initial treatment that preserves the larynx[2].

On the other hand, a recent article by Lefebvre et al. [3] concluded that it was impossible to give general therapy recommendations because the selected therapy approach should involve a combination of a treatment guideline and a careful patient management process.

Further understanding of the molecular alterations of laryngeal SCC may allow providing more accurate and useful prognostic markers.

Molecular and immunohistochemical diagnostics can detect abnormalities in lesions not yet appreciated histologically and thus predict early recurrences [4,5].

Since the discovery of the tumor suppressor gene p53, more than 15 different tumor suppressor genes have been identified [6] .Maspin (mammary serine protease inhibitor), a 42-kDa protein, is known to have a tumor- suppressor function. It belongs to the serpin (serine protease inhibitor) super-family which is categorized to inhibitory and non- inhibitory serpins [7]. Maspin expression has been demonstrated in several normal human tissues including breast, prostate, placenta, skin, oral cavity and lung [8].Previous findings supported the inhibitory effects of maspin as anti –invasive[9], inhibiting angiogenesis by blocking it both in vitro and in vivo models [10,11] and sensitizing apoptosis [12].In spite of being regarded as a tumor suppressor gene, paradoxically, both decreased and increased maspin levels have been described to parallel tumor progression [13,14]. This could be contributed to the fact that maspin demonstrates different subcellular localization that appears to be important in its function. It localizes primarily to the cytoplasm, but may also be located in

the secretory vesicles and the cell surface. Nuclear maspin has only recently been recognized[8,15]. Moreover, it has been noted that maspin nuclear localization is associated with favorable prognosis particularly in lung, gastric, and pancreatic carcinomas[9,14,16] .

The present study investigated the expression of maspin and its value in predicting carcinoma prognosis and recurrence in relation to its subcellular localization in a homogeneous group of laryngeal SCC patients concerning the treatment modality. All cases were treated by total laryngectomy with neck dissection without primary radiotherapy. The relationship between cell proliferation marker; Ki-67, microvessel density-assessing marker; CD-105, and recurrence of laryngeal SCC have also been considered. This might influence decision regarding extent of local excision, lymph node dissection, boundaries of estimated defects, and possible reconstruction tools.

2. Patients and Methods:

This study was carried out on 28 cases of primary laryngeal SCC, 19 males and 9 females, ranged from 32-70 years (median 50). Thorough clinical evaluation, routine preoperative, neck ultrasonography, head and neck contrast enhanced computerized tomography or/and magnetic resonance imaging were performed. All cases underwent total laryngectomy without primary radiotherapy at the departments of General Surgery and ENT, Zagazig University Hospital from May 2007 to January 2010. In ten cases the hypopharyngeal tissues left after total laryngectomy were sufficient, so we were not in need of reconstruction technique. In eight of our cases pectoralis major flap based on thoracoacromial vessels used as myocutaneous flap with adding part of the abdominal fascia and overlying skin. We had used this flap as a tubed one[17].

Four cases of our series, was operated by usage of free radial forearm flap. This flap raised on the radial artery, paired venae comitants and cephalic vein[18].

Two cases operated by free jejunal flaps for reconstruction of cervical esophagus. The pedicle of free jejunum is located within the mesentry. While the patients were, supine and two teams were operating simultaneously to decrease time and blood loss[19].

Four cases were reconstructed by the use of gastric pull up technique, and all our cases were followed up for about 10-36 months. All specimens were immediately sent to Pathology Department, processed, diagnosed and evaluated for tumor differentiation according to Geelen et al. [20] as 15 well, 9 moderately ,and 4 poorly differentiated cases.

According to TNM classification of Malignant Tumor of International Union Against Cancer[21] the pathological staging of primary laryngeal SCC (T) was T2 in 16, and T3 in 12 cases. Regional lymph node staging (N) was N1 in 17 and N2 in 11 cases

Immunostaing

It was performed on 4 microns formalin-fixed, paraffin-embedded tissue sections using the avidin – biotin peroxidase complex (ABC) procedure as described by Marioni et al.[22]. Antigen retrieval was performed for each section (microwave 750 w, 10mM citrate buffer, pH 6.0 for 15 minutes).Non specific binding was blocked with 10% normal rabbit serum.

Commercially available antibodies used were as follows: maspin antibody (clone G167-70,BD-Biotechnology, CA, USA, dilution 1:500) , Ki-67 (clone MIB-1,Dako,Glostrup,Denmark, dilution 1:100), and CD105(clone 4G11,Novocastra Lab., Ltd, UK, dilution 1:100). The immuno-staining was developed using diamino-benzidine as chromogen and Meyer's hematoxylin as counter stain.

As negative controls, the primary antibody was replaced by non – immune rabbit serum. Human normal breast tissue sections were used as positive control for maspin while sections of invasive breast cancer as controls for Ki-67 and CD105.

Evaluation criteria

Maspin subcellular pattern of distribution was scored as: negative, cytoplasmic, nuclear, and nuclear-cytoplasmic. The presence of strong cytoplasmic reactivity was investigated at x40 magnification. Weak cytoplasmic staining was considered negative[23].

Proliferative index (PI) was assessed by scoring the percentage of Ki-67 labeled nuclei in at least 5 high power fields (x 400 magnification). A cut-off value of 20% was chosen to separate cases with high and low index[24].

CD105-assessed MVD was measured by counting individual micro-vessel at x200 power field in three areas with the highest density (hot spots). Any brown- stained endothelial cell or cell cluster was considered a single microvessel while vessel lumen was not necessary to define a vessel .The mean count for each specimen was recorded and the median MVD was considered as a cut off value to determine high and low MVD[22].

Statistical analysis was performed using the SPSS program for Windows (SPSS Inc. Chicago, IL, USA), to assess the association between variables, chi-squared or Fisher exact test were used. P < 0.05 was considered significant.

3. Results

Normal squamous epithelial cells near SCC showed only weak cytoplasmic maspin expression (Fig. 1). In SCC cases, two positive immunostaining patterns were recognized: cytoplasmic and nuclear-cytoplasmic. Three out of 28 cases (10.8%) showed no or weak cytoplasmic reactivity that was considered negative .Strong cytoplasmic expression was found in 9 cases (32.1%)(Fig.2). Statistical analysis revealed no significant association between cytoplasmic maspin expression and age (p=0.79), sex (p=0.73), pathological grade (p=0.67), Tumor stage (p=0.77), Nodal stage (p=0.39) ,Ki-67 index (p=0.2),and MVD (p=0.73) (Table 1).

Nuclear-cytoplasmic pattern appear-ed in 16/28 cases (57.1%) (Fig.3 A&B). This pattern was not statistically correlated with the studied clinicopathological parameters {age (p=0.66), sex (p=0.77), pathological grade (p=0.93), T stage (p=0.50), and nodal stage (p=0.7)}. However, a significant inverse correlation was apparent between nuclear-cytoplasmic maspin expression and both Ki-67 index (p=0.049) and CD105 –assessed MVD (p=0.016) as shown in Table 1.

Only 11/28 (39.3%) cases developed loco-regional recurrence after a mean period of 25 +/-13 months. Table 2 showed comparison between the two patients' groups with (pR+) and without (pR-) postoperative recurrence. No significant difference was found with patient's age, sex, pathological grade, tumor stage, nodal stage and cytoplasmic maspin expression (all P>0.05).

However, nuclear-cytoplasmic expression was significantly higher in the pR- group (P= 0.018). On the other hand, both high Ki-67 index (Fig. 4) and CD105-assessed MVD (Fig. 5) were significantly associated with the pR+ group (P= 0.007 and 0.004 respectively)

Table(1) Relationship between clinicopathological, immunohistochemical results and subcellular maspin expression in laryngeal squamous cell carcinoma.

Variables	Score	+ ve maspin expression (n=25)				
		Cytoplasmic (n=9)		P value	Nuclear – cytoplasmic (n=16)	P value
		No.	%		No.	%	
Age	**≤ 50**	5	55.6	0.79	8	50	0.66
	> 50	4	44.4		8	50	
Sex	**Male**	7	77.8	0.73	11	68.8	0.77
	Female	2	22.2		5	31.2	
Pathological grade	**Well**	4	44.5	0.67	9	56.3	0.93
	Moderate	3	33.3		5	31.3	
	Poor	2	22.2		2	12.5	
Tumor stage	**T$_2$**	3	33.3	0.77	6	37.5	0.50
	T$_3$	6	66.7		10	62.5	
Nodal stage	**N1**	7	77.8	0.39	9	56.2	0.7
	N$_2$	2	22.2		7	43.8	
Ki-67 index	**≥ 20%**	5	55.6	0.2	3	18.8	0.049*
	< 20%	4	44.4		13	81.2	
Microvessel density	**High**	3	33.3	0.73	2	12.5	0.016*
	Low	6	66.7		14	87.5	

Table (2) Evaluation of clinicopathological and immunohistochemical parameters in laryngeal squamous cell carcinoma patients according to presence (pR+) or absence (pR-)of recurrence.

Variable	Score	PR + (n=11)		PR - (n=17)		P value
		No	%	No	%	
Age	≤ 50	6	54.5	10	58.8	0.86
	> 50	5	45.5	7	41.2	
Sex	Male	7	63.6	12	70.6	0.57
	Female	4	36.4	5	29.4	
Pathological grade	Well	5	45.5	10	58.8	0.48
	Moderate	4	36.3	5	29.4	0.97
	Poor	2	18.2	2	1.8	0.93
Tumor stage	T$_2$	6	54.5	10	58.8	0.86
	T$_3$	5	45.5	7	41.1	
Nodal stage	N1	7	63.6	10	58.8	0.59
	N$_2$	4	36.4	7	41.2	
Maspin expression	Negative	2	18.2	1	5.9	0.68
	Cytoplasmic	6	54.5	3	11.8	0.09
	Nuclear.-cytoplasmic	3	27.3	13	82.3	0.018*
Ki-67 index	≥ 20	9	81.8	2	11.8	0.007**
Microvessel density	High	7	63.6	1	5.9	0.004**
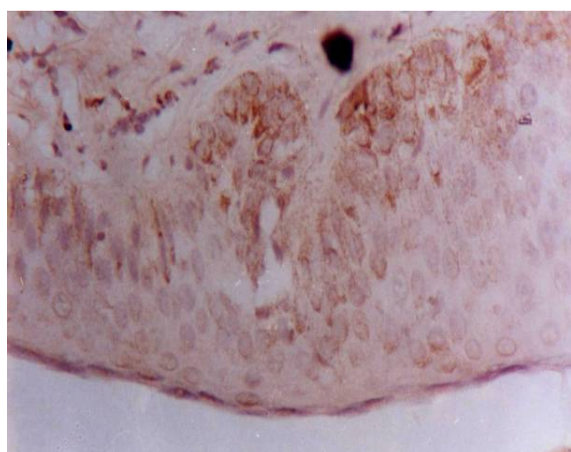
Fig (1): Normal laryngeal epithelial cells near SCC showed weak cytoplasmic maspin staining (ABC, Meyer's hematoxylin counter- stain, original magnification X400).
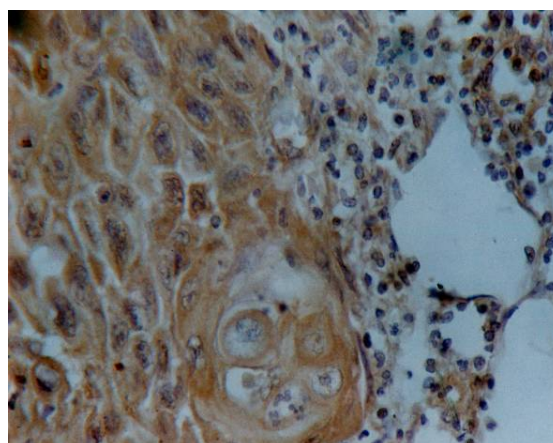
Fig. (2): Strong maspin cytoplasmic pattern of expression in a well differentiated laryngeal SCC (ABC, Meyer's hematoxylin counter-stain, original magnification X400).
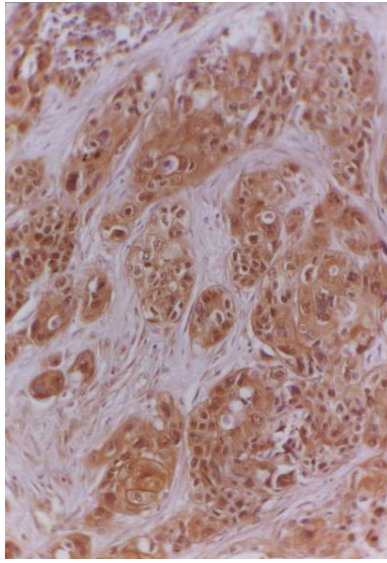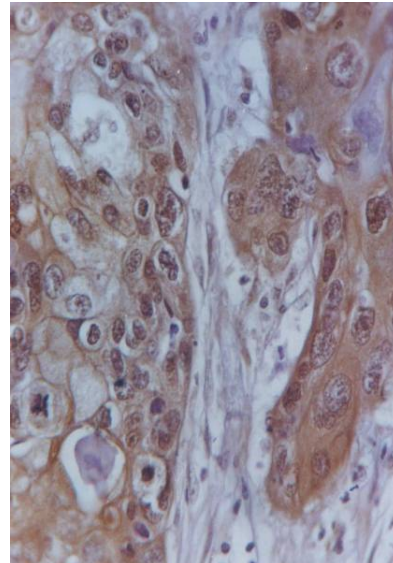
| A | B |

Fig. (3): Maspin nuclear-cytoplasmic pattern of expression in a moderately differentiated (A) and a poorly differentiated (B) SCC (ABC, Meyer's hematoxylin counter-stain, original magnification X200&X400 respectively).
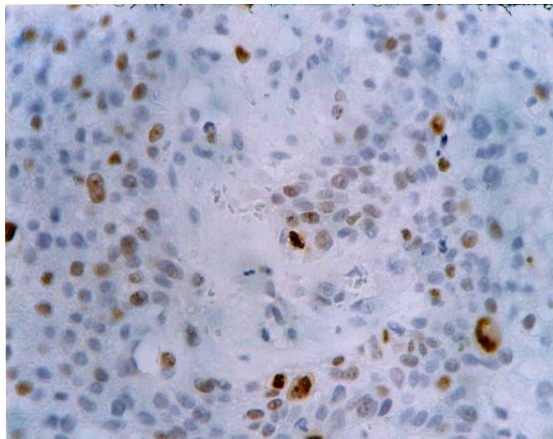
Fig. (4): Ki-67 immunoreactivity in a poorly differentiated SCC showing diffusely scattered positive nuclei with high labeling index (ABC, Meyer's hematoxylin counter-stain, original magnification X400).
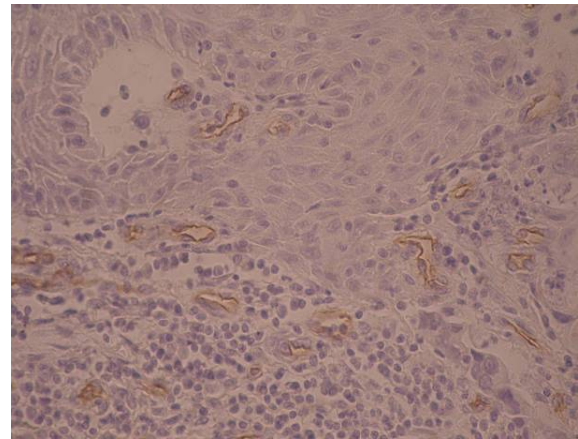
Fig. (5): CD105 immunoreactivity in a well differentiated SCC showing high microvessel density (ABC, Meyer's hematoxylin counter-stain, original magnification X400).
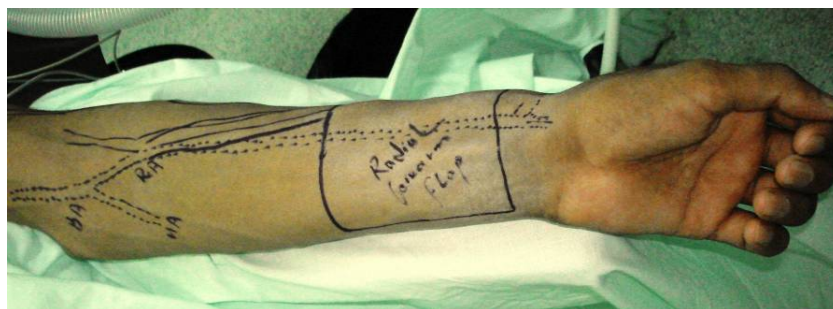
Fig. (6): Drawing of radialforearm flap
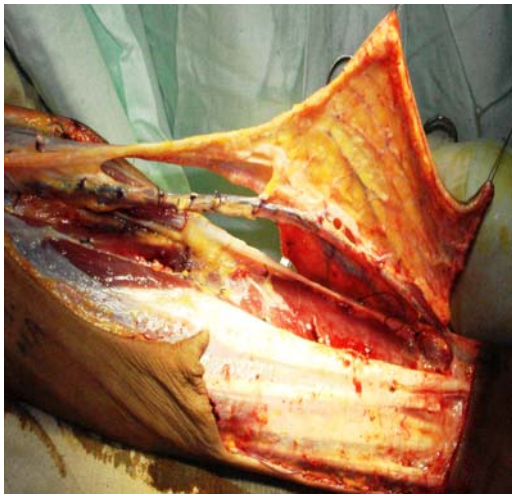
Fig. (7): Raising of radialforearm flap.
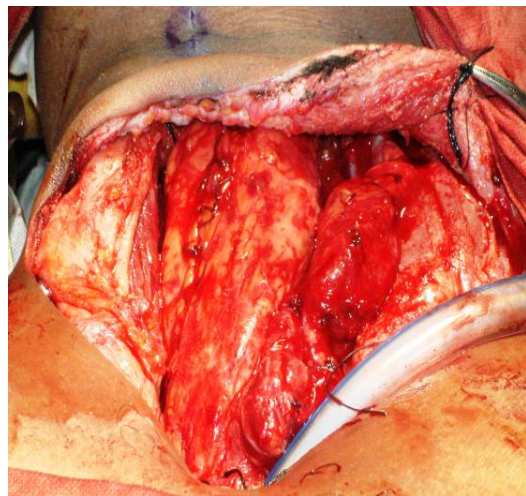
Fig. (8): Insetting of the flap after microsurgery anastomosis.

Fig. (9): postoperative.

4. Discussions

The cure rates of laryngeal SCC have improved little over the last few decades. Despite the higher number of therapeutic and histopathologic studies performed, there are no specific parameters available to predict the outcome in these patients [25]. Maspin expression is down-regulated or absent in breast and esophageal cancers [13,26].Conversely, it is up-regulated in pancreatic, ovarian and gastric cancers [9,14,27]. Concerning maspin expression in head and neck SCC, Yoshizawa et al.[28] reported that higher maspin expression in oral SCC was correlated with the absence of lymph node metastasis and better prognosis.

In this current study, both cytoplasmic (32.1%) and nuclear- cytoplasmic (57.1%) patterns of maspin expression were demonstrated in laryngeal SCC. Cytoplasmic maspin expression was not significantly associated with the studied clinicopathological

parameters including the pathological grade (P>0.05). This finding was not completely in accord with other head and neck SCC whereas low maspin expression was found to be associated with high tumor grade [29]. The precise mechanism of significant maspin expression in the cytoplasm of laryngeal SCC cells remains unclear. A possible explanation may be that during laryngeal carcinogenesis, some trigger factors stimulate the regulation mechanism of maspin. It is known that maspin expression results in tumor suppression, however, the high cytoplasmic concentration of maspin may result in auto-inhibition of its activity by polymerization[30].

Maspin function in the nucleus is less investigated and probably more complex. In the absence of a nuclear localization signal, maspin must either be chaperoned to the nucleus or cross the nuclear membrane by passive diffusion[31].

Nuclear-cytoplasmic expression of maspin in the present study showed no significant association with the clinico-pathological parameters, while a significant inverse correlation was found with both Ki-67 index (P=0.049) and CD105-assessed MVD(P=0.016).In agree-ment with these results, Marioni et al [23] concluded that nuclear localization of maspin was associated with less proliferative laryngeal SCC. MVD was also found to be significantly lower in laryngeal SCC with nuclear maspin than in carcinomas with cytoplasmic pattern[11]. These results confirmed the crucial role of nuclear maspin in reducing the proliferative activity and angiogenesis of laryngeal SCC.

Comparing the two groups of patients with and without postoperative carcinoma recurrence (pR+ versus pR-), a statistically significant difference in maspin nuclear-cytoplasmic expression was found in the present study (p=0.018), whereas this pattern was higher in the pR- group. This finding is consistent with the results achieved by Marioni et al. [12] who observed a highly significant lower recurrence rate in the group of patients with nuclear maspin localization (P=0.0086). Their study supported the hypothesis of an apoptosis-sensitizing effect of nuclear maspin in laryngeal carcinoma with the potential perspective of a clinical use of the tumor suppressive pro-apoptotic function of maspin.

Similar results were reported in other carcinomas. Lee et al. [14] evaluated that gastric carcinoma cases with nuclear-cytoplasmic maspin expression survived longer than those with only cytoplasmic expression. Also, Frey et al. [32] concluded that nuclear maspin in stage I lung carcinoma is an important predictor of improved survival supporting the hypothesis that maspin tumor inhibitor properties may be linked to its nuclear localization.

Regarding the Ki-67 PI, pR+ group in the current study had a statistically significant correlation with higher proliferating laryngeal SCC (P=0.007). Similarly, Calgaro et al. [24] and Cordes et al.[33] observed that the patients' group with low proliferating laryngeal SCC had a statistically longer absolute and recurrence –free 5-year survival time than patients with a highly proliferating cancers.

Angiogenesis is one of the critical mechanisms to postoperative recurrence and metastasis in carcinomas. CD105 (endoglin) was not expressed in the vascular endothelial cells of normal tissue in contrast to CD34 that reacts with normal vessels trapped within the tumor. So, the use of antibodies to CD105 had a higher specificity than pan-endothelial markers in the identification of new microvessels and could reduce the false positive staining spots[34].

The results of Marioni et al [22] and Zvrko et al. [35] revealed a strong association between high CD105 –assessed MVD and recurrence in laryngeal SCC (P=0.009 and 0.012 respectively). The present study results were consistent with the previous studies (p= 0.004) reflecting the role of this marker in identification of high risk patients for recurrence. Interestingly, CD105-assessed MVD was correlated with prognosis and recurrence of other cancers including hepatocellular and breast carcinomas[36,37].

5. Conclusion:

Nuclear expression of maspin may be useful to identify patients at lower risk of malignancy recurrence and less aggressive laryngeal SCC. On the other hand, high Ki-67 PI and CD105-assessed MVD can be valuable parameters for selecting patients who should be treated with more aggressive therapies.

So, we recommend that using preoperative biopsies of laryngeal SCC immunostained for maspin, Ki-67 and CD105 may be useful to predict patients at risk for developing regional recurrence. This might influence decision regarding therapeutic management.

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Environmental Studies of Domestic Wastewater Treatment Using Integrated Anaerobic/Aerobic System

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Abstract: Conventional aerobic technologies based on activated sludge processes are dominantly applied for the treatment of domestic wastewater due to the high efficiency achieved, the possibility for nutrient removal and the high operational flexibility. Anaerobic pre-treatment of domestic wastewater can serve a viable and cost-effective alternative due to its relatively low construction and operational cost, operational simplicity, low production of excess sludge, production of energy in form of biogas and applicability in small and large scales. A viable alternative is the sequential anaerobic–aerobic systems. The performance of the integrated anaerobic/aerobic wastewater treatment system (AAWTS) for domestic wastewater treatment has been investigated. The domestic wastewater and activated sludge were collected from Ras El-Bar wastewater treatment plant. The overall removal efficiency of the suggested system, is in the order $TSS < TN < BOD < Cl^- = TDS < COD < NH_3$. The deficiency of the applied AAWTS may be due to the limiting effect of salts on the biological treatment of saline influents.
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1. Introduction:

In developing countries where access to safe drinking water is not guaranteed for a majority of the population, it is important to maintain the quality of surface water sources for drinking purposes, industrial development or agricultural expansion. It is obvious that integrated management programs for the available water resources are required. Meanwhile, the responsible authorities should urgently consider a strategy for the proper management and development of non conventional water resources (Tawfik *et al*, 2004 and 2006). Consequently, wastewater like domestic sewage, apart from being sanitized, can become an important source of re-usable water, fertilizer, soil conditioner and energy. Currently, built-up and usually expensive and sophisticated systems for wastewater treatment usually fail, especially in developing countries: no manpower, no finances for operation, maintenance of equipment, etc. Thus, need to develop reliable technologies that treat domestic wastewater is a must. The degree of treatment required varies according to the specific reuse application and associated water quality requirements (Tandukar et al, 2005 and 2007).

Anaerobic digestion presents a high potential in most developing countries for domestic wastewater treatment, and thus is a suitable and economical solution (Foresti, 2001 and Halalsheh, 2002). The anaerobic process can serve as a viable alternative, compared to conventional aerobic processes. The fact that the process can be carried out in decentralized mode means also that this application can lead to significant savings in investment costs of sewerage systems (Gavala and Lyberatos, 2001 and Lettinga, 2004).

The efficiency of an anaerobic digester depends on the dynamics and kinetics of the microbe populations within the reactor and on the narrow limits that thermodynamics places on the ensuing reactions. Acetate is a major product of the fermentation of intermediate organic molecules. About 70% of methanogenesis is through the acetate route (De Smedt et al, 2001 and Fuchs et al, 2003). Very few known species of the methanogenes make use of the acetate route of methane production, but almost all are able to use the alternate H_2/CO_2 route which has the fundamental importance in keeping the H_2 pressure of a system low (El-Mitwalli et al, 2002). Recently, however, more efficient anaerobic systems have been developed, and they are being successfully applied for treatment of low-strength wastewaters such as domestic wastewater, particularly under tropical conditions where artificial heating can be avoided, to cut down on the costs (Foresti, 2001, Carr et al, 2004, Ernst et al, 2006 and Asano, 2007).

Ras El-bar is a major city and resort on the Mediterranean Coast, in Damietta Governorate. The city covers about 200 km north of Cairo and is distinguished by its unique position since it is the point where the Nile River meets the Mediterranean Sea. In addition to that it enjoys a good moderate weather, crystal clear water and numerous tourist places. The climate of Ras El–bar is Mediterranean, and contains a high percentage of iodine. There are approximately 15,000 to 25,000 inhabitants in Ras El Bar. However, during summer season (July-August),

it becomes a well known local resort and receives between 200,000 and 250,000 visitors during that period. Thus, organic load increases which contain a high level of salts and this cause an elevation of wastewater salinity. Sodium chloride (NaCl) and other salts may reach wastewater by several means. Seawater has been used as an alternative water source for toilet flushing in some coastal cities, resulting in a high salt content in the sewage.

        Also, in coastal areas, infiltration of saline water into sewers is associated with a subsurface water rise and contributes a high concentration of chloride to wastewater. In addition, certain industrial wastewater contains high inorganic salts because of specific technologies, such as cheese, pickling, canning and dye manufacturing. The activated sludge process is usually used to treat wastewater for its low cost, so it is thought that the shock salt loading may influence the process when saline wastewater enters wastewater treatment systems.

        The aim of this work is to study the efficiency of domestic wastewater treatment of Ras El–Bar by applying integrated anaerobic/aerobic system as an alternative of the conventional aerobic activated sludge process. To achieve that, the physico-chemical characteristics of domestic wastewater were investigated before and after applications of anaerobic/aerobic treatment system.

2. Material and Methods

        All chemicals used were of analytical reagent grade quality. The experimental method involved the collection of composite samples in clean plastic containers of 5 liter capacity at three different units of the treatment plant, namely, a) Influent to the treatment plant, b) Effluent of an aeration tank and c) Final effluent for seven months (monthly sample) for assessment of Ras El–Bar treatment plant performance. The samples were analyzed using the Standard Methods (Adams, 1990; APHA, 2005; and EPA, 2000). The primary parameters included Temperature(T), pH, total dissolved solids (TDS), settlable solids (SS), electrical conductivity(EC), alkalinity, ammonia(NH_3), dissolved oxygen (DO), 5-day biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), chlorides and sulfide; while secondary parameters are mixed liquor suspended solids (MLSS), sludge volume index (SVI). The pH was estimated using pH meter (3305, Jenway, UK).

Experimental set-up and design

        Figure (1) shows a schematic diagram of anaerobic/aerobic wastewater treatment system (AAWTS) which was designed and fabricated by the project team. This module consists of a reactor with capacity of 300 liter volumes, water jacket, stirrer blade, electric heater and stirrer, wastewater filling hole, control valves, variable speed pump and settling tank with 250 liter volumes, and fixed speed return pump. The anaerobic reactor was provided with 5 holes for measuring instruments such as temperature and pH. A separate aeration section with a dimension of 21x 54x90cm and a precipitation section with a volume of 45L were used. Aeration was performed with an air pump and diffusers. The domestic wastewater and activated sludge were provided from Ras El-Bar Sewage Wastewater Treatment Plant. All experiments were conducted in batch-wise at room temperature (25C°±5 C°). After 180 L domestic wastewater and 36 L activated sludge (6 g/L MLSS) were added into the reactor (pH 7.2), stirring started to acclimate activated sludge. During the acclimation period, 180 L supernatant was withdrawn and 180 L fresh domestic wastewater was refilled in the reactor every 24 hours for one month. After acclimation, 180L supernatant was withdrawn and 180 L fresh domestic wastewater was refilled in the reactor. The wastewater samples were harvested for complete analysis. The characteristics of sludge were determined after it was squeezed by distilled water.

3. Results and Discussion:

        Special consideration has been given in the current study to the organic content, characterized by BOD_5, COD and the COD/ BOD_5 ratio as shown in Tables (1 and 2). Based on the performance study conducted for different primary and secondary parameters for a period of seven months, Colmenarejo et al. (2006) determined the general efficiency indicator to compare overall performances of the different plants in terms of average TSS, COD, BOD_5 and ammonia removal efficiencies. Similarly, the efficiency of plants is generally measured in terms of removal of organic matter (CPHEEO, 1993). The pH directly affects the performance of a secondary treatment process (Metcalf and Eddy, 2003) because the existence of the most biological life is dependent upon a narrow and critical range of pH. Since, the solids removal is an important measure for the success of a primary treatment unit (McGhee, 1991) and the dissolved solids content of the wastewater is of concern as it affects the reuse of wastewater for agricultural purposes, by decreasing the hydraulic conductivity of irrigated land if the total dissolved solids content in the water exceeds 480 mg/l (Bouwer, 1978). Also, BOD removal is indicative of the efficiency of biological treatment processes (Sincero and Sincero, 1996).

1	Reactor with 300 litre volum
2	Water Jacket
3	Steirre Blade
4	Electric Heaters
5	Wastewater Filling Hole
6	Controle Valves
7	Electric Steirrer
8	5-Holes For Measuring Instruments
9	Water Filling Hole
10	Variable Speed Pump
11	Settling Tank With 250 litre Volum
12	Fixed Speed Return Pump
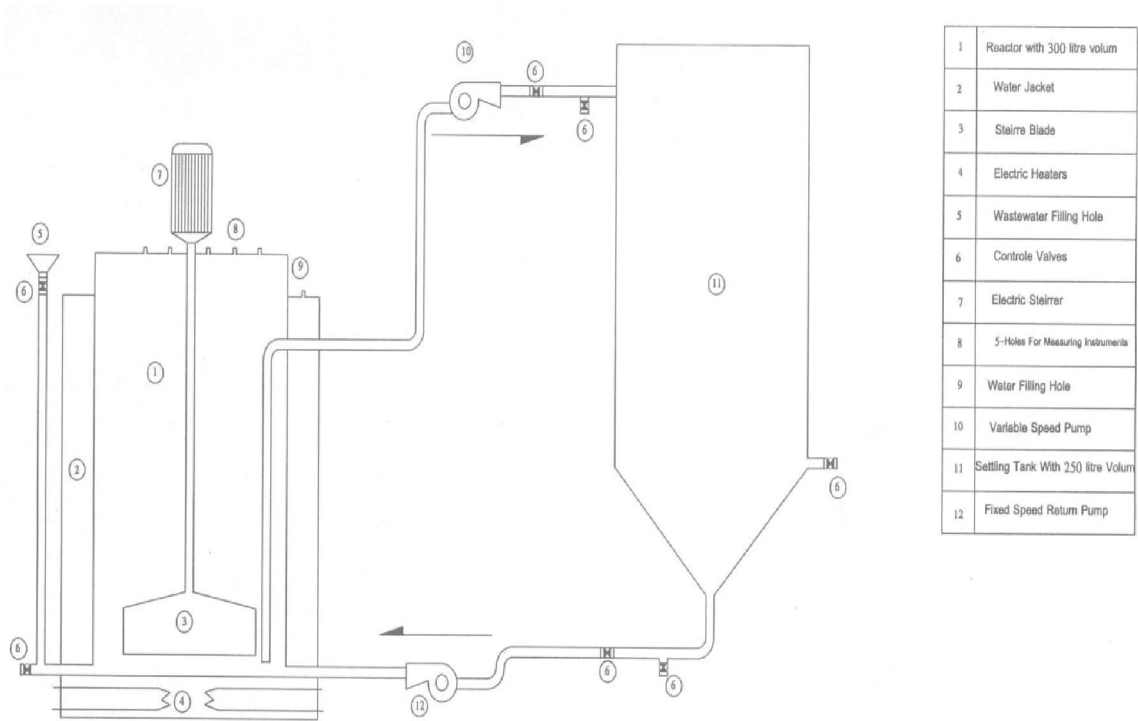
Figure (1): Schematic Diagram of Anaerobic Wastewater Treatment System (AAWTS).
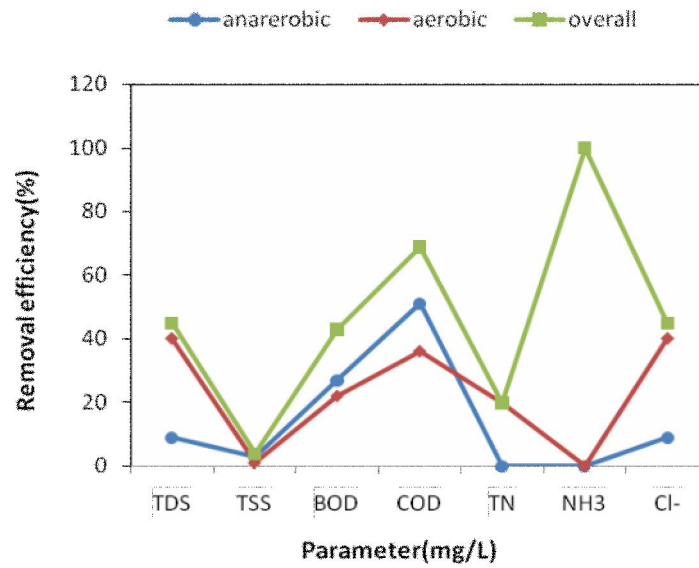
Figure (2): Removal efficiency (%) of the suggested anaerobic/aerobic treatment system (AAWTS).

Influent wastewater characteristics to the inlet of the Ras EL-Bar treatment plant

As shown in Table (1), the wastewater varied in their characteristics, where the pH ranged from 6.6 to 7.72. The concentration of total dissolved solids, settlable solids, BOD_5 and COD were 3325-7504 mg/L, 116-263 mg/L, 126-225 mg/L, 303-542 mg/L, respectively, while the average chlorides and sulfide were 3015 mg/L and 8.8 mg/L, respectively

(Table 1). Out of seven months of performance study, the highest value of total dissolved solids (7504 mg/L), settlable solids (263 mg/L), BOD_5 (225 mg/L) and COD (542 mg/L) is attributed to heavy organic and inorganic loading with less liquid content (Kumar, et al., 2010). In the treatment plant, the DO was "nil" at the inlet, stimulated by oxidation of sewage ammonia to nitrates, septic condition, heavy organic loadings.

Table (1): Average wastewater characteristics at the inlet and primary effluent of Ras EL-Bar treatment plant for seven months

Parameter	Raw wastewater (Influent)	Primary effluent	Removal efficiency (%)	Law 48 (1982)
Temperature (C°)	23.7	23.7	-	35
pH	7	7.1	-	6 - 9
Electrical Conductivity (µS/cm)	7678	6157	20	-
TDS(mg/L)	4532	3864	15	-
Settleable solids(mg/L)	212	104	51	50
DO(mg/L)	0	0	-	4
BOD(mg/L)	185	117	37	60
COD(mg/L)	446	223	50	80
S^{-2} (mg/L)	8.8	2.9	67	1
NH_4(mg/L)	18.5	1.1	94	0
Alkalinity(mg/L)	347	311	10	-
Cl^-(mg/L)	3015	2291	24	-

Effluent wastewater characteristics of the aeration tank

The aeration tank in the treatment plant is considered a most important step in the activated sludge process, and the priority was intended to increase the dissolved oxygen level of sewage so that the efficient aerobic digestion facilitates decomposition of organic matter. This has to be ensured because of low dissolved oxygen content (nil) in the influent. In Ras EL-Bar Treatment Plant, the DO showed a slight increase and ranged from 1.1to 2.3 mg/L in the aeration tank, indicating an inefficient and unsatisfactory working. The pH varied from 6.7 to 7.9 (Tables 2 and 4). Efficiency of the aeration tank was calculated by considering percentage reduction of BOD_5. The average influent value of BOD_5 in the aeration tank was 185 mg/L while the average effluent value from this tank is 120 mg/L. The percentage removal of BOD_5 in the treatment plant is 35.2 % against the expected value of 70-85%, illustrating that BOD_5 reduction is little less than the expected. This slight decrease is attributed to the recycling of old sludge that contained fewer microorganisms, besides insufficiency of MLSS for the aerobic digestion of the organic matter. The DO during the aeration was absorbed by the microorganisms due to less availability of fresh organic matter. As shown in

Tables (2 and 4), the MLSS concentration in the aeration tank ranged between 555-3752 mg/L against the expected concentration of 1500-3000 mg/L, confirming suitability of secondary clarifier in terms of microbial content. A SVI value of 46-69 mL/gm indicates bad settling of suspended solids that can be achieved for proper MLSS concentration. The SVI of Ras EL-Bar Treatment Plant was quite low compared to the expected value (60-90 mL/gm).

Table (2): Effluent characteristics of the aeration tank of Ras EL-Bar treatment plant.

Parameter	Aeration tank
Temperature (C°)	23.7
pH	7.3
Sludge Volume(ml/L)	104
MLSS(mg/L)	1723
Sludge Volume Index(mg/L)	53
DO(mg/L)	1.1 - 2.3
BOD(mg/L)	120

Wastewater characteristics of the suggested anaerobic/aerobic wastewater treatment system (AAWTS)

During the working period, samples were taken from: the tank (raw wastewater), which feeds the anaerobic reactor, the effluent of the anaerobic reactor (the influent to the aerobic reactor), the

effluent of the aerobic reactor and from the aeration tank (Tables 3 and 4). The removal efficiency of BOD5, COD, TDS and chlorides after anaerobic treatment was 27 %, 51 %, 9%, and 9%, respectively, confirming inefficiency of the anaerobic treatment and its unsuitability to be discharged. However, after aerobic treatment, the removal efficiency of BOD5, COD, TDS, TN and chlorides were 22 %, 36 %, 40%, 20% and 40 %, respectively (Tables 3 and 4).

The overall efficiency of Ras EL-Bar Treatment Plant was calculated by considering the TDS, TSS, COD, TN, NH_3, chlorides and BOD_5 of the influent (raw wastewater) and the final effluent from the suggested anaerobic/aerobic treatment system. Tables (3 and 4) indicated that the reduction in COD is 69 % while the percentage reduction of total dissolved solids was 45 % much below the expected removal of 70-80% indicating poor efficiency in terms of total dissolved solids removal. However, the removal of total suspended solids and BOD_5 was found to be unsatisfactory. The reduction in total suspended solids is 4% against the expected value of 85-90 %, while the reduction in BOD_5 is 43 % against the expected value of 85-90 %. Kassab et al. (2010) stated that the range of TSS removal efficiencies by UASB-AS and UASB –SBR reactors were 85 – 92 % and 84 - 98 %, respectively. On the other hand, the anaerobic SBR or aerobic SBR system cannot remove the TSS content of the wastewater. According to that, our results were agreed with this finding. Suspended solids in wastewater are known to affect anaerobic digestion adversely (Gijzen, 2001; Bodik et al., 2003). They decrease sludge activity due to adsorption and entrapment, limit substrate transfer, lead to the formation of scum layers, inhibit granulation, and enhance sludge production, causing the frequent need to de-sludge reactors (Angelidaki and Sanders, 2004; Kumar et al, 2010).There is usually no correlation between BOD_5 and COD in wastewater with slowly biodegradable organic suspended solids and in complex waste effluents containing refractory substances (Eckenfelder, 1989; Coskuner and Ozdemir, 2006). Hence, treated effluents may exert virtually no BOD and yet exhibit a substantial COD. Since, the COD represents virtually all organic matter, either partially degradable or non-biodegradable and BOD_5 the total oxygen demand, it is necessary to develop a relationship between BOD and COD. Accordingly, the average influent and effluent COD/BOD_5 ratios for the suggested anaerobic/aerobic treatment system were calculated, and it is observed that, the COD/ BOD_5 ratio frequently varied for effluents compared to untreated wastes (Eckenfelder, 1989; Coskuner and Ozdemir, 2006).

The influent wastewater exhibited a ratio of 4 and this value is not comparable to those presented by Metcalf and Eddy (2003). As shown in Tables (3 and 5), the typical COD/BOD_5 ratio of domestic wastewaters is usually in the range 1.25 to 2.5. However, for treated effluents of this study, it was 2.24. This indicates a relatively higher proportion of the non-biodegradable content in treated effluent than raw wastewater. As a consequence, the efficiency of BOD_5 removal is lower than that of COD removal. However, the physical removal of COD by sedimentation and filtration in the sludge bed without anaerobic degradation may be the reason for obtaining higher value of maximum COD removal. Thus, the obtained results using the integrated system of this study was relatively low compared to Kassab et al., (2010), who indicated that the removal efficiency of COD from wastewater treatment using upflow anaerobic sludge blanket (UASB) with activated sludge (AS) or sequencing batch reactor (SBR) system generally in the range of 79 to 85 % They attribute that to the integration UASB with SBR which especially enhance the removal efficiency of organic content of effluent. The removal efficiency of TN after anaerobic treatment was found to be zero. Overall removal efficiencies of TN and ammonia were 20 and 100 %, respectively (Tables 3 and 5). A similar result were reported by Lema and Omil (2001), who found that the ammonia was removed under moderate to low temperature of sewage treatment using UASB reactor. Moreover, the anaerobic reactor removed only the particulate nutrients by sedimentation and filtration and, therefore, it had relatively low removal of nutrients. Luostarinen et al., (2006) obtained a similar result, and they found that percentage of nitrogen removal in the range of 71-77 % under anaerobic and at low temperature conditions. Moreover, Tables (3 and 5) show that the average concentration of chlorides was 3746 mg/L of raw wastewater with anaerobic and aerobic removal efficiencies of 9 and 40%, respectively, and the overall removal efficiency was 45%. However, the high salt content of these wastewaters makes biological treatment with a conventional microorganism difficult. Thus, the biological removal of organic compounds from hypersaline wastewaters requires the use of halophilic organisms that are well adapted to hypersaline media. In order to overcome the inhibitory effect of the salt a specific flora, capable of decomposing organic compounds in high concentrations of salts (>30%), was adapted to saline wastewater by gradual increases in organic and salt concentrations.

The high salinity of industrial waste-waters is one of the causes of the difficulty in treating these wastewaters by conventional systems. As shown in

Tables (1, 3 and 5) the high salt concentrations disrupt metabolic functions and cause plasmolysis and/or loss of activity of microbial flora; hence the biological treatment of saline wastewaters with conventional microorganism results in low chemical oxygen demand (COD) removal efficiency. The effects of salt on the performance of biological processes in the treatment of wastewaters have been studied previously. Wang et al., (2005) have studied the effect of salt concentration on biological treatment of synthetic saline wastewater by feed batch operation. They found that increasing salt concentration reduced the COD removal rate and efficiency. Accordingly, it was necessary to dilute the influent wastewater with fresh or irrigation water to decrease the salinity and enhance the dissolved oxygen. AAWT system can use the anaerobic growth and support medium for biomass, which was retained in a high concentration, thus prolonging the sludge retention time (SRT) of the system, and provided a suitable environment for the growth and activity of slow-growing microorganisms. The limiting effect of salt on the biological treatment of saline effluents may also be overcome if conventional activated sludge is replaced by microorganisms that are well adapted to hypersaline media (Eckenfelder, 1989; Wang et al., 2005).

Table (3): Descriptive statics of the wastewater characteristics from the suggested anaerobic/aerobic treatment system (AAWTS).

	Parameter	pH	T (C°)	EC (µS/cm)	Alkalinity (mg/L)	TSS (mg/L)	TDS (mg/L)	DO (mg/L)	BOD (mg/L)	COD (mg/L)	TN (mg/L)	NH$_3$ (mg/L)	Cl$^-$ (mg/L)
	No Samples	10	10	10	10	10	10	10	10	10	10	10	10
Raw	Mean	7.33	23.5	9910	700	930	6342	0	220	892	44	16	3746
	Max.	7.8	24	9930	710	987	6438	0	253	920	49	19	3905
	Min.	7.1	23	9896	694	890	6117	0	200	850	40	11	3611
	Range	0.7	1	34	16	94	321	0	53	74	9	8	294
Anaerobic	Mean	7.6	-	9037	756	903	5771	0	160	440	44	17	3409
	Max.	8	-	9098	763	921	5823	0	173	476	56	19	3578
	Min.	7.38	-	9012	734	892	5713	0	145	427	38	15	3397
	Range	0.62	-	86	29	29	110	0	28	49	18	4	261
Aerobic	Mean	7.5	-	5422	690	893	3463	4.5	125	280	35	0	2046
	Max.	7.8	-	5571	702	900	3563	4.7	127	294	37	0	2157
	Min.	7.3	-	5489	681	886	3459	4.3	119	273	31	0	2035
	Range	0.5	-	82	21	4	104	0.2	6	21	6	0	122

Table (4): Sludge characteristics from the aeration tank of the suggested anaerobic/aerobic treatment system (AAWTS).

	MLSS(mg/L)	SVI (ml/L)
No Samples	5	5
Mean	680	59
Max.	687	61
Min.	673	54
Range	14	7

Table (5): Removal efficiency (%) of the suggested anaerobic/aerobic treatment system (AAWTS).

Parameter	Removal efficiency of Anaerobic treatment (%)	Removal efficiency of Aerobic treatment (%)	Overall removal efficiency (%)
pH	-	-	-
EC(µS/cm)	-	-	-
TDS(mg/L)	9	40	45
TSS (mg/L)	3	1	4
BOD(mg/L)	27	22	43
COD(mg/L)	51	36	69
TN(mg/L)	0	20	20
NH$_3$(mg/L)	8.8	0	100
Cl$^-$(mg/L)	9	40	45

4. Conclusion:

The overall removal efficiency of the suggested anaerobic/aerobic wastewater treatment system (AAWTS), is in the order TSS < TN < BOD < Cl⁻ = TDS < COD < NH₃. The results obtained in this study revealed that, the domestic wastewater of Ras El-Bar Treatment Plant was very saline. These conditions adversely affect the total biological efficiency of the activated sludge during the different stages of biological treatment. This decline of efficiencies may due to the plasmolysis phenomenon of non-halophilic microorganisms and thus responsible microorganisms for biological degradation of organic matter were inhibited. Accordingly, it was necessary to dilute the influent wastewater with fresh or irrigation water to decrease the salinity and enhance the dissolved oxygen. AAWT system can use the anaerobic growth and support medium for biomass, which was retained in a high concentration, thus prolonging the sludge retention time (SRT) of the system, and provided a suitable environment for the growth and activity of slow-growing microorganisms. The limiting effect of salt on the biological treatment of saline effluents may also be overcome if conventional activated sludge is replaced by microorganisms that are well adapted to hypersaline media.

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H$_2$/H$_\infty$ Controller Design for Singular Perturbation Systems

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Abstract: In this paper the synthesis of logic-based switching H$_2$/H$_\infty$ state-feedback controller for singular perturbation systems is considered that achieves a minimum bound on the H$_2$ performance level, while satisfying the prescribed H$_\infty$ performance. The proposed hybrid control scheme is based on a fuzzy supervisor which manages the combination of two controllers. A convex LMI- based formulation of the two fast and slow subsystem controllers leads to a structure that ensures a good performance in both the transient and the steady state phase. It is shown that the system with the proposed controller remains globally stable despite the configuration (controller) changing. [Fatemeh Jamshidi, Afshin Shaabany. H$_2$/H$_\infty$ Controller Design for Singular Perturbation. Journal of American Science 2011;7(3):493-499]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Singular perturbation system; Fuzzy supervisor; Linear Matrix Inequality (LMI); Switching H$_2$/H$_\infty$ control.

1. Introduction

There has been increasing interest in hybrid control in recent years, due to its potential to overcome limitations of adaptive control and benefits in controlling of systems that cannot achieve the desired performance by a single controller. Indeed, hybrid control scheme provides an effective mechanism when facing large modelling uncertainty and highly complex systems. Even for simple linear time invariant systems, controllers switching can be utilized in improving the performance (Sun, 2005, Feuer, 1997, and McClamroch, 2000). To date, Morse, Hespanha and Liberzon have established a theoretical backbone for hybrid controllers (Morse, 1997, Hespanha, 1999, and Liberzon, 2003). By now, stabilizing a continuous system via hybrid output feedback has attracted a number of authors, such as (Santarelli, 2008) where a comparison between the responses of the switching controller and two other forms of LTI control have been made. An experimental assessment of controller switching with state and control magnitude constraints is carried out in Kogiso, 2004. In Zheng, 2006, the multi-objective robust control of an induction motor with tracking and disturbance rejection specifications is proposed via switching. In Essounbouli, 2006, DeCarlo, 1988, and Jamshidi, 2010 controller switching has been proposed to improve the trade-offs in design multi objectives.

Supervisory control employs logic-based switching for adaptation, instead of continuous tuning of parameters as in conventional adaptive control. This type of switching-based supervisory control scheme consists of the following subsystems: a plant to be controlled, a bank of controllers, and a switching logic. Dwell-time method is representative of the trajectory independent switching logic for supervisory control (see Yoon, 2007 and its references). On the other hand, Lyapunov functions are employed in such trajectory dependent switching methods as in Yoon, 2007.

Systems with slow and fast dynamics, described mathematically by singular perturbations, are studied extensively in numerous papers and books; see for examples (Kokotovic, 1986, Tan, 1998). For robust control of singular perturbation systems, the controller is usually derived through indirect mathematical programming approaches (e.g. solving Riccati equations), which encounter serious numerical problem linked with the stiffness of the equations involved in the design. To avoid this difficulty, several approaches (Oliveira, 1999, Pan, 1993) have been developed to transform the original problem into ε -independent sub-problems, among which, the time-scale decomposition (Oliveira, 1999) is commonly adopted. As an alternative to Riccati equation solution, LMI formulation has been attracting more and more attention of robust control researchers. However, up to the present, it remains an open area solving mixed H$_2$/H$_\infty$ control problems for singular perturbation systems through LMI approach. Garcia et al. Garcia, 1998 proposed a solution to the infinite time near optimal regulator problem (H$_2$ control) for singular perturbation systems through an LMI formulation. A time scale-decomposition was employed on the overall system as well. In (Li, 2001) the problem is formulated into a set of inequalities independent of ε . An algorithm is given to solve this set of inequalities through LMI formulation. But extension of this method to mixed H$_2$/H$_\infty$ control is very difficult. In (Li, 2007) a same approach is used for solving problem with static output feedback instead of state feedback. Combination of different techniques to obtain the different performances is

widely used today (Essounbouli, 2006, Pan, 1993, and Peres, 1994). This method results in hybrid dynamical systems which include continuous and discrete dynamics and a mechanism (supervisor) managing the interaction between these dynamics. In the present paper, the switching mixed H_2/H_∞ state feedback control problems for continuous-time linear singular perturbation systems are solved. The simple design methods of Garcia, 1998 are applied to derive the state-feedback gains, separately for two fast and slow sub-systems. A fuzzy supervisor is proposed for hybrid combination of these controllers to use their advantages and to ensure the required performances and the stability of the closed loop system.

The contribution of the presented work is combining fast and slow sub-system controllers using a supervisor, which manages the gradual transition from one controller to another. This method is applied to use the advantages of each controller. The control signal is obtained via a weighted sum of the two signals given by the slow and fast sub-system controllers. This weighted sum is managed thanks to a fuzzy supervisor, which is adapted to obtain the desired closed loop system performances. So, the fast sub-system controller mainly acts in the transient phase providing a fast dynamic response and enlarging the stability limits of the system, while the slow sub-system controller acts mainly in the steady state to reduce chattering and to maintain the tracking performances. Furthermore, the global stability of the system even if the system switches from one configuration to another (transient to steady state and vice versa) is guaranteed.

The structure of the paper is as follows. Section 2 presents the system definition and the controllers used. In Section 3, the fuzzy supervisor and the proposed control law are described. Stability analysis is demonstrated in Section 4. The design procedure is explained in Section 5 and an example is given to illustrate the efficiency of the proposed method, followed by conclusions in Section 6.

2. Problem Statement

Consider the following linear singularly perturbated system Σ with slow and fast dynamics described in the "singularly perturbated" form:

$$\Sigma : \begin{cases} \dot{x}_{slow} = A_1 x_{slow} + A_2 x_{fast} + B_1 u + B_{w_1} w \\ \varepsilon \dot{x}_{fast} = A_3 x_{slow} + A_4 x_{fast} + B_2 u + B_{w_2} w \\ z = C_{z_1} x_{slow} + C_{z_2} x_{fast} + D_z u \end{cases} \quad (1)$$

where x_{slow}, x_{fast} are the states; $u \in R^{m_1}$ is the control input; $w \in R^{m_2}$ is the disturbance input; $z \in R^{l_z}$ is the output to be regulated; and ε is a

small positive parameter. By introducing the following notations:

$$x = \begin{bmatrix} x_{slow} \\ x_{fast} \end{bmatrix}, A_\varepsilon = \begin{bmatrix} A_1 & A_2 \\ \dfrac{1}{\varepsilon} A_3 & \dfrac{1}{\varepsilon} A_4 \end{bmatrix}$$

$$B_\varepsilon = \begin{bmatrix} B_1 \\ \dfrac{1}{\varepsilon} B_2 \end{bmatrix}, B_{w_\varepsilon} = \begin{bmatrix} B_{w_1} \\ \dfrac{1}{\varepsilon} B_{w_2} \end{bmatrix}, C_z = \begin{bmatrix} C_{z_1} & C_{z_2} \end{bmatrix}$$

(2)

The system Σ can be rewritten into the following compact form:

$$\Sigma : \begin{cases} \dot{x} = A_\varepsilon x + B_\varepsilon u + B_{w_\varepsilon} w \\ z = C_z x + D_z u \end{cases} \quad (3)$$

Applying a static state feedback control:

$$u = Kx \quad (4)$$

leads to the following closed-loop system:

$$\Sigma_{cl} : \begin{cases} \dot{x}_{cl} = A_{cl} x_{cl} + B_{cl} w \\ z = C_{cl} x_{cl} \end{cases} \quad (5)$$

where $A_{cl} = A_\varepsilon + B_\varepsilon K$, $B_{cl} = B_{w_s}$, $C_{cl} = C_z + D_z K$.

Denote the transfer function of the closed-loop system Σ_{cl} from w to z as: $T(s,K) = C_{cl}(sI - A_{cl})^{-1} B_{cl}$. The H_2 norm of $T(s,K)$ is defined by:

$$\left\| T(s,K) \right\|_2 = \frac{\left\| z \right\|_\infty}{\left\| w \right\|_2} \quad (6)$$

and the H_∞ norm of $T(s,K)$ is defined by:

$$\left\| T(s,K) \right\|_\infty = \frac{\left\| z \right\|_2}{\left\| w \right\|_2} \quad (7)$$

2.1. Slow and fast sub-systems

If A_4 be a non- singular matrix, we can decompos original singularly perturbated system (1) to two slow and fast subsystems. The slow subsystem defined letting $\varepsilon = 0$ in second equation of (1) and computing x_{fast} in terms of x_{slow}, u and w, then substituting it in the first equation. Therefore, slow subsystem obtained as follows:

$$\dot{x}_{slow} = A_s x_{slow} + B_s u + B_{w_s} w$$
$$z_{slow} = C_{z_s} x_{slow} + D_s u + D_{w_s} w \quad (8)$$

where [6]

$$A_s = \left(A_1 - A_2 A_4^{-1} A_3 \right), B_s = \left(B_1 - A_2 A_4^{-1} B_2 \right)$$
$$B_{w_s} = \left(B_{w_1} - A_2 A_4^{-1} B_{w_2} \right), C_{z_s} = \left(C_{z_1} - C_{z_2} A_4^{-1} A_3 \right)$$
$$D_s = \left(D_z - C_{z_2} A_4^{-1} B_2 \right), D_{w_s} = -C_{z_{fast}} A_4^{-1} B_{w_2}$$

The fast subsystem of (1) is defined by [6]:

$$\dot{x}_{fast} = A_f x_{fast} + B_f u + B_{w_f} w$$
$$z = C_{z_f} x_{fast} + D_f u \tag{11}$$

Therefore, the overall system is decomposed into slow and fast subsystems. In sequel, these subsystems are used to design slow and fast controller and then are mixed using a fuzzy supervisor to produce a controller for the overall system. In this paper we focus on the suboptimal mixed H_2/H_∞ static state feedback control problem in terms of linear matrix inequalities (LMI).

Lemma 2. 1. [1] (H_2 control problem): Consider overall system (1). The static state feedback control law (4) stabilize closed loop system (5) and achieves a prescribed H2-norm bound $0 < \nu$ for it, if and only if there exists $Q = Q^T > 0, T, Z$ with appropriate dimensions such that:

$$\begin{bmatrix} A_{11} & QC_z^T + T^T D_z^T \\ C_z Q + D_z T & -I \end{bmatrix} < 0$$

$$A_{11} = A_\varepsilon Q + Q A_\varepsilon^T + B_\varepsilon T + T^T B_\varepsilon^T \tag{10}$$

$$\begin{bmatrix} Q & B_{w_\varepsilon} \\ B_{w_\varepsilon}^T & Z \end{bmatrix} > 0, trace(Z) < \nu$$

By solving mentioned LMI's, Q, T and Z will be found and control law (4) is calculated as:

$$K = TQ^{-1} \tag{11}$$

It guarantees that closed loop system is asymptotically stable and H_2-norm (6) is less than ν.

Lemma 2. 2. [1] (H_∞ control problem): The control law (4) stabilize closed loop system (5) and achieves a prescribed H∞- norm bound $0 < \gamma$ for it, if and only if there exists $Q = Q^T > 0$ and T with appropriate dimension such that:

$$\begin{bmatrix} A^{11} & B_{w_\varepsilon} & QC_z^T + T^T D_z^T \\ B_{w_\varepsilon}^T & -I & D_{cl}^T \\ C_z Q + D_z T & D_{cl} & -\gamma^2 I \end{bmatrix} < 0 \tag{12}$$

$$A^{11} = A Q + Q A^T + B_\varepsilon T + T^T B_\varepsilon^T$$

By solving LMI (12), Q and T will be found and control law (4) is calculated from (11).

Lemma 2. 3. [1] (Mixed H_2/H_∞ control problem): The control law (4) satisfies mixed H_2/H_∞ control problem if and only if the following LMI's for $Q = Q^T > 0, T, Z$ and a given positive scalar $\gamma > 0$ are satisfied:

$$\begin{array}{ll} \min & \nu \\ \text{subject to} & (12) \text{ and } (10) \end{array} \tag{13}$$

By solving (13), Q, T, Z and ν are found and the control law (4) is computed from (11).

3. Fuzzy Supervisor

The approach used in this paper for solving mixed H_2/H_∞ control problem for linear singular perturbation system is different from former approaches. We start with an overall linear singular perturbation system and decompose it to slow and fast subsystems. Then we solve mixed H_2/H_∞ control problem for each slow and fast subsystems and find K_{slow}, K_{fast} by solving corresponding LMI's. It is well known that fast subsystem can be a good approximation for transient time of overall system response and slow subsystem can be a good model for steady state time of overall system response. Therefore, fast subsystem controller K_{fast} can be used during the transient time and slow subsystem controller K_{slow} can be used during the steady state, their control actions are combined by means of a weighting factor, $\alpha \in [0 \quad 1]$, representing the output of a fuzzy logic supervisor that takes the tracking error e and its time derivatives $\dot{e}, \ddot{e}, \dots, e^{n-1}$ as inputs.

The fuzzy system is constructed from a collection of fuzzy rules whose jth component can be given in the form:

If e is H_1^j And … And e^{n-1} is H_n^j Then $\alpha = \alpha_j$. Where H_i^j is a fuzzy set and α_j is a singleton.

The fuzzy implication uses the product operation rule. The connective AND is implemented by the minimum operation, whereas fuzzy rules are combined by algebraic addition. Defuzzification is performed using the centroid method. Since the membership functions that define the linguistic terms of the output variable are singletons, the output of the fuzzy system is given by

$$\alpha = \frac{\sum_{i=1}^{m} \alpha_i \prod_{j=1}^{n} \mu_i^j}{\sum_{i=1}^{m} \prod_{j=1}^{n} \mu_i^j}$$

where μ_i^j is the degree of membership of H_i^j and m is the number of fuzzy rules used. The objective of the fuzzy supervisor is to determine the weighting factor, α which gives the participation rate of each control signal. Indeed, when the norm of the tracking error e and its time derivatives $\dot{e}, \ddot{e}, \dots, e^{n-1}$ are small, the plant is governed by the slow subsystem controller K_{slow} ($\alpha = 1$). Conversely, if the error and its derivatives are large, the plant is governed by the fast subsystem controller K_{fast} ($\alpha = 0$). The control action u, is determined by:

$$u = (1-\alpha)u_{fast} + \alpha u_{slow} \tag{14}$$

where

$$u_{slow} = K_{slow} x_{slow}, u_{fast} = K_{fast} x_{fast} \tag{15}$$

Structure of proposed controller with a fuzzy supervisor has been shown in Figure 1.
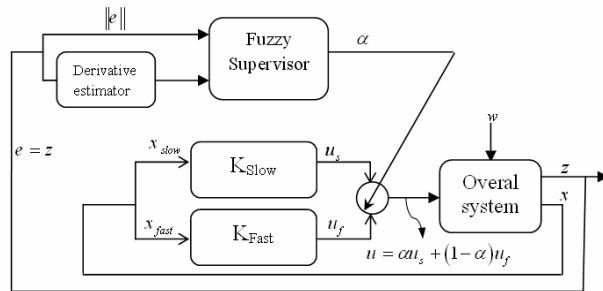
Figure 1. The structure of the proposed controller

4. Stability Analysis

The theorem of Essounbouli et al. [7] is used to prove the global stability of the system. Similar to [7], this theorem is rewritten as follows:

Theorem 4.1. Consider a combined fuzzy logic control system as described in this work. If:

1. There exists a continuously differentiable and radially unbounded scalar function $V > 0$ for each subsystem,

2. Every subsystem gives $\dot{V} < 0$ in its active region,

3. The weighted sum defuzzification method is used, such that for any control input u

$$\min(u_{slow}, u_{fast}) \leq u \leq \max(u_{slow}, u_{fast})$$

Then the resulting control u, given by (14), guarantees the global stability of the closed loop system.

Proof: Satisfying two first conditions guarantees the existence of a Lyapunov function in the active region which is a sufficient condition for ensuring the asymptotic stability of the system during the transition from the fast subsystem controller to the slow subsystem controller. Consider the Lyapunov function $V_{fast} = \zeta^T P_{fast} \zeta$ where P_{fast} is a positive definite matrix and the solution of (13) for fast subsystem and the Lyapunov function $V_{slow} = \zeta^T P_{slow} \zeta$ where P_{slow} is a positive definite matrix and the solution of (13) for slow subsystem. To satisfy the second condition it is enough to choose P_{slow}, P_{fast} such that:

$$P_{slow} \leq P_{fast} \qquad (20)$$

This condition guarantees that in the neighbourhoods of the steady state, the value of the Lyapunov function V_{fast} is greater than that of V_{slow}. To guarantee the third condition, the balancing term α takes its values in the interval [0 1]. Consequently, the three conditions of the above theorem are satisfied and the global stability of the system is guaranteed.

So, The Problem formulation (switching H_2/H_∞ control) will be as:

$$\begin{cases} \min & \left(\|T(s,K)\|_2 \right)_{slow} \\ subject\ to & \left(\|T(s,K)\|_\infty \right)_{slow} < \gamma_{slow} \end{cases} \quad and$$

$$\begin{cases} \min & \left(\|T(s,K)\|_2 \right)_{fast} \\ subject\ to & \left(\|T(s,K)\|_\infty \right)_{fast} < \gamma_{fast} \end{cases}$$

$$while : P_{slow} \leq P_{fast}$$

5. Design Procedure

The design procedure can be summarizing as follows:

Compute slow and fast subsystems of overall system from (8) and (9). Solve control problem (16) for each subsystem with given positive scalars γ_{slow} and γ_{fast} to find K_{slow} and K_{fast} from (11). Compute u_{slow} and u_{fast} from (15). Calculate overall control signal u from (14) that α is governed by fuzzy supervisor according to error and its derivatives. Apply this control signal to (1) and construct closed loop system (5). To construct the fuzzy supervisor, firstly, the fuzzy sets are defined for each input (the error and its derivatives) and output; then, the rule base is elaborated. The error vector is computed and then is injected in the supervisor to determine the value of α to apply to the global control signal.

6. Simulation

To demonstrate the solvability of the various LMIs, simplicity and low conservatives of the proposed method, The formulation of the switching H_2/H_∞ control of the singularly perturbed system is now applied to control the longitudinal flight dynamics of F-8 aircraft model. The longitudinal dynamics of the aircraft exhibits two- time scale properties identifiable by the phugoid (slow) and the short period (fast) mode. The H_2/H_∞ controllers are designed for the longitudinal axis dynamics of the aircraft for the cases of full- order control, fast control and slow control.

The linearized small-perturbation longitudinal equations of the motion and aerodynamics stability derivations are provided in [8]. The longitudinal F-8 aircraft model is for a flight condition of Mach 0.6 ($V_0 = 620\, ft\, s^{-1}$) altitude of 20000 feet, and angle of attack of 0.078 rad. The state variables are v : velocity ($ft\, s^{-1}$); α : angle of attack (rad); q : pitch rate ($rad\, s^{-1}$); θ : pitch angle (rad); and the input variable is: σ stabilator deflection (rad). Here, the slow states are the forward air speed and pitch angle,

while the fast states are the angle of attack and pitch rate. The space model is obtained as [6]:

$$A_1 = \begin{bmatrix} -3 & 1 \\ 1 & -1 \end{bmatrix}, A_2 = \begin{bmatrix} 1.5 & 1 \\ 1 & 1 \end{bmatrix}, A_3 = \begin{bmatrix} 0.5 & 1 \\ 1 & 2 \end{bmatrix}$$

$$A_4 = \begin{bmatrix} -5 & 1 \\ 3 & -4 \end{bmatrix}, B_1 = \begin{bmatrix} 0 & 0 \\ 0 & 0 \end{bmatrix}, B_2 = \begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix}$$

$$B_{w_1} = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \end{bmatrix}, B_{w_2} = \begin{bmatrix} 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix}$$

$$C_{z_1} = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \end{bmatrix}^T, C_{z_2} = \begin{bmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix}^T$$

$$D_Z = \begin{bmatrix} 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix}^T, \varepsilon = 0.1$$

The input disturbance w is zero mean white noise process with $E\left(w\left(t\right)w\left(\tau\right)\right) = \delta\left(t - \tau\right)$ is injected into the system in the interval $t \in \begin{bmatrix} 10 & 30 \end{bmatrix}$.

Following the proposed design method in section 5, the following results are obtained:

$$K_{H_2/H_\infty} = \begin{bmatrix} -1.8265 & -3.9832 & 1.0093 & -0.8187 \\ -1.5087 & -3.2663 & -0.7951 & 0.7210 \end{bmatrix}$$

$$K_{fast} = 10^{-7} \times \begin{bmatrix} 0.4230 & 0.3809 \\ -0.3759 & 0.2860 \end{bmatrix}$$

$$K_{slow} = \begin{bmatrix} -2.1457 & -4.6675 \\ -1.7237 & -3.7761 \end{bmatrix}$$

$$K_{switching} = \begin{bmatrix} \left(1-\alpha\right)K_{fast} & \alpha K_{slow} \end{bmatrix}$$

Only first time derivative of tracking error is used because in practical system, it is difficult to measure the higher order time derivatives of the tracking error. The fuzzy supervisor is constructed by using three fuzzy sets zero, medium and large for the norms of the tracking error and its time derivative. The corresponding membership functions are triangular. For the output, five singletons are selected; very large (VL), large (L), medium (M), small (S) and zero (Z), corresponding to 1, 0.75, 0.5, 0.25 and 0, respectively. Rules are defined in Table 1., for example, a rule in the table can be stated as follows: "IF the norm of the error is medium AND the norm of the error derivative is large, THEN α is zero".

From obtained simulation results in Table 2., it is clear that the proposed method gives better response than conventional overall design method for full order system. In our proposed switching method, with a smaller γ for H_∞ constraint, the H_2 norm is smaller. But both of H_2 and H_∞ norms are increased in conventional overall method. From Figure 2, it is clear that output regulation in our proposed controller is better related to conventional overall controller.
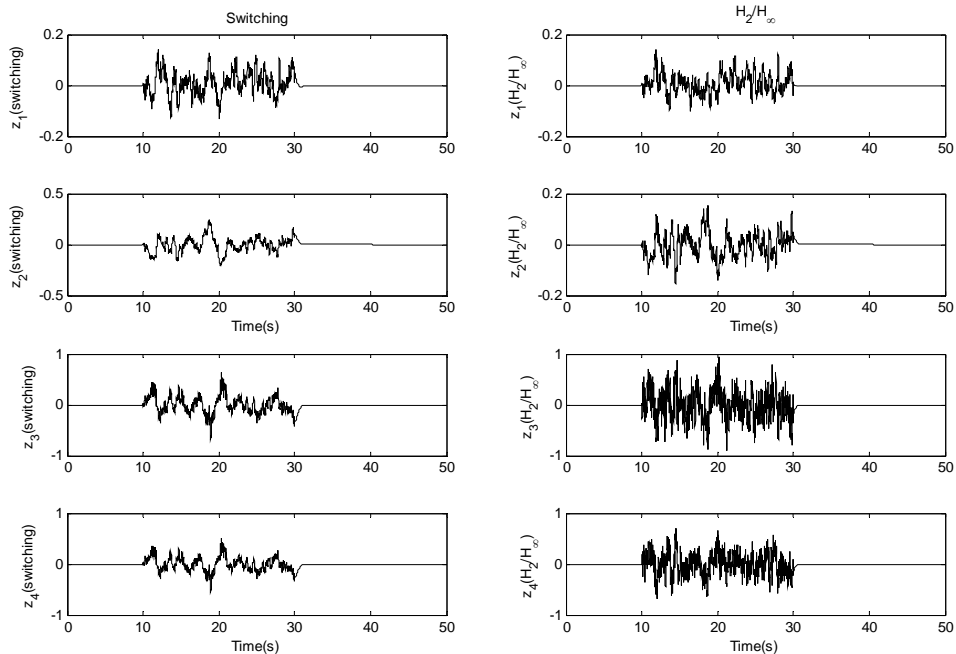
Figure 2. The State Response of simulation

7. Conclusion

In this paper, convex optimization method is used to design the logic based switching H_2/H_∞ controller for a linear singular perturbation system. Proposed controller guarantees the stability of the closed loop system and satisfies the prescribed level of performance indexes for both of H_2 and H_∞ norms. Using the reduced-order fast and slow mode controllers instead of one full-order overall controller with higher order is the main contribution of this paper. A fuzzy supervisor manages both of fast and slow controller performance efficiently such that in spite of switching nature of control scheme, stability of closed loop system is guaranteed and the performance criterion is satisfied. In reality, fast mode controller has a good performance in transient mode (low energy impulse response) and slow mode controller affects the steady mode section and attenuates the low frequency disturbances. Simulation results show that the proposed controller causes the considerable improvement in the overal performance of the closed loop system.

Table 1. The rules of the proposed Supervisor

	|de/dt|			
	Z	VL	L	M
|e|	M	S	S	Z
	L	Z	Z	Z

Table 2. The results of the simulation

	$\sup_w \dfrac{\|z\|_\infty}{\|w\|_2}$	$\sup_w \dfrac{\|z\|_2}{\|w\|_2}$
K_{H_2/H_∞}	10.2309	0.3062
$K_{switching}$	6.341	0.2361

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Politics and Education: A case study of political participation of women in Iran

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Abstract: This article studies the structural obstacles of women's political participation in Iran. The objective of the study has been achieved by answering the question; how structural factors (Education and knowledge) act as obstacles to women's participation in top political positions in Iran? A qualitative case study method is used in the paper and the primary data are collected mainly through in-depth interviews with five informants by using purposive technique. The analysis of the study is supported by resources theory. The findings of the article suggested that inadequate intellectual resources act as the structural obstacles of Iranian women in politics.
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1. Introduction

Political participation is understood as a prerequisite for political development. Accordingly, considering the political participation of women, who constitute half of the world population, can help to advance political development. It has been accepted that real development would not be possible if women were kept away from the policy-making process (Panday, 2008). In fact, "without the active participation of women and the incorporation of women's perspective at all levels of decision making, the goals of equality, development and peace cannot be achieved"(FWCW, 1995; Art 181).

The involvement of women in the political process is also necessary to strengthen and sustain democracy. According to Sun (2004) "...the legitimacy of democratic governance highly depends on the equality of gender representation in the political decision-making process" (p.148). Zeleke (2005) asserts that "...it cannot be denied that a democracy that lacks equal involvement of both men and women is, in effect, a nominal democracy" (p. 8). Furthermore, the European Network of Experts (1997) observed how "a balanced representation of women and men at all levels of decision-making guarantee better government. Because increased participation of women in decision-making will create a new culture and shed new light on how power should be exercised" (p. 8).

Regarding the importance of the presence of women in politics, from inception of the United Nations (UN) in 1945, it has concentrated its efforts in securing, promoting and ensuring women's political participation (Bello, 2003; Jahan, 2006; Khan, 2000).The Universal Declaration of Human Rights in 1948, the International Covenant on Civil and Political Rights in 1966, the Declaration on the Elimination of Discrimination against Women in 1967, the International Women's Year in 1975, Decade for Women: Equality, Development and Peace in 1981, the Beijing Declaration and Platform for Action in 1995 in China and the Millennium Declaration in 2000 are among mechanisms the UN has employed to enhance women's political participation throughout the world (Bello, 2003; UNFPA, 2003; Zeleke, 2005). Although most of member states of the UN signed and committed to perform its goals, regarding the improvement of women's positions in politics, women still remain underrepresented in political power positions in many parts of the world (Uphoff, 2003; Vissandjee, Abdol, Apale, & Dupere, 2006).For instance, the Inter Parliamentary Union (IPU) asserts that in 2003 only twelve women were heads of the states. Accordingly; as a worldwide average, in 2010 women represented 19.2 percent of the seats in congress and 11.3 percent of national cabinet and senior management positions (IPU, 2010).

In most third world countries there has also been great disparity between males and females with respect to their representation in politics (UN, 1989; UNDP, 1995). Islamic Republic of Iran is no exception. Although women constitute half of Iran's total population, their importance and potential role in the country's political and socio-economic development has been overlooked. The constitution of the Islamic Republic guarantees women the right to social and political equality yet a great gap still exists between what the constitution guarantees and actual representation of women on different decision making bodies. A glance at the statistics of the eight terms of the Parliament in Iran illustrates a lower level of political participation of women in comparison with other countries. As table 1 in note shows the rate of participation of Iranian females during the last 8 Parliaments did not exceed 5.6 percent

In addition, political empowerment presence rate of women in political administration and managerial positions is lower in the developing world including East Asia, Latin America and Sub-Saharan Africa compared with developed countries (Jaquette, 1997).Besides the above-mentioned facts, another proof of the disappointing condition of women should be sought in managerial and vocational divisions. From the total number of 17563 positions of governmental management, women occupy only two percent while, the number of male managers in Iran is thirty times more than female managers (Davani, 2005; Khalili, 2007; Naji- Raad, 2003).

Therefore, based on these statistics, that will indicate the unsatisfactory status of women in various aspects only partially, what should be pointed out is that women's negligible participation in various social and political divisions can have disastrous consequences, deprive women from important rights and responsibilities as citizens, discard their viewpoints in making decisions and passing and executing rules as half the population of members of the society, deprive them of their right in national budgets and resources, and most importantly, render the society deprived of their skills, knowledge and viewpoints (Molaverdi, 2005). Consequently, the present paper focuses on the political participation of women after the Iranian Revolution and attempts to study structural factors that inhibit the participation of women in political decision-making. Our analysis is supported by resources theory that provided by Blood & Wolf (1960).

The findings of this article will benefit women' studies development and will draw much attention of governments and civic institutions to rethinking women who play important role in political development of societies. Also this study will contribute to future research on similar topics.Our argument is developed in four sections. First, we explain the method that is applied in this study. In our second section, we describe concept of political participation. In third section, we study resources theory and how we can approach its using critical frame analysis. In the forth, we discuss our findings. Last, we state the conclusion the study.

2. Methodology

The researcher elected qualitative case study method as the suitable research method to answer the research questions, which flowed from the research problem. This method made it possible for the researcher to utilize a flexible approach in her attempt to explore the experiences of informants about obstacles of political participation of Iranian women in high level of decision making. Purposive technique also enabled the researcher to include participants in the context to which the study was related, answering the research question through informant in the field of political participation of women in Iran. The informants of the study are selected from presidential advisors and political organizations such as; political party, city council, and Parliament.

In addition, data collection took place by means of in-depth interviews. Five individual interviews were carried out by the researcher during one month; from 3 October 2009 to 3 November 2009. The interviews were tape-recorded, then transcribed, and field notes were added to the transcripts. Following this, descriptive data analysis method was employed to transform the data into a workable form. Trustworthiness and ethical practice received attention during the whole process. Furthermore, the data of this study is gathered through printed and unprinted media, published and unpublished articles, journals, books, and reports. Holloway and Wheeler (1997) are of the opinion that the literature should be used as an aid to compare and contrast with the themes that emerge from the study. The literature was therefore used as a verification tool, and enabled the researcher to verify the major themes with the relevant literature.

3. Concept of Political Participation

Political participation is an interesting topic that has drawn much attention of sociologists and political scientists. According to Orum (1983, p. 131), "One of the foremost concerns of contemporary political sociology is the manner and the degree of citizen participation in politics". In the other hand, Lerner (1958) distinguishes traditional societies from modern societies on the basis of "participation (P 51).

Besides, Huntington and Nelson (1976) regard political participation as "Any activities by private citizens designed to influence government decision-making. They may be individual/collective, organized/spontaneous, sustained/sporadic, peaceful/violent, legal/illegal and effective or ineffective" (p. 3). In addition, according to Weiner(1971) political participation refers to "Any voluntary action, successful or unsuccessful, organized or unorganized, employing legitimate or illegitimate methods intends to influence the choice of public policies, the administration of public affairs or the choice of political leaders at any level of government, local or national"(p. 164). Verba and Nie (1972) also, define political participation as activities by private citizens that are directly aimed at influencing the election of government personnel and/or the actions they take.

Lastly, Milbrath(1965) classifies political participation in three kind of activities; 1) *Gladiatorial* activities relating to those most active in politics and set at the pinnacle of various kinds of political participation. Being a candidate for office and holding public and party office are embraced in gladiatorial

activities; 2) *Transitional* activities, in this type of political participation; people are less active in politics. Some activities such as attending a political meeting, wearing a button or putting a sticker on the car and making a monetary contribution to a party or candidate include in transitional activities and, 3) *Spectator* activities involve those who don't interfere in politics, except in voting and exposing oneself to political stimuli. Since the focus of this article will be political participation of women who occupy political top positions in Iran, therefore for the purpose of this study the term political participation will be limited to persons held positions of leadership at upper levels of the political decision making.

4. Theoretical Framework

Decision making power of women in politics is influences by resources including intellectual (education, knowledge, information, and ideas) resources. Resources theory is developed by Blood and Wolf (1960) to explain the relationship between resources (intellectual and information) and decision making power. The main argument of this theory is that in society if a person has more resources he or she will has more power to exercise in decision making within the politics. This theory is supported by various authors as well (Buric & Andjelka, 1960; kandel & Lesser, 1972; Lamouse, 1969; Michel, 1967).In fact, discussed theory is employed to examine the structural factors (education and information) that act as obstacles of women's participation in high political positions in Iran.

5. Findings and Discussion

The findings of this study are constructed using the researcher's interpretation of the experiences of five women who hold high-level positions in the politics of Iran. In fact the findings of the study present the main theme, which has been obtained from the participants, their follow-up comments, and the researcher's notes.

There is a main theme that is identified by this study: educational obstacles. This main theme came from the informants, who were asked to describe their experiences of working in high political positions in government, their best and worst experiences of working as a politician woman, the lessons they have learned from work, and their suggestions to improve the conditions of women in political arenas in Iran.

According to Nijhoff (1992) structural barriers refer to educational and also financial levels. From the in-depth interviews with high level women in the politics of Iran it was found that most of them believed that Iranian women to achieve high levels of political decision-making are facing with structural barriers. Therefore in this article, was reviewed insights and experiences of informants regarding to structural

barriers of political participation of women in one main theme; educational obstacles.

5.1 Educational Obstacles

It needless to say that educated individuals have more information and knowledge on government and politics and because of it develop an ability to enter politics. Therefore it is important to study the role of education on the entry of women into politics from informants' point of views. It is need to say that in this study purpose of training is educational level, awareness and knowledge of women in politics that named intellectual resources of women.

When the researcher asked informants about the role of education on women's political participation in high level of decision-making, most of them knew that so effective and emphasized women without education, awareness and capabilities cannot achieve to political posts. One of the informants said;

> With training and awareness to women, their performance will be increased because if women do not be training, strong and no interning to management field, women cannot obtain experiences, as a result they ignore of taking a political post. (Political party member).

Another informant asserted that "Education as one of the greatest factors enhances influences women's chances of obtaining her exercise of legal and political rights and her chance of acquiring political power". (City council member) Hence she concludes that "when women's educational level is lower on average than men it reduces women's chances of holding office because it leads to a smaller pool of potential activities".Another informant also mentioned that "Education provides skills that are necessary to comprehend and participate in the political world. Individuals with higher education have more complex cognitive capabilities and thus are able to learn more easily about the political world than less educated individuals". (Advisor to President).

Above statements were supported by literature. From the literature it seems evident that formal education should be strongly associated with political participation for women and for men. For example, the American sociologists Burns, Schlozman and Verba (2001) assert that education is an "especially powerful predictor of political participation" (p. 286). Besides, they identify a range of direct and indirect effects that formal education has upon political participation. According to them, its direct effects of education include an increase the communication skills that is useful to political analysis and its indirect effects are

include the benefits of voluntary engagement that provide people for politics

In addition, the data suggested less experience and lack of confidence as reasons for the negligible presence of women in high level decision-making positions. The study showed these elements are related to a lack of education and awareness of women in politics. As an example, A member of political party explained how in a managerial section a woman without any educational and management experience becomes a colleague with a man with 20 years experience in management. As a result, the community's trust in women's success in management will be negative, while interest in women's employment in managerial levels will be low. As a consequence, women should engage gradually and continuously with management issues and take such managerial experiences with them, and then with their increasing confidence and experience, women can get themselves into the higher level posts.

On the other hand, some of the informants stressed on education role in the family,where school and university increase a women's role for taking key responsibilities in high levels in the public sphere. This is reflected in the statement of advisor to president when she said mainstream education is essential to improving a women's knowledge, but has less relevance to this fundamental factor, namely, developing forms of political sociability through education. Iranian people do not instruct their children, especially their daughters in how to become a capable, competent and responsible manager. This Informant also mentioned the role of educational institutions in enhancing women's experiences for politics;

> …there are not any comprehensive and targeted programs in (Iranian) universities for enabling girls and even is not provided suitable areas for management practice and experience… if the research has been done in this field in universities it is not practical and applied school and university to increase women's role for taking important responsibilities in high levels in public spheres.

The literature also approved the role of educational centres in increasing women's presence in politics. The Study of Enayat (2001) carried out in Iran showed educational institutions are important agents of political socialization of women for participation in politics. Data in this research collected through structured questionnaires and interviews revealed more than half the informants (58.0 percent) were interested in politics when they were high school students or had obtained a high school degree. According to this study

another 42.0 percent have written a treatise in politics, either when they were university students or had acquired the B.A or B.S level of education.

The data also showed education is an important variable in overcoming traditional gender ideology. Some informants noted education can change the traditional view of society which considers different roles for men and women, and overlooks any places for women in the category of political decision-making and power. Most researchers (Choudry, 1995; Khanam, 2003; Salfilios, 1969) also argued education is key when it comes to tackling traditional ideas on gender. Informant I confirmed this idea with an experience:

> Education and awareness are the most important tools for changing traditional attitudes and patriarchal culture about political participation of women. For example, in elections publicity I explained to people that candidate should be expert and capable so the gender is not important. I remember this statement caused many women could participate in the campaign publicity.

From the data that was also found that education is an important variable in overcoming traditional gender ideology. Some of informants noted that education can be change the traditional view of society which considers different roles for men and women, and no consider any places for women in the category of political decision-making power. Most of researchers (Choudry, 1995; Khanam, 2003; Salfilios, 1969) also argued that education is an important variable in overcoming traditional gender ideology.

This theme indicated intellectual resources have an important role in the process of political participation of women. Education at different levels, from family to school and then university, can help raise awareness, enabling women to gain experience and self-confidence for interning the political decision-making area. It also will gradually change gender stereotypes, the patriarchal culture and traditional attitudes about the political participation of women. The data also shows how education is one of the greatest forces for change in women's political life. Indeed, a higher level of formal education facilitates involvement in political activities. Higher educated women have more political decision making power and also have a greater chance to enter the higher political circles such as parliament, the ministry and so on. For that reason, when a woman's educational level is lower on average than a man's, it reduces women's chances of holding office.

Appendix
Table 1: Female and male members of the Iranian Parliament during 8 terms
(Statistics Center of Iran, 2009)

Period	Year	Total Seats	Female		Male	
			No.	%	No.	%
First	1980	270	4	1.4	266	98.6
Second	1984	270	4	1.4	266	98.6
Third	1988	270	4	1.4	266	98.6
Fourth	1992	270	9	3.3	261	96.7
Fifth	1996	270	14	5.2	256	94.8
Sixth	2000	270	12	4.4	258	95.6
Seventh	2004	290	14	4.8	276	95.2
Eighth	2008	290	8	2.7	282	97.3

6. Conclusion

The purpose of this study was to identify the obstacles of high level women's participation in the politics of Iran. In this article was applied the resources theory by analyzing the prevailing conditions in Iran.

The study is based mainly on primary data collection. It used in-depth interviews regarding the structural obstacles of high level women's participation in the politics of Iran. The study population was purposively selected from the two political zones and limited to presidential advisor and political organizations such as political party and parliament. Informant interviews were recorded, and after the tapes had been transcribed, important themes were identified and interpreted.The objective of the article was to identify the structural obstacles of political participation of women in high decision- making power in Iran. The data claimed that lack of adequate intellectual resources act as the structural obstacles of Iranian women in top political positions.

From the data it was observed that education is one of the greatest forces for change in women's political life. Indeed, a higher level of formal education facilitates involvement in political activities. The higher educated women have more political decision making power and also have more chance to enter in high political spheres such as parliament, ministry and so on. For that reason, when women's educational level lower on average than men's it reduces women's chances of holding office. This study also found agreement among the informants that women faced by structural obstacles in their entrance into the top political position. The most important contributions of this study were the highlight that the level of education of women was significantly associated with their roles in political power decisions-making. This finding provides strong support to the resources theory, as the findings indicated that when women are educated they are more involved in power decision making. Therefore, educational resources together appear to contribute more effectively to increase women's self- reliance and enhance their role in decision making within political power. And this study promoted a better theoretical understanding and knowledge of the area of political participation of women; therefore, this study also contributed in broader terms to women's studies.

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Effect of Social Capital on welfare of Rural Households in South-western States, Nigeria

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Abstract: This study conducted an empirical investigation on the effects of social capital on welfare of rural households in the southwestern, Nigeria. Multistage sampling technique was employed. The data for the study were collected with the aid of structured questionnaires from three hundred and ninety nine households in Ekiti and Osun states. The data were analyzed using descriptive and regression techniques. The average age of the households head in the study areas was 41.3 years. Households belong to at least two associations and the most important one is religion association. Average household size is 5.0 members and has about 66.7 percent index of participation. However, the level of heterogeneity index is 54.7 percent while meeting attendance index of the households represents halves of the maximum recorded. Cash contribution index is surprisingly low with value of 16.8 percent while labour contribution index is 66.3 percent and with mean social capital value of 15.21. The result of regression show that location, marital status, household size, primary occupation cash contribution index and heterogeneity index of households significantly impacted welfare. The use of instrumental variable lead to an increase in the value of adjustment R^2 from 0.2302 to 0.2564 compared with the use of the actual social capital index. Policy that enhances better strong social ties of poor households is recommended for poverty alleviation.

Introduction

Today, as other continents continue to register sustainable economic growth and development, Africa is not only lagging behind but is trapped in a vicious circle of borrowing and donor dependency syndrome which is one of the major causes of poverty and lack of development. Africa has perpetually failed to focus its development efforts on the optimum utilisation of the immense natural resources that many countries are endowed with to turn it into wealth to propel their economies and people towards a high level of economic and social development and as a consequence eliminate pervasive poverty. Records reveal that more than one billion people across the world today live in poverty with Nigeria harbouring over 60 million of this number, six percent of the total figure (Nwachukwu, 2006). As the most populous and one of the largest countries in sub-Sahara Africa, the issue of poverty in Nigeria is of concern not only in itself but also as a challenge for poverty reduction mandate in the entire African continent. Though, Nigeria is blessed with abundant physical and human resources, there had been progressively worsening welfare and poverty condition of its nationals (Okunmadewa, 2001). Statistics from the National Bureau of Statistics (NBS) indicate that the poverty situation in the country which has been increasing since 1960 (15.0 percent), 1980 (28.1 percent), 1985 (46 percent), 1992 (42.8 percent), and 1996 (65.5 percent)

respectively, dropped to 54.4 percent in 2004. At the 2006 International Day for the Eradication of Poverty (IDEP) event in Abuja, tagged 'Working Together out of poverty', Magnus Kpakol, National Coordinator of National Poverty Alleviation Programme (NAPEP) affirmed that poverty rate in Nigeria was as high as 54.4 percent identifying the North East region of the country as the poorest in the country, rating about 72.2 percent on the poverty ladder. It is followed closely by the North West zone with 71.2 percent; North Central, 67.0 percent, South-West 43.0 percent, South-South 35.1 percent, and South East 26.7 respectively. However, research findings and empirical evidence have shown that significant poverty reduction are possible and have indeed occurred in many developing countries where on the average majority of the population is considered to be poor.

The effort to alleviate poverty traditionally has used and was based on natural capital, physical or produced capital, and human capital (Ismawan, 2000). Together they constitute the wealth of nations and form the basis of economic prosperity. The missing link three types of capital is social capital. Putnam, (1993) views it as a set of "horizontal associations" between people: social networks ("networks of civic engagement") and associated norms that have an effect on the productivity of the community. Two empirical presumptions underlie this concept are norms and networks are empirically associated, and these have

important economic consequences. The key feature of social capital is that it facilitates coordination and cooperation for the mutual benefit of the members of the association. The most encompassing view of social capital includes the social and political environment that enables norms to develop and shapes the social structure. There is growing evidence that social capital can have an impact on development outcomes – growth, equity, and poverty alleviation. Associations and institutions provide an informal framework to organize information sharing, coordination of activities, and collective decision-making. There is growing evidence that social capital is an element for sustainable development due to the role it plays in managing risks, shocks and opportunities. It is therefore holds strong position to confront poverty and vulnerability (Narayan, 1997), resolve disputes (Schafft and Brown, 2000) and share beneficial information(Isham, 1999; Rauch and Casella, 2001).crucial to understanding economic performance (North,1990), reduces transaction costs (Ben-Porath, 1980; Pollack 1985), provides contract enforcement (Johnson et al., 2000), enables credit constrained households access to funds (Besley, 1993), fosters adoption of new production technologies (Narayan and Pritchett, 1997; Isham, 2002), and more importantly, provides avenues for risk sharing (Rosenzweig, 1988). In 1994, government and the civil society in Nigeria, with the support of the donor agencies devoted considerable resources at reducing poverty and the outcome of which led to the formulation of the draft national strategy for poverty alleviation code named "Community Action Programme for Poverty Alleviation" (CAPPA) in 1996 (Okunmadewa et al., 2005,Yusuf, 2008). However, efforts at poverty reduction have largely remained unfelt by the poor. While the emphasis in most of the interventions is on provision of physical infrastructure to support the poor and the acquisition of human capital, there has been little or no consideration for the institutional development of local level institutions or mechanism to ensure delivery of support (financial services) to the poor. The absence of such institutions and the weakness of existing ones largely disenfranchised the poor from participating in the decision making process of interventions and issues that affect their welfare (Okunmadewa et al., 2005 and Yusuf, 2008). Some recent studies do indicate that local institutional strengthening through the active participation of the poor in project design and implementation is a necessary factor in poverty reduction in Nigeria. This recognition probably explains the promotion of group formation as an important requirement for the poor to benefit from some of the public instituted poverty reduction programme. This study examines the effects of social capital on welfare of rural households in the south western, Nigeria.

Materials and Methods

The south-western part of Nigeria represents a geographical area covering between Latitude 50° and 90° N and has a land area of approximately 114,271 km^{2} representing 12% of the country's land mass and comprises of six States Ekiti, Oyo, Osun, Ogun, Ondo and Lagos. The South west of Nigeria falls on Latitude 6^{0} to the North and Latitude 4^{0} to the South. It is marked by Longitude 4^{0} to the West and 6^{0} to the East. The total population is 15,456,789 and more than 96% of the population is Yorubas (NPC, 2006). This study was carried out in Ekiti and Osun states, southwest part of Nigeria. The states were chosen because they rank high among the poor states in south western part of Nigeria. They are both Community Poverty Reduction Programme (CPRP) states (Ekiti state being funded by the World Bank and Osun State by the African Development Bank). Ekiti and Osun were carved out of Ondo and Oyo states on 1st October 1996 and 27th of August, 1991 respectively. Ekiti State has 16 Local Government Areas (LGAs), while Osun has 30 LGAs. Ekiti and Osun states have population of about 2,384,212 and 3,423,536 and cover areas of 5,433.00 and 8,882.55sq km respectively (NPC.2006). Agriculture is a dominant economic activity and main source of employment in the states providing employment and income for more than 75.0 per cent of the population. The people are predominantly farmers, while women engage in food processing, trading and in addition, farming. The states have distinct wet and dry seasons, which characterize its humid tropical climate, with the dry season extending from November to March. Annual rainfall varies from about 500 mm in the northern belt to 1,100 mm in the forest belt.

Sources of Data and sampling procedure:

Primary data were collected for the purpose of this study by use of structured questionnaires drawn microcredit household groups of States' National Poverty Alleviation Programme (NAPEP).   The questions were based on both personal and household/dwelling characteristics, membership of associations, participation in the local level institution activities, productive activities and household consumption expenditure details in the last one-month prior to survey in these states.

Multistage sampling technique was employed for this study. Ekiti and Osun states were randomly selected among the states in the South western geopolitical zone. The second stage involves the random selection of two Local Government Areas from each of the three senatorial areas of the two states. This was necessary for equal representation of the

households of the micro credit groups. The third stage involves the random selection of microcredit groups in each of the selected local government areas depending on the number in each LGA. Hence, the expected number of microcredit groups to choose is function of the number of micro credit groups available in a particular local government area (probability proportionate to size). The proportionality factor used in the selection of micro credit groups is stated as:

$$X_i = n/N*30 \quad \text{……………………………………………… (1)}$$

Where i= number of micro credit groups to be sampled
n = number of micro credit group in the particular local Government Area
N = total number of micro credit in all the local government Areas

Hence, the last stage of sampling involved the random selection of households in each of selected micro credit groups based on proportionate to size and the interview was carried on them. In all, a total of four hundred and fifty (465) households head were interviewed. Out of the total of Four hundred and fifty questionnaire distributed, only three hundred and ninety nine that were retrieved have meaningful information for analysis.

Analytical Technique:
The analytical framework earlier applied by Narayan and Prichett (1997) and Grootaert (1999) and was used by Okunmadewa et al, (2005), Okunmadewa et al, (2007) and Yusuf, (2008) was used to analyze social capital and its influence on welfare of rural micro credit household. The conventional model of household economic behaviour under constrained utility maximization was used to relate the level of household expenditure (as money – metric indicator of welfare) directly to the exogenous asset endowments of the household and variables describing the social and economic environment in which the household makes decision. The micro credit household welfare is hypothesized to be influence by the independent variables as represented in the equation below:

$Ln\beta_i = \mu_i + SC_i + HC_i + OC_i + X_i + Z_i + _i$ ………………………………….(2)

Where β_i = Household expenditure per capita of micro credit household i
SC_i = Household endowment of social capital
HC_i = Household endowment of Human Capital
OC_i = Household endowment of other assets
X_i = a vector of household characteristics
Z_i = a vector of village characteristics
$_{ii}$ = error term

Social capital Variable:
S_1 = Heterogeneity index of associations (%)
S_2 = Meeting attendance index of households to associations (%)
S_3 = Decision making Index (%)
S_4 = Membership density of households in association (%)
S_5 = Cash contribution index of households to associations (%)
S_5 = Labour contribution index of households to associations (%)
Human Capital:
HC_1 = Years of formal educational of household head (years)
OC_1 = Household asset endowment (total assets value of household) (Naira)
Household Characteristics:
H_1 = Age of household head (Year)
H_2 = Age squared of household head to capture life cycle of household welfare (Year)
H_3 = Gender of household head (D=1 for male, otherwise D=0)
H_4 = Household size (Continuous)
H_5 = Marital status (D=1 if Married, 0=Otherwise)
H_6 = Primary occupation (D=1 if Farming, 0= otherwise)
Regional Characteristic:
Z_1 = Locality (D= 1 if rural, 0=Otherwise)

The key feature of the model is the assumption that social capital is truly "capital" i.e. a stock, which generates a measurable return (flow of income) to the household. Social capital has many "capital features: it requires resources (especially time) to be produced and it is subject to accumulation and

destruction. The effect of destruction of social capital is evident in the work of Rose (1995) on Russia and former Yugoslavia. Much social capital is built during interactions, which occur for social, religious, or cultural reasons. The key assumption is that the network built through these interactions has measurable benefits to the participating individuals, and lead, directly or indirectly, to a higher level of well-being. There is an impact assumption that social capital is embodied in the members of the household. This conforms to the position advocated by Portes (1998), which highlights that, although the source of social capital is the relationship among a group of individuals, the capital itself is an individual asset.

Variable Definitions:

The effectiveness with which social capital, in the form of local associations, can fulfill its role in disseminating information, reducing opportunistic behaviour, and facilitating collective decision making depends on many aspects of the association, reflecting its structure, its membership and its functioning. For this study we focus on six of the indices adopted by Grootaert and Narayan, (2000),,Okunmadewa et al, (2005), Okunmadewa et al (2007) and Yusuf, (2008)
The social capital (SC) variables that were used in the regression analysis include: density of membership, heterogeneity index, labour contribution, cash contribution, meeting attendance index and decision making index. The measurement of each is as described below.
(1) Density of membership. The number of memberships of each household in existing associations measures this. Household members were asked which associations they were members of.
(2) Heterogeneity index. The questionnaire identifies the three most important associations for each household. For those associations, a number of supplementary questions were asked including about the internal homogeneity of the group. This was rated according to eight criteria: neighbourhood, kin group, occupation, economic status, religion, gender, age, and level of education. On that basis, we constructed a score ranging from 0 to 8 for each of the three associations (a value of one on each criterion indicated that members of the association were "mostly from different" kin groups, economic status, etc.). The score of the three associations was averaged for each household and the resulting index was re-scaled from 0 to 100 (whereby 100 correspond to the highest possible value of the index).
(3) Decision making index. It has been argued that associations which follow a democratic pattern of decision making are more effective than others. The questionnaire asked association members to evaluate subjectively whether they were "very active"

"somewhat active" or "not very active" in the group's decision making. This response was scaled from 2 to 0 respectively, and averaged across the three most important groups in each household. The resulting index was re-scaled from 0 to 100.
(4) Cash contribution: This was obtained by the summation of the total cash contributed to the various associations which the household belong. The actual cash contribution for each household is rescaled by dividing this amount by the maximum fee amount in the data and multiplying the resultant fraction by 100.
(5) Labour Contribution score: This is the number of days that individual members belonging to institution claimed to have worked for their institutions. This represents total numbers of days worked by household members. This is also rescaled to 100 using the same method of cash contribution.
(6) Meeting Attendance: This is obtained by summing up of attendance of the household members at meeting and relating it to the number of scheduled meetings of the associations. The value is multiplied by 100.
Aggregate social capital index: This is obtained by the multiplication of density of membership, heterogeneity index and decision making index otherwise
The Human Capital (HC) is highest level of education attained by the micro credit household head.
Household Asset (OC) defines as a proportion of total listed asset that the household is endowed with. It is captured by finding the natural logarithm of the percentage asset endowment.

Result and Discussions

Among the most important demographic variables and social capital considered are: the respondent location, age, gender, marital status, occupation, asset endowment household size, membership of association and SC (Density of membership, Meeting attendance, heterogeneity index, Labour contribution score, Cash contribution score, Decision making index) The descriptive analysis of socioeconomic and demographic characteristics of respondents is given in Table 1. The average age of the households head in the study areas was 41.3 years. This indicates that a higher proportion of sampled household heads in the South West Nigeria area are in their active and productive years. Household size in the area of study is about 5 persons per household. Majority of households were married. The results of respondent's educational status reveal that majority of the respondent household heads have no formal education.

Table 1: Socio economic characteristic of households

Variable	Respondent Household		
Age	Frequency	%	
< than 30	70	17.5	
30 – 40	159	39.8	
41 – 50	93	23.3	
> than 50	77	19.3	
Total	399	100.0	Mean=41.3, SD=10.2
Household Size			
1 – 3	74	18.5	
4 – 8	293	73.5	
> than 8	32	8.0	Mean=5.1, SD=0.9
Total	399	100.0	

Field Survey 2007

The table 2 shows the activities of the households in the Local Level Institutions (LLIs). Six dimensions of social capital were examined. These are: membership density of the household in local level institution, cash contribution, heterogeneity index, labour contribution and decision making index. The result shows that household belong to at least two associations and has about 66.7 percent index of participation at decision making. However, the level of heterogeneity is 54.7 percent while average household attend half of total meetings. Cash contribution index is surprisingly low with value of 16.8 percent while labour contribution index of 66.3 percent The result also shows the mean social capital value of 15.21.

Table 2 Socioeconomic Characteristic and Dimensions of Social capital

Social Capital	Mean	Minimum	Maximum	Standard Deviation
Density of Membership	52.3	25.0	100.0	10.7
Decision Making Index	66.7	20.0	70.5	0.1
Heterogeneity Index	54.7	20.0	80.0	15.5
Meeting Attendance Index	49.8	0	100.0	32.0
Cash Contribution Index	16.8	20.0	100.0	10.0
Labour Contribution Index	66.3	25.0	100.0	30.5
Social capital score	15.2	2.9	42.8	30.6

Table 8 below shows the effect of human capital, multiplicative and additives social capital indices on household welfare proxied by per capita expenditure. The use of both multiplicative and social capital and additive social capital indices is premised on the fact that conceptual and theoretical underpinning of social capital is not as develop to proffer justification for the use of one method instead of the other. Narayan and Prichett (1997), Okunmadewa (2005) and Yusuf (2008) used both approaches and conclude that additive and interactive variables are valid approaches for introducing social capital in household behavioral model.

Column one of the result in the table 3 below indicates that the basic reduced form model of the household welfare excluding an social capital variables explain 22.4 percent of variance in the household welfare (human capital and demographic factors)

The result indicates that farming as an enterprise reduces welfare of the household. Households with large family size have their welfare reduces for instance; the result indicates that an additional member to household will decrease their welfare by 6.8 percent. Married households, all things being the same have their welfare improved by 6.2 percent. The significant coefficient of the location variable indicates that there remain important location specific determinants of household welfare which were not captured by the model.

Column two shows the result of introduction of a multiplicative social capital variable. This variable led to the slight improvement in the value of explanatory power of the model to 23.02 or 0.7 percent point. At a mean social capital index 15.2 percent, the coefficient of the variables shows that a one unit increase in social capital would increase household per capita expenditure by 0.05 percent.

Column three introduces the six social capital dimensions separately. This leads to a slight increase in the models ability to explain the variance of welfare relative to the apex approach. This reveals the importance of the various dimensions of the social capital specified in the model. The result shows that household increase cash contribution score is associated with a 0.34% higher level of household welfare. Higher heterogeneity index of the

household indicates increased welfare. The significance of the heterogeneity index and cash contribution index are positively affect welfare of households.

Heterogeneity enhances flow of information (credit information) as people of different background come together in group. Hence, a unit increase in both cash contribution and heterogeneity index would induce a very low 0.34 and 0.37 respectively but significant improvement in per capital expenditure.

In the column four, cash contribution was removed from the social capital dimensions because of it endogenous nature. Heterogeneity index indicates highly significance value at 10% level. The explanatory power of the model declined and hence the coefficient of heterogeneity index slightly decreases.

Table 3: Results of social capital on welfare

	Basic Model	Social Capital Multiplicative	Social Capital Additives	Social Capital Additives[a]
Variable	Coefficient	Coefficient	Coefficient	Coefficient
Constant	3.89810	3.84991	3.674408	3.769256
	(19.54)***	(19.25)***	(11.33)***	(11.31)***
Age	0.00020	0.0003747	-0.000078	-0.0016771
	(0.02)	(0.04)	(-0.01)	(-0.20)
AgeSquared	-9.35E-06	-1.24E-05	-2.27E-06	0.0000164
	(-0.10)	(-0.14)	(0.05)	(0.19)
Location	0.06258	0.0652072	0.0311938	0.0482076
	(2.03)*	(2.13)*	(1.05)	(1.58)
Gender	-0.02288	-0.2057744	-0.0170431	-0.0142869
	(-0.74)	(-0.69)	(-0.60)	(-0.49)
Marital status	0.06272	0.0569381	-0.0448358	0.049571
	(1.86)*	(1.69)*	(1.26)	(1.50)
Household size	-0.068967	-0.0696629	-0.0649601	-0.0671322
	(-10.26)***	(-10.39)***	(-10.17)***	(-10.18)***
Primary occupation	-0.109708	-0.1104499	-0.1065245	-0.12017152
	(-3..24)***	(-3.28)***	(-3.31)***	(-3.64)***
Asset value	0.0567271	0.0571847	-0.0102973	0.0258948
	(0.88)	(0.890	(-0.20)	(0.41)
Social capital		0.0005536		
		(2.06)*		
Density of membership			-0.00554499	-0.0045207
			(-0.38)	(-0.33)
Cash contribution			0.0034236	
			(4.79)***	
Labour contribution index			0.0041925	0.0061887
			(0.40)	(0.53)
Decision index			-0.393529	-0.7422624
			(-0.21)	(-0.39)
Heterogeneity			0.0037774	0.0047467
			(4.09)***	(5.11)***
Meeting attendance index			-0.000131	-0.001591
			(-0.31)	(-0.39)
F-Statistic	15.33	14.23	13.71	12.21
R Adjusted	0.2336	0.2302	0.3087	0.2680
Observations	399	399	399	399

a. Cash contribution score was removed because of its dependence on income and by extension the per capita expenditure

Social capital and household welfare: Two-Way Causality?

In order to test empirically whether social capital is truly consumption good like human capital and it is also an input in households production function (Grootaert 1999). The instrument chosen is a multiplicative index of the

members of the three most important local level institutions that household belongs to whether they are of the same religion culture or trust using the aggregate social capital by instrument variable index of religion. Index of religion is independent on income of individual. This provides the basis for determining the direction of causality between social capital and welfare. This informs the use of religion in this study. The table 4 below presents the result of instrumental variable. The use of instrumental variable lead to an increase in the value of adjustment R^2 from 0.2302 to 0.2564 compared with the use of the actual social capital index in addition the instrument variable method leads to the higher coefficient (0.003936) for the social capital index than in the OLS method where it was (0.000554). The higher social capital index is an evidence of improvement and this confirms the exogeneity of social capital. Hence, this result is in agreement with Narayan and Pichett (1997) and Yusuf (2008). The result shows that one percent increase in the level of instrumented social capital leads to 0.39 increase in household expenditure. The increase recorded represents 0.034 percent point higher than the value for the OLS estimation

Table 4: Social capital and household welfare: Two-Way Causality?

Variable	With Multiplicative Social Capital	Instrumental Variable
	Coefficient	Coefficient
Constant	3.84991 (19.25)***	3.865053 (1966)***
Age	0.0003747 (0.04)	-0.0021563 (-0.26)
AgeSquared	-1.24E-05 (-0.14)	0.0000116 (0.13)
Location	0.0652072 (2.13)*	0.0769791 (2.54)*
Gender	-0.2057744 (-0.69)	-0.0245983 (-0.84)
Marital status	0.0569381 (1.69)*	0.0580471 ((1.75)*
Household size	-0.0696629 (-10.39)***	-0.0697274 (-10.58)***
Primary occupation	-0.1104499 (-3.28)***	-0.1160796 (-3.50)***
Asset Value	0.0571847 (0.890	0.0473046 (0.75)
Social capital	0.0005536 (2.06)*	0.0039360 (2.40)*
F-Statistic	14.23	14.72
R-Adjusted	0.2302	0.2568
Observation	399	399

Conclusion of the study:

Based on the empirical evidence emanating from both descriptive and inferential statistics employed for this study, the following conclusions can be drawn on the findings: Six dimensions of social capital were examined. These are: the percentage of members of the household belonging to local level institution, cash contribution index, heterogeneity index, labour contribution index and decision making index. Household belong to at least two associations. The most important association in the area is religion association followed by cooperative and community based association respectively. Average household size is about 5.0 members and has about 66.7 percent index of participation in decision making. However, the level of heterogeneity index is 54.7 percent while meeting attendance index of the households represents halves of the maximum recorded. Cash contribution index is surprisingly low with value of 16.8 percent while labour contribution index is 66.3 percent. The result shows the mean social capital value of 15.2. Households that put more into LLIs decrease their probability of being poor. The result indicates that farming as an enterprise reduces welfare of the household. The result shows that household increase cash contribution score is associated with a 0.34% higher level of household welfare

The use of instrumental variable lead to an increase in the value of adjustment R^2 from 0.2302 to 0.2564 compared with the use of the actual social capital index. Based on the findings of this study, policy for the poverty alleviation effort should be focused toward empowerment and support of poor social capital needs.

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Identifying Contexts of Application of Electronic Education in Training of Youth Rural in Ilam Province, Iran

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Abstract: Rural youth are only producer of future in providing the raw materials and food and industrial, productions in society. Youth are most number in society in terms of unfavorable living and less of suitable state of Training and improvement naturally; they could not advanced producer to progress agriculture sector. Rural youth immigrated to cities in result of unemployment and loss of training the cities have problem with rural emigration. The new technologies in rural area causes that youth can provide education needs. For this purpose, this research is accomplished with The Feasibility of E- learning Application for Training rural youth in the City of Ilam. It is applied research and research method is correlation. Questionnaire is main instrument in research and to study the observing research instrument, guide professor and experts and advisors got questionnaire. For the measure of questionnaire constant we take primary test with 20 questions and Cronbach's Alpha coefficient is calculated 87 percent. Statistical population of research is 7950 people of Ilam rural youth. According to Cochran formula 150 people are sample number and with helping of class accidental sampling method is selected best descent statistical analysis is done with Spssversion12 computer software. Descriptive conclusion of this research is presented that social and economic factors are most effective factor in performance of E-learning. Training and searching factors are less effect in performance of e-learning. Results of regression analysis presented that social and training factor are most effects in E-learning for rural youth.
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Keywords: E-learning; rural youth; electronic education; training

1. Introduction

Majority of the population in the developing world lives in rural areas and they have no or little access to information. Iran is no exception and about one third of its population which lives in rural areas have limited access to information.

During the last two decades, the world witnessed an unprecedented growth in area of information and communication Technology (ICT). ICT helps people to communicate effectively, overcomes the limitations of time and space, empowers people by providing information and knowledge, provides income generating and learning opportunities, increases government transparency and efficiency and enables people to express their concerns and to actively participate in decision making processes (Asian Development Bank, 2004).

In terms of rural development, ICT can play an important role in improving the quality of life for rural people. However, the promise has yet to be realized due to the lack of connectivity and accessibility to universal service and markets among rural communities. Therefore, it is necessary to remove the impediments faced by the developing rural economy and provide basic infrastructure in rural areas to enable the spread of ICT. This would enable ICT to be part of a comprehensive socio-economic development strategy for rural development as a means, not an end (Lee and lee, 2004).

Perhaps the most obvious advantages of ICTs is development of e learning system which is dynamic interaction and flexible schedule. In terms of dynamic interaction, the web-based instruction presents an enormous amount of information through various interconnections that offer students a rich exploration environment (Chenn, 2005).

ICT can play an important role in facilitating students to acquire knowledge. However, the promise has yet to be realized due to the lack of connectivity and accessibility to this service. Therefore, it is necessary to remove the impediments in developing and providing basic infrastructure to enable the spread of ICT.

Bridging the digital divide between urban and rural areas is one of the challenges facing governments and policy makers today. Factors that contribute and exacerbate this divide include economic: ICT infrastructure remains cost-prohibitive for many communities and nations; geographic: terrain, distance and infrastructure; technological: increasing skills required to participate in the ICT economy; cultural: inequalities in access and participation and political: long-term investment

versus short-term political cycle (Kushner and Chong, 2004).

In Iran due to centralized planning and as a part of national development program, a radical approach to establish the community e-centers (CeCs) by the government in the rural areas is underway. For instance, the Ministry of Agriculture along with the Ministry of Communication and Information Technology have established more than 6000 CeCs in rural areas, and more than 52000 villages in Iran have access to telephone. The goal is to establish more than 12000 CeCs by the end of Fifth National Development Program. (Iran ICT News, 2008).

Many studies have identified important factors in affecting the application of the ICT in an education system [Rusten and Ramirez, 2003; Dirr; 2001; Barajas and Owen, 2006; Surry, 2002; Ebadi, 2005; Sribhadung, 2005;]. Some of those results summarized in Table1:

Table 1: Factors Affecting the Application of ICT in an Education System

Factors
Infrastructure, hard ware, soft ware, skills, cultural and organizational.
Evaluation, interface design, technological, pedagogical, institutional, ethical, resource support, management.
Physical infrastructure, intellectual infrastructure, data and telematic services.
Resources, infrastructure, human, strategies, learning, evaluation, support.
Soft ware, hard ware, communicative hard ware, human resources, data and sources.
Technical, legal and cultural infrastructures.
hard ware, soft ware, human ware and network.
Strategically planning, curriculum and content, use of the internet and acceptable use policies, connectivity infrastructure and network, intellectual property and copy right, intergovernmental issues and cost , finance and partnership.
Connectivity and access, capacity building, content and application development, good governance and policy.
Learner, instructor, course, technology, design and environmental dimension.
Online instructional tool and design.

2. Material and Methods

The methodology used in this study involved a combination of descriptive and quantitative research and included the use of correlation, regression and descriptive analysis as data processing methods. The total population for this study was 7950 rural youth in Ilam City and by using Cochran formula, 150 were selected through random sampling method.

Measuring respondent's attitudes towards e learning has been achieved largely though structured questionnaire surveys. The usual questionnaire approach to measure attitude is to include a range of semantic-differential (with good/bad options for example) and Likert items (with agree/disagree options for example) to operationalize the attitude construct. The final questionnaire was divided into several sections. The first section was designed to gather information about personal characteristics of respondents. The second section was designed to measure the attitudes of respondents about the factors influencing the application of e learning. The respondents were asked to indicate their agreements by marking their response on a five point Likert-type scale. The next section explored the barriers on application of e learning items were presented in a 5-point Likert format with responses from 1—completely disagree to 5—completely agree. The variables and their measurement scale are presented in table 2

Table 2: Variables and their measurement scale

Variables	Measurement Scale
Attitudes about Factors Influencing the Application of e learning	Five- point Likert
Barriers about Application of e learning	Five- point Likert
Gender	Categorial
Age	Categorial
Educational Level	Categorial

Content and face validity were established by a panel of experts consisting of faculty members at Islamic Azad University, Science and Research Branch and some specialists in the Ministry of Agriculture. Minor wording and structuring of the instrument were made based on the recommendation of the panel of experts.

A pilot study was conducted with 20 persons who had not been interviewed before the earlier exercise of determining the reliability of the questionnaire for the study. Computed Cronbach's Alpha score was 87.0%, which indicated that the questionnaire was highly reliable.

Key dependent variable in the study included application of e learning which were measured by perception of respondents about 11 statements. The independent variables in this research study were social, economic, cultural, research and educational factors which influences the application of e learning. For measurement of correlation between the independent variables and the dependent variable correlation coefficients have been utilized and include person test of independence.

3. Results

The results of descriptive statistics indicated that 53.3 percent of respondents were male and less than 50 percent were female. Majority of rural youth had high school diploma and slightly more than 31 percent had at least an associate degree. Almost half of youth indicated that they have access to computer.

In order to finding the perception of rural youth about impact of e learning, respondents were asked to express their views. As can be seen (Table 3) the highest mean refers to increasing their motivation to study (mean=3.82) and the lowest mean to having better access to educational materials (mean=2.97).

Table 3: Means of respondents' views about impact of e learning (1=strongly disagree; 5=strongly agree).

Statement	Mean	SD
Increasing their motivation to study	3.82	0.92
Increasing involvement in planning and implementing the projects	3.56	0.88
Improving their knowledge level	3.47	0.93
Providing more opportunities to stay and work in rural areas	3.44	0.90
Helping to reduce the digital divide between urban and rural areas	3.36	0.99
Feeling better about their place residence in rural areas	3.30	0.95
Saving time and cost	3.26	0.83
Increasing their income level	3.17	0.90
Improving job opportunities	3.07	0.109
Having better access to educational materials	2.97	0.120

The perception of respondents about factors which influence the application of e learning by rural youth shows that the highest mean number refers to social factors (mean=3.60) and the lowest mean number was for economic factors (mean=3.45) (Table 4).

Table 4: Means of respondents' views about factors influencing the application of e learning (1=strongly disagree; 5=strongly agree).

Statement	Mean	SD
Social Factors	3.60	0.62
Cultural Factors	3.58	0.67
Research Factors	3.47	0.72
Educational Factors	3.46	0.75
Economic Factors	3.45	0.61

Pearson coefficient was employed for measurement of relationships between the perception of rural youth and factors influencing the application of e learning. Table 5 displays the results which show that there was relationship between perception of respondents and economic, education, research, social and cultural factors.

Table 5: Correlation measures between independent and dependent variable

Independent variables		r	Sig
Education factors		0.633	0.000**
Cultural factors		0.552	0.000**
Social factors	Application of e-learning	0.540	0.000**
Research factors		0.468	0.000**
Economic factors		0.394	0.000**

**p<0.01.

Table 6 shows the result for regression analysis by stepwise method. Independent variables that were significantly related to perception of rural youth about factors that influence the application of e-learning were subjected to regression analysis. The result indicates that 41% of the variance in the perception of respondents could be explained by two variables of education and social factors.

In the first step, the variable education factors was entered and result shows that 39% of variance for perception of rural youth about factors which influence the application of e-learning is accounted by education factors. In the second step, the variable social factors were entered and along with education factors, these two variables accounted for 41% of variance for respondents' perception.

Table 6: Multiple Regression Analysis

Variables	B	Beta	t	Sig.
Constant	1/327	-	6/005	0.000
Education factors (X1)	0.392	0.500	5.703	0.000
Social factors (X2)	0.181	0.191	2.178	0.031

$R^2 = .41$

Y=0.392(X1) +0.181(X2) +1.327
Y=0.5 (X1) +0.192(X2)

4. Discussions

Based on the findings, social factors are the most important issue. The findings are in accordance with the studies by Sullivan (2002), Samak (2006) and Tyan (2003).

Handling of New technology needs constant maintenance, technical proficiency and training is considered to be a critical aspect in the application of e learning. Many developing countries lack enough skillful experts to train users in new technology. Moreover, continuous training of manpower to adapt them with changing technology is necessary. These often act as constraints before the smooth growth of ICT (Usun, 2004).

Based on the findings, economic factor is considered as one of the most important issues. Developing countries have to ensure that such a technology be accessible to the target group and also fulfills their needs. Such a scenario essentially implies that an advanced technology is not necessarily the best technology. However, developing countries often invest in the latest technologies without considering whether the target audience are effectively reached or are interested in the technology (Usun, 2004).

To achieve the goal of application of e learning, financial, social, human, and organizational sustainability need to be assured over a period of time. Technology options that provide affordable access need to be carefully examined.

Therefore innovative technologies and applications need to be developed that cater specifically to rural areas. The financial burden on developing e learning for rural areas has been mainly on the governments and it is important to help and introduce NGOs and private sector to participate in developing e learning in rural Iran.

The issue is not only the access to technology, but it is equally critical to provide training, tools and guidance to make rural population aware of what technology can do for them, and what they can do with technology.

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Determining Causal Model Role of ICTs in Improving Food Security of Iran's Rural Households

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Abstract: Access to desirable, sufficient, safe and nutritious food is one of the basic components of the development and health of a society. Information and communications technologies (ICTs) represent an important strategy that can be used in attaining food security. The main purpose of this research, performed in 2006-2007, was to identify the effectiveness of ICTs in improving food security of Iran's rural households. A descriptive methodology was applied in this research, through questionnaires. The statistical population for the study included 253 agricultural extension experts; from this population, 170 persons were selected. The results showed that, according to the experts' point of view, the situation of food security in Iran's rural households was unfavorable, but that ICTs could play an important role in improving this situation. The results of stepwise regressions showed that providing information about food, increasing food production, helping to market agricultural products, considering clientele needs, improving interactions and communications, ensuring appropriate ICTs, providing access to old technology and accessing the content of this type of technology, were determined to account for 78% of the variance of the food security of Iran's rural households. Moreover, the path analysis technique demonstrated that the improvement of interactions and communications had the greatest influence on determining the causal model of improving food security of Iran's rural households (=0.992).
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Key words: Information and communications technologies, food security, rural households, Iran

1. Introduction

Access to desirable, sufficient, safe and nutritious food is a basic component of development and health of a society. Thus, when developing country goals and priorities, food security is of utmost importance. Most observers of rural development believe that, currently, the necessary condition for obtaining food security is information. Knowledge and information are important factors to ensure food security, and ICTs have the ability to present the information required for improving food security. According to the definition determined by the World Food Summit (1996), Food security exists when all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life. Food security for a household means access by all members at all times to enough food for an active, healthy life (CTA 2005). In other words, food security is the guarantee of the physical availability of and economical accessibility to sufficient food (produced with bioenvironmental and sustainable social methods) in terms of quantity (amount, distribution, calories) and quality (safe, nutritious, balanced), while cultural admittance for all people at all times means having healthy and active lives to preserve human places and degrees (Temu and Msuya 2004).

Food security can be summarized according to three factors: food availability, food accessibility and food utilization. Food availability is achieved when a sufficient amount of food is constantly available for all members of society. This kind of food can be obtained through household production, local production, imports or food aids. Food accessibility is obtained when households and individuals have sufficient sources to consume a suitable diet. In other words, food accessibility is possible if the household income allows for the preparation and purchase of enough food (Bakhtiari and Haghi 2003). Food utilization refers to suitable biological uses of food that depend on a household knowledge of techniques for storing and processing food and basic principles of nutrition and caring for children (Sustainable Development Department 2006).

Different strategies exist for obtaining food security; the use of information and communications technology is one of these strategies. ICTs consist of various collections of resources and technical tools that are used for connecting, spreading, storing and managing information (Pigato 2004). In other words, ICT represents the collection of hardware and software that is used for producing, preparing, transferring and storing data via devices such as computers, radios, televisions, etc., and it includes an

extensive scope of traditional and modern media (Norad 2002). In general, ICTs can be classified into three groups:

1. New ICTs: This group consists of computers, satellites, one-on-one connections, wireless phones (mobile), the internet, e-mail, the web, internet services, video conferences, CD-ROMs, personal computers (PC), distance control systems, informational-geographical systems, global positioning systems (GPS), electronic cameras, databases, etc. The hidden concept behind these technologies is that they are not automatically considered to be new, but their common and inexpensive availability has resulted in them being regarded as new.

2. Old ICTs: This group consists of radios, televisions, telephones, telegraphs, audio and video cassettes, films and slides. This group of technologies has been used for several decades.

3. Very Old ICTs: This group of technologies has been used for several centuries and includes newspapers, books, photo albums, posters, theater, human interactions, markets and plays (Obayelu and Oyunlade 2006).

According to Chowdhury (2001), ICTs play an important role in food security through facilitating accessibility to related policies and information for market communication, improving market profitability, helping farmers to make decisions, increasing diversity in rural economies and reducing the cost of living. In general, some of the important capacities of ICTs in food security are related to improving communications between research systems, farmers and extension, improving accessibility to information regarding inputs, introducing technologies, providing more rapid accessibility to high quality information, ensuring information about the appropriate times and places for optimized sales of agricultural products, increasing agricultural products and decreasing agricultural waste products (Balakrishna, 2003, Maoz, 2004, Temu and Msya 2004).

Many studies have been carried out in relation to the role of ICTs in improving the food security of rural households. The main result of the FAO research (1998:9) focused on creating an agricultural communication network project in Italy has helped to ensure agricultural inputs and product marketing. The results of Indonesia's participatory video project (1998:11) have been considered to help with clientele needs. The findings from the research of Fortier and Van Crowder (2000:4) about the electronic diffusion of agricultural information projects in rural communities of Kenya can improve the ability for individuals to acquire information,

increase food production and develop the local capacity of rural community building. The research of Gerster and Zimmermann (2003:6) focused on a radio program project aimed at improving financial decisions and increasing food production. The findings of Uganda's knowledge system and agricultural information project (2000:7) are related to improving the power of acquiring individual information and attending to clientele needs. The results of PCARRD (2003:4) research regarding the Philippines' information services and agricultural technology were used to improve the marketing of agricultural products and to increase production. The findings of Bangladesh's rural ICT project (2001:9) resulted in better marketing of agricultural products, decreased costs of accessing information and the creation of jobs. The main results of Malaysia's E-bario project pertained to the improvement of interactions and communications and responses to clientele needs.

In development fourth program of Iran, 10000 ICT rural offices have been predicated, but 2500 ICT office has been mobilized at the present. There was no ICT rural office in Iran in 2000, but the quantity of ICT office in 2005 was 963, in 2006, 2287 and in 2007, 2446 (information technology company, 2007:23).

The results of FAO research in relation to situation of food security in Iran showed that food security indicator in rural households has been decreased during 1985-2005. Therefore, in recent years for ensuring food security in Iran, different programs have been carried out, including increasing food production in 1945-1948, ensuring rate of strategic products in 1973-1981 and investing in agricultural sector in 1983-1987 (ministry of hygiene, remedy & medical education 2004).

In addition, above mentioned solutions, using ICT for improvement food security of rural households can be an important option, because the key element in rural development in general and food security in particular, is information.

The main purpose of this research was the identification of the effective capabilities of information and communications technologies for improving the food security of rural Iran's households. With this purpose in mind, the following objectives were compiled:

1- The study of the personal and professional characteristics of extension experts.

2- The study of the situation of food security in rural Iran's households, from the extension experts' point of view.

3- The examination of the role of information and communications technologies in improving the food security of Iran's rural households.

4- The determination of the causal model role of information and communications technologies in improving the food security of Iran's rural households

2. Material and Methods

The methodology of this research was descriptive, and it was carried out as a survey. The instrument that was used for data collection was a questionnaire. The research independent variables consisted of: (A) ICT capability in improving food security (B) ICT tools(C) implications of the use of ICTs for improving food security (as you see in figure 1) and (D) personal characteristics of extension experts: gender, age, job record, level of education, major and workplace. The dependent variable was the experts' point of view about food security; to assess it, forty-four statements were used in the form of a five-point Likert scale (from very unsuitable to very suitable), and the mean score of the answered questions was identified as the respondent's attitude. After computing the statements, they were examined on an interval scale. Some of these statements were related to the rate of food production by rural households, the rate of government investments in agricultural sectors, the amount of farming lands, the yield per hectare of agricultural products, government policies regarding the avoidance of changes in farm operations, government functions related to land consolidation, government policies related to the guaranteed sales of agricultural products, the rate of the application of scientific principles in agricultural production, the amount of foreign food imports, the volume of agricultural waste products, etc.

Content and face validity were established by a panel of experts consisting of faculty members at Islamic Azad University, Science and Research Branch and some specialists in the Ministries of Agriculture and Health. Minor wording and structuring of the instrument were made based on the recommendation of the panel of experts. A pilot study was conducted with 30 persons who had not been interviewed before the earlier exercise of determining the reliability of the questionnaire for the study. Computed Cronbach Alpha score was 90.0%, which indicated that the questionnaire was highly reliable.

The statistical research personnel consisted of 253 extension experts from agricultural organizations in eight provinces of Iran: Qom, Ilam, Kerman, Semnan, Qazvin, Kordistan, Tehran and Lorestan. The required research sample size was also calculated to be 170 people by using the Cockran

formula. Thus, in a pre-test, 30 questionnaires were distributed, and the variance of the dependent variable (food security) was calculated as $S^2 = 0.26$. Using N = 253, d = 0.05 and t = 1.96, the required sample size was determined to be 155 persons; to increase certainty; it was increased to 170 persons. The research sampling method was stratified.

A series of in-depth interviews were conducted with some senior experts in the Ministries of Agriculture and Health to examine the validity of questionnaire. A questionnaire was developed based on these interviews and relevant literature. The questionnaire included both open-ended and fixed-choice questions. The open-ended questions were used to gather information not covered by the fixed-choice questions and to encourage participants to provide feedback.

The final questionnaire was divided into several sections. The first section was designed to gather information about personal characteristics of respondents. The second section was designed to measure the attitudes of extension experts about potential of ICTs in improving food security. The respondents were asked to indicate their agreements by marking their response on a five point Likert-type scale. The next section explored the impact of using ICTs on improving food security were presented in a 5-point Likert format with responses from 1—completely disagree to 5—completely agree. Eight attitudes were presented in a 5-point Likert format.

To maintain the proportion between research personnel size N = 253 and sample size N = 170 in each province, the necessary sample size was chosen randomly, according to the number of experts in those provinces.

To analyze the collective data, the software SPSS 13 was used. For descriptive statistics, mean, median, mode and coefficient of variation and inferential statistics methods such as correlation, regression and path analysis were used.

3. Results

In order to assess the current food security situation of rural Iran's households, 44 statements were used. The results of the research indicated that most of the respondents (81.2%) assessed the food security situation of rural Iran's households as unsuitable.

To determine the role of ICTs in improving the food security of rural Iran's households, a total of 48 statements were used. The results indicate that most respondents (36.5%) assigned an important role to ICT capabilities in improving the food security of rural Iran's households.

In order to determine the variance in the improvement of food security of rural Iran's

households, stepwise regression analysis was used. The analysis results are shown in Tables 1 and 2.

According to Table 1, the providing information about food, increasing food production, helping to market agricultural products, considering the clientele needs, improving interactions and communications, ensuring appropriate ICTs, providing access to old technologies and accessing the content of old technologies were entered as stepwise regressions.

In the first step, the practice of providing information about food was entered in the regression equation, and it was determined that 24% of the variance changed with respect to the dependent variable (food security).

In the second step, the increasing food production and the previous variable represented 32% of the changes, and in the third step, the practice of assisting in the marketing of agricultural products

and the two previous variables were determined to represent 41% of the changes.

In the third step, the variable related to considering the clientele needs and the three previous variables were determined as 48%, and in the fifth step, the variable related to improving interactions and communications and the previous variables were determined as 57%; in the sixth, seventh and eighth steps, the practices of ensuring appropriate ICTs, providing access to old technologies and accessing the content of old technologies were determined as 63%, 69% and 78% of the variance changes in food security, respectively.

In total, when entering all of these variables, the result was $R^2 = 0.783$. This coefficient shows that 78.3% of the food security of rural households' variance changes was related to these eight variables.

Table 1- Stepwise regression in improving food security of Iran's rural households

Steps	R	R Square	Adjusted R Square
1	0.56	0.311	0.245
2	0.63	0.391	0.323
3	0.66	0.433	0.416
4	0.73	0.533	0.485
5	0.77	0.598	0.573
6	0.82	0.682	0.636
7	0.87	0.751	0.697
8	0.91	0.836	0.783

According to the results shown in Table 2, the regression equation according to the B and β quantities were, respectively:

$$Y= 89.667 + 0.865x + 0.774x + 0.694x + 0.612x + 0.531x + 0.472x + 0.384x + 0.311x$$
$$Y = 0.794x + 0.732 x + 0.684 x + 0.592 x + 0.481x + 0.374 x + 0.284x + 0.211x$$

Table 2- Standardized & unstandardized coefficients of improving food security

Variables	Unstandardized Coefficients B	Standardized Coefficients Beta	Sig
Constant	89.667	------------	
Informing about food (X)	0.865	0.794	
Increasing food producing (X)	0.774	0.723	
Agricultural marketing (X)	0.694	0.684	
Considering to clientele needs (X)	0.612	0.592	
Improving interactions & communications (X)	0.531	0.481	
Ensuring appropriate ICTs (X)	0.472	0.374	
Accessing to old technologies (X)	0.384	0.284	
content of old technologies (X)	0.311	0.211	

To determine the causal model of effective capabilities of ICTs in improving the food security of rural households, a path analysis technique was used. To determine the path coefficients and calculate the direct and indirect influences of the variables, a regression technique was used. In each step, one variable is the dependent variable, and the other variables of the regression analysis are independent variables, thus allowing for the calculation of the direct and indirect influences (Table 3).

Table 8- Direct & indirect influences of independent variables on food security

Independent variables	Indirect influences	Direct influences	Total influences
Informing about food (X)	046/0	947/0	0.841
Improving food production (X2)	----	723/0	0/723
Agricultural marketing (X3)	134/0	684/0	818/0
Consideration to clientele needs (X4)	272/0	592/0	846/0
interactions and communications (X5)	51/0	481/0	991/0
Ensuring appropriate ICTs (X6)	43/0	374/0	804/0
Accessing to old technologies (X7)	----	284/0	284/0
Content of old technologies (X8)	126/0	0/85	211/0

4. Discussions

This research, carried out to study the role of information and communications technologies in improving the food security of rural Iran's households, has shown that the food security situation of rural households is unsuitable. This means that factors such as the rate of unemployment in agricultural sectors, the rate of inflation in the country and also the volume of agricultural waste products are not only problematic but that they also threaten the food security situation of rural Iran's households. In the experts' view, information and communications technologies can have an important role in improving the food security of rural households. The practices of providing information about food, increasing food production, helping to market agricultural products, considering clientele needs, improving interactions and communications, ensuring appropriate ICTs, providing access to old technologies and accessing the content of old technologies could play an important role in improving the food security of rural households. Information about food related to the manner of storing food processing food, optimizing food consumption, improving food distribution, supplying food and providing food safety played a direct and important role. On the other hand, the improvement of the food security of rural households was strongly influenced by the improvement of interactions and communications; this rural means that practices such as increasing the quality of studies in the agricultural section, improving interactions and communications among various production factors, improving presentations of extension services, improving communications among researchers, extension personnel and farmers, and decreasing the gap between rural people and researchers can increase and improve the food security of rural Iran's households. This research confirmed the results of Fortier (2000), Zimmermann and Gerster (2003), PCARRD (2003), rural ICT of Bangladesh (2001) and E-bario Malaysia (2003). The results obtained here are consistent with the results of the VERCON project in Egypt (2000), the Indian global center of agricultural information (2000) and E-bario in Malaysia. It can be concluded that:

To achieve improvements in the food security of rural households, more consideration should be paid to creating jobs in the agricultural section, to controlling and decreasing the rate of inflation in the country and also to managing the agricultural waste products.

According to most of the experts' point of view, much more precise considerations regarding the use of information and communications technologies in improving the food security of rural households are completely necessary and logical. Actions such as identifying and assessing appropriate ICTs for fulfilling participatory needs, ensuring appropriate ICTs for improving food security, ensuring appropriate software and hardware, providing equal access to ICTs for all people, considering clientele needs in presenting programs and information, investing in ICTs and promoting technical-information infrastructures for this purpose are essential.

To improve the role of information and communications technologies in increasing the food security of rural households, solutions such as the use of appropriate content from old technologies, for example, radios and televisions, for presenting information about storing food, processing food, optimizing food consumption, ensuring the safety of food, increasing food production, marketing agricultural products and considering clientele needs are highly recommended; this requires that rural households have access to old technologies.

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Predicting manpower productivity promotion factors in Guilan University of Medical Sciences using Structural Equation Modeling (Iran)

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Abstract: Manpower productivity issues have attracted increasing interest among researchers during the last decade. There are various factors affecting human resources productivity. This study elaborated the human resources productivity promotion factors in Guilan University of Medical Sciences using structural equation modeling. The research was cross-sectional, descriptive and analytical. The study was carried out in two stages during three month of fall season in 2009. In quality stages of research, 45 specialists in management were involved. In the quantity stage, 321 members of the faculty, educational and human resources experts affiliated to Guilan University of Medical Sciences were selected and the data has been collected using the questionnaires. Expert panel has been used for content validity and exploratory factor analysis and confirmatory factor analysis were performed for construct validity. Finally, path analysis carried out in order to identify human productivity promotion factors. Manpower productivity promotion factors identified in path analysis were included organizational culture with 0.51 path coefficient, motivational factors with 0.25 path coefficients, environmental status with 0.17 path coefficient, faculty member's empowerment with 0.11 path coefficient and leadership style with 0.08 path coefficient. The results indicated that organizational culture, motivational factors, environmental conditions, empowerment and leadership style were the most important human productivity factors for Guilan University of Medical Sciences.

Introduction

Productivity issues have attracted increasing interest attention amongst researchers during the last decade (Vurinen et al. 1998, Parasuraman, 2002, Sahay, 2005). However, the term of productivity was used over two century ago in the Journal of de 1" Agriculture (Tangen, 2005). The concept of productivity is deeply rooted in the context of mass manufacturing and this may be the reason for the prolonged neglect of the productivity issue on service management (Adam and Jonson, 1995, Adam and Gravesen 1996). Productivity defined as the ratio of output to input or as the relationship between inputs and outputs (Singh et al, 2000). Published research shows that productivity and similar terms are not used consistently (Kinnader and Grondahl, 1999). Different factors impact the ratio of growth and productivity of organizations (Ramsey, 1983). Nowadays, productivity and human resource element are one of the main issues that assure stability in organizations and keep succeeding with consistency (Eastaugh 2002, Dehghan Nayeri et al, 2006). Implementing culture of productivity will lead organizations to make the best use of human and material resources to approach competencies and optimum potentials of organization. Suitable productivity is not achievable if we just focus on the changes of layouts, adding new technology, documenting work instructions and procedures. Human resources and manpower are the most important factor in individual productivity, social and organizational activities and to improve productivity (Soltani, 2007, Abtahi and kazemi, 1999). Efficient manpower is the main factor to obtain organizational goals and keep succeeding with consistency (Eastaugh, 2002). Human factors may waste or make the most use of resources (Soltani, 2007). It is stated that organizations that locate manpower on top of the list of their agenda as vital factor would succeed to a desirable level (Abdolahi and Navehebrahim, 1999). For that reason identifying significant factors to upgrade manpower productivity is the main objective of many researchers (Alam, 2009). Likewise, almost all researchers believe that promoting manpower productivity may not result from only one special cause (or case), but a combination of factors should be considered (Taheri, 2007).

Present statistics indicates medical schools and organizations as well as medical training centers unlike industrial and commercial organizations have scarcely considered suitable methods to increase productivity among staff in Iran not to mention the models devised in industrial and commercial areas are

not suitable for medical and health sectors (Jordan, 1994). Furthermore, due to some differences in cultural, social and economic circumstances, studies conducted in other countries are not feasible and proper to achieve suitable models for Iranian organizations either. (Dehghan Nayeri et al, 2006). Reports specify that manpower productivity indexes in Iran are lower than the other countries of Middle East along with the eastern Asian countries. (Taheri, 2007).

In comparison with other members of Asian productivity organization countries, average productivity manpower growth index of Iran was % 2.03 at the period of 2000 to 2006 means locating at ninth level amongst the 14 members of Asian productivity organization (kameli, 2009). Universities as the most important center to produce knowledge in order to perform important tasks and improve the levels of productivity require determining the factors affecting human resources productivity (khodayari, 2008). Hence, in this study researcher used path analysis to identify factors influencing manpower productivity in Guilan University of Medical Sciences to increase.

Methods

In this research, descriptive, analytical and cross-sectional studies were carried out during three months of September, October and November of 2009 in two stages (cross-sectional and qualitative). At quality stage, 45 experts in manpower productivity participated in the research sample population to determine manpower productivity dimensions. Data collection instruments at qualitative stage were interview and questionnaires. Issues that appear through research include empowerment of staff, method of leadership, organizational support, clarifying, documenting services and staff intention and motivation, Likert scale (Andaleeb,2004), completely agree(5), agree(4), no comment(3), disagree(2) and completely disagree(1)was used. After determining the score for each component, the results were fed into SPSS software. Then the agreement extent for each component among experts was calculated. Next, the components on which 70% of experts had identical agreement, chosen to be beneficial for manpower productivity. At last, the rest of the components as well as newly-proposed issues were negotiated again among those experts in order to reach to a total agreement.

At cross sectional stage, research society were contain of scientific group, training experts and human resource experts from medical faculty, dentists faculty, health care center, nursing center, midwife, medical laboratories and international unit of Guilan university of medical sciences. At this stage, data gathering tool was questionnaires which consist of two sections. Section one consist of 8 questions in relation

to personal and demographic information including sex, age, marital status, employment status, work experiences, education level, management experiences and scientific group membership. Section two consists of 42 questions in relation to manpower productivity variables and Likert measurement method has been used for marking of every question with 5 degree, so that 5 indicated very much, 4 indicated a lot, 3 indicated averages, 2 indicated little and 1 indicated very little. Creditability of manpower assessment has been conducted using library studies and item analysis; content creditability using expert panel,. Reliability of this questionnaire calculated by test- retest method was 0.98 and internal consistency was 0.89 using Alpha Cronbach method. Content validity conducted by expert panel. structure creditability using exploratory factor analysis considering main issues with Varimax rotation method and volume sufficiency using Kaiser-Meyer-Olkin(Dixon,2001).KMO method was employed in order to assess sample population volume, the logical result achieved was 0.96 in which it was found that the result was 0.8 more than the ideal value.The suitability of data was also carried out by using Bartlet test (Bartlett, 1954) which indicated the suitability at the $p = 0.000$ level. As it can be seen, this suitability disclosed recognizable relations between those variables subjected to factor analysis .questionnaires carried out in Sept and Oct. Necessary explanation in relation to research objective was carried out and 347 persons received questionnaire which out of all, 321 persons completed them accordingly.

Results

Initial conceptual model of human productivity with 6 components designed using previous models and findings of other researchers (Figure 1):

Basic suggested model examined using 45 experts. Staff empowerment with 100% agreement, leadership style with 100% agreement, organizational support with 91.1% agreement, documentation services with 82.2% agreement, staff motivation with 97.7% agreement and decisions validity with 86.7 agreements as an effective component of manpower efficiency were selected.

Since at previous stage some experts believed that other components such as environmental conditions, organizational culture, and innovation and creativity can be effective in manpower productivity in addition to the previous components, new components including organizational culture with 91.1% agreement, organizational structure with 86.7% agreement, innovation and creativity with 73.3% agreement and environmental condition with 71.1% agreement were

selected as components which might affect manpower efficiency.

Finally at this stage a model with ten components as a logical model of manpower productivity was designed. The components of this model included leadership style, staff empowerment, staff motivation, organization support, organizational culture, decision validity, organizational structure, transparency, innovation and environmental condition.

Then to identify the most effective components which influence the efficiency of human resources and also to identify the amount of variables loading on each component, exploratory factor analysis has been used. Bartlett test showed the fitness of the data significantly

In exploratory analysis the main issue with 42 variables identified as follow:

1. Organizational culture with 18 variables, 29.26 % variance and Eagan value of 21.62

2. Environmental conditions with 7 variables, 12.96 variance and 2.63 Eagan values.

3. Motivation factors with 10 variables, 12.84 % variance and Eagan value of 1.58

4) Empowerment with 4 variables, 7.47 % variance and Eagan value of 1.34.

5) Leadership style with 3 variables, 5.50 % variance and Eagan value of 1.21.

These 5 issues with 67.60 % variance defined changes of manpower productivity.

In order to confirm and fit obtained issues in exploratory factor analysis and the loaded variables, described under each issue(fig2), LISREL 8.80 has been used (Schomacker, 2004)
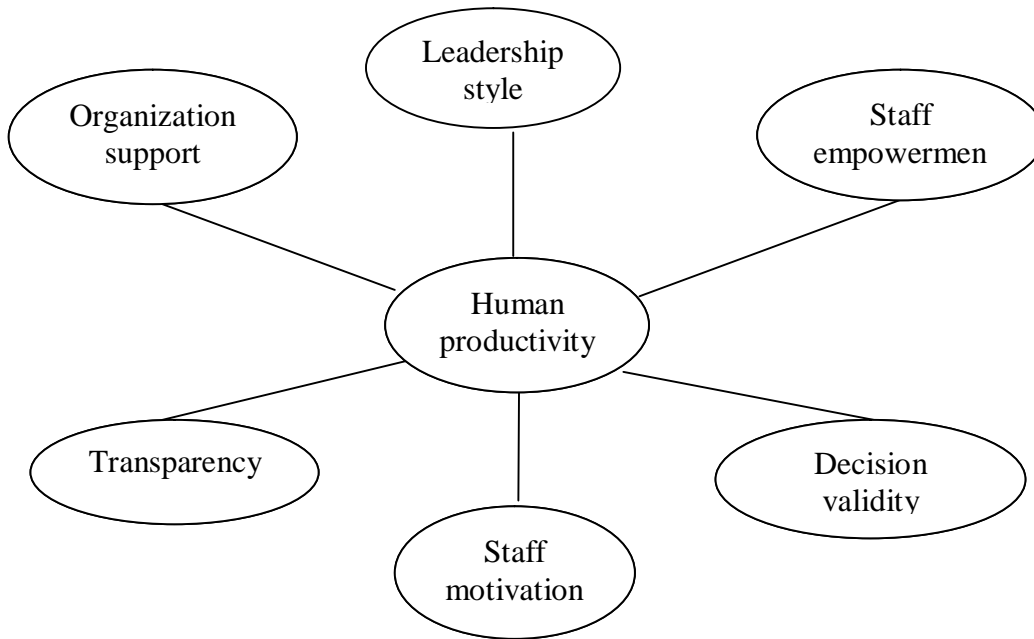
Figure1. Initial conceptual model of manpower productivity
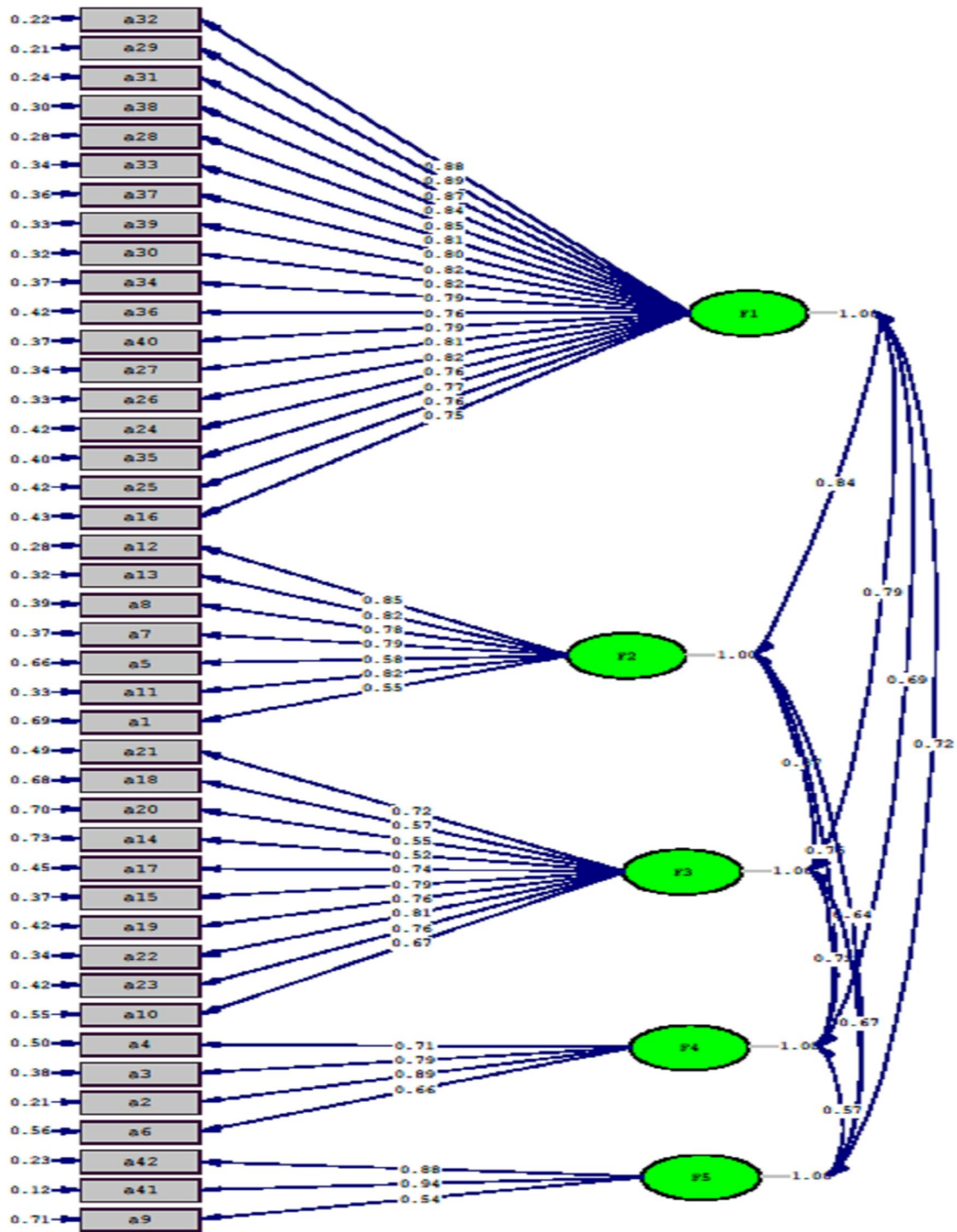
Figure2. Index model in confirmatory factor analysis and loaded variable

Fit-index

1 – Root mean square error of approximation is equal to 0.090 and because this figure is less than 0.1, then we can consider this is an acceptable result for model used in factor analysis. In other words, degree of variables loaded under every issue is higher than 0.5, accordingly the model is approved (Norris, 2005).

2- Comparative fitting index was equal to %0.97 and because this is higher than 0. 9, therefore, this indicates suitable index factor analysis model in comparison with similar models (Norris, 2005).

3. Standardized root mean residuals (SRMR) was equal to 0.039

4. Adjust goodness fit index (AGFI) was equal 0.075

5. Goodness of fit index (GFI) was equal to 0.90

6. Normed fit index (NFI) was equal to 0.96

7. Relative fit index (RFI) was equal to 0.96

Correlation and productivity power of five factors including organizational culture, motivational factors, environmental conditions, empowerment and leadership style with human productivity have been assessed using path analysis. Model was perfectly fit the saturated model. In this model organizational culture with path coefficient of 0.51 was the most important predictor of human productivity. The next predictor was staff motivation with path coefficient of 0.25. Environmental conditions with path coefficient of 0.17 were third factor .Empowerment with path coefficient of 0.11 was the fourth factor that changes the representation of manpower productivity. Leadership with path coefficient of 0.08 was the last predictor of the manpower productivity (Figure 3):
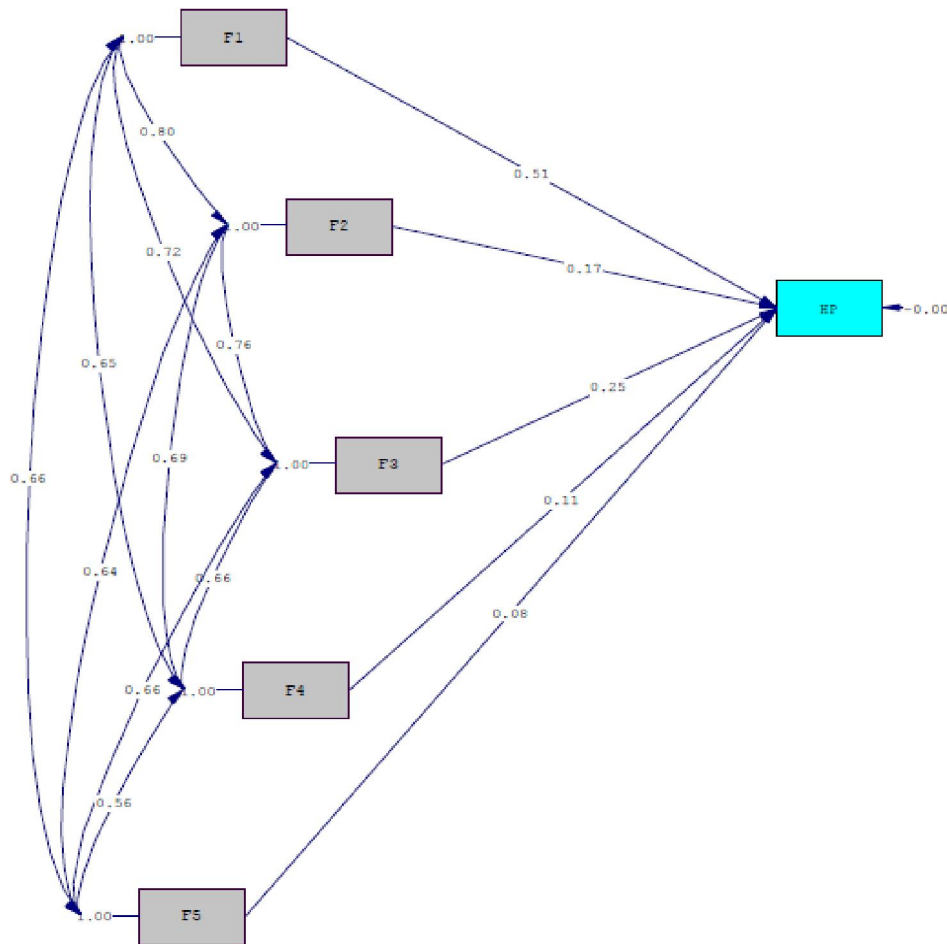
Figure3. Human resources productivity fit model of the Guilan University of Medical Sciences (GUMS)

Discussion and Conclusion

The results of this study indicated that structural model which fit with the data of Guilan University of Medical Sciences had five factors. The comparison of fit model with a logical model (qualitative phase) indicated that organization culture as an effective factor was the most important predictor of the manpower productivity in GUMS. While in logical model, this factor located at fourth level affecting manpower productivity. Shermerhorn, (1999) also reported that organizational culture affect on all aspects of an organization. Nassiripour (2009) also found that there is a significant relationship between organizational culture and manpower productivity in the hospitals of Iran University of Medical Sciences.

The second important factor in this model was motivational factors with 7 variables and 0.25 path coefficient. The comparison of this model with logic model confirms the fact that in the logical model motivational factor was also as an effective component of human resources utilization. Sadeghi (1998) also examined the factors affecting the productivity of human resources, amongst 1,300 employees of the headquarters of welfare organization and found motivational factor as third important component affecting the efficiency of manpower productivity. Meanwhile, Alvani and Ahmadi (2001) designed the productivity of management pattern using 8 components and introduced motivational factor as one of the main effective factors affecting manpower productivity.

The third factor that identified in the structural model of manpower productivity was environmental condition with 7 variables and 0.17 path coefficient. Analysis of findings in this study indicates that environmental condition is an effective component of manpower productivity in the logical model as well. However, in the logical model this component was less important than other components. In the Achiu model, environment has been considered as an effective component in manpower productivity in addition to other components such as capability, transparency, organizational support, motivation, evaluation and validation. Janalinejad (2001) conducted research regarding factors affecting manpower productivity in Tarbiat Modarres University among 108 employees and he also concluded that environmental conditions were significant components in productivity manpower. In the present model with 4 variables, staff empowerment with the path coefficient of 0.11 was the fourth predictor of manpower productivity. However, in the logical model, staff empowerment had been introduced as the most important component of manpower productivity. While this component was at the fourth priority in the structural model. Findings of research conducted by Asgari (2005) regarding effective factors in manpower productivity in Steel Company indicated that human resources training i.e. empowerment operate as an effective component in manpower productivity.

The fifth and last component effecting manpower productivity in structural model was leadership style with 3 predictor variables and the path coefficient of 0.08. This component has been introduced as important factor in both structural and logical models. However, in the logical model, leadership style was the second important component of manpower productivity, while in the structural model this component had fifth priority affecting manpower productivity. Meanwhile, Anbari, Sadaghiani and Tabibi (2003) introduced leadership style as effective component in Arak hospitals. Therefore, the current study indicated that 5 components including organizational culture, motivational factors, environmental conditions, empowerment and leadership style, are the most effective factors to increase human resources productivity in Guilan University of Medical Sciences, respectively.

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Bit-Interleaved Turbo-Coded over Wireless Channels

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Abstract: This paper presents an improved version of bit-interleaved turbo-coded modulation (BITCM) scheme designed for bandwidth efficient transmission over wireless channels. The proposed scheme consists to apply signal space diversity (SSD) to conventional BITCM and a rotated modulation. At the receiver side, an iterative demapping and decoding is proposed in order to optimize the error performance. Simulation results carried out on 2 bit/s/Hz 64-QAM BITCM indicate that is possible to obtain a gain exceeding 0.5 dB at a BER = 10^{-7} compared to the classical 64-QAM BITCM scheme. It is also shown that, the error floor can be significantly lowered using SSD technique at a little cost in terms of system's complexity.
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Keywords: BICM, DVB-RCS turbo code, signal space diversity, iterative demapping, error floor, wireless channels.

1. Introduction

Bit-interleaved turbo-coded modulation (BITCM) is bandwidth-efficient coding designed using the bit-interleaved coded modulation (BICM) approach. It consists on a serial concatenation of turbo coding, bit-by-bit interleaving and higher-order modulation according to Gray mapping. BITCM scheme have been shown to achieve bit error rate (BER) performance close to the capacity limit over additive white Gaussian noise (AWGN) channels The mapping used in , the opposite mapping strategy is used, where the most protected bit positions are dedicated to the systematic bits. Due to the systematic information bits in the turbo decoding algorithm, the latter mapping strategy outperforms the former at only of what is known as the waterfall region. Recently, it is shown that, the performance of bit-interleaved coded modulation (BICM) can be greatly improved through iterative information exchange between the soft-input soft-output (SISO) damper/demodulator and the (SISO) convolution decoder at the receiver. This system, introduced in is usually referred to as BICM with iterative decoding (BICM-ID). The purpose of this paper is to apply the BICM-ID concept to BITCM scheme. An initial exploration of this concept was presented in which proposed an additional feedback from the (SISO) turbo decoder to the damper. Furthermore, only indicate a performance gain of 0.1 dB compared to classical BITCM. In this contribution, we propose to modify the BITCM structure so as to improve their error performance for Rayleigh wireless channels, with respect to low error floor and early convergence threshold. For that, an iterative decoding process is implemented in the receiver side which incorporates the soft-input soft-output (SISO) demodulator into the iterative turbo decoding loop. Moreover, the

diversity order can be maximized by optimally rotating the constellation and separately interleaving the signals in each coordinate such technique is also known as signal space diversity (SSD). As far communications on wireless channels are considered, we show that the diversity order can be increased using (SSD) technique. Throughout this work, we focus on the design of a 2 bit/s/Hz BITCM system employing the well known DVB-RCS turbo code and a 64-ary quadrature amplitude modulation (QAM) constellation. The DVB- RCS turbo code is the double binary 8-state turbo code which is adopted in the digital video broadcasting (DVB) standards for return channel via satellite (DVB-RCS) (DVB-RCS 2000) and the terrestrial distribution system (DVB-RCT) (DVB-RCT 2000). The proposed system improves the iterative process convergence and lowers the error floor. The paper is organized as follows: in Section 2, we provide a brief review of BITCM schemes focusing on the reason behind the degradation in performance for Rayleigh channel. The proposed BITCM scheme with signal space diversity is presented in Section 3. Simulation results and performance comparisons are shown in Section 4. Finally, Section 5 presents conclusions of this contribution.

2. Review of BITCM
2.1. BITCM Structure

Conventional BITCM can be modeled as a serial concatenation of a turbo encoder which may stand for binary turbo encoder or duo-binary turbo encoder, a bit interleaver π and an M-ary memory less modulator (where $M = 2^m$) as shown in Figure 1. At the transmitter, the sequence of information bits u is first encoded by a turbo encoder to produce the output coded sequence c before being bitwise

interleaved to v. The purpose of the bit interleaver π (required only for the Rayleigh wireless channel) is to break the fading correlation and increase the diversity order to the minimum Hamming distance. After the interleaver π, m consecutive bits of the interleaved sequence v can be grouped as a channel symbol $\underline{v}_t = \left(v_t^{(1)}, \ldots, v_t^{(m)} \right)$, where $v_t^{(i)}$ denotes the ith bit in the bit pattern at time index $t, t = 1, \ldots, T$. The complex transmitted signal $s_t = \mu(\underline{v}_t)$, is then chosen from the M-ary constellation χ to carry m coded bits over each symbol duration. Here, μ denotes the mapping scheme from the bit patterns to constellation points. We assume a frequency nonselective Rayleigh wireless channel and coherent detection, the received discrete-time base band signal can be written as

$$r_t = \rho_t s_t + n_t \qquad (1)$$

Where   is the Rayleigh-distributed fading coefficient with $E\left(\rho_t^2 \right) = 1$ and n_t is a complex white Gaussian noise sample with independent in-phase and quadrature components having two-sided power density the variance $\sigma^2 = {N_0}/{2}$. For AWGN channel, $\rho_t = 1$. Throughout this work, we assume perfect channel state information (CSI) so that ρ_t is perfectly estimated and available to the receiver.
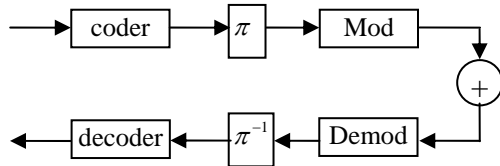

Figure 1. Structure of the classical BITCM.

The receiver of BITCM system depicted in Figure 1 includes three elements: the demodulator, the de-interleaver π^{-1}, and the turbo decoder. For each received r_t, the Logarithm of Likelihood Ratio (LLR) $\Lambda\left(v_t^i\right)$ associated with each bit $v_t^i, i \in \{1, \ldots, m\}$, is computed by demapper μ^{-1} and used as relevant soft decision by the turbo decoder. The LLRs $\Lambda\left(v_t^i\right)$ are obtained as

$$\Lambda\left(v_t^i\right) = \log \frac{\sum_{s_t \in \chi_i^1} p\left(r_t \mid s_t, \rho_t\right)}{\sum_{s_t \in \chi_i^0} p\left(r_t \mid s_t, \rho_t\right)} \qquad (2)$$

Where χ_b^i denotes the subset of symbols $s_t \in \chi$ whose bits labels have the binary value

$b \in \{0, 1\}$ at i th bit position. The function $p\left(r_t \mid s_t, \rho_t\right)$ is the probability density function of the revived signal r given the fading amplitude ρ_t and signal s_t was transmitted. With M-ary signal constellation χ, $p\left(r_t \mid s_t, \rho_t\right)$ is given as

$$p\left(r_t \mid s_t, \rho_t\right) = \frac{1}{2\pi\sigma^2} \exp\left(-\frac{d_{rs}^2}{2\sigma^2}\right), \qquad (3)$$

With

$$d_{rs}^2 = \left\| r_{I_t} - \rho_t s_{I_t} \right\|^2 + \left\| r_{Q_t} - \rho_t s_{Q_t} \right\|^2 \qquad (4)$$

For practical implementation, the computational complexity of the demapper can be considerably reduced by approximating (2) with the following expression:

$$\Lambda\left(v_t^i\right) \approx \min_{s_t \in \chi_i^0} \left\| r_t - \rho_t s_t \right\|^2 - \min_{s_t \in \chi_i^1} \left\| r_t - \rho_t s_t \right\|^2 \qquad (5)$$

For a square M-ary QAM constellation with Gray mapping, LLRs are correlated over ${m}/{2}$ values.

Thus, it necessary to use the de-interleaver π^{-1} to ensure efficient turbo decoding.

2.2. Performance Evaluation

A comparison of the performance of BITCM scheme over AWGN and Rayleigh wireless channels shows a higher gap to the channel capacity limit over Rayleigh wireless channels. Moreover, an error floor is observed at higher value of BER over Rayleigh wireless channels. To confirm these results, we have represented in Figure 2 and 3, the BER performance achieved over AWGN and Rayleigh channels respectively, for 2 bit/s/Hz 64-QAM BITCM scheme using DVB-RCS turbo code operating on either large (51200 bits) or small (1504 bits) frames.

DVB-RCS turbo code. MAP decoding with 8 iteration. From Figure 2 and 3, it is seen that, at a BER=10⁻⁵, the gap (≈ 0.5 dB) between the capacity limit and the performance of 64-QAM BITCM using scheme A on Rayleigh channel is higher to that obtained on AWGN channel. However, in the case of Rayleigh channel, we can notice that 64-QAM BITCM scheme suffers from an early error floor occurring at a BER level equal to 10⁻⁵.

This degradation in performance could explained by the fact that the transmitted QAM constellation signal is subject to the same fading coefficient on both component axes. The proposed
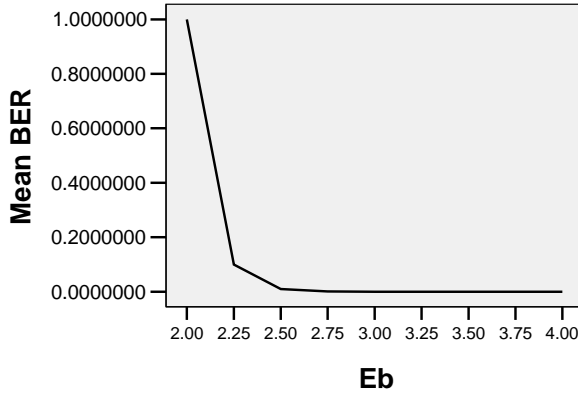
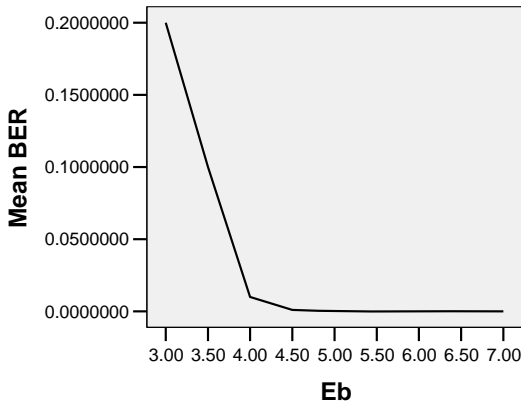Figure 2. BER performance over AWGN channel of a 2 bit/s/Hz 64-QAM BITCM using a 1/2-rate DVB-RCS turbo code.

Figure 3. BER performance over Raleigh channel of a 2 bit/s/Hz 64-QAM BITCM using a 1/2-rate

BITCM In this section, we start by presenting the (SSD) technique which is suitable for wireless channels. It is followed by the structure of the proposed BITCM system for Rayleigh channel.

3.1 Diversity Improvement

Signal space diversity can provide performance improvement over wireless channels by increasing the diversity order of communication system it combats wireless channel by rotating the constellation and adding independent interleavers for the in-phase (I) and quadrature (Q) components of a transmitted symbol chosen from a properly rotated constellation after a modulator. The purpose of coordinate interleaving is to make the I and Q component of a modulated symbol uncorrelated during the same fading interval. To maximize the diversity order, the constellation should be properly rotated such that all distinct signal symbols are separable on every coordinate. Figure 4 shows the system model of $\pi/8$-rotated encoded 64-QAM with

an IQ interleaver, which is adopted in our system. It can be seen as a serial concatenation of a memory less modulator and an IQ interleaver (π IQ). Therefore, the diversity order of BITCM can be achieved by adding independent interleavers for the I and Q components of a transmitted signal *st* chosen from a $\pi/8$-rotated 64-QAM constellation after a modulator. In practice, it is possible to replace the IQ interleaver with a time delay in only one of the quadrature (Q) components. For a memory less wireless channel, a delay of at least one symbol period is sufficient.

3.2 Structure of the Proposed BITCM Scheme

The block diagram of the BITCM system using a rate-1/2 DVB-RCS turbo code and 64-QAM constellation is shown in Figure 5. A modulator maps each channel symbol $\underline{v}_t$ according to Gray mapping to a complex symbol $s_t = \mu(\underline{v}_t) = s_{I_t} + js_{Q_t}$, chosen from a $\pi/8$-rotated 64-QAM constellation $\tilde{\chi}$. With IQ interleaver, the complex transmitted signal is given by

$$\tilde{s}_t = \begin{cases} s_{I_t} + js_{Q_{t-1}} & t \in [2, \ t] \\ s_{I_t} + js_{Q_T} & t = 1 \end{cases} \tag{6}$$

Thus, the received channel symbols $\tilde{r}_t$ can be written as

$$\tilde{r}_t = \rho_t \tilde{s}_t + n_t \tag{7}$$
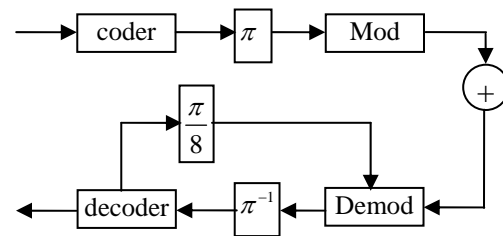
Figure 5. Structure of the proposed BITCM.

In order to optimize the error performance of the receiver, demapping and turbo decoding are performed according to an iterative algorithm. This receiver is made up of three (SISO) modules, connected through interleavers/de-interleavers, that exchange information in order to improve the reliability of the LLRs flowing through them. A particular SISO module process LLR sequence, called *a priori* information, produced by the other modules in order to generate updated versions of those input sequences, called *a posteriori* information. An *extrinsic* knowledge is then obtained by subtracting an *a priori* LLR from the

corresponding *a posteriori* LLR. This *extrinsic* information is then passed as an *a priori* knowledge to the other modules for further iterative steps. In our receiver, there are two iterative loops operating in parallel and allowing for information exchange between demapper and turbo decoder, and both constituent decoders inside turbo decoder. Based on sample r_t and the corresponding *a priori* LLRs $\Lambda_{\mu^{-1}}^{a}\left(v_t^i\right)$, generated by the turbo decoder, demapper μ^{-1} calculates an *extrinsic* LLR $\Lambda_{\mu^{-1}}^{e}\left(v_t^i\right)$ associated with bit $v_t^i, i \in \{1, \ldots, m\}$, by using a calculation similar to that as follows

$$\Lambda_{\mu^{-1}}^{e}\left(v_t^i\right) = \ln \frac{\sum_{\tilde{s}_t \in \tilde{\chi}_i^1} p\left(r_t \mid \tilde{s}_t, \rho_t\right) \prod_{l=1, l \neq i}^{m} e^{\Lambda_{\mu^{-1}}^{a}\left(v_t^l\right).\tilde{s}_l}}{\sum_{\tilde{s}_t \in \tilde{\chi}_i^0} p\left(r_t \mid \tilde{s}_t, \rho_t\right) \prod_{l=1, l \neq i}^{m} e^{\Lambda_{\mu^{-1}}^{a}\left(v_t^l\right).\tilde{s}_l}}$$

(8)

where $\tilde{\chi}_i^b$ denotes the subset of symbols $\tilde{s}_t \in \tilde{\chi}$ whose labels have the binary value $b \in \{0, 1\}$ at i th bit position, and $\tilde{s}_l$ is the value of the label of symbol $\tilde{s}$ in position l. The LLR $\Lambda_{\mu^{-1}}^{a}\left(v_t^i\right)$ represents the extrinsic LLR generated by the turbo decoder at the previous iteration. In case of IQ interleaver, the probability density $p\left(r_t \mid \tilde{s}_t, \rho_t\right)$ describing the channel model is given by

$$p\left(r_t \mid \tilde{s}_t, \rho_t\right) = \frac{1}{2\pi\sigma^2} \exp\left(-\frac{d_{r\tilde{s}}^2}{2\sigma^2}\right)$$

(9)

, with $d_{r\tilde{s}}^2 = \left\| r_{I_t} - \rho_t \tilde{s}_{I_t} \right\|^2 + \left\| r_{Q_t} - \rho_{t-1} \tilde{s}_{Q_t} \right\|^2$ (10)

Without IQ interleaver (10) simplifies to $d_{r\tilde{s}}^2 = \left\| r_{I_t} - \rho_t s_t \right\|^2$. After appropriately de-interleaving the LLRs $\Lambda_{\mu^{-1}}^{e}(v)$ by π^{-1} to $\Lambda_{dec}^{a}(c)$. The LLRs $\Lambda_{dec}^{a}(c)$ are fed into the turbo decoder (as *a priori* information), which computes extrinsic LLRs $\Lambda_{dec}^{e}(c)$ on both parity and systematic bits. For the next iteration, the LLRs $\Lambda_{dec}^{e}(c)$ are interleaved again by π^{-1} to LLRs $\Lambda_{\mu^{-1}}^{a}(v)$ in order to be fed into the demodulator. The number of metrics to be computed at the demapper in n the case of a classical BITCM with non-rotated 2^m-QAM is 2×2^m. Each metric is computed over one dimension according to the in-phase I or quadrature Q component. This is due to the independence induced by the Gray mapping. Whereas, in the case of the proposed BITCM with a rotated 2^m-QAM, the number of metrics to be computed at the demapper becomes 2^m.

Owing to correlation between the I and Q components introduced by the rotation, each metric is now computed over two dimension corresponding to the I and Q components of the rotated constellation signal. The computational is multiplied by a factor of $\sqrt{2^m}$ with respect to the classical demmaper case. 4. Simulation results We now consider a 2 bit/s/HZ BITCM system using a rate-1/2 DVB-RCS turbo code and 64-QAM constellation. The iterative decoding at the receiver side is performed in 8 iterations (with a single local iteration of turbo decoding for each global iteration of BITCM), and the MAP algorithm is used for the decoding of the component decoders. Interleaver $\pi/8$ is designed. Figure 6 illustrates the bit error rate (BER) performance versus signal-to-noise ratio E_b/N_0 with and without iterative demapping, obtained with the classical 2 bit/s/Hz 64- QAM BITCM schemes using two different types of bit allocation, scheme A and Y
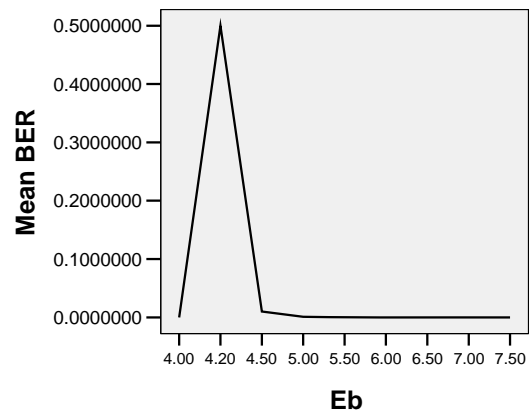
Figure 6. BER Performance achieved over Rayleigh channel with and without iterative demapping,

BITCM schemes operating on small (1504 bits) frames. All schemes employ a 1/2-rate DVB-RCS turbo code. Turbo decoding is performed in 8 iterations. From Figure 6, it is seen that, the iterative demapping can be only beneficial for BITCM systems operating at sufficiently low SNR values, i.e., at the asymptotic region. For instance, the scheme Y outperforms the scheme A, at a BER below to 5×10^{-7}. Figure 7 shows that, the proposed BITCM scheme results in significant improvement performance compared to all classical BITCM schemes with a better convergence threshold and a lower error floor. At a BER=10^{-7}, our scheme outperforms the classical 64-QAM BITCM schemes by more than 0.5 dB. We believe that this significant performance gain is mainly due to the application of

the signal space diversity technique. However, we point out that the convergence threshold, above the iterative decoding becomes effective, shifts by more than 0.5 dB to a higher E_b/N_0 for the proposed 64-QAM BITCM scheme without iterative demapping. Nevertheless, such scheme can still be of interest in some applications for which the asymptotic error-rate performance is the target.
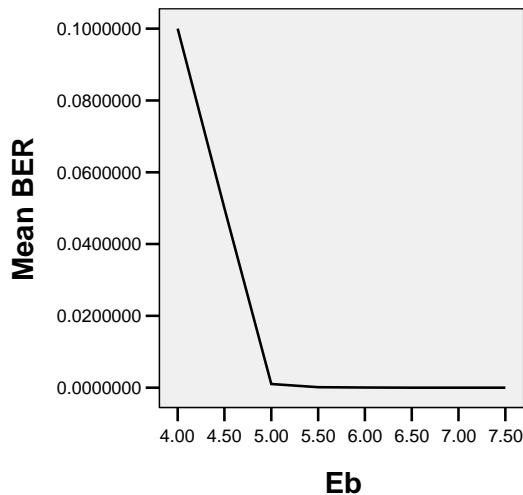
Figure 7. BER Performance comparison over Rayleigh channel between several 2 bit/s/Hz 16-QAM

5. Conclusion

In this paper, we investigated the gain which can be obtained by applying signal space diversity and implementing an iterative decoding algorithm at the receiver side, to the design of BITCM over Rayleigh wireless channels. We showed that the error performance of a 2 bit/s/Hz 64-QAM turbo coding scheme can be significantly improved, especially in what is known the asymptotic region, without loss in the convergence threshold. The proposed modifications lead only to a slight increase in the system complexity. Finally, note that the method introduced here can be applied in straightforward manner to BITCM systems employing high-order modulations in place of 64-QAM. We also think that further error performance improvement of BITCM schemes on wireless channel is possible by using constellation shaping and an iterative receiver. This will be the subject of future research.

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Analysis of P53 Gene Mutations in Peripheral Blood Lymphocytes of Egyptian Spray Workers Exposed to Multiple Pesticides

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Abstract: The advanced research of the molecular genetic toxicology is focused on the fundamental molecular mechanisms involved the risk of mutations in genes related tumor. Mutations of the p53 tumor suppressor gene plays an important role in the development of common human malignancies. Previous reports revealed that the tumor suppressor gene p53 is considered to be the most frequently mutated gene in human tumors. The present study was designed to investigate the association among three factors, which focusing on occupational exposure to pesticides, aging and smoking habit and their effect on p53 gene mutation in fresh blood lymphocytes of workers occupationally exposed to a mixture of pesticides in outdoor fields in El-Fayoum governorate, Egypt. Because p53 gene mutation is associated with many factors not one factor effects on the result but may be many factors affect on this result. So we used a multiple Linear Regression statistical test to give the effect of each factor individual as well as the interaction among these factors and the results showed that there was a significant Linear Regression of p53 mutations with age and smoking factors with ($P = 0.005$) and ($P = 0.002$) respectively but there is no significant with pesticide exposure factor ($P = 0.528$). P53 genetic mutation occurs relatively with age after 40 years old and the workers in any occupation when they are smokers will be prone to P53 gene mutations than other environmental factors. In conclusion, PCR-"cold" SSCP is a rapid and sensitive method for identifying p53 genetic mutation and useful as biomarker but at least should be used with many other biomarker tests to give a clear picture about environmental genotoxicity.

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Key Words: Occupationally exposed, Pesticides, Age, Smoking, p53, SSCP

1. Introduction:

In recent years the pesticide problem has been focused of public interest. The reason is that these chemicals have a very important role in agriculture but in the same time the possible threat to human health has also been increased. Most of the pesticide sprayers in Egypt are not protected by safety measures when using the pesticides in the fields; therefore they are directly exposed to these compounds whose toxicity is ranged from moderate to hazardous. Many pesticides have been tested for mutagenicity by a variety of *in vitro* and *in vivo* assays, and mutagenic compounds have been found among all major categories of pesticides, including insecticides, fungicides and herbicides.

A limited number of field studies also have been done aiming to evaluate their genetic risk, at least at the molecular genetic level. In recent years, p53 tumor suppressor gene mutation has been found to be the most common genetic alternation in human cancers (Hollstein *et al.,* 1991). P53 protein appears to function in a cell cycle control as a negative regulator of cell division in the G1-S phase (Hartwell, 1992; Kastan *et al.,* 1991; Livingstone *et al.,* 1992).

The location and type of p53 gene mutation can reflect exposure of humans to certain types of carcinogenic agents according to (Wang *et al.,* 1996).Genetic mutations in genes controlling cell growth and proliferation, have been potential in discovering crucial biological alterations associated with long-term exposure to carcinogens; consequently, they may aid in leading to tumor formation. In this context, mutations of the tumor suppressor gene p53, which encodes a multi factorial transcription factor controlling cellular response to DNA damage (Levine, 1997) represent a potentially useful biomarker in the search for etiology, molecular mechanisms, and, hopefully, prevention of environmental cancers (Harris, *et al.,* 1996, Sidransky, *et al.,* 1996 and Hollstein *et al.,* 1997). So the present study was designed to investigate the association between three factors, which focusing on occupational exposure to pesticides, aging and smoking habit and their effect to induce mutation in the p53 gene. We supposed the mutations occurring at the molecular genetic level may be useful as biomarker of the DNA damage after exposure to pesticides. So we test the genetic damage in DNA of fresh blood lymphocytes for a population of workers occupationally exposed to a

mixture of pesticides in outdoor fields with addition two factors aging and smoking habit.

2. Material and Methods

2.1 Subjects and blood samples

Prior to the study, a questionnaire was obtained from each subject to determine the lifestyle of each subject, age, smoking, smoking years, exposure to pesticides and exposure years. Seventy four subjects were selected for this study. They were lived in the same geographical area located in El-Fayoum governorate, Egypt. They were subjected to various mixtures of pesticides by inhalation, skin and eye contact. On the other hand, twelve healthy young individuals living in the same area and with no history of occupational exposure to pesticides or smoking habits were selected as a control group. The blood samples collected from each subject using heparinized vacutainers.

2.2 DNA extraction from fresh blood lymphocytes

Lymphocytes and other mononuclear cells were isolated from 2mL heparinized peripheral blood sample using a standard method (Histopaque-1077, Sigma). Genomic DNA was isolated from Lymphocytes using a standard method described by (Gustincich et al., 1991) with few modifications by authors. The suspended lymphocytes was added to 1.5ml microfuge tube containing 400μl lysis solution (25 mM Na_2EDTA, 2 % SDS) then pipetted up and down approximately 10 times to lyse the cells. 150μl Protein precipitation solution (10 M Ammonium Acetate) was added to cell lysised. The tubes were inverted gently no more than 3 times and place into an ice bath for 10 minutes. The tubes were centrifuged at 10000 rpm for 3 minutes. The precipitated proteins formed an irregular brown pellet. The supernatant (which containing the DNA)was pipette into a clean 1.5ml microfuge tube containing 600 μl 100% Isopropanol then mixed by inverting gently no more than 3 times. The tubes were centrifuged at 12,000 rpm for 6 minutes; the DNA

will be visible as a small transparent pellet. The supernatant was poured off. A 300 μl of 70% ethanol was added and the tubes were inverted several times to wash the DNA pellet. The tubes were centrifuged at 12,000 rpm for 3 minutes. Carefully, the ethanol was pour off by draining each tube on clean absorbent paper and allowed them to air dry for 10-15 minutes. Finally, 50μl of TE buffer was added to each tube (this gave a concentration of 1μg/2μl). The solubilization of the genomic DNA pellet was facilitated by incubated for an hour at 55°C.

2.3. PCR amplification of the p53 gene

Most of the mutations reported in human p53 gene are clustered in exons 5-8, which are highly conserved among different species (Levine et al, 1991 and Hollstein, et al, 1991). The sequences of the primers and the annealing temperature are listed in Table (1). Oligonucleotide sequences of primers were cited from (Thongsuksaia et al., 2003). PCR reactions were performed in a 50μl volume containing 39.5?l sterile dd water, 5?l 10X optimized reaction buffer (DyNAzyme, Finnzymes), 2?l of 10mM dNTP Mix, 0.5?l of each sense and antisense primer(100 ?M stock), 2?l of DNA sample (0.5 ng/?l), 0.5?l of DyNAzyme Taq polymerase (2U/?l). Amplification was carried out in a DNA Thermal Cycler (PTC-100, MJ Research).

A hot-start PCR is used to reduce primer-dimer formation by added Taq DNA Polymerase after the reactions heated to 94°C for 5 min then run the PCR conditions, denaturation at 94°C for 1 min, annealing temperature (below 2 degree than melting temperature) for 1 min, and 72°C extension for 1 min. followed by 35 cycles. The final extension was conducted at 72 °C for 5 min. 10?l of the PCR product was added to 2?l of 6x loading dye then loaded onto a 1% agarose gel, stained with ethidium bromide, and photographed under UV light. This step was important to ensure the specific molecular weight of the p53 exons were obtained.

Table (1) the sequences of the primers of p53 exons 5-8

Primers	Sequences		Fragments length (bp)	Annealing Temperature
Exon 5 Sense	5` TCTTCCTACAGTACTCCCCT	3`	205	55 °C
Antisense	5` AGCTGCTCACCATCGCTATC	3`		
Exon 6 Sense	5` GATTGCTCTTAGGTCTGGCC	3`	136	57 °C
Antisense	5` GCAAACCAGACCTCAGGCGG	3`		
Exon 7 Sense	5` TTATCTCCTAGGTTGGCTCT	3`	130	59 °C
Antisense	5` GCTCCTGACCTGGAGTCTTC	3`		
Exon 8 Sense	5` TCCTGAGTAGTGGTAATCTA	3`	157	58 °C
Antisense	5` GCTTGCTTACCTCGCTTAGT	3`		

2.4. Molecular analysis of P53 mutations

A rapid method for single-strand conformation polymorphism analysis of PCR products (SSCP) that allows using ethidium bromide staining was firstly described by (Hongyo et al.1993). This method is based on the PCR-amplified products can be separated into single strands by denaturation and electrophoresed on non-denaturating polyacrylamide gels. Under non-denaturating conditions, single-stranded DNA adopts a secondary structure that is dependent upon its sequence. Changes in the sequence (eg, point mutations) can cause a shift in the electrophoretic mobility of the analysed conformations compared with wild type DNA. In SSCP, detectable mutations include base substitutions, small insertions, deletions, and rearrangements (Peltonen, et al., 2007). Thus, the (PCR-SSCP) is a powerful approach for qualitative analysis of the DNA (Yap and McGee, 1992and Mohabeer et al., 1991). For SSCP analysis, 10?l of PCR product was mixed with 10?l gel loading dye (98% formamide, 10mM NaOH, 20mM EDTA, 0.05% bromophenolblue, 0.05% xylenecyanol) in 0.2ml ultra thin reaction vial. Denature the mix at 100°C, 7 min. in a heating block then putted in ice for 5min. The mixture was loaded on the 12% polyacrylamide gel (10 × 8× 0.1 cm3). The ratio of acrylamide/bisacrylamide was (37.5:1). Electrophoresis was carried out in 1.5x TBE buffer, initially at 200 V for two minutes, followed by 120 V for 4.5 hours, using the refrigerator as cooling system at 10°C. After the gel run was completed, the gel stained with 0.5mg/ml solution of ethidium bromide in 1 X TBE buffer for 15 minutes followed by washing in distilled water. The bands were visualized by using a 340 nm UV transilluminator and photographed.

3. Statistical Analysis

Statistical analyses were performed using the Multiple Linear Regression test. For all statistical tests, P-values < 0.05 were considered significant. Data were analyzed using SigmaPlot, Ver. 11 Statistics software. The obtained data by PCR and SSCP techniques were analyzed using Gel-Pro analyzer Ver. 3.1 software

3. Results

As shown in Fig. (1) The clear bands of PCR product (double-stranded DNA) show the specific molecular weight base pair (bp) of exons 5-8 of human p53 gene.

Mutations in exons 5–8 of the p53 gene were screened by cold SSCP analyses, and the mutation detects by monitoring the mobility shift of the normal two single strand bands of each exon which illustrated in Fig (2). Our results are summarized in Table(2), the results related to the age factor revealed that the high percentage of p53 gene mutation among workers with age ≥ 40 years, 22 cases (48.5%) and 1case (3.4%)

among the workers with age < 40 years. The high significant multiple Linear Regression between the age and p53 mutation was high significant (p= 0.005). On the other hand, the negative control group consisting of young adult people with average age 18 years old did not show any mutation in the p53 gene.

The results related to exposure factor revealed that, the workers who occupationally exposed to pesticides more than 15 years were 15cases (51.7%) have mutations in p53 geneand8 cases (17.8%) for workers who exposed to less than 15 years and there is no significant regression between the mutation and the pesticide exposure factor (p= 0.528).

However, the results related to smoking factor investigate that 11 cases of workers who were smoking more than 20 years exhibited mutations with 84.6% than workers were smokers less than 20 years that showed only 7 cases with 28.0%. The smoking factor indicated high significant multiple Linear Regression (p= 0.002) but the non smoking workers revealed only 5cases of mutation (13.9%). Furthermore, according to our results the p53 gene mutation occurred only in exons 5 and 7, and we did not find any mutations either in exon 6 or 8.
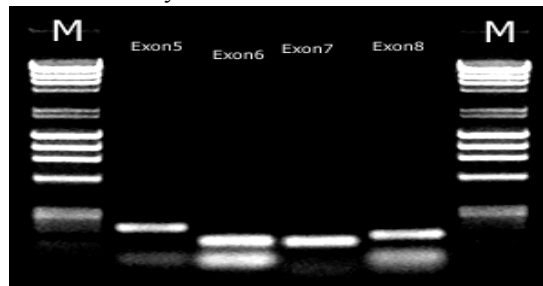
Fig. (1) PCR product shows molecular weight of exons 5-8 of human p53 gene (bp). (lane 1,6) DNA Marker a mix of Hind III digest and Hae III digest.
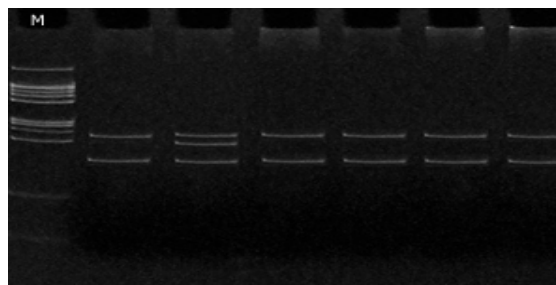

Fig. (2) SSCP analysis of P53 exon5. The band pattern reveals whether a mutation exists (lane 3) with heterozygous wt/mut in the same case; (lane 2) has no mutation and seems similar to (lanes 4, 5, and 6) which belong to control group; (lane 1) DNA marker. mix of Hind III digest and Hae III digest.

Table (2) the regressions between factors related to subjects and p53 Gene mutations(N=74)

Factors	No of subjects (%)	P53gene Mutations		P-values*
		Positive (%)	Negative (%)	
Age(y)				
<40	29 (39.2)	1 (3.4)	28 (96.5)	0.005
≥40	45 (60.8)	22 (48.9)	23 (51.1)	
Exposure duration				
< 15	45 (39.2)	8 (17.8)	37 (82.2)	0.528
≥15	29 (60.8)	15 (51.7)	14 (48.3)	
Non- Smoking	36 (48.6)	5 (13.9)	31 (86.1)	
Smoking				
< 20	25 (33.8)	7 (28.0)	18 (72.0)	0.002
≥20	13 (17.6)	11 (84.6)	2 (15.4)	

4. Discussion:

The incidence of P53 mutation varied in different population, which may be partly a result of the inclusion of people from different geographic areas and differences in the technique used to analyze the mutations (Hiseh et al., 2001). In the present study, we studied the probability of three factors to induce mutations in the p53gene as a biomarker of genotoxicity. These factors were focusing on occupational exposure to the pesticides, aging and the smoking habits. The statistical correlation test between pairs of variables was not performed because the investigation of our study (p53 gene mutation) is associated with many factors not one factor effects on the result but may be many factors affect on this result. So a Simple or multiple Linear Regression is the most commonly statistical test used in this case. By this test, we can discuss each factor as individual and then concluded the effect of all the factors together.

The age factor

Our results indicated the increase of percentage in p53 mutation by age older than 40 years. These results is appeared to be in agreement with (Tiwawechac et al., 2010) from 2.01 to 2.63 fold by age >40 to >50 years in the patient have Nasopharyngeal carcinoma (NPC) in Thailand. The study also revealed the polymorphism of gene p53 carriers with age of >40 years. our results are also in agreement with (Rugge et al., 2000)who found The rate of p53 gene mutationsin gastric cancers patients < 40 years are low versus (Hsieh et al., 2000) found the increasing in frequency of p53 gene mutations in intestinal-type tumors in elderly populations. Also studies related to age and p53 mutations seem to suggest the same idea(Pollack, et al., 2001) studied the relationship between frequency of p53 gene mutation exons 5-8 and age in childhood malignant

gliomas and reported that children <3 years of age the frequency of mutations (2 of 17 tumors, 11.8%) and between 3 and 10 years old the mutations were 12 of 27 tumors (44.4%) and12mutations of 33 tumors (36.4%)from children between 10 and 18 years old at diagnosis. Therefore the age factor may be apparent has an essential role in the p53 gene mutation.

The pesticide exposure factor

There is an association between occupational exposure to complex of pesticides and the genotoxicity which have been established in a number of studies in long ago (Sorsa et al., 1990) and (Hagmar et al., 1994). Also previous studies have shown that loss of P53 function leads to genomic instability (Livingstoneet al., 1992). Recently Loyant et al., 2005 investigated the P53 mutations in exons 2-11 by denaturing high performance liquid chromatography (DHPLC) and they explored the hypothesis that occupational exposures to pesticides and organic solvents could raise the frequency of P53 mutations in brain tumour cells. These mutations are present in approximately 30% of brain tumours. On the other hand our results indicated no significant between pesticide exposure factor and the incidence of mutation in p53 gene this result seems to in parallel with previous study(Pastor et al., 2001) reported that no statistically significant differences in the frequencies of cytogenetic damage were detected between exposed pesticide worker and control individuals in lymphocyte cultures but the multiple linear regression analysis indicated that the cytogenetic endpoints were inversely influenced by other factors which included also in the control group e.g (alcohol, tobacco, and coffee, etc.), and may be these factors together affect on the result not only one factor .

Our results showed high significant multiple Linear Regression between p53 gene mutations and

smoking factor than any other factors. These results are in agreement with several other studies (IARC, 1986: Hackshaw et al., 1997) reported that the smoking is the most important risk factor for lung cancer, but passive smoking increases the risk as well as. Also these results appear to be in agreement with the previous report (Bennett et al., 1999; Hainaut and Pfeifer, 2001; Pfeifer et al., 2002) they reported that Both qualitative and quantitative data link To P53 gene mutations at CpG sites in lung cancer with smoking and the incidence of P53 mutations is10–26% in non-smokers and about 60% in smokers with overrepresentation of G to T base pair shift mutations. Thus, the existing molecular epidemiology studies on different aspects of smoking-related lung cancer support theP53 gene as a target of carcinogens in cigarette smoke and a tobacco-specific P53 mutation spectrum at a population level. However, so far no studies exist on P53 mutations specifically in lung cancers putatively due to occupational exposure to passive smoking. Versus studies have been noted the passive smoking effect on P53 mutations by (Husgafvel-Pursiainen et al., 2000) showed that lung cancers of passive smokers have an increased risk for p53 mutations. In western Chinese's population, the high incidence of P53 mutation (42.56%) is associated with known risk factors, such as tobacco smoking and alcohol drinking (Ostwald. et al., 2000). Recently (TANG et al., 2010) investigated that the high incidence of P53 mutation has proven to be related to tobacco smoking in most oral squamous cell carcinoma patients. It seems that, P53 gene mutation frequency is increased in smoking, the effect may be strong enough to overcome the effect of other carcinogens. Furthermore, recent studies have shown that various kinds of carcinogens produced by smoked cigarettes as polycyclic aromatic hydrocarbon (PAH) might be responsible for different p53 gene mutations and p53 over-expression (Taghavi, et al., 2010). Therefore, it has been hypothesized that continuous exposure to specific carcinogenic components of tobacco smoke may cause mutations in some important cell cycle genes, such as p53, leading to over-expression and abnormal accumulation of the translated proteins. This specific mutation may result in the formation of a dysfunctional protein, which is sequestered and accumulated in the cell, leading to cancer development. This means that workers in any occupation when they are smokers or exposed to tobacco smoke will be prone to P53 gene mutations and at risk to cancer initiation through the inactivation of p53.

5. Conclusion

It can be concluded that PCR with cold-SSCP analysis is a rapid and sensitive method for identifying p53 gene mutation. Although p53 genetic mutation polymorphisms play an important role to explore the effects of gene-environment interaction, probably not a lone is useful as biomarker but at least should be used with many other biomarker tests to give a clear picture about environmental genotoxicity.

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Assessment of Sport Practice among Adolescent School Students and Its Effect on Perceived Health in Sharkia Governorate –Egypt

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Abstract: Sedentary lifestyle is a major contributing factor to increasing health problems among adolescents. Inactive youth have a high probability of becoming obese adults with increased risk for coronary heart disease, hypertension, and diabetes. This study aimed to assess the frequency of practice of adolescent students towards sport practice, to study some of the risk factors that affect sport practice and to measure the subjective direct and indirect effects of sports participation on perceived health. The sample was selected by multistage simple random sample technique from students of preparatory and secondary schools. The tool in our study was questionnaire which was included data about practice of sports and its association to socioeconomic condition, some lifestyle factors, knowledge, attitude, , the direct and indirect effect on perceived health, then we measured body weight and height of the students and calculated their Body Mass Index. Obtained data revealed that 81.8% our sample practice sports but the majority of them with low level of practice 75.2%, study work were the most barriers against practicing sports 29.4% while fun and social role were the most common motives (30.5%& 23.5%respectively). Low levels of knowledge, attitude as well as negative perception of health & high level of anxiety, feeling depression and psych-physiological score were significant among those not practice sports. In addition; smoking, obesity, female sex were significant associated with non practice of sports. In conclusion; feeling anxiety, depression, negative attitude, low level of father and mother education were the most common predictor factors of not practice sport, so we recommend health education programs, social mobilization to eliminate barriers and increase motives toward sport practice and incorporated more students into sports programs into schools or community.
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1. Introduction:

Physical activity has considerable health benefits for children and adolescents. During the 20th century, the main leading causes of death shifted from infectious to chronic diseases: cardiovascular disease, cancer, and diabetes which they are now considered the most prevalent, costly and preventable of all health problems. Physical activity is considered one of the important issues that may favourably affect some risk factors for cardiovascular disease such as body mass index (BMI), blood lipid profiles and resting blood pressure (Jafar et al 2008). Weight-bearing exercise increases bone mass density among young healthy people and improves aerobic endurance and muscle strength (Marwaha&Sripathy 2008).

Morales and active involvement in sport and exercise has beneficial effects relating to psychological well-being and lowered risk of negative health behaviors including smoking, alcohol use and longevity. exercise is negatively associated with stress, anxiety and depression in adolescents (Seefeldt et al2001) . Physical activity also makes a significant contribution to the overall quality of life at any age and especially in older adults (Brownson et al 2000).

Wasfi et al (2008) reported that World Health Organization (WHO) in 2003emphasized the importance of behaviour risk factor surveillance as a first step for prevention of non communicable diseases.

Physical activity is defined as any body movement produced by a contraction of skeletal muscle those results in a substantial increase over resting energy expenditure. For children, physical activity often consists of play, recreational activities, and competitive sports. Seventy percent of children engage in some vigorous physical activity (activity at 60% or more of maximum heart rate for age), but only 42% of males and 30% of females are vigorously active by the end of adolescence (Anne et al 1998).

In a sample of Icelandic 15- and 16-year-old adolescents, Thorlindsson and colleagues' (1990) reported that sports participation had a direct effect on perceived health. Furthermore, sports participation affected perceived health indirectly through

decreasing smoking, anxiety, feeling depression and psycho- physiological symptoms (Pastor et al 2003).

Despite the reported benefits of sport and exercise, scientists and educators repeatedly report that many young people are physically inactive (Seedfeldt et al 2001). So, this study was conducted to get information about the practice of adolescent students of sport and study some of the risk factors that affect it. Also, this work will threw light the direct and indirect effects of sports participation on perceived health.

2. Subjects and Methods:

The present work was carried on a sample of adolescent school students who were selected from the preparatory and secondary schools.

The school sample was selected by multistage simple random sampling technique from Sharkia governorates. Two districts were randomly selected and from each one it was divided into urban and rural strata, from each area we randomly selected schools for boys and others for girls from which a simple random sample was selected. Proportion allocation was putted into consideration during selection of our sample.

The sample size was calculate by assuming that the estimated non practice of sport among adolescent student about 22 % (Wasfi et al 2008). The confidence level was 95 % with a power of the study was 80 % and the population size of school student age 12-18 years in Sharkia Governorate was 588,747 (Centraland General Package Statistics System 1996). Putting into consideration10% non response rate, the calculated sample size was (374) that selected according to the proportion allocation of the different stage and gender.

Schools were approached and asked to take part in research on adolescents' health-related lifestyles. Administrative approval was taken from selected school. Adolescents were informed that participation in the study was voluntary and asked to complete the questionnaire with the researcher. The data were collected through:

A- questionnaire: Include:

1) socio-demographic data: as age, sex, residence, parental and maternal education and socio-economic status.

2) Tobacco consumption were measured and classified as: smoker (currently smoked at least 1 year before); passive smoker (continuous exposed to tobacco smoke exhaled by smokers in an enclosed environment); ex-smoker (smoked at least 1 cigarette/day over the year and had not smoked for 6 months or more at the time of study); and non-smoker (never smoked .

3) The knowledge in our questionnaire included 8 closed-ended questions; 3 about the benefit of sport on physical, mental and social health and 5 to assess knowledge regarding the dangerous effects of not practising sport on body weight, diabetes, hypertension, lipid profile and coronary heart disease. Level of knowledge regarding sport benefit was classified as high (66%–100%); acceptable (33% < 66%); and low (< 33%) (Wasfi et al 2008).

4) Attitude towards sport practice was classified as: positive (student agreed that sport practice has an effect on obesity or diseases such as diabetes or heart disease); negative (student agreed that sport practice leads to loss of money, effort or time); and indifferent (student neither agreed that there were beneficial effects nor that there were disadvantages). Sports participation was measured by asking the students how often they participated in sports, types of activities and duration. Then Actual sport practicing of students was classified into: good (student did vigorous exercise ≥ 3 times/week for about 20 min/session and also > 30 min of moderate physical activity most days of the week , acceptable (student did vigorous exercise < 3 times/week for about 60 min and > 30 min of moderate physical activity most days of the week); poor (student did no vigorous activity or irregularly practised vigorous exercise < 60 min/week and 30 min of moderate physical activity most days of the week; and none (did not practise physical activity at all).

Regularity of sport practice were classified into: regular (student practised sport regularly every day or every other day or at least 3 times/week); irregular (student practised sport in an irregular manner, not constantly and not every week or month throughout the year); and occasional (student practised sport occasionally ≤ 1 time/month throughout the year).

Perceived physical fitness was measured by using the single item 'How would you rate your physical fitness? This was assessed on a four point scale that ranges from 1 (not good at all) to 4 (very good).

The three dimensions of psychological distress employed in this work were: subjective feelings of anxiety and subjective feelings of depression and psycho- physiological symptoms. Feelings of anxiety were assessed by asking the students how often they felt nervous (range: 1='almost never' to 5='almost every day'). Feelings of depression were measured by asking the students how often they felt depressed (range: 1='almost never' to 5='almost every day'). These two variables were used only to measure subjective feelings of anxiety or nervousness and bad moods, it was not our intention to measure anxiety disorders or clinical depression. The psycho-

physiological symptoms variable is a mean score from four items measuring how often students suffered from headache, stomach pains, back pains or felt dizzy. Students rated each item on a five-point likert scale (range: 1='almost never' to 5='almost every day (El Sherbini 1996 and Guidelines for promoting physical activity and reducing sedentary living among youth 1997)

Perceived health status, was measured by asking students to assess their health on a four point scale which ranging from 1 (this mean not healthy at all) to 4 (very healthy). The items used in this paper came from a WHO cross-national survey of Health Behavior in School-children (Pastor et al 2003).

Anthropometric measurements as weight and height was measured and body mass index were classified according to WHO classification into underweight, normal, overweight or obese (WHO 1995).

Ethical consideration:

An orientation about the objectives of the study was carried out followed by verbal consent taken from every participant. Confidentiality was maintained through the study.

Data analysis

The data were analysed using Odds Ratio and confidence interval categorical data, and logistic regression analysis was done for significant risk factors. The data were coded and analysed using *SPSS*, version 15 (Statistical Package for social science for windows 1996).

3. Results:

As shown in (table 1) of the results, Most of our sample was practice sports 81.8% but the majority had low level of practice according to our classification (75.2%).

As regard to the regularity of practice sports, regularity was found in 17.6 % of those practice sports and 44.8 % had irregular practice while 37.6 % of them were occasionally practice sports as shown in (table 2)

As shown in (table 3), the most common place of practice sports in this study sample were at road and schools (48.0 % & 43.5% respectively).

On studying the barrier and motives toward practice sports we found that study work load and loss interest were the most frequent barrier against practice sport (29.4%& 27.9%) while tendency to fun and social role were the most frequent motives (30.5% & 23.5%). (Table 4)

Table (1) Practice of sport and perception toward the practice among the studied group

	Frequency	Percentage %
Practice sport:		
Not practice	68	18.2
Practice:	306	81.8
Level of practice:		
Low	230	75.2
Acceptable	43	14.0
Good	33	10.8

Table (2) Regularity of practice sports

	Frequency	Percentage
Irregular	137	44.8
Occasionally	115	37.6
Regular	54	17.6

Table (3) the most common place of practice physical activity:

	Frequency	Percentage
Roads	147	48.0
School	133	43.5
Club	26	8.5
Home	0	00.0

Table (4) common barriers and motives toward practice sports

	Frequency (68)	Percentage
Health problem	4	5.8
Social	15	22.1
Economic	5	7.4
Familial	5	7.4
Study work load	20	29.4
Loss of interest	19	27.9
Motives	Frequency (306)	Percentage
Fun	93	30.5
Social aspect	72	23.5
Skill development	67	21.9
Self esteem	54	17.6
Fitness	20	6.5

As shown in (table 5), Non practice of sport was more significant common among females OR 2.91 (1.64-5.17), obese OR 4.31 (1.95-9.67), smokers OR 1.98(1.1-3.59), and those who their father and mothers were of low educational levels OR 10.2(2.7-41.7) & 8.5 (3.18-23.5) respectively.

Table 6 showed that non practice of exercise was significant more among those with low level of knowledge OR 8.14(33-20.3), negative attitude OR 8.5 (3.5-20.7),feeling anxiety almost every day OR 11.5(5.3-25.2),feeling depression almost every day OR16.196.8-38.7), high psychophysiological score almost every day OR 9.8(4.4-22.2) and those of low bad perception of their health OR 3.58(1.15-11.83) .

According to logistic regreesion analysis in (table 7) we found that non practice of sport was more significant predicting among those feeling depression and feeling anxiety almost every day, those their fathers were illiterate,had high pychophysiological score almost every day ,had negative attitude,had illiterate mothers and had poor level of knowledge.

Table (5) association between practice physical activity and some biological, socioeconomic and life style factor.

Variable	Non practice sport		Practice sport		
	No	%	No	%	OR (CI)
Sex					
Male	27	11.8	201	88.2	
Female	41	28.1	105	71.9	2.91(1.64-5.17)
BMI					
Normal weight	12	10.1	107	89.9	1
underweight	7	14.0	43	86.0	1.45(0.48-4.32)
Overweight	19	16.8	94	83.2	1.8(0.78-4.19)
Obese	30	32.6	62	67.4	4.31(1.95-9.67)
Smoking:					
Smokers	46	22.7	157	77.3	1.98(1.1-3.59)
Non smoker	22	12.9	149	87.1	
Residence:					
Rural	40	18.8	173	81.2	1.1(0.6-1.9)
Urban	28	17.4	133	82.6	
Father education					
Illiterate	7	53.8	6	46.2	10.2(2.7-41.7)
read &write	17	39.5	26	60.5	5.8(2.4-14.3)
basic	19	52.8	17	47.2	10.06(4-25.6)
secondary	10	7.6	122	92.4	0.74(0.3-1.82)
university	15	10.0	135	90.0	1
Mother education					
Illiterate	23	44.2	29	.55.8	8.53(3.18-23.5)
Read and write	19	38.8	30	61.2	6.81(2.49-19.12)
Basic	5	13.9	31	86.1	1.73(0.45-6.46)
Secondary	13	9.1	130	90.9	1.08(0.4-2.98)
University	8	8.5	86	91.5	1

Table (6) association between practice physical activity among the studied sample and their knowledge, attitude, and their direct and indirect perception of health

	Non practice sport		Practice sport		OR(CI)
	No	%	No	%	
Knowledge: good	8	7.7	96	92.3	1
Average	20	11.7	151	88.3	1.59(0.6-4.1)
Bad	40	40.4	59	59.6	8.14(3.3-20.3)
Attitude: positive	35	12.6	242	87.4	1
Indifferent	17	25.0	51	75.0	2.3(1.1-4.6)
Negative	16	55.2	13	44.8	8.5(3.5-20.7)
Feeling anxiety: -Never	13	5.9	204	94.1	1
- To sometimes	22	27.8	57	72.2	6.06(2.7-13.6)
-Almost every day	33	42.3	45	57.7	11.5(5.3-25.2)
Feeling depression:-Never	9	4.3	198	95.7	1
-To sometimes	24	28.6	60	71.4	8.8(3.65-21.7)
-Almost everyday	35	42.2	48	57.8	16.1(6.8-38.7)
Psychophysiologoical score:					
-Never	11	5.8	180	94.2	1
-To sometimes	24	25.3	71	74.7	5.53(2.43-12.8)
- Almost everyday	33	37.5	55	62.5	9.8(4.4-22.2)
Perception of their health					
-Very good	5	11.6	38	88.4	1
-Good	16	14.4	95	85.6	1.28(0.40-4.33)
-Average	23	19.3	96	80.7	1.82(0.60-5.92)
-Bad	24	32.0	51	68.0	3.58(1.15-11.83)

Table (7) Logistic regression analysis of factors predicting practice of sport:

Variables	Beta ±SE	Wald	P
Feeling depression(almost every day)	-4.97±1.49	11.07	0.001
Feeling anxiety(almost every day)	-2.45±0.87	7.88	0.005
Father education(illiterate)	2.89±1.07	7.32	0.007
Pychophysiological score(almost every day)	-1.3±0.51	6.68	0.01
Attitude(negative)	1.16±0.54	4.56	0.03
Mother education(illiterate)	2.36±1.1	4.57	0.03
Knowledge(poor)	2.39±1.2	3.96	0.047

4. Discussion:

Health-related behavior in early life influences later risks for lifestyle-related disorders. It is therefore important to investigate the health behavior among young people in order to improve health promotion activities targeting this group. It would also help to develop health education initiatives targeting students, which requiring a detailed knowledge about the health of students, their health related behaviors, and factors that influence these behaviors (Afifi 2006).

This study revealed that 18.0% of the students did not practice sport while among those who practice sports 75.2 % of them had a poor level of practice. Only 8.9 % of school students had a good level of sport practice ,this may be related to that although children and adolescents are more physically active than adults. Also, many young people do not engage in moderate or vigorous physical activity (at least 3 days a week) (Afifi 2006).

Regular sport practice among our sample was only constituted 17.6 % while other study reported that regular practice of sport among local UAE students (50.6%) which was significantly higher than among non-UAE students (Wasfi et al 2008).This variation in regularity explained as sports clubs are more affordable for UAE , the UAE students' houses are larger and more suitable for sport practice than in our locality ,also The level of knowledge about the benefits of sport was also higher than among our sample.

This investigation clarify that the risk of non participation among female was significant higher about three times more than male. Most investigators of gender differences in physical activity have attributed this to the impact of socialization into sport or exercise involving family, school, or peer group ,20 other found that boys are more likely to have physically active friends and friend's physical activity is among the strongest correlates of one's own physical activity (Vilhjalmsson andThorlindsson1998). Also schools have a role in this differentiation through physical education (PE) classes. Thus, more girls than boys are believed to have negative physical exercise experiences that lower their interest and involvement in subsequent leisure time physical exercise (Ennis et al 1999) This is consistent with results of the Centers for Disease Control in the Youth Risk Behaviour Surveillance 1996.

The majority of collected sample had positive attitudes towards sport practice while participation of sports was about 8 times more among those with positive attitude toward practice of spots. In agreement with our study ;(Zakarian 1994) and (Ann et al 1998) revealed that positive attitudes toward physical education was positively associated with physical activity among young people.

the most motives for practice sports among this study was fun (30.5%) , socialization aspect to form friends and team work (23.5%) ,acquiring skills (21.9%) and for development self esteem (17.6%) this is agreeing with Gill, Gross and Huddleston who found that basic motives for involvement in sport were: achievement/status, team atmosphere, fitness, energy release, skill development, friendship and fun (Biddle 1997).

Fewer children of illiterate fathers practised sport (46.2 %) than did children of higher educated fathers (90%). The level of sport practice was significantly related to father's more than mother's level of education. The results can be explained on the basis that socioeconomic level and economic accessibility to sport practice mainly depends on the father's education level (Afifi 2006).

A high study workload was the main reason given for not practising any kind of sport among our study sample and this is consistence with many researches Also, lack of interest, social role of family or community, economic and health problems played a role in lack of sport practice. The explanation for social and economic problems is attributed to the unavailability of places for sport practice at home due to the small size of apartments, the high cost of sport clubs (Chinna et al 2006), this was evident in our research as most of the students practiced sports in the street or in schools. In agreement with the present study, Kelder et al (1995) concluded that lack of time is negatively associated with physical activity among adolescents . WHO 2007 revealed that many factors prevent young people from regular physical activity, including lack of time and motivation,

insufficient support and guidance from adults, feeling of embarrassment or incompetence, lack of safe facilities and locales for physical activity and simple ignorance of the benefit of physical activity.

Also, Hamlin and Ross (2005) found that social, behavioural and physical changes that characterize adolescence act as barriers to physical activity during this period. Major barriers included a reduction in active transport, altered community design, less physical education time at school and a shift away from active to passive leisure.

Our study found that sport practice among average weight students was significantly better than obese ones as the risk of not practice sports increase by about more than four times among this group compared to the average or normal weight. This result was agreement with Fogelman (2004). In fact obesity may be a risk for non practice sport and otherwise may be the direct result of not practice sport as Henry et al (2004) in their study concluded that the amount of physical activity undertaken by adolescents was very low and attribute this to Cultural and weather restrictions and social change of the community that are not conducive to physical activity and play a major role in levels of physical inactivity. This may explain, in part, the rise in the incidence of obesity in this population (Nerin et al 2004).

Non Smokers had about two fold more of practise sport than smokers. The practice of physical exercise during adolescence as part of a health prevention programme might interfere with the factors that lead young people to start smoking and thereby contribute to a reduction in the prevalence of tobacco use in the population as a whole. Sports participation could reduce smoking and alcohol use in adolescents which in turn would enhance health perceptions (Malek and Bakir 2007).

Also, sports participation might lessen feelings of depression, feelings of anxiety and reported psycho- physiological symptoms, and thus lead to improvements in health perceptions, this result was significant in our study as we found subjective feeling of anxiety, depression and psycho-physiological symptom were significant increase among non participants of sports (Pastor et al 2003).

The present study showed that non practice of sports was significant increase among those who had low level of knowledge about the benefit of sport while Wasfi et al (2008) in their study found that overall mean knowledge score about the benefits of sport among the studied sample was high this difference may be explained due to their believes as they considered that no knowledge is more crucial than knowledge about health, without health, no other life goal can successfully be achieved

Good perception of health was significant about seven times more among those practice sport In spite of that results, Henry et al (2002) reported that the significant direct effect of sports participation on perceived health is not altogether clear. Other variables might be mediating this relationship. For example, it is suggested that sports participation could improve self-esteem by increasing self-efficacy in the performance of specific sport activities and the different components of physical self-concept . It might be that with increasing self-esteem, the more optimistic for increasing the health assessment.

Other explanations of the effect of sport on perceived health could be the improvement of physiological functions (i.e. oxygenated blood for heart muscle needs, heart rhythm disturbances, blood pressure, beta-endorphin concentrations, mono-amines synthesis, etc.). Further research is required to shed light on processes involved in the effect of sports on perceived health.

5. Conclusion and Recommendation

The current study concluded that there was a strong relationship between non sport practice and feeling of depression, anxiety, level of education of father (illiteracy), psycho-physiological symptom, positive attitude toward practice of sport, educational level of father and mother of participants, knowledge about perception of health. So we recommend that health education intervention program to improve perception of health, knowledge and attitude toward sport practice among young people which must be applied in schools, also social mobilization must be enhanced as society must be worked to decrease the influence of barriers against practice sports and enhance and support opportunities for young people to become physically active.

Also, organized sport programs in schools could recruit more children and adolescents into sport and exercise, and reduce or eliminate gender disparities. This would be an important step towards equalizing the life chances of young people and enhancing the health of the public and schools must be increasing area of backyards and tools of practiced spots to motivate and make practicing sports easy.

More research is needed to find the most suitable methods of enhancing practice of sports with low cost.

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Relationship between Impulsivity and Coping Strategies among Psychiatric Outpatients at Assiut University Hospital

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Abstract: Routine assessment of impulsivity and accompanying coping skills is essential for planning care and appropriate management of patients identified as impulsive. Impulsivity is one of the defining characteristics of many adult psychiatric disorders and is also a key component in the clinical risk assessment of anger and aggression. This study aimed to assess the impulsivity and coping skills among psychiatric patients and to determine the relationship between impulsivity and coping skills. The study was carried out in psychiatric outpatient clinic at Assiut University Hospital. The study sample comprised 120 psychiatric patients (70 males) and (50 females), diagnosed with schizophrenia, mood disorders and delusional disorder. Three tools were used for data collection, namely: Patient's assessment structured interview schedule, Impulsivity scale, and Coping scale. the main results yielded by the study proved that, concerning the level of impulsivity , the majority sample had moderate impulsivity, highest among patient's aged from 30–39 years, males than females (51%), married, illiterate (33%), and farmers (32%). The study recommended teaching nurses to understanding the effect of impulsivity on personality, behavior and coping strategies is essential for the accurate assessment and appropriate management of impulsive individuals.
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Key Words: Impulsivity, Coping Strategies, Psychiatric Patients

1. Introduction:

Impulsivity among psychiatric patients appears to be a growing problem and is a subject of importance and concern to clinicians and researchers. Impulsivity has highlighted the importance of the defining characteristics on various psychiatric disorders and is a key component in the clinical risk assessment of anger and aggression [1]

Impulsivity seems to be a basic part of some psychiatric disorders such as personality disorder, conduct disorder, aggression, bipolar disorder, suicidal behavior, attention deficit, hyperactivity disorder and psychoactive substances [2].

Subjects with a history of suicide attempts have impulsive errors on the immediate memory tasks and had shorter response latencies especially for impulsive responses [3]. Respectively impulsive behavior is a central feature of many psychiatric disorders which can affect personality, behavior and coping abilities of the clients .It is essential for accurate assessment and appropriate management of impulsive individual [1].

Impulse – control disorders are characterized by failure to resist an impulse, derive or temptation to perform an act that is harmful to the person or to others. The individual feels an increasing sense of tension, or arousal before committing the act, and then experiences pleasure, gratification or relief at the time of committing the act [4].

Coping defined as constantly changing cognitive and behavioral efforts to manage specific external and / or internal demands that are appraised as taxing or exceeding the resources of the person [5]. Patients who have not developed healthy coping responses are vulnerable and at high risk of continuing to react to stressful events in destructive ways. Using an appropriate coping measurement can help impulsive patients to identify and monitor feelings and become more attuned to triggers that signal the onset of impulsive behavior. Therefore, it is important for patients to know that they are capable of controlling their behaviors. If impulsive patients acquire essential and effective coping skills, this will lead to healthier life styles, decreased hospitalizations and improved quality of life [1].

As nurses are the key persons in giving care for patients with impulsivity , it is important to know ways of coping skills during assessing patients to cope with their impulsivity , develop self – controlling , seeking social support , accepting their responsibility and able to problem solving [1]. The aim of the study was to assess the impulsivity and coping strategies and to determine the relationship between impulsivity and coping skills among psychiatric patients.

2. Materials and Methods
Materials
Research design:

The design followed for this study is a descriptive correlational design.

Setting:

The study was conducted at psychiatric outpatient clinic at Assiut University hospital. The hospital is serving Assiut City and all Upper Egypt governorates.

Subjects:

Subjects of the study comprised all psychiatric patients attending to outpatient psychiatric clinic within a period of four months from March to June 2010 which included: patients diagnosed with schizophrenia, mood disorders, and delusional disorder, both sexes and agree to participate in the study. The study subjects mounted to 120 patients (70 males and 50 females).

Tools of the study:

Three tools were used for data collection:

1- Tool (1): Psychiatric Patient's Sociodemographic Data Structured Interview **schedules:** This interview schedule developed by the researchers included the sociodemographic data of the study subjects such as age, sex, occupation, marital status, education and diagnosis.

2- Tool (2): Impulsivity scale:

It was developed by Barratt (2000) ([6]). It comprises 30 items used to measure impulsive personality traits. It is divided into two order factors, the first order factors contain 6 factors as attention (5 items) , motor (7 items) , self – control (5 items) cognitive complexity (5 items) perseverance (4 items) and cognitive instability (3 items) .The second order factors contain 3 factors as attention (5 items) motor (11 items) and non planning (11 items). The scale is a four points likert type. The rate in each statement ranged from 1 (rarely/never) 2 (occasionally) 3 (often) to 4 (almost always/always).A total score is obtained by summing the first or second – order factors. A higher summed score for all items indicate higher levels of impulsivity. The degree of impulsivity was categorized according to the following scores: low impulsive ranges from 30 to 59, moderate impulsive ranges from 60 to 89, and severe impulsive ranges from 90 to 120.

3 – Tool (3): Coping scale it was developed by Jalowiec and Powers (1988)

This scale is used to measure coping strategies, which composed of:

A – Problem – oriented coping strategies, which primarily aimed at solving problems or handling stressful situations. There are classified into 2 factors:

Factor 1: active role of coping strategies (10) items.

Factor 2: passive role of coping strategies (6) items.

B- Affective – oriented coping strategies, which is used to manage emotions accompanying stressful situations, and they are classified into 5 factors:

Factor 1: coping strategies related to withdrawal (7) items.

Factor 2: coping strategies related to projection and displacement (5) items.

Factor 3: coping strategies related to neurotic reactions (4) items.

Factor 4: coping strategies related to daydream and fantasy (3) items.

Factor 5: coping strategies related to resign the self to the fate (5) items.

This scale is a five point likert scale with response options of always (5), often (4), about half the time (3), occasionally (2), never (1). A high score indicates greater use of that particular coping strategy. Al – Mahdy ([7]) translated it and established content validity and reliability of the Arabic version of this tool.

Methods:

1- An official permission was obtained from the dean of Faculty of Nursing – Assiut University and from the hospital director to the head of Psychiatric Department at Assiut University Hospital.

2- Tool two translated into Arabic language. Both the Arabic and English items were submitted to five experts from the English section, Faculty of Art, Assiut University to be reviewed for its translation. A jury of five experts in the psychiatric field examined the content validity. Reliability done by using Crombach alpha coefficient, it was 0.98.

3- Explanation the purpose of the study for patients before starting data collection.

4- Each patient has been interviewed once on an individual basis at outpatient psychiatric clinic (the outpatient clinic worked three times /week)

5- The patient was oral informed consent about the aim of the study and ensured about the confidentiality and privacy for them.

6- The data were collected by the researchers during the period of four months from the first of March to the end of June 2010.

7- The patient was interviewed for about 30 – 45 minutes at one time.

Statistical analysis

The data were computerized and verified using the SPSS (statistical package for social science) version 11.5 to perform tabulation and statistical analysis. Data were presented using descriptive statistics in the form of numbers and percentages. Qualitative variables were compared using chi – square test (X^2) and quantitative variables were compared by using the ANOVA (F - test). Statistical significance was considered at p – value <0.05.

3. Results:

Results of the present study showed that:

In the present study, 37.5% of the studied group was in the age group 30-39 yrs. While 28.33% of them were less than 30 yrs and small percentage of them (10.83%) were 50 yrs and more. The mean age regarding studied group were averaged 35.82 ± 9.26 years. As regard sex, 58.33 % of the studied groups were men (Table1).

Regarding educational level, 37.5% of the studied groups were illiterate. According to marital statues, nearly half of the studied groups were married, 36.66% were farmers and 23.33% of them were unemployed. As regard diagnosis, 42.5% of the studied groups were diagnosed schizophrenia and 25.82% of them were mania, while 23.33% and 8.33% of the studied groups were depression and paranoid, respectively.

Moderate impulsivity was recorded in 88.33 %, low impulsivity in 8.33%, while severe impulsivity was recorded only in 3.33% (Table 2).

Table (3) shows the correlation between impulsivity and coping. There was a highly significant differences between different coping strategies and impulsivity, while active role in coping strategies only significant which increased when the impulsivity decreased.

Table (4) shows the relationship between the level of impulsivity and coping strategies. It can be observed that impulsive patients more used of projection as a coping skills (F= 20.80, P = 0.01).

Table (5) shows the relation between the level of impulsivity and coping strategies. As regards diagnosis, there was a significant differences were present between level of impulsivity and coping strategies among schizophrenic disorders were had moderate impulsivity that used neurotic reaction (p= 0.007), dream and fantasy (p=0.016), resign self (p= 0.001) and passive role (p=0.023) as a methods of coping skills than other diagnosis.

Table (1) Demographic characteristics of the studied patients.

Characteristic of studied group	Frequency (No = 120)	Percent (%)
Age :-		
- (30 yrs)	34	28.33
- 30 yrs	45	37.5
- 40 yrs	28	23.33
-(50 yrs)	13	10.83
Mean ± SD	35.82 ± 9.26	
sex :-		
Males	70	58.33
Females	50	41.67
Education :-		
- Illiterate	45	37.5
- Read & write	14	11.66
- Primary school	19	15.83
- Prep school	2	1.66
- Secondary school	25	20.83
- University	15	12.5
Marital statues :-		
- Single	49	40.83
- Married	59	49.17
- Divorced	5	4.16
- Widow / Separated	7	5.83
Occupation :-		
- Unemployed	28	23.33
- Worker	23	19.16
- Farmer	44	36.66
- Student	13	10.83
- Professionals	12	10
Diagnosis:-		
- Schizophrenia	51	42.5
- Mania	31	25.82
- Depression	28	23.33
- Delusional disorder	10	8.33

Table (2): Level of impulsivity among the studied group.

Level of impulsivity	Studied group (N=120)	
	NO	%
- Low impulsive	10	8.33
- Moderate impulsive	106	88.33
- Severe impulsive	4	3.33
Total	120	100%

Table (3): Correlation between impulsivity and coping scale

Coping scale	Impulsivity	
	r	P
Withdrawal	0.48	**
Projection	0.59	**
Neurotic reaction	0.43	**
Dream &fantasy	0.46	**
Resign self	0.25	**
Active – role	-0.21	*
Passive – role	0.28	**

* P= 0.05   Significant ** P = 0.01   highly significant

Table (4) Relationship between level of impulsivity and coping strategies among studied group

Coping strategies	Level of impulsivity (Mean ± SD)			F- value	Signif.
	Low level (n= 20)	**Moderate level (n=76)**	**High level (n=24)**		
Withdrawal	15.80± 3.52	18.41± 4.52	23.21±3.59	18.60	**
Projection	8.80±2.63	11.82±4.19	16.38±4.22	20.80	**
Neurotic reaction	8.45±3.78	1.62±3.71	13.92±2.72	12.97	**
Dream &fantasy	7.00±2.34	8.42±2.57	11.13±2.56	15.95	**
Resign self	10.15±2.11	12.36±3.25	13.33±3.43	5.94	**
Active – role	26.65±9.22	23.91±7.39	20.50±5.32	3.90	*
Passive – role	14.40±4.68	14.67±3.36	17.13±3.54	4.60	*

* P= 0.05   Significant   ** P = 0.01   highly significant

Table (5) Relation between level of impulsivity and coping strategies in relation to diagnosis.

Coping strategies	Level of impulsivity	Diagnosis								CHI	Sign.
	Low	Schizophrenia		Mania		Depression		Paranoid			
		No	%	No	%	No	%	No	%		
Withdrawal		7	5.83	7	5.83	7	5.83	0	0.00	8.64	0.373
	Moderate	31	25.83	20	16.67	18	15.00	9	7.50		
	severe	13	10.83	4	3.33	3	2.50	1	0.83		
Projection	Low	8	6.67	9	7.50	9	7.50	1	0.83	12.25	0.14
	Moderate	35	29.17	14	11.67	16	13.33	6	5.00		
	severe	8	6.67	8	6.67	3	2.50	3	2..50		
Neurotic reaction	Low	12	10	12	10.00	7	5.83	3	2.50	21.05	0.007**
	Moderate	33	27.50	16	13.33	8	6.67	6	5.00		
	severe	6	5	3	2.50	13	10.83	1	0.83		
Dream& fantasy	Low	8	6.67	9	7.50	11	9.17	3	2.50	18.74	0.016*
	Moderate	22	18.33	17	14.17	15	12.50	5	4.17		
	severe	21	17.50	5	4.17	2	1.67	2	1.67		
Resign self	Low	7	5.83	10	8.33	1	0.83	5	4.17	24.49	0.001**
	Moderate	39	32.50	18	15.00	19	15.83	4	3.33		
	severe	5	4.17	3	2.50	8	6.67	1	0.83		
Active-role	Low	11	9.17	7	5.83	3	2.50	1	0.83	8	0.433
	Moderate	28	23.33	19	15.83	21	17.50	7	5.83		
	severe	12	10	5	4.17	4	3.33	2	1.67		
Passive-role	Low	3	2.50	3	2.50	10	8.33	4	3.33	17.7	0.023*
	Moderate	45	37.50	25	20.83	16	13.33	5	4.17		
	severe	3	2.50	3	2.50	2	1	1	.83		

4. Discussion:

Impulsivity has been defined as a predisposition toward unplanned reactions to internal or external stimuli, without regard to the negative consequences, which more common in some mental disorders (**[8]**). Individuals with mental illness may have difficulty appraising stressful events as well as deficient cognitive and behavioral strategies necessary to manage stress., if unsuccessful in coping with a stressful events , impulsive behaviors may emerge , so it is a way of impulsive individuals can cope with difficult or crisis situations (**[1]**).

The present study aimed to assess the impulsivity and coping skills among psychiatric patients and to determine the relationship between impulsivity and coping skills. The highest percentage of the studied patients were in the age group from 30 to 39 years old , males than females , married than single , patients with low level of education (illiterate) and farmers . This result consistent with other studies (**[9-10]**) who stated that the impulsivity levels in males have a higher propensity than females, 42 % had low education and 54 % were unemployed and farmers, while 42 % of the studied sample had never been married. **Petry ([11])** found that the impulsivity levels were a highly common among the age of adolescence. Some studies does not go with the findings of **Petry ([11])** who stated that women with high impulsive were less likely to delay gratification during are ward for performance task and were likely to respond aggressively toward individuals whom they perceived as being in their way.

In relation to impulsivity and coping strategies, the present study found that the impulsive patients were highly using of emotional – oriented coping strategies as projection than problem – oriented coping strategies. This is accordance with **Nagata et al., ([12]),** who stated that impulsive patients with bulimia nervosa had significantly higher of emotional coping scores than problem coping scores. Also, the findings of **Lightsey and Hulsely ([13])** found that a high positive correlation between ineffective coping strategies among the pathological gamblers and problem that the emotional coping conjunction with high impulsivity.

Concerning diagnosis as a factor in coping methods, there were a significant differences between the level of impulsivity and coping strategies among schizophrenic patients were highly used of emotional – oriented coping strategies than problem – oriented coping strategies. This finding consistent with the study of **Nagata et al., ([12]),** who stated that, the highest levels of impulsivity were found among schizophrenic patients. This finding is also goes with **Dervaux et al., ([14]),** who stated that impulsivity associated with substance abuse among individuals with schizophrenia or schizoaffective disorder. **Lancu et al., ([15])** found that high impulsivity in schizophrenic patients is significantly etiology of suicide in schizophrenia. Also this consistent with the findings of **Mohamed Agoub, ([16]).** He showed that high levels of impulsivity were found among schizophrenic patients. Clearly, impulsivity is a personality trait of importance not only among psychiatric patients, but in the general population as well.

5. Conclusion:

Based upon the study results, it is concluded that the majority of sample had moderate level of impulsivity , and more in age groups from 30 – 39 years old , males than in females , married than single/ separated , low educational levels (illiterate) and in farmers than other occupations . Highest in schizophrenic disorders than other diagnosis, patients were used emotional - coping strategies than problem – oriented coping style.

Recommendations

In the light of the study findings, it is recommended to:

- Teach nurses to understanding the effect of impulsivity on personality, behavior and coping strategies is essential for the accurate assessment and appropriate management of impulsive individuals.
- Using an appropriate coping measurement can help nurses and patients identify specific coping styles and strategies.
- Nurses can help patients identify and monitor their feelings and become more attuned to triggers that signal the onset of impulsive behavior.
- Encourage patients to know they are capable of controlling their behaviors.
- Routine assessment of impulsivity and accompanying coping skills is critical for planning care of patients identified as impulsive.

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2/2/2011

Control of Construction - Associated Nosocomial Invasive Aspergillosis Outbreak at Kuwait Cancer Control Centre

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Abstract: Outbreaks of nosocomial invasive aspergillosis may occur in association with construction/renovation activities. Outbreak of Nosocomial invasive aspergillosis had been declared at July 2010 at Kuwait Cancer Control Centre coinciding with different construction and renovation activities. A total of four cases of aspergillosis were identified .An urgent meeting of a multidisciplinary team comprising infection control staff, clinicians, hospital deputy director, engineering department staff and the director of nursing staff was established with subsequent implementation of different control measures including: Sealing of construction sites with impermeable barriers , face-masking of patients with N95 mask, frequent wet cleaning around construction area and posaconazole antifungal prophylaxis for high risk patients.
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Keywords : Aspergillosis; Outbreak; Construction

Introduction:

Nosocomial outbreaks of aspergillosis are a well known complications of construction, demolition or renovation activities in or near hospital wards housing immune-suppressed patients [1] .

Construction activities inside hospitals may cause aspergillus spores aerosol pollution which resulted from disturbance of settled aspergillus spores or disrupting a locus of growth . These aerosolized spores will be carried by air to be inhaled by immune compromised patients (malignancies receiving intensive immunosuppressive , allogeneic haematopoietic stem cell transplants or solid organ transplants) causing pulmonary aspergillosis that may proceed to invasive disease [2].

Invasive aspergillosis is difficult to diagnose and treat. Moreover it has high mortality rate ranging from 25% to 90% in-spite of new therapies, so prevention of such a condition is of a high priority in managing at risk patients during construction activities [3,4] .

Ideally several preventive measures must be under- taken in the pre-planning stage for imminent hospital construction in order to protect at risk patients from exposure to aspergillus spores. These measures include demolition, construction and ventilation measures, infection control measures and chemoprophylaxis[5] .

This article is conducted in order to highlight our   successful experience in managing   and controlling construction - associated invasive aspergillosis outbreak at Kuwait Cancer Control Centre (KCCC) during the year 2010 with the following objectives :

To describe aspergillosis outbreak that occurred at KCCC

To describe the measures that was taken to control this outbreak

Methods
Setting

Kuwait Cancer Control Centre (KCCC) is 200 bed hospital. It is the only specialized oncology centre in Kuwait serving all population residing Kuwait, whether Kuwaiti or of other nationality; approximately 3.5 million according to 2009 census [6].

KCCC provides inpatient medical oncology, haematology, surgical, radio-diagnosis, laboratory and radiotherapy services in addition to outpatient clinics for most of the previous services.

The inpatient haematology ward s are located at the first and the second floors of the old building while the inpatient medical oncology wards are located at the first and the second floors of the new building, the third floor of the new building accommodates autologus bone marrow transplant patients (BMT).

The inpatient hematology wards are serviced by standard air filtration with pre-filters to remove large particulate matter using standard deep-bed filters, which are checked and washed monthly and replaced

annually. While the inpatient oncology and autologus BMT wards are served by high efficiency particulate air (HEPA) filter .

Fluconazole 200 mg daily (antifungal prophylaxis) was administered to patients with leukemia and undergoing to induction treatment and to patients with high grade lymphoma receiving aggressive therapy.

Construction and Renovation Works at KCCC

Construction period extended from the first of June up to the end of November in an area connecting the old building with the new one. The following works were done: replacement of ceiling, demolition, electrical rewiring, floor works, building walls, joinery, painting, plumbing and plastering.

Definitions of Aspergillosis Cases

According to European Organization for Research and Treatment of Cancer (EORTC) definitions [7]. Proven invasive fungal infections: Histopathologic or cytopathologic examination showing hyphae from needle aspiration or biopsy-specimen with evidence of associated tissue damage (either microscopically or unequivocally by imaging) or positive culture result for a sample obtained by sterile procedure from normally sterile and clinically or radiologically abnormal site consistent with infection, excluding urine and mucous membranes, Probable invasive fungal infections : At least 1 host factor criterion and 1 microbiological criterion and 1 major (or 2 minor) clinical criteria from abnormal site consistent with infection , Possible invasive fungal infections : At least 1 host factor criterion and 1 microbiological or 1 major (or 2 minor) clinical criteria from abnormal site consistent with infection.

Laboratory and Radiological Work- up

The identified cases were subjected to the following:
1- Polymerase chain reaction (PCR) for identification of Aspergillus fungus type from positive filamentous fungus sputum samples was performed at Kuwait Faculty of Medicine.
2- Galactomannan antigen assay was performed at Kuwait Faculty of Medicine.
3- Fungal culture for sputum at Ibn Sina Hospital laboratory
(Kuwait).
4- CT scan for chest and sinuses at Kuwait Cancer Center .
5- Tissue biopsy at Ibn Sina Hospital Histopathology laboratory.

Data Collection:

Medical files, laboratory reports and nursing notes of the identified cases were reviewed in addition to reviewing that of hematology, medical oncology and BMT patients treated prior to the outbreak.

Administrative Consideration:

A permission from the Director of the Kuwait Cancer Center and the Director of Infection Control Directorate of Kuwait, Ministry of Health for collecting and releasing the data of this research was taken.

Outbreak Description and Management:

Aspergillosis Outbreak was declared at 11 July after identification of two cases of aspergillosis and after revealing that there is no previous reported invasive aspergillosis cases in the last four years infection control departments, surveillance reports .

A total of four cases of aspergillosis were identified between 23 June 2010 and 25 November 2010; one proven, two probable and one possible case according to EORTC definitions (Table 1&Figure1). Aspergillus terreus was identified in the sputum of three case, while Galactomannan assay was positive among 2 cases only. The identified cases were prospectively followed up by active surveillance.

Immediately after declaring the outbreak a multidisciplinary team comprising infection control staff, clinicians, hospital deputy director, engineering department staff and the director of nursing staff was established. Then an urgent meeting of this team was held on, during this meeting the situation was described and discussed in details. After that and in accordance with guidelines published by the Healthcare Infection Control Practices Advisory Committee of the CDC [8,9] , recommendations and procedures to control the situation were clearly settled ,outlined and assigned to all the meeting personnel.

These measures included the following : establishing airtight barrier around the construction area , using of negative pressure ventilation in the construction area, covering the air supply and exhaust vents in the construction zone, redirection of construction traffic to be away from patient area through the back doors of the hospital , regular removal of construction debris from the construction site in sealed containers, frequent cleaning around the construction area more than usual using wet mop. Using N95 face masks for patients during their transport near the construction area.

Active daily surveillance of infections in patients who are at increased risk for aspergillosis infection was performed by infection control personnel in addition to daily following up of

implementation of the multidisciplinary team recommendations.

As the construction period was long extended from June up to November; Posaconazole was given to patients stratified by their treating clinicians to be

at high risk (autologus and allogenic bone marrow transplant patients during neutopenic period , prolonged neutopenia more than 14 days after chemotherapy). Posaconazole is a new triazole drug that recently showed efficacy for fungal prophylaxis in high-risk patients[10,11].
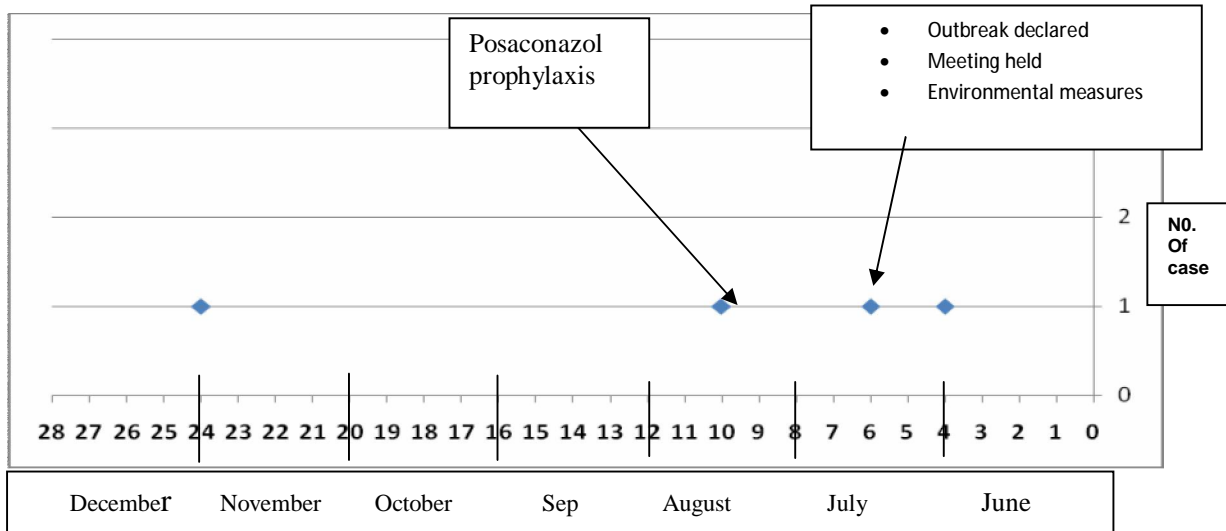

Figure (1): Timeline of invasive aspergillosis outbreak

Table (1) Clinical, Radiological and Laboratory Data of Invasive Aspergillosis Cases

case	EORTC definition	Underlying disease	Chemotherapy /radiotherapy	Neutopenia period	Clinical symptoms	CT chest-CT sinuses	Pathology/Microbiology/molecular	outcome
1	possible	AML	3 cycles	>14days	Moderate respiratory symptoms	Multiple nodular consolidations in the lung parenchyma Pan sinusitis with mucosal thickening	Sputum Culture negative Galactomannan negative	Alive and underwent for allotransplant
2	proven	Non hodjekins lymphoma	2 cycles	3days	Severe respiratory symptoms	CT was not done CXR: Rt. sided pneumonia	Sputum Culture positive Galactomannan positive Positive brain pathology	Deceased.
3	probable	Refractory AML	3 cycles	>14days	Moderate respiratory symptoms	Consolidation involving the Rt. upper lobe with multiple enlarged sup. Mediastinal L.N and hilar L.N	Sputum Culture positive Galactomannan positive	alive
4	probable	CNS lymphoma	radiotherapy	No	Severe respiratory symptoms with respiratory failure	Consolidation and three cavities involving Rt. upper and lower lobes	Sputum Culture positive Galactomannan negative	Deceased with multi-organ failure

EORTC: European Organization for Research and Treatment of Cancer; CT: computed tomography; AML: acute myeloid leukemia CXR: chest radiograph

Discussion

Construction in or around hospitals is a never-ending phenomenon in modern-day healthcare. Construction, renovation and demolition are the main causes of nosocomial aspergillosis outbreaks [1]. Different construction and renovation activities started at different areas of KCCC without implementation of preliminary preventive measures to reduce the risk of aspergillosis. Construction period extended from June up to November 2010.

After declaring aspergillosis outbreak at July2010 and establishing a multidisciplinary prevention and control team, different measures were implemented to contain the situation. These measures included environmental sealing of construction areas, masking, wet cleaning, reducing unnecessary traffic through affected areas. Regular follow up of the implementation of the preventive measures was held daily by infection control team to ensure that construction sites were sealed well, doors and windows remained shut, and cleaning was adequate .

At this outbreak four cases of aspergillosis were identified during the follow up period that extended from time of outbreak declaration (11july2010) up to three months after finishing construction (1st week of march 2011) as the incubation period of aspergillosis is not well defined and may extend up to three month[12,13] . Two of the reported cases died; one from CNS aspergillosis and the other from multi-organ failure.

This outbreak was not our first experience regarding aspergillosis; during the year 2005, three probable cases of invasive pulmonary aspergillosis were reported at hematology ward during construction of a new nearby hospital building. One of the cases died and the others survived and the outbreak was controlled after the application of different preventive and control measures.

Moreover our finding is consistent with many invasive aspergillosis outbreaks that had been described worldwide in different cancer centers; Spanish cancer hospital (2000)[14], Israel cancer Hospital (2001)[15], Jabalpur Cancer Hospital (2002, India)[16], Japanian Cancer Centre (2003)[17],Geelong cancer Hospital (2006, Australia,)[18] , etc.

Regarding air sampling for aspergillus fungus, we did not perform it as its role is controversial. Air sample collection provides a "snapshot" for what is occurring in the air at the time of sample collection missing peaks in fungal spores.. Moreover Outbreaks of invasive aspergillosis with negative air-sampling data have been described previously ([1,19]).

We gave Posaconazole as an antifungal prophylaxis for high risk patients during the period of construction (200 mg 3 times daily), none of them developed aspergillosis however we diagnosed one

patient as a probable case of aspergillosis. This patient was suffering from chronic renal failure with central nervous lymphoma and was not on posaconazole prophylaxis as he was not classified as high risk patient.

After construction activities were finished, clinicians re-established Fluconazole as a routine antifungal

As education of medical staff on the basic infection control measures particularly at outbreak times may prevent the occurrence of such event in the first place in the future[20]. Different aspergillosis educational sessions were prepared and conducted by infection control team with the help of KCCC Staff Development Unit. These sessions involved ; health care workers, cleaning staff supervisors and engineering department staff .

Conclusion:

Hospital construction work is a clear risk factor for development of aspergillosis. A multidisciplinary approach involving clinicians, hospital management and engineering personnel with a proper plan is essential in prevention and control of nosocomial aspergillosis outbreaks.

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2/1/2011

EVALUATION OF SOME ESSENTIAL OILS AGAINST *SESAMIA CRETICA* LED. UNDER FIELD CONDTIONS

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Abstract: Efficacy of some volatile plant oils against the corn borer, *S. cretica* Led. was investigated under field conditions throughout 2010 early summer corn season . Oils of four plants, namely; Cinnamon, Clove, Marjoram and Ginger Essential oils were used at concentrations 2.5 and 5%. Also, Eugenol (aromatic fragment) which was found in all the essential oils was used in 4 concentrations. The recommended pesticide Diazinox ® was used in addition to the control treatment. It was found that Cinnamon at 5% achieved the highest percentage reduction in egg masses, larvae and dead heart being 95.2, 85.5 and 92.1, respectively. The heaviest yield of maize ears was obtained as a result of treating plants with cinnamon 5% and eugenol 0.4% being 89.9 and 86.2% increase than control, respectively.

Key words: Essential oils - *Sesamia cretica* Led**.**

1. Introduction:

Maize (*Zea mays* L.) is one of the most economic cereal crops in Egypt. Flour is an essential food for the majority of farmers, while green maize plants are used as fodder for livestock animals and rabbits. Also, grain flour is added as main component of maize food industries. It is, also, used for human food after being mixed with wheat flour for bread production. In addition, it plays an important role in several industries important to Egypt's economy such as corn oil, fructose and starch production.

Maize plants are attacked by many insect species of which *Sesamia cretica* Led., which considered one of the most important, as larvae bore in the stems of seedlings causing deterioration of plants in the seedling stage, (Awadallah, 1974).

Many workers reported that many plants are considered to contain materials efficient for pests control. Such agents may be used as toxicants, repellents, synergists, growth regulators or antifeedants for many insect pests (Kubo and Clocke, 1982).

Essential oils may have attractive or repellent effects and in some cases they showed insecticidal action against certain insect pests. Oils isolated from plants which consist of cyclic and monocyclic mono-terpens proved effective repellents against insects (Rodriguez and Levin, 1975). It was found that these bioactive compounds are potentially toxic to insects and mites but relatively safe to human and wildlife. Recently, there is a great need to find alternative pesticides instead the traditional chemical insecticides which proved to have toxic effect on human, animal and on the whole environment. Some essential oils are extracted from natural sources, such as eugenol which is extracted from clove fruits (Farag *et al*., 1991). There was no significant difference between the retention of clove oil and Eugenol solutions, indicating that differences in their phytotoxicity to broccoli leaves was not due to differential foliar retention (Luke *et al*., 2006).

This study aimed to evaluate the efficacy among different plant extracts; Cinnamon, Cloves, Marjoram and Ginger, also Eugenol as (essential oils) against S*. cretica* infesting maize seedling in the field.

2. Material and Methods:
1-Experimental design:

This study was carried out throughout 2010 early summer corn season at the Experimental Farm of the Faculty of Agriculture at Moshtohor, Kalubiya Governorate. An area of about quarter feddan was chosen to be sown with maize seeds of the common commercial variety Giza 2. The area was divided to 48 plots of 3.5x3 meters each. Each plot contained five rows at a distance of 70 cm. sowing was done at rate of 3-4 grains/ hill, leaving a distance of 30 cm. between hills. Sowing took place on April, 17th as it was recorded to be the date of the highest natural infestation with *Sesamia cretica* Led, to maize plants (Awadallah, 1974 and Shalaby, 1996).

2-Materials used

Essential oils of each of the following materials were applied on four replicates/ treatment.

English name	Scientific name	Family	concentration
Clove buds	*Eugenia aromaticum*	Myrataceae	2.5 and 5%.
Cinnamon	*Cinramomum zeylanicum*	Lauraceae	2.5 and 5%.
Marjoram	*Majorana nortensis*	Labiolae	2.5 and 5%.
Ginger	*Zingiber officinale*	Zingiberaceae	2.5 and 5%.

Essential oils of the mentioned plant species were purchased from the local market.

Eugenol, 2-methoxy-4-(2-proporyl) phenol isolated from clove buds *Eugenia aromaticum* was purchased from (Shanghai Medical Instruments Co., ltd.). It was applied at 4 concentrations (0.1, 0.2, 0.3, and 0.4%) in the field.

The recommended pesticide (Diazinox) ®, (Organophosphorus- Diazinon) was applied at 5% concentration; i.e., 6 Kg /feddan and control treatment. A complete randomized block design with four replications was used. The assayed materials were sprayed by using one liter hand sprayer in case for the liquid materials while, Diazinox® granules was applied in the whorl of plant. Spraying was applied two times, the first 16 days after sowing, while, the second spray took place 7 days later. Data concerning the infestation by *S. cretica* egg-masses (20 randomly picked plants/plot) was recorded after 24 hours of first spray. Larvae (20 randomly picked plants/plot) were recorded after 24 hours of second spray. The perforated leaves and dead hearts were estimated per 50 plants /plot, after 35 days from sowing. At harvest all maize ears of each plot were collected, weighed and adjusted to find out the ear yield per feddan expressed as (ardab/feddan).

3-Statisical analysis

Data were subjected to ANOVA (Costat Statistical softwere, 1990). Means were compared using L.S.D.

3. Results and Discussion:
1. Number of egg-masses:

Results in Table (1) clearly showed that, all treatments caused significant reductions in the number of *S. cretica* egg-masses. The untreated plants harboured the highest number being 21 egg-masses /20 plants. The highest efficacy was obtained from treatment with cinnamon 5% being only one 1 egg- mass/ 20 plants, showing 95.2% reduction than control, followed insignificantly by marjoram 5% and Eugenol 0.4% (1.3 egg-masses/20 plants) indicating 94.1% reduction than control and Ginger 5% which gave 91.7% reduction. Treatments including Cinnamon 2.5 %, Ginger 2.5% Eugenol (0.3&0.2%) and Euegnol 0.1% achieved intermediate efficacy in which the egg-masses were 2.3, 2.5, 2.5, 2.8 and 3.3 egg-masses /20 plants as 89.3, 88.1, 88.1, 86.9 and 84.5% reduction than control, respectively. On the

contrary, treatments that showed low efficacy were the marjoram 2.5%, clove 5&2.5% and the recommended chemical pesticide (Diazinox) (75, 75, 69.1 and 42.9% reductions, respectively).

In agreement with the present results, Abdel Samea (1998) indicated that jojoba oil inhibited hatchability of *O. nubilalis* eggs. The same author indicated also that jojoba oil had a promising ovicidal activity against *S. cretica* and *O. nubilalis*.

2. Number of *S. cretica* larvae:

Number of *S. cretica* larvae was counted in 20 plants for each treatment and presented in (Table (1). Untreated maize plants (control) received the highest infestation rate with *S. cretica* (38 larvae/20 plants). All assayed materials caused different efficacies on the *S. cretica* larval counts in maize plants leading to reductions in larval counts than control. The high efficacy was obtained by Cinnamon 5% and Marjoram 5% treatments which caused 85.5 and 83.6% reductions than control, respectively. Remaining treatments may be arranged descending by as Marjoram 2.5% (9 larvae/ 20 plants) indicating 76.3% reduction than control. Ginger 5% and Eugenol 0.4% and 0.3% caused 76.3, 74.3 and 73.7% reduction with averages of 9, 9.8 and 10 larvae /20 plants, respectively. Treatments of intermediate effect were Diazinox, Cinnamon 2.5%, Eugenol 0.2%, Ginger 2.5% and Eugenol 0.1% (12.0, 12.5, 14, 14 and 17 larvae /20 plants). On the contrary, Clove 2.5 and 5% resulted in the least efficacy reduction in the number against larvae (15.8 and 2.6% reductions than control, respectively; Table, 1).

These results agreed with Abdel-Aziz *et al.* (1995) who found that hexane and chloroform extracts of *Dodonaea viscose* gave the highest larval mortality against *Spodoptera littoralis.* Also Isman *et al.* (1991) revealed that neem products used as insecticide against the pyralid *O. nubilalis* showed moulteing disturbance, antifeedant and insect growth regulatory activities.

3. Numbers of plants containing perforated leaves:

As shown in table (1), all treatments gave reductions in plants with perforated leaves than control. This received 20 plants containing perforated leaves/50plants. Reductions in plants with perforated leaves than control ranged between 62.5 (after application of marjoram 2.5%) to 92.5 (Eugenol 0.4% treatment). Treatments which caused high efficacies

may be arranged in descending order as Eugenol 0.3% (1.8 plants with perforated leaves; 91.3% reduction than control) Cinnamon 2.5%; (2.5, 87.5%), Ginger 5%; (3.5, 82.5%), Cinnamon 5%; (3.8, 81.3) Clove 5%; (3.8, 81.3), Eugenol 0.2%; (3.8, 81.3) and Marjoram 5%; (4.0, 80.0), respectively.

4. Numbers of dead heart cases:

As for the main symptom for *S. cretica* infestation is the dead hearted plants, which was recorded after 37 days from sowing. The untreated plants contained 19 dead heart cases/50 plants. Cinnamon 5%, Marjoram 5% and Eugenol 0.4% caused high reduction percentages than control, being 92.1, 90.8 and 90.8%. Eugenol 0.3% and Cinnamon 2.5% came next (82.9 and 86.8% reductions than control, respectively). The remaining treatments may be arranged in ascending order as Eugenol 0.1%, Marjoram 2.5%, Cloves 2.5&5%, Ginger 5%, Eugenol 0.2% and Diazinox (50.0, 57.9, 64.5, 71.1, 72.4, 75.0 and 79.0% reductions, respectively.

These results may be considered in harmony with those of Yacoub *et al.* (2010) who stated that the treatments which caused the best results against *S. cretica* egg-masses, larvae, number of dead hearts were Nat-com 40 (jojoba oil) and Tresser, water dodonia extract and sour orange extracted in petroleum ether. Subsequently, gained the highest yield of 26.7 ardab/fedan. Also, El-Hosary *et al.* (2010) data demonstrated that treatment with Runner alone, mixture of water radish extract and Protecto achieved the highest percentage reduction in number of *S.cretica* egg-masses, number of larvae, and plants containing perforated leaves and dead hearts, and led to the highest yield.

5. Dry ears yield

All treatments led to increases in the weight of harvested ears. The highest ears yield resulted from Cinnamon 5% treatment (24.5 ardab/feddan, showing 89.9% increase than control), followed by treatments with Eugenol 0.4%, Cinnamon 2.5% and Eugenol 0.3 which achieved 24.0, 22.5 and 22.2 ardab/feddan, respectively opposed to 12.9 ardab/ feddan from the control treatment. The remaining treatments may be arranged descending by according to the obtained yields as: Marjoram 5%; (21.8 ardab), Cloves 5%; (19.4 ardab) , Marjoram 2.5%; (18.9 ardab), Diazinox; (17.0 ardab), Eugenol 0.2%; (16.8 ardab), Cloves 2.5%; (13.5 ardab), Ginger 5%; (15.1 ardab), Ginger 2.5%; (14.2 ardab) and Eugenol 0.1%;(13.0 ardab) indicating 68.7, 50.5, 46.8, 31.8, 29.9, 4.5, 16.8, 9.8 and 0.4 %increase in yield of maize ears than control (Table,1).

In this respect, El-Hosary *et al.* (2010) and Yacoub *et al.* (2010) data showed that there were

significant negative correlation between yield and each of *S. cretica* traits (egg-masses, number of larvae, plants containing perforated leaves and dead hearts).

Constituents of each of the assayed essential oils were determined according to studies by different authors found in the literature. Oils were fractionated and identified using the Gas Liquid Chromatography (GLC) technique. These components may be demonstrated as follows:

1- Cinnamon essential oil

The chemical components of Cinnamon essential oil could be classified into 9 chemical categories namely; monocyclic terpen, bicyclic terpenes, aromatic hydrocarbons, aromatic ehydes, alcohols, phenol & phenol ethers , terpene esters , aromatic alchols and aromatic esters. These identified compounds counted for 90.4% of the composition of cinnamon essential oil. The Cinnamon essential oil phenol and phenol ethers which contains one compound namely; eugenol. These findings comply with those obtained by Lawiess, Julia (1992) and Burdock (1995), who reported that phenol content (as eugenol) represents from 4% to 10% from cinnamon by essential oil.

2- Clove essential oil

The chemical constituents of clove essential oil could be classified into 4 chemical categories namely; monocyclic terpen, ketone, sesquilerpene and phenol & phenol ethers. These identified compounds accounted for 99.12% of the composition of clove essential oils. The fourth chemical group was phenol and phenol ethers which consisted of 2 compounds namely; eugenol and eugenol acetate. These compounds were reported previously as constituents of clove essential oil by Furia and Bellanca (1975), Masada (1980), who found that eugenol acetate percentages in clove essential oil ranged from 75% to 95%.

3- Marjoram

Among 45 compounds recorded by gas chromatography of the water-distilled essential oil of marjoram (*Origanum majorana* L.), GC–MS and GC–FTIR 43 components were identified. This essential oil was found to be rich in terpinen-4-ol, cis-sabinene hydrate, p-cymene and γ-terpinene Vera and Chane-Ming (1999).

4- Ginger essential oil

The chemical constituents of ginger essential oil could be classified into 9 chemical categories namely; α cyclic terpenes, cyclic terpenes, bicyclic terpenes, oxides, alcohols, aromatic esters, phenol &

phenol ethers, sesquterpenes and aromatic hydrocarbons. These identified compounds accounted for 86.62% of the composition of this oil. The ginger essential oil was phenol and phenol ethers which

consisted 2 compounds namely; benzene,1-methoxy-4-(2-propenyl) and eugenol. These compounds were reported as constituents of ginger essential oil by Masada (1980).

Table 1. Effect of some essential oils on *S. cretica* infestation in corn plants

Treatments	No. of egg masses /20 plants		No. of larvae /20plants		No. of plants with perforated leaves /50 plants		No. of dead heart / 50 plants		Yield (ardab /Fedden)	
	Average	% reduction	Average	% reduction	Average	% reduction	Average	% reduction	Average	% increase
Cinnamon 2.5%	2.3	89.3	12.5	67.1	2.5	87.5	2.5	86.8	22.5	74.4
Cinnamon 5%	1.0	95.2	5.5	85.5	3.8	81.3	1.5	92.1	24.5	89.9
Cloves 2.5%	6.5	69.1	32.0	15.8	4.3	78.8	6.8	64.5	13.5	4.5
Cloves 5%	5.3	75.0	37.0	2.6	3.8	81.3	5.5	71.1	19.4	50.5
Marjoram 2.5%	5.3	75.0	9.0	76.3	7.5	62.5	8.0	57.9	18.9	46.8
Marjoram 5%	1.3	94.1	6.3	83.6	4.0	80.0	1.8	90.8	21.8	68.7
Ginger 2.5%	2.5	88.1	14.0	63.2	6.3	68.8	8.0	57.9	14.2	9.8
Ginger 5%	1.8	91.7	9.8	©74.3	3.5	82.5	5.3	72.4	15.1	16.8
Eugenol 0.1%	3.3	84.5	17.0	55.3	6.3	68.8	9.5	50.0	13.0	0.4
Eugenol 0.2%	2.8	86.9	14.0	63.2	3.8	81.3	4.8	75.0	16.8	29.9
Eugenol 0.3%	2.5	88.1	10.0	73.7	1.8	91.3	3.3	82.9	22.2	72.0
Eugenol 0.4%	1.3	94.1	9.8	74.3	1.5	92.5	1.8	90.8	24.0	86.2
Diazinox	12.0	42.9	12.0	68.4	6.0	70.0	4.0	79.0	17.0	31.8
control	21.0		38.0		20.0		19.0		12.9	
F value	192.2		148.3		119.7		52.5		31.5	
L.S.D.	1.1		2.6		1.2		1.8		2.2	

The results obtained in this study may be in harmony with those obtained by Tiwari *et al.* (1998) who reported insect repellent activity of the Cinnamomum essential oil. These results may, also, agree with those previously reported by Chaieb *et al.* (2007). They tested the biological activity of *Eugenia caryophyllata* on several microorganisms and parasites, including pathogenic bacteria, Herpes simplex and hepatitis C viruses. In addition to its antimicrobial, antioxidant, antifungal and antiviral activity, the authors indicated also that clove essential oil possesses antiinflammatory, cytotoxic, insect repellent and anaesthetic properties.

Abd El-Aziz and El hawary (1997) mentioned that basil, *O. basilicum* showed an insecticidal action against *Spodoptera littorals* larvae (80% larval mortality) and that basil, *O. basilicum* gave 94.34%repellency to *S .littoralis* moths.

Isman (2000) reported that certain plant essential oils and/or their constituents have a broad spectrum of activity against insect and mite pests, plant pathogens and other fungi and nematodes. Accordingly, they have considerable potential as crop protectants and for pest management in other situations (e.g. urban pest control). Current information indicates that they are safe to the user and the environment, with few qualifications. As a cautionary note, the essential oils that are most efficacious against pests are often the most phytotoxic; this latter property requires serious attention when formulating products for agricultural and land-scape use. Also, selectivity among invertebrates is not well documented. Honey bees appear somewhat susceptible (Lindberg *et al,* 2000), and the susceptibility of various natural enemies has yet to be reported, although the lack of persistence of essential oils under field conditions, they could

provide some measure of temporal selectivity favoring these non- target species.

Essential oils may have attractive or repellent effects and in some cases they showed an insecticidal action against insects. Essential oils isolated from plants and consisting of cyclic and monocyclic monoterpes are effective repellents against insects. Rodriguez and Levin (1975) reported that the ideal essential oil insecticide would consist of compounds that are active against pest arthropods, while being harmless to beneficial and safe for human and the environment.

(Cavalcanti *et al*. 2004) reported that the essential oils of *O. arnericanum* and *O. gratissimun* had larvicidal activity against *Aades aegyptii* Masquito and caused 100% mortality at a concentration of 100ppm. *O. americanum* showed toxicity to the hairy caterpillar, *Euproctis fraternal* (Mc Indoo, 1983).

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Study of Resistin and Leptin in patients with Thyroid Dysfunction

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Abstract: Background: Leptin and resistin are adipocytokines associated with body mass, insulin resistance and inflammation. Data linking adipokines with thyroid hormones are confusing. **Aim:** Evaluation of leptin and resistin in patients with thyroid dysfunction. **Subjects and methods**: 28 patients with hyperthyroidism, 26 patients with hypothyroidism and 24 age and gender matched control subjects were included in the study. BMI was calculated. Serum concentrations of TT3, FT4, TSH, resistin and leptin were measured by ELISA. **Results**: A higher BMI (29.4 ± 2.1)kg/m^2, TSH (21.7 ± 2.4) Mu/L and leptin (34.9 ± 2.8) ng/ml were found in the hypothyroid group compared with the hyperthyroid group BMI (23.7 ± 2.7)kg/m^2, TSH (0.07 ± 0.03) Mu/L and leptin (9.7 ± 1.8)ng/ml. The hyperthyroid group exhibited a significant increased TT3 (6.6 ± 1.6)nmol/L, FT4 (2.6 ± 0.1)Pmol/L and resistin $(13.8 + 3.7)$ng/ml compared with the hypothyroid group TT3 (0.3 ± 0.1)nmol/L, FT4 (0.68 ± 0.04)Pmol/L and resistin (6.3 ± 3.4)ng/ml. Resistin correlated significantly and negatively with TSH $(P<0.01)$ and BMI $(P< 0.01)$ and positively with TT3 $(P<0.01)$ and FT4 $(P< 0.05)$. Leptin correlated positively with TSH $(P< 0.01)$ and BMI $(P<0.01)$ and negatively with TT3 $(P<0.01)$ and FT4 $(P<0.05)$. Factors affecting resistin level in a multivariate logistic regression analysis were sex, TT3 and FT4. Leptin is affected only by sex and TSH. The cutoff level of leptin associated with hyperthyroidism is 15.3 ng/ml with sensitivity of 100%, and specificity of 60%. **Conclusion:** Thyroid hormones have direct effect on resistin but not leptin. Leptin may affect the thyroid function indirectly through its central action on TSH independent of the BMI. Leptin level of 15.3 ng/ml is associated with hyperthyroidism.

1. Introduction

For decades, the adipose tissue was considered as a mere store for fat that plays a passive role in energy metabolism. Now, it is widely accepted that the adipose tissue is the largest ever endocrine organ [1,2]. It secretes a lot of hormones, named adipocytokines or adipokines that control feeding, thermogenesis, immunity, reproductive hormones, and neuroendocrine function[2]. This new look towards the adipose tissue has emerged after the discovery of the obese gene and leptin[3].

Resistin is a cysteine-rich 92 amino acid protein. It is an adipocytokine, discovered in 2001, and has been linked to insulin resistance and the development of type 2 diabetes mellitus [4]. In addition, it has been linked to the obesity associated inflammatory state[5].

Leptin is a messenger of satiety from the fat cells to the brain, a regulator of insulin and glucose metabolism and plays a role in energy balance and body weight by neuroendocrine mechanisms[6]. It is, another adipocytokine, produced exclusively in proportion to fat mass specially the subcutaneous fat. It circulates in the plasma in a free form or bound to leptin-binding proteins**.** Leptin is produced in larger quantities in subcutaneous adipose tissue than in visceral adipose tissue[7]. A fall in leptin mediates weight gain through the hypothalamus to increase appetite, decrease energy expenditure, and modify neuroendocrine functions [8].

Thyroid function, even within the reference range, is associated with changes in body weight [9,10]. However, the pathogenesis of this link between thyroid function and body weight is not clear and it must consider not only changes of thyroid hormones, but also body fat distribution, obesity duration and the state of low grade inflammation[11].

Thyroid hormones act on several aspects of metabolic and energy homeostasis controlling body weight, thermogenesis, as well as lipolysis in adipose tissue. Similarly, adipocytokines have multiple effects on several tissues acting on the energy homeostasis. Hence the increased concern about the

possible relationship between adipocytokines, thyroid status, and thyroid dysfunction[12].

Aim of the work

The purpose of the present study is to evaluate the relationship between leptin and resistin and thyroid hormones in patients with thyroid dysfunction.

2. Subjects and Methods
Subjects:

The protocol for this study followed the ethical standards of this institution. Patients were selected from the outpatient clinic, Internal Medicine Department, Faculty of Medicine, Menofia University. Fifty four patients (23 men and 31 women) aged 34-53 years, and 24 apparently healthy controls (10 men and 14 women) aged 36-58 years which represent the control group. Patients were classified according to their thyroid status into two groups; the hyperthyroid group which included 28 patients (13 men and 15 women) and the hypothyroid group which included 26 patients (10 men and 16 women)**.** Patients with clinical symptoms of thyroid eye disease (other than mild forms), other co-morbidity or who were receiving medications were excluded from the study. In all patients and controls measurements of height and body weight were carried out. BMI was calculated as the ratio of body weight to body height squared (kg/m^2).

Sample collection and preparation:

Five milliliters of blood were obtained at 9 AM from all participants under aseptic condition by venipuncture on their routine clinical visits after overnight fasting for at least 8 hours. Samples were centrifuged and serum stored at - 20 C until assayed. All patients were subjected to the following:

Laboratory methods:

The serum concentrations of TT3, FT4, and TSH were measured by commercial ELISA Kit (ALPHA DIAGNOSTIC INTERNATIONAL, USA).

The serum concentration of resistin was measured using a commercial enzyme immunoassay kit (Phoenix Pharmaceuticals Inc., Belmond, CA, USA). The assay was performed on 96-well polystyrene plates pre-coated with secondary antibody that can bind to the Fc fragment of the primary antibody; the Fab fragment of the primary antibody binds specifically with resistin.

Serum concentration of leptin was measured using a commercial LINCO Human Leptin ELISA Kit (Cat.#EZHL-80SK) This assay is a direct Sandwich ELISA based, sequentially, on capture of human leptin by a polyclonal rabbit anti-human leptin

antibody immobilized on a 96-well microtiter plate. Interassay and intra-assay reproducibility was analyzed by the manufacturer by determining the coefficients of variation, which ranged between 3.6 and 7.8 and between 4.1 and 5.4%, respectively.

Statistical analysis

IBM SPSS Statistics 19 software (SPSS Inc., Chicago, IL, USA) was used for statistical analyses and P value 0.05 (two-tailed) was considered statistically significant for all analyses. Descriptive statistics are given as mean $\pm$ S.D. for all variables. Group differences were analyzed by Student t test and Mann Whitney test, for normally distributed and non-normally distributed. Pearson's correlation (2-tailed) coefficient was used to evaluate the association between serum leptin with clinical measurement and thyroid hormones. All variables which associate with serum leptin were included in a univariate analysis. All significant variables at p<0.05 were included in a multivariate analysis. ROC curve was used to detect cutoff levels associated TSH using medcalc software.

3. Results

This is a cross sectional study to detect factors that affect both serum leptin and serum resistin levels in patients with thyroid dysfunction. No significant difference was found between all groups as regarding the age and sex.

As expected, BMI shows statistically significant higher level in the hypothyroid group (29.4 ± 2.1) kg/m^2 than the control group (25.4 ± 2.6) kg/m^2 which was higher than the hyperthyroid group (23.7 ± 2.7) kg/m^2. Similarly, TSH level was statistically higher in the hypothyroid group (21.7 ± 2.4) mU/L than the control group (2.8 ± 0.9) mU/L which was higher than the hyperthyroid group (0.07 ± 0.03) mU/L. As regarding FT_4, It was higher in the hyperthyroid group (2.6 ± 0.1) ng/L than the control group (1.75 ± 0.49) ng/L which was higher than the hypothyroid group (0.68 ± 0.04) ng/L. Similarly, TT3 was higher in the hyperthyroid group (6.6 ± 1.6) nmol/L than the control group (2.1 ± 0.8) nmol/L which was higher than the hypothyroid group (0.3 ± 0.1) nmol/L (Table 1).

A highly significant difference was found among all groups as regarding serum leptin with the highest value in the hypothyroid group (34.9 ± 2.8) ng/ml followed by the control group (11.2 ± 2.7) ng/ml and the lowest value in the hyperthyroid group (9.7 ± 1.8) ng/ml. Serum resistin was statistically higher in the hyperthyroid group (13.8 ± 3.66) ng/ml than both the hypothyroid (6.316 ± 3.413) ng/ml and the control group (6.900 ± 1.968) ng/ml. However, no significant difference was found between the

hypothyroid and the control group (Table 1). Figure 1 shows the mean leptin and resistin levels in all groups.

Correlative analysis between both serum leptin and resistin with both clinical measures and thyroid hormones:

To identify factors that may associate with both serum leptin and serum resistin levels, we performed correlative analysis between both serum leptin and serum resistin from one side and both clinical and thyroid hormones from the other side. A highly significant positive correlation was found between serum leptin and both BMI (r=0.770, P< 0.01) and TSH (r=0.980, P< 0.01). A highly significant negative correlation was found between serum leptin and both FT4 (r=0.884, P< 0.01) and TT3 (r=0.773, P< 0.01) (Table 2). Figure 2 shows the correlation between serum leptin and TSH. On the other hand, serum resistin positively correlated with FT4 (r=0.641, P< 0.01) & TT3(r=0.805, P< 0.01). Serum resistin also significantly but negatively correlated with both BMI (r=-0.571, P< 0.01) and TSH (r=-0.502, P< 0.01) (Table 2).

Multi-variate logistic regression analysis:

In a multivariate logistic regression analysis serum leptin with the correlated variables in addition to age and sex as confounding factors, only sex and TSH were highly significant with odd ratio of 0.105 and 0.920 respectively (Table 3).

Receiver Operating Characteristic (ROC) curve:

When plotted the serum leptin concentration against TSH level as a classifying variable, it gives a significant value with area under the curve (AUC) of 0.874. Positive cases were defined by TSH below normal control values. The best cutoff value was 15.3 ng / ml with a sensitivity of 100% and specificity of 60%. Figure 3 shows the ROC between serum leptin and TSH in the whole population.

4. Discussions

The present study is a cross sectional study designed to demonstrates the adipocyte hormones namely leptin and resistin in relation to thyroid functional status. The findings of this study showed that resistin concentrations are statistically increased in hyperthyroid patients and decreased in hypothyroid patients in comparison to the control group. These results agree with two studies who reported a significant high resistin level in hyperthyroid patients [13,14]. On the other hand, Iglesias et al., found a reduced resistin level in hyperthyroid patients [15]. These conflicting results may be due to the small number of patients (20

patients) in the latter study which lack any significant statistical power.

In the hypothyroid group resistin level was similar to the control group but significantly lower than the hyperhyroid group. Similar to the present results, Krassas et al., concluded that hypothyroidism is not associated with changes of resistin level [16]. Meanwhile, Owecki et, al. in 2008 showed a significant reduction in resistin level after thyroxin withdrawal [17]. Botella et al., reached to an opposing result with an increase in resistin level after thyroxin withdrawal [18]. However, these studies were short term studies on a small number of patients.

In the present study, a significant positive correlation was found between resistin and thyroid hormones and a significant negative correlation with TSH. This correlation may be causal or association. Many studies tried to explain these results. In rats, changes in resistin level are secondary to thyroid function. This may partially explain the insulin resistance state in hyperthyroidism[19]. Pedro and Juan, thought in a different way and concluded that hyperthyroidism is associated with weight loss despite increased appetite and elevated metabolic rate. Weight loss is associated with increased endogenous resistin in human [20,21]. In a multivariate logistic regression analysis, factors which affect resistin level were sex, TT3 & FT4. Similar studies demonstrated clearly the significant higher resistin level in males[23].

Several groups have studied the relationship between serum leptin and thyroid function with conflicting data [22-26]. The present study showed that leptin concentrations were increased in hypothyroid patients and decreased in hyperthyroid patients. Serum leptin correlated positively with TSH and BMI. On the other hand, it correlated negatively with TT3 & FT4. This was in agreement with several studies[7].

In 2003, Iglesias et al., demonstrated a low leptin level in patients with both hyperthyroidism and hypothyroidism[15]. However, they concluded also the increased level of leptin in hypothyroid patients after treatment. Yatura et al., confirmed the association between thyroid function and resistin but they didn't find any relation between leptin and thyroid function[13]. This opposing data may be related to the sampling. The authors mentioned that they withdrew the samples in the morning not at a fixed time. They also didn't mention if the patients were fasting or not. These two points are critical as serum leptin level has a circadian rhythm reaching a peak in the early morning hours and a lowest level in late morning [26]. Serum leptin also is markedly changed with the fed state[27].
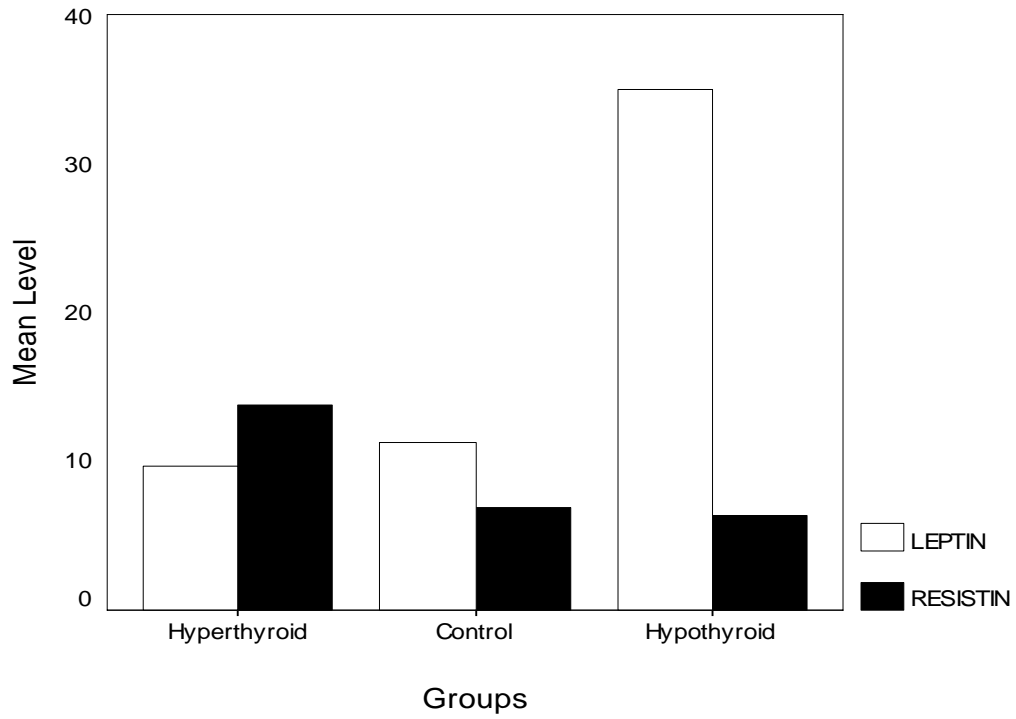
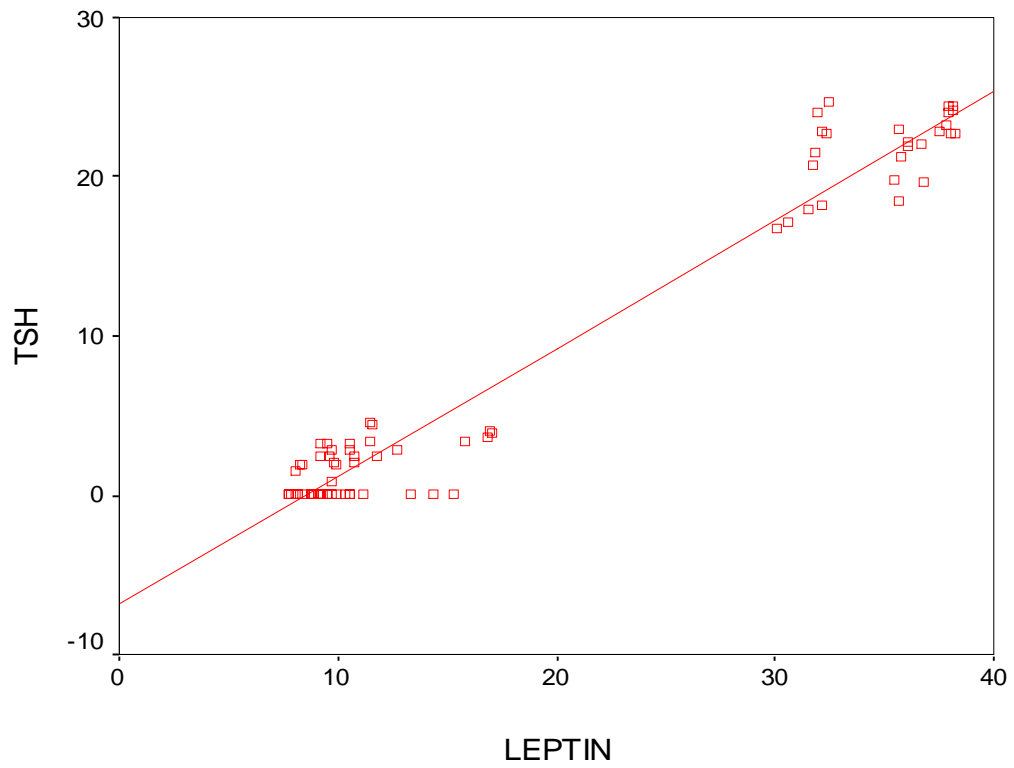**Figure 1: Mean leptin and resistin concentrations in different groups.**

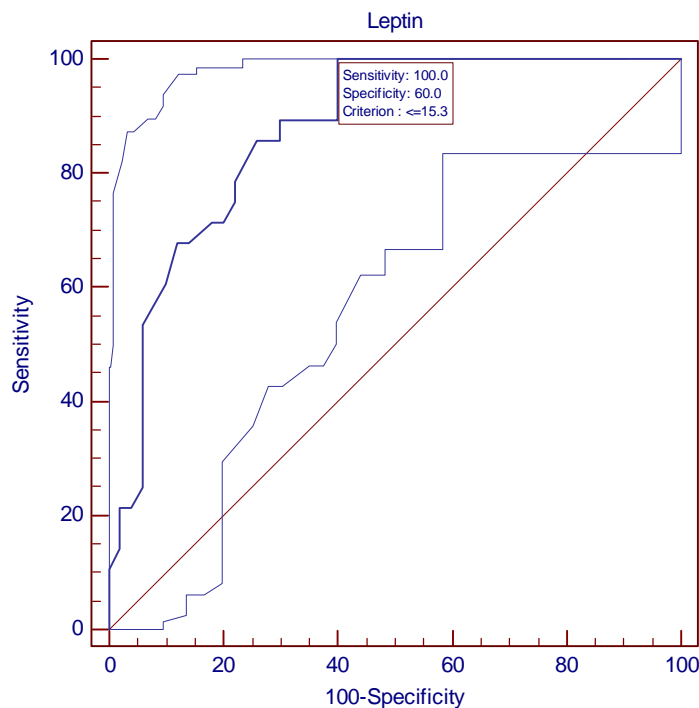**Figure 2: Correlation between serum leptin and TSH in all groups.**

Figure 3: ROC curve between serum Leptin and TSH in the whole group.

Table (1): A comparison between the three groups regarding the anthropometric and Laboratory parameters (mean +SD).

Groups	Hyperthyroid Gp (No=28)	hypothyroid Gp (No=26)	control Gp (No=24)	P 1	p2	p3
Age (years)	43.9+8.5	45.2+4.7	45.6+7.6	0.455	0.808	0.508
Gender (Male /Female)	13 / 15	10 / 16	10/14	0.405	0.157	0.267
BMI (Kg/m^2)	23.7+2.7	29.4+2.1	25.4+2.6	**0.025**	**0.000**	**0.000**
TSH (mU/L)	0.07+0.03	21.7+2.4	2.8+0.9	**0.000**	**0.000**	**0.000**
FT4 (ng/L)	2.6+0.1	0.68+0.04	1.75+0.49	**0.000**	**0.000**	**0.000**
TT3 (nmol/L)	6.6+1.6	0.3+0.1	2.1+0.8	**0.000**	**0.000**	**0.000**
Resistin	13.8+3.66	6.316+3.413	6.900+1.968	**0.000**	**0.000**	0.467
Leptin (ng/ml)	9.7+1.8	34.9+2.8	11.2+2.7	**0.019**	**0.000**	**0.000**

P1: Significance between the hyperthyroid and hypothyroid groups.
P2: Significance between the hyperthyroid and control groups.
P3: Significance between the hypothyroid and control groups.

Table2. Pearson correlation analysis between both serum Leptin and Resistin with clinical and biochemical results

Parameter	S. Leptin		S. Resistin	
	Correlation	P value	Correlation	P value
AGE	0.003	0.981	0.083	0.469
BMI	**0.770**	**0.000**	**-0.571**	**0.000**
TSH	**0.980**	**0.000**	**-0.502**	**0.000**
TT3	**-0.773**	**0.000**	**0.805**	**0.000**
FT4	**-0.884**	**0.000**	**0.641**	**0.000**

Table 3: Multivariate logistic regression analysis of all markers with both serum leptin and Serum Resistin

Parameter	S. Leptin			S. Resistin		
	B	OR	P Value	B	OR	P Value
AGE	7.600	-0.045	0.320	1.938	0.465	0.643
SEX	**2.518**	**0.105**	**0.000**	**2.726**	**-5.038**	**0.000**
BMI	0.161	0.047	0.185	0.174	1.209	0.231
TSH	**1.117**	**0.920**	**0.000**	2.883	0.433	0.666
TT3	0.362	0.088	0.080	**2.201**	**9.014**	**0.000**
FT4	1.301	0.094	0.186	**3.116**	**-2.667**	**0.009**

In accordance with the present results, Baig et al., demonstrated a significant correlation between BMI and serum leptin but they didn't find a correlation between serum leptin and patients with hypothyroidism. A drawback of this study is the small number of patients investigated (only 21 males) [24].

The relationship between serum leptin and thyroid hormones may be bidirectional and it needs a lot of studies to explain it. Several studies suggested that thyroid hormones affect serum leptin through its effect on the body fat composition which is the main source of leptin[28]. Others suggested that thyroid hormones are regulators of leptin mRNA expression which may mediate the weight changes associated with thyroid dysfunction[29]. Others suggested that both changes in thyroid function and leptin levels are secondary to changes in body fat composition and no causal relationship is found between them[20,30,31].

To further evaluate factors which may affect serum leptin, multivariate logistic regression analysis was done. Only sex and TSH were significant while age, BMI and thyroid hormones had insignificant effect on serum leptin level. This is in agreement with Mantzoros et al., who confirmed the close pulsatile association between TSH and serum leptin. They concluded that leptin may regulate TSH pulsatility and circadian rhythmicity [26]. Of interest, patients with congenital leptin deficiency demonstrate central hypothyroidism. Moreover, this hypothyroid state is corrected by intervention with leptin administration[32].

From another point of view, TSH also can influence leptin release. Menendez et al., demonstrated that TSH stimulates leptin secretion by a direct effect on adipocytes[33]. This opinion is further supported by the changes in leptin level with intervention with the administration of thyroid hormone[15].

For detection of the cutoff level of serum leptin with hyperthyroidism as TSH the classifying variable a ROC curve was done. The best cutoff level was 15.3 ng/l with a sensitivity of 100%, Specificity of 60% and

an area under the ROC curve (AUC) of 0.874. To the best of our knowledge this is the first study to detect this cutoff level.

5. Conclusion

The relationship between adipokines, namely, resistin and leptin with thyroid dysfunction is bidirectional and dynamic. In other words, changes in one arm are associated with changes in the other arm independent of body fat composition. Although both markers correlated with thyroid function, only sex and TSH has an effect on leptin while sex, TT3 and FT4 affect the resistin level. A cutoff level of 15.3 ng/l is associated with marked changes in TSH with a sensitivity of 100%, Specificity of 60% and an area under the ROC curve (AUC) of 0.874.

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IT IS NOT ENDOCANNABINOIDS BUT THE TYPE AND AMOUNT OF FOOD ARE THE MAIN CAUSE OF METABOLIC DISTURBANCES IN RATS

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Abstract: Obesity has been identified as a major global health problem. A major cause of the obesity is the changes in feeding behaviour. Many controversy data concerning the role of endocannabinoid system in regulation /or disturbing of the metabolic parameters. The aim of this research is to identify the effect of methanandamide (as a one of CB1 selective agonist) on some metabolic parameters in rats fed by different types of food to clarify which is the cause of metabolic abnormality in obese ?.Design: A total number of 56 healthy adult male albino rats were used to study the effect of different types of diet and daily i.p injection methanandamide (CB1 agonist) in a dose of 0.5 mg/kg BW for 6 weeks on some metabolic parameters using pair feeding paradigm.Results: a significant increase in final body weights and a significant dyslipidemia and hyperglycemia with insulin resistance was in both HFD and HFrD fed groups when compared with that of standard chow diet fed group. Moreover, a significant dyslipidemia and hyperglycemia with insulin resistance was observed in methanandamide treated ad libitum group. In addition, our study revealed an insignificant change in all parameters measured between HFD and HFrD fed groups except for TG and VLDL parameters which are significantly higher in HFrD-fed group in comparison with that of HFD fed group. Interestingly, an insignificant change in serum levels of all previously mention parameters in the three different methanandamide treated pair fed groups in comparison with that of the three different fed control groups respectivelyConclusion: we can conclude that endocannabinoid system is not the main responsible for metabolic disturbance in obese rats.
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Key Words: high fat, high fructose, endocannabinoid, insulin resistance, dyslipidemia.

1. Introduction:

The history of marijuana and its medicinal use go back thousands of years, but the endogenous counterparts of cannabis, the endocannabinoid (ECs) have only been known about 18 years ago, their discovery have been triggered by the identification of specific cannabinoid receptors in the brain[1].

Two subtypes of cannabinoid (CB) receptors have been identified to date. Both CB_1 and CB_2 receptors couple to the Gi/o subtypes of G proteins, but can also activate additional, G protein-independent pathways[2].

It is common knowledge that marijuana use improves appetite, presaging the role of ECs as endogenous orexigenic factors. However, findings as early as the 1970's suggested that [9]-tetrahydrocannabinol ([9]THC), the psychoactive ingredient of marijuana, has additional metabolic effects unrelated to appetite[3].

CB_1 receptors are present in skeletal myocytes and are upregulated in obesity[4] and may be one of the targets for cannabinoid-induced insulin resistance. ECs may also influence insulin secretion in the endocrine pancreas, although there are conflicting reports on CB_1 receptors mediating a decrease[5] or an increase in

insulin release[6], so ECs may share in the regulation of glucose homeostasis and insulin sensitivity.

Metabolic studies have confirmed that calories obtained from fat have a greater role in obesity and have been considered as dietary risk factors for metabolic syndrome (MS)[7,8], moreover, fructose as a common supplement of the packed food has been implicated as a possible cause of the MS[9].

CB_1 receptor inhibition by AM251 is capable of countering insulin resistance in adipose tissue, muscle and liver[10,11]. However, similar beneficial effects on insulin sensitivity were also observed in pair fed animals[12]. The lack of significant improvement in metabolic status in AM251 treated, in comparison to pair fed animals does not mirror this finding. Thus, further studies are necessary to confirm these initial observations. Interestingly, AM251 also increased locomotor activity in agouti mice[13].

Recently, **Sink et al.** [14] reported that all clinically available cannabinoid receptor antagonists (e.g. AM 251 and rimonabant " SR141716A") are inverse agonists that can target CB_1 receptors located in both central circuits regulating appetite and motivation and in peripheral organs regulating metabolism and energy expenditure. This profile (inverse agonism)

complicates understanding of cannabinoid CB_1 receptor blockade as a therapeutic strategy in obesity and metabolic disorders.

Unlike rimonabant, chronic adminstration of LH-21 (selective CB_1 antagonist only) reduces feeding but does not improve hypertriglyceridaemia or hypercholesterolaemia; nor does it reduce liver fat deposits in Zucker rats. These data explain why AM251 increased locomotor activity in agouti mice at low doses[13].

It is still uncertain whether an overactive ECS is an early cause[15] or just one of the several consequences, of HFD and the subsequent development of overweight and obesity.

The present study was done to identify the effect of methanandamide (as a one of CB1 selective agonist) on some energy metabolic parameters concerning glucose homeostasis and lipid in rats fed by different types of food (commercial, high fat and high fructose diet) using pair feeding paradigm in a trial to clarify which is the cause of metabolic abnormality in obese, it is ECS alone, type of food, amount of food intake or crosstalk between them?.

Materials And Methods
Animals
This study was carried out on a total number of 56 adult (4 months; body weight, 180-200 gm) healthy male albino rats. Under hygienic conditions, in the animal house of the faculty of medicine Zagazig University, all rats had free access to water and chow, supplied in separate clean containers. Rats were kept at comfortable temperature (20 to 24 ℃) and were maintained on a 12 hr light/dark cycle[16].

Diet
Normal (standard) diet: consists of commercial rat standard chow [it was consisted of 25.8 % protein, 62.8 % carbohydrate and 11.4 % fat[17].

High fat diet (HFD): was consisted of 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[17.18].

High fructose diet (HFrD): commercial rat laboratory chow containing 60% fructose[19].

Methods
The rats were accommodated to the new laboratory conditions for three weeks before the beginning of the experimental regimen[20].
Grouping of the animals
Group I "normal fed group": to study the effect of 6 weeks normal diet. It is consists of 24 rats which further subdivided into 3 equal subgroups (n= 8):
Group IA: vehicle (saline) treated group with access to ad libitum standard chow.

Group IB: Methanandamide (dissolved in sterile saline), treated (0.5 mg/kg BW i.p, daily)[21,22], pair fed group. Rats were given a weighed amount of standard chow each day corresponding to the amount consumed by vehicle treated rats on the previous day[12].
Group IC: Methanandamide treated (0.5 mg/kg BW i.p, daily), ad libitum group, rats had access to ad libitum standard chow.
Group II "HFD fed group: to study the effect of 6 weeks HFD diet. It consists of 16 rats which further subdivided into 2 equal subgroups (n= 8):
Group IIA: vehicle treated group with Access to ad libitum HFD.
Group IIB: Methanandamide treated (0.5 mg/kg BW i.p daily), HFD pair fed group.
Group III " HFrD fed group: to study the effect of 6 weeks HFrD diet. It consists of 16 rats which further subdivided into 2 equal subgroups (n= 8):
Group IIIA: vehicle treated group with Access to ad libitum High fructose diet.
GroupIII B: Methanandamide treated (0.5 mg/kg BW i.p, daily), HFrD pair fed group.
For all groups, body weight was recorded at the beginning and the end of the study period (6 weeks).

Sampling of blood
At the end of the experimental period (at the end of 6th week) after overnight fasting, at 8:00 a.m, blood samples were obtained from sinus orbitus vein of each rat after ether inhalation[23]. The blood samples were allowed to clot at room temperature before centrifuging at approximately 3000 rpm for 15 minutes. The serum was stored at -20° C.

Serum analysis
Determination of serum glucose level: According to **Trinder**[24] using glucose enzymatic **(GOD-PAP)-** liquizyme Kits (Biotechnology, Egypt).

Determination of serum insulin level: By a solid phase enzyme amplified sensitivity immunoassay according to **Starr et al.** [25] using KAP1251-INS-EASIA (Enzyme Amplified Sensitivity Immunoassay) Kits (BioSource Europe S.A., Belgium).

Determination of the Serum total cholesterol (TC): by enzymatic colorimetric method according to **Allain**[26] using Cholesterol RTU 61218 kits: (bioMerieux S.A., Lyon, France).

Determination of the Serum high density lipoprotein cholesterol (HDL): by enzymatic colorimetric method according to **Warnick et al.**[27], using Stanbio HDL-cholesterol procedure No. 0599

kits (Stanbio laboratory Inc., San Antonio, Texas, USA).

Determination of the Serum Triglyceride levels: It was carried out according to **Naito**[28] using triglycerides ESPAS SL kits (Elttech S.A., Sees, France.).

Calculation of very low density lipoprotein cholesterol (VLDL) and Low density lipoprotein cholesterol: According to **Friedewald et al.** [29].
HOMA-IR was assessed by homeostasis model assessment (where HOMA= fasting serum insulin (μIU/mL) x [fasting serum glucose (mmol/L)/22.5][30].

The data obtained in the present study were expressed as mean ± SE for quantitative variables and statistically analyzed by using SPSS program (version 18 for windows) (SPSS Inc. Chicago, IL, USA). P value <0.05 was considered statistically significant.

3. Results:

Table 1&2 and histograms 1, 2, 3, 4, 5, 6, 7, 8 & **9** show the final body weight (gm), serum glucose (mg/dL), insulin (μIU/mL), the HOMA index of insulin resistance (HOMA-IR), total cholesterol (CHO) (mg/dl), HDL-C(mg/dl), triglyceride (TG) (mg/dl), VLDL (mg/dl) and LDL-C(mg/dl) levels in all studied groups. In group IB "Methanandamide treated normal diet pair fed group" the mean values were found to be non significant (P > 0.05) when compared with that of group 1A"normal diet control group", however group

IC "Methanandamide treated normal diet ad libitum" were found to be significantly higher than that of both group 1A and group IB (P < 0.01, P < 0.001, P < 0.001, P < 0.001, P < 0.001, P <0.001, P < 0.001, P < 0.001 & P < 0.001 respectively) except HDL-C levels was significantly lower. In addition, the mean values of group IIB "Methanandamide treated HFD pair fed group" and III B "Methanandamide treated HFrD pair fed group" were found to be non significant (P > 0.05) when compared with that of group IIA "HFD control group" and IIIA "HFrD control group".

It was found that both of group IIA "HFD" and group IIIA "HFrD" showed a significant increase in final body weights, serum glucose, insulin, the HOMA-IR, CHO, TG, VLDL-C LDL-C levels (P <0.001) and HDL-CHO (P < 0.01 & P < 0.05)when compared with that of group IA normal diet group". While, group IIIA "HFrD" showed an insignificant change (P > 0.05) in final body weights, serum glucose, insulin, the HOMA-IR, CHO, and LDL-C levels, however, it had significant increase in TG and VLDL (mg/dl) (P < 0.001) when compared with that of group IIA"HFD".

In addition, there were significant positive correlations between final body weights and serum glucose, serum CHO, serum TG, serum VLDL-C, serum VLDL- C, LDL-C levels and calculated HOMA-IR, accompanied by significant negative correlation between serum HDL-C levels and final body weights in all studied groups.

Table 1: Final body weights, serum glucose, insulin, the HOMA-IR, levels in all studied groups.

parameter	N= 8	Normal Diet			HFD		H Fr. D	
		Group IA	Group IB	Group IC	Group IIA	Group IIB	Group IIIA	Group IIIB
Final body weight (gm)	$\overline{X}$ ± SE	241.25± 2.13	243.63± 2.93	255.25 ± 3.47	266.86 ± 3.47	267.25 ± 3.06	264.50± 2.49	264.87 ± 2.81
	P	NS		<0.01*$	NS		NS	
Glucose (gm/dl)	$\overline{X}$ ± SE	89.63 ± 6.0	93.13 ± 7.75	219.63± 3.64	272.0± 6.67	273.5 ± 5.5	281.75± 4.41	280.75 ± 4.89
	P	NS		< 0.001*$	NS		NS	
	r / P	+.752 / < 0.05	+.795 / < 0.05	+ .771 / < 0.05	+ .890 / < 0.01	+ .948 / < 0.01	+ .866 / < 0.01	+.881 / < 0.01
Insulin (IU/mL)	$\overline{X}$ ± SE	21.04±0.93	21.05± 0.69	36.44± 1.78	40.25± 1.61	39.17± 1.21	38.68± 1.18	39.76± 1.22
	P	NS		< 0.001*$	NS		NS	
	r / P	+ 0.749 / < 0.05	+ 0.804 / < 0.05	+ 0.920 / < 0.01	+ 0.932 / < 0.01	+ 0.977 / < 0.01	+ 0.775 / < 0.05	+ 0.932 / < 0.01
HOMA index	$\overline{X}$ ± SE	4.69 ± 0.44	4.88± 0.49	20.12±1.26	27.57± 1.71	26.93± 1.34	27.35± 1.22	28.00± 1.19
	P	NS		< 0.001*$	NS		NS	
	r / P	+ 0.901 / < 0.01	+ 0.921 / < 0.01	+ 0.920 / < 0.01	+ 0.937 / < 0.01	+ 0.981 / < 0.01	+ 0.832 / < 0.05	+ 0.848 / < 0.01

* VS group IA $ VS group IB

Table 2: Serum CHO, TG, VLDL-C LDL-C levels in all studied groups.

Parameter	N= 8	Normal Diet			HFD		H Fr. D	
		Group IA	Group IB	Group IC	Group IIA	Group IIB	Group IIIA	Group IIIB
CHO	$\overline{X} \pm$ SE	106.0± 2.66	101.50± 2.24	133.88± 5.30	186.75± 3.87	190.63± 3.87	189.38± 4.17	191.88± 2.68
	P	NS		< 0.001*$	NS		NS	
	r P	+ 0.839 < 0.01	+ 0.828 < 0.05	+ 902 < 0.01	+ 0.724 < 0.05	+ 0.793 P < 0.05	+ 935 < 0.01	+ 0.925 < 0.01
HDL-C (mg/dl)	$\overline{X} \pm$ SE	51.0± 1.65	47.75± 3.08	38.63± 1.55	38.25±1.75	37.75± 1.33	39.63± 1.05	38.13± 1.74
	P	NS		< 0.001*$	NS		NS	
	r P	- 0.800 < 0.05	- 0.734 < 0.05	- 0.955 < 0.01	- 0.958 P < 0.01	- 0.886 < 0.01	- 0.865 < 0.01	- 0.877 < 0.01
TG (mg/dl)	$\overline{X} \pm$ SE	56.86± 1.8	57.75±2.31	91.37± 4.85	125.63± 2.90	130.13± 2.49	150.75± 4.19	161.5±3.91
	P	NS		< 0.001*$	NS		NS	
	r P	+ 0.716 < 0.05	+ 0.834 < 0.05	+ 0.968 < 0.01	+ 0.737 < 0.05	+ 779 < 0.05	+ 0.831 < 0.05	+ 0.970 < 0.01
VLDL-C (mg/dl)	$\overline{X} \pm$ SE	11.38±0.36	11.55±0.46	18.28±0.97	25.13±0.58	26.0±0.50	30.15±0.84	32.30±0.78
	P	NS		< 0.001*$	NS		NS	
	r P	+ 0.716 < 0.05	+ 0.834 < 0.05	+ 0. 968 < 0.01	+ 0.737 < 0.05	+ 779 < 0.05	+ 0.831 < 0.05	+ 0.970 < 0.01
LDL-CHO (mg/dl)	$\overline{X} \pm$ SE	43.63±4.04	42.20±4.11	76.98±5.75	122.25± 5.94	126.85± 4.54	119.60±4.41	121.45±3.53
	P	NS		< 0.001*$	NS		NS	
	r P	+ 0.813 < 0.05	+ 0.908 < 0.01	+ 0.925 < 0.01	+ 0.822 < 0.05	+ 0.853 < 0.01	+ 0.933 < 0.01	+ 0.919 < 0.01

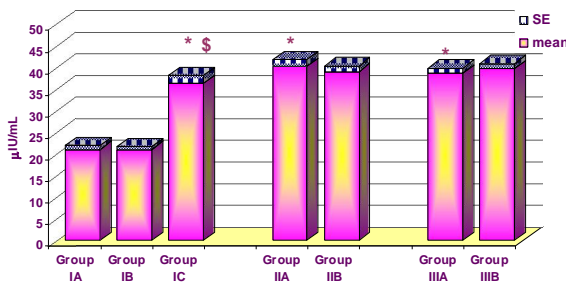* VS group IA $ VS group IB
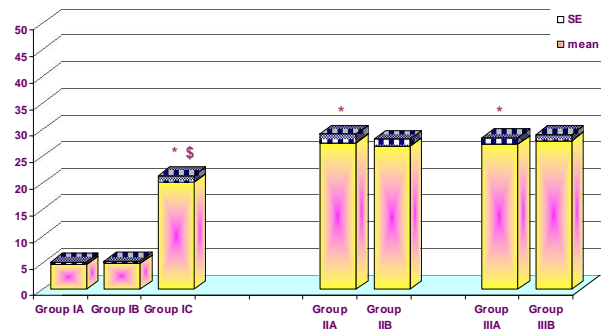

Histogram 1 : The final body weights (gm) in all studied groups.
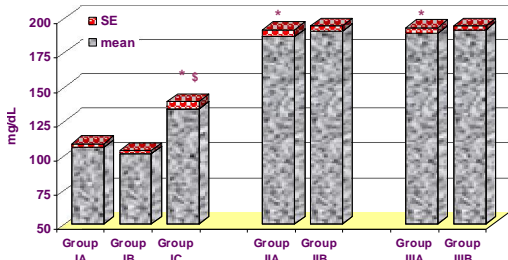* VS group IA $ VS group IB

Histogram 2: illustrates serum glucose levels (mg/dl) in all studied groups.
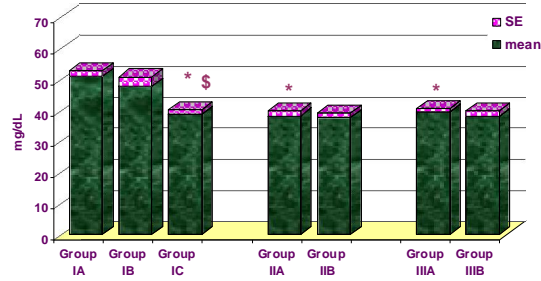* VS group IA $ VS group IB

Histogram 3: illustrates serum insulin levels µIU/ml in all studied groups.
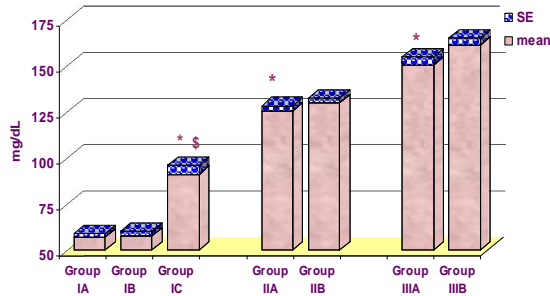* VS group IA $ VS group IB

Histogram 4: illustrates HOMA index of insulin resistence in all studied groups.
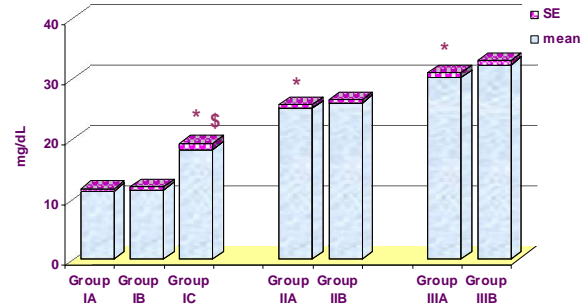* VS group IA $ VS group IB

Histogram 5: illustrates total serum cholesterol levels mg/dl in all studied groups.
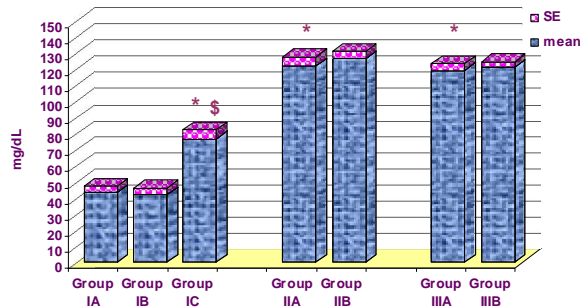*** VS group IA   \$ VS group IB**

Histogram 6: illustrates serum HDL-C levels (mg/dl) in all studied groups.
* VS group IA   \$ VS group IB

Histogram 7: illustrates serum TG levels (mg/dl) in all studied groups.
* VS group IA   \$ VS group IB

Histogram 8: illustrates serum VLDL levels (mg/dl) in all studied groups.
* VS group IA   \$ VS group IB

Histogram 9: illustrates serum LDL-C levels (mg/dl) in all studied groups.
* VS group IA   \$ VS group IB

4. Discussion:

In mammals, body weight and composition are maintained within a narrow range by the integrated control of energy intake, storage and expenditure. The endocannabinoid system (ECs) which consists of cannabinoid receptors, the endogenous lipid ligands (endocannabinoids), and the machinery for their biosynthesis and metabolism share in this control[31].

To explore the exact role of diet and ECs in metabolic disturbance, the present study used the 6 weeks pair-feeding paradigm to ascertain if the metabolic effect of ECs were related to type and/or amount of food intake or not.

As regard HFD ad libitum group, It showed a significant increase in final body weight with preservation of its correlation with all metabolic parameters, and a significant disturbance in glucose metabolism, distinguished by significant increase in fasting serum glucose levels, fasting serum insulin levels, and HOMA index of insulin resistance. Ad libitum HFD fed group also showed dyslipidemia proved by significant increase in serum total cholesterol, TG, VLDL-cholesterol levels, and LDL-cholesterol levels, accompanied by a significant decrease in HDL-cholesterol levels when compared with that of ad libitum normal diet group.

These data are in consistent with **Alsaif and Duwaihy**[32] who found that in general, HFD significantly increased weight gain, impaired glucose tolerance and increased insulin resistance.

Buettner et al.[33] stated that long term HFD feeding often impairs lipid metabolism by reducing insulin sensitivity of peripheral tissue.

HFD decrease insulin-stimulated glucose disposal in skeletal muscle and increase TG, long-chain acyl-CoA and diacylglycerol contents[34]. In addition, Glut 4 mRNA in WAT is down-regulated by HFD[35].

The HFD induced glucose intolerance and dyslipidemia which proved in the present study can be explained by disturbance of some metabolic hormones such as insulin resistance which has been proved in our results. In addition, **Naitoh et al.[36]** confirmed that mRNA expression level of adiponectin in WAT and plasma adiponectin levels were found to be decreased in HFD-fed mice compared with commercial diet-fed mice. This hypoadiponectinemia was closely linked to insulin resistance and low HDL cholesterol[37], as adiponectin enhance lipid clearance from plasma and increasing fatty acid beta oxidation in muscle[38].

It was found that, diet induced obesity in humans and rodents have very high amounts of circulating leptin, this hyperleptinemia is associated with leptin resistance which neither reduces appetite nor increases energy expenditure[39].

Apelin has been reported as a beneficial adipokine up-regulated in obesity as an attempt to overcome either insulin resistance or obesity-related cardiovascular diseases[40], elevated plasma apelin has been estemated in moderately[41] and in severe obese[42]. It has also been shown that plasma apelin levels were increased in diabetic subjects and positively correlated with BMI, HOMA-IR and fasting plasma insulin[43], suggesting a role of apelin in the pathogenesis of type II diabetes induces by overweight[44].

Watanabe et al.[45] stated that disturbed ECs levels that accompanied HFD are related to onset, duration and its fatty acid composition.

Cannabinoids promote lipogenesis and the storage of adipose tissue via CB_1, and the expression of CB_1 in adipose tissue is up-regulated in rodent models of obesity[15,46].

On the other hand, antagonists of CB_1 promote lipolysis and fatty acid oxidation and increase in insulin sensitivity[47,48].

As regard the comparison between the HFrD and the normal diet fed groups, HFrD fed group showed a significant increase in final body weight with preservation of its correlation with all metabolic parameters and a significant increase in fasting serum glucose levels, fasting serum insulin levels, and HOMA-IR, HFrD fed group also showed dyslipidemia proved by significant increase serum total cholesterol, serum triglyceride, VLDL-cholesterol levels, and LDL-cholesterol, accompanied by a significant decrease in HDL-cholesterol.

These data are in line with studies on rodents that stated that HFrD increases intra hepatocellular lipid and stimulates hepatic de novo lipogenesis within a few days and induce hyperlactatemia and hypertriglyceridemia[49].

Studies of pure fructose fed to laboratory animals show increased plasma free fatty acids, and abdominal adipose tissue, as well as impaired insulin sensitivity[50].

Fructose consumption by adult rats has been shown to produce diminished glucose tolerance and insulin sensitivity as well as elevated TG, cholesterol, and body fat[51]. The insulin resistance in this animal model probably results from the impairment of insulin-stimulated glucose uptake in insulin-responsive tissues as well as changes in hepatic glucose metabolism[52] Insulin resistance may also contributed to this hypertriglyceridemia by reducing the inhibitory effect of insulin on TG secretion rate in the liver of fructose fed rats[53].

The increase in TG level may be due to stimulation of hepatic VLDL-triacylglycerol synthesis and secretion and decreased VLDL-triacylglycerol clearance[54]. **Stanhope et al.[55]** indicate an increase in weight gain on diets rich in fructose, and a correlation between body fat and circulating TG has been established. In addition, HFrD induce leptin and insulin resistance. Taken together, leptin or insulin resistance and elevated TG serum levels may cause food over-consumption and contribute to the corresponding obesity, moreover, ingesting fructose-sweetened food raises calorie intake resulting in an over-consumption of energy which is not balanced by energy output, leading to weight gain[55].

As regard the comparison between HFD and HFrD fed groups, both groups showed dyslipidemia and glucose intolerance, however there were no significant differences between both groups in final body weight, fasting serum levels of glucose, insulin, total cholesterol, HDL-cholesterol, and LDL-cholesterol and HOMA-IR. While a significant increase in serum triglyceride, VLDL-cholesterol in HFrD fed group when compared with HFD fed group. These data are in consistent with that of **Liu and Manson[57]** who proved that diet high in carbohydrates is associated with glucose intolerance and obesity.

One of the main differences between glucose and fructose metabolism is that glucose must advance through a negatively regulated step using phosphofructokinase which regulates glycolysis in the liver. Fructose can bypasses this regulatory step and continue to be metabolized in the liver into glycerol-3-phosphate and acetyl coenzyme, these latter metabolites serve as substrates for glyceride synthesis leading to increased formation of VLDL and TG in the liver[58]. In contrast to glucose, when large amounts of fructose are ingested, the glycolytic pathway becomes saturated, and TG production is facilitated[59].

The present results showed an insignificant disturbance in all metabolic parameters measured in methanandamide treated normal diet pair fed group and methanandamide treated HFD pair fed group and methanandamide treated HFrD pair fed group when

compared with that of normal diet fed, HFD fed, and HFrD fed control groups respectively. However, methanandamide treated normal diet; ad libitum showed a significant disregulation of glucose metabolism "significant hyperglycemia, hyperinsulinemia, and increase HOMA-IR and significant dyslipdemia when compared with that of both ad libitum normal diet and methanandamide treated normal diet pair fed groups.

Taken together, this results proved that the ECS affects the metabolism by increasing the substrates that are needed for the lipogenesis, through increasing food intake and this concept is in line with **Williams and Kirkham**[21], as they proved that AEA injections in rats activated CB1 and promoted overeating, similar results were reported in rats injected with 2-AG[60]. ECS is considered to be primarily involved in the regulation of food intake via effects in the hypothalamus and nucleus accumbens[4]. CB_1 is selectively expressed in ventro-medial hypothalamus neurons. Absence of these neurons leads to weight gain, and their excitability is decreased in the presence of CB_1 agonists and increased by leptin[61].

ECs appear to interact with several other anorexigenic and orexigenic factors, clearly implicating the ECS in appetite regulation in a central control mechanism[2]. Cannabinoid and leptin signals are integrated in lateral hypothalamic neurons[62]. **Di Marzo et al.**[63] demonstrated that injecting mice with leptin, an anorexigenic adipokine that acts on the hypothalamus, resulted in a significant decrease in both AEA and 2-AG in the hypothalamus. They also showed that defective leptin signaling in the hypothalamus of obese db/db, ob/ob mice and Zucker rats was associated with an increase in endocannabinoid content.

Some studies stated that CB_1 antagonism has shown an improvement in insulin resistance and plasma glucose parameters, and a decrease in insulin and free fatty acid levels[12,64].

The absence of significant disturbance in methanandamide treated pair fed groups in comparison to other ad libitum control groups is in line with **Irwin et al.**[12] who suggested that the effect of ECS on disturbing the metabolism is due to increase food intake, depending in their study on the anorectic effect of the CB_1 blocker (AM 251) they observed that subchronic AM251 treatment in ob/ob mice caused weight reduction, improved the impaired metabolism and decrease insulin resistance, but these observations were found also in pair-fed control animals suggesting that the beneficial actions of CB_1 receptor antagonism is due to reduced food intake.

Specific central CB_1 blockade decreased body weight and food intake in diet induced obese rats, but had no beneficial effects on glucose metabolism; on the other hand, peripheral CB_1 blockade also reduced food intake and body weight but, in addition, enhanced insulin sensitivity. However, this improvement in insulin sensitivity was also detected in vehicle pair-fed rats, which suggest that decreased energy intake was the major factor responsible for these effects[65].

Finally, collecting data from previous researches concerned with ECS and ECs benefits revealed that ECs have been used therapeutically for alleviating pain[66], And induces neuroprotection in ischemic brain areas[67]. Moreover, endocannabinoid signaling was proposed to protect against the consequences of stress in a certain dose range as low doses of methanandamide was proved to induce anxiolytic effects which are CB_1 receptor-mediated. In addition, CB_1 receptor activation at amygdala promotes fear extinction[68]. Cannabinoids are also effective in treatment of models of nausea and vomiting[69], gastric ulcers, irritable bowel syndrome, ulcerative colitis, Crohn's disease, secretory diarrhea, paralytic ileus and gastro-esophageal reflux disease[70].

In fact, many side effects related to the use of CB_1 receptor antagonists were proved as impairs fear extinction, increase anxiety-related behaviours in the elevated plus maze. In addition, both pharmacological antagonism and genetic inactivation of CB_1 receptors impair extinction of conditioned fear memories[71]. Moreover, acute injections of rimonabant or AM251 (CB1 antagonists) also increase both basal and stress-induced serum corticosterone levels[72]. Furthermore, mice lacking CB_1 receptors are impaired in actively coping with stress in a model predictive for antidepressant-like activity[73]. Thus, CB_1 receptor antagonism may induce psychiatric side-effects, in humans, mainly anxiety- and depression-like states, being in accordance with the notion that the endocannabinoid system acts to keep a set point counteracting aversive emotions[71]. Obese patients already suffer from anxiety or depression more frequently than non-obese subjects[74].

Taken together with the results of the present study, it can be concluded that ECS should not be palmed for metabolic disturbances but the type and amount of diet is the main cause and its receptors antagonists must not be the main target for treatment obesity.

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The role of information and communication technologies (ICT) in agricultural development

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Abstract: Policy makers and service providers have increasingly come to view information and communication technologies (ICT), and particularly the Internet, as an important tool in providing disadvantaged groups and areas with access to information, services and markets that would otherwise be inaccessible. The concept of development of the rural, today, is not just project initiatives and governance; it is much more beyond that. This paper uncovers a whole plethora of ICT emergence as a technology of the new millennium. Against the backdrop of the ongoing ICT boom, this paper makes an attempt towards studying its applications and usage planning process and policy making for the rural communities focusing on how it helps in aligning the key factors and reduce the problems of alienation, fragmentation and dislocation of knowledge.

Introduction:

The importance of communication in the development process has been acknowledged for many years by the development community. FAO has spent at least thirty years pioneering and promoting - both in thinking and practice - the centrality of communication in development. The most essential ingredient of good communication – putting people at the centre of the communication process - has similarly been understood and documented for many years.

Agriculture extension and farmer-outreach programs face three major challenges – cost-effective outreach, solutions tailored to needs of individual farmers and an image that is farmer-friendly. The internet and mobile networks have the potential to provide agro-information services that are (i) affordable, (ii) relevant (timely and customized), (iii) searchable and (iv) up to date. Large sections of the farming community, particularly the rural folk, do not have access to the huge knowledge base acquired by agricultural universities, extension-centers and businesses. While telecenters are beginning to dot the rural landscape [1], one of the big barriers remains the lack of agro-content that (i) is in the language of the farmers (ii) is relevant to their needs and (iii) is delivered in a form that is of immediate use to them.

Information Technology, more precisely the Information and Communication Technology (ICT), has emerged world over as a technology of the new millennium. By augmenting the process of information exchange and reducing the transaction costs, this ubiquitous technology is instrumental in increasing productivity, efficiency, competitiveness and growth in all spheres of human activity. The potential benefits of, however, can be harnessed only if the technology diffuses across the different sectors of the society. Unfortunately, we are living in a world of 'digital divide' wherein half of the world population have never made a telephone call . The digital divide is not only an international problem, but for most developing nations including is also a national phenomenon. Nonetheless, it has been argued that in an era of globalization, the ability to harness this technology for the 'rural' improves the capability of the developing country.

Information technology (IT) has connected the world globally and is now changing our lifestyle and social consciousness dynamically. Of late, it has emerged as a best tool for information sharing and mutual communication. None of the walks of life have been left untouched by the IT sector be it grain threshing or global business. Agriculture has also been greatly influenced by IT in the present era though the share of IT in agriculture is only 1.3%.

P roblems faced is that:

- The population of the earth is burgeoning every minute and there is sufficient evidence of impending food

crisis, especially in the developing countries even after attaining self-sufficiency.

- Even the major powers in the world are finding it difficult to balance the agricultural productivity with the environmental

requirement and meet the expectation of the millions round the world.

- The politics and economics in any country and the world trade mechanism are now dependent on the balance of supply and demand of the food.
- Inefficient recording and storage of data in spite of huge data collection.
- Lack of timely forecasting of weather and agriculture productivity.

In the current scenario, the role of IT assumes great importance and only with proper integration of IT with agriculture, the problem of food crisis can be solved and the world can move towards a sustainable production.

Integration of IT with agriculture must be done with following main objectives in mind:

- Develop multi-level decision support models for synergising the natural resource system with economic and social imperatives.
- To develop indicators of sustainability for agricultural production system.
- Based on the above scientific assessment, suggest alternatives to conserve and improve the health of natural resource system.

Two fundamental steps exist in establishing an innovation as a valuable, readily used tool: diffusion and adoption. Both diffusion and adoption must occur in order for an innovation to successfully reach its target user and be implemented (Mahajan, et al.). First, diffusion, the process by which an innovation "is communicated through certain channels over time among the members of a social system" (Rogers), must occur. In this study, the StratSoy project was a major factor in the IT diffusion process in state soybean organizations. Other factors that influenced diffusion included the media, word-of-mouth, and experiences of friends, associates and family members.

In addition to individuals having access to a new technology, adoption must also occur, which means individuals accept the innovation as valuable and use it. Numerous factors could influence IT adoption and use in agricultural organizations and can be grouped into five categories: access to IT, demographic, IT training/education, trust, and time. It is possible for adoption factors to fit into more than one category.

In the case of IT, access to the technology means an individual must have access to a computer equipped with IT such as e-mail and access to the WWW. The category "access to IT" would not only include the use of a computer with IT ability, but would also include the ability to upgrade computer hardware and software to facilitate IT use. The price of needed computer equipment and the expense of Internet use are also related to access to IT. It is predicted that the higher the level of access to IT, the higher the level of IT use by an individual.

The demographic category includes adoption factors such as age, education level, gender, and income level. It is hypothesized that factors in the demographic category will not significantly influence IT adoption and use. Although previous literature suggests that IT use will be higher for younger, more educated individuals (Batte, et al.), 1997 survey results suggest that demographic factors have little influence on IT adoption and use. This may reflect that demographic factors may influence the decision to adopt a new technology, but once that decision to adopt is made, demographic factors may have little influence on use.

Another category of IT adoption factors is IT training/knowledge. This IT adoption factor can be measured with variables such as type of IT training, days of IT training, and the level of knowledge on IT use. It is hypothesized that as the quality and level of IT training increases, the use of IT will also likely increase.

An important factor influencing the adoption of any new technology is an individual's perception of that technology. It is hypothesized by this research that one of the key perception aspects influencing the adoption of IT is the level of trust that the potential adopter has in the IT system and in those who use IT. Trust can be defined as "an individual's optimistic expectation about the outcome of an event" (Hosmer 1995). There are different aspects of trust related to IT.

An individual must first trust that information technologies will work and that IT will be beneficial in accomplishing his/her goals and in completing his/her tasks. An individual must also trust that the information they obtain via IT is accurate and the information they send via IT will not be tampered with and privacy levels will be maintained.

Trust proves to be a difficult variable to measure. Factors included in the trust category include an individual's perception of the ease of use of IT as well as the benefit of IT. In this study, trust is measured by variables such as helpfulness of IT for work-related communication, problem solving ability, and banking and shopping via the Internet. Some individuals, either due to their background or current environment, have a fear of IT and feel that it is difficult to use. It is hypothesized that an individual will use IT more if they have a positive perception or high trust level in IT.

The final IT adoption category proposed by this research is the passage of time. It is hypothesized that individuals will increase their use of IT over time, as access to IT becomes more commonplace. In this

study, the same group of people were surveyed twice to evaluate their changes in IT use over time. Time was measured by establishing a dummy variable where each survey response from the 1997 survey was assigned a value of zero and each survey response from 1998 was assigned a value of one. Time-interaction variables were also created for each variable by multiplying the original variable by the time variable. For example, the "days of training" variable (tdays) was multiplied by the time variable and became the "timeinfluenced days of training" variable (tdayst).

Managerial Implications

Identifying the determinants of IT adoption and use will help industry participants, especially managers, use information technologies to increase information flow and increase the level of trust in the firm and the demand for the firm's products or services. For example, if a livestock company promotes the use of IT to its producers, it will open up more efficient means of communicating product information and providing other services to its customers. As consumers increase their use of IT, firms will be able to communicate more effectively with them, and demand for the firm's product may increase.

Determining the factors that influence IT adoption can assist companies in determining the IT use profile of their customers based on the significant adoption factors identified in this study. Knowledge of the factors that influence IT adoption can also help companies target individuals, who due to their progressiveness and use of IT, may be potential customers of the company's products and/or services. The company can then focus marketing and advertising campaigns on attracting these individuals to their business. This research is also important because IT can possibly substitute for trust with an organization just as trust often substitutes for contracts. A customer's comfort and trust level with a company may increase as they are able to gain more information about a company via IT. For example, a customer's trust level with a company will increase if he is able to track his shipment order via the Internet.

In addition to the general managerial implications of identifying IT adoption factors, this research also suggests specific ways in which a manager can promote IT adoption that can lead to more efficient communication and increased demand for the firm's products and services.

First, the research shows that IT training increases IT adoption and use. Therefore, firms may benefit from providing training on information technologies for both employees as well as customers.

Second, managers should proactively use IT to promote the trust their employees, customers, and other business associates have in IT, and thus increase the overall use of IT. The positive coefficient on the variable "e-mail is helpful for work related communication" suggests that the more those with whom you communicate use e-mail, the more helpful e-mail is in communicating with them. An agricultural producer might consider using e-mail to communicate with the firm because she observes that her well-respected chemical sales representative uses IT successfully.

This research also suggests that an individual's use of IT is greater when the individual's access to IT is not restricted. Therefore, managers may want to provide greater access to IT by providing each employee his or her own computer hardware equipped with Internet capabilities.

The employees will be free to use IT at their convenience and will be less concerned with privacy or security problems related to sharing a computer. Managers should promote the use of IT in all aspects of employees' and customers' personal lives and work. The significance of the variable "time-influenced do job related work at home" indicates that employees use e-mail more when they are physically separated from work.

The implication for managers is that IT use is greater when people work outside the office, or have flexible work relationships such as telecommuting. Increasingly, individuals will turn to IT when they need information or to communicate with the firm for personal or work-related reasons.

Certainly many individuals and organizations within society at large still have a fear or mistrust of IT. At the same time, agriculture constantly experiences advances in technology and the use of information technologies is becoming more common place each day. Therefore, it is essential for firms and managers to understand the reasons for IT adoption to remain competitive and to best serve their industry and customers.

Information Technology and its Components

Induction of IT as a strategic tool for agricultural development and welfare of rural requires that the necessary IT infrastructure is in place. The rapid changes and downward trend in prices in various components of IT makes it feasible to target at a large scale IT penetration into rural. Some of the broad factors to be noted with respect to various components of IT are listed below :

1. Input devices :

Radical improvements are witnessed with respect to the means of communication by human

beings with computers such as key boards, mouse devices, scanners. The advent of touch screen monitors that allow users to give input to computers by touching on the appropriate location of the monitor has made it possible to develop user-friendly interface for farmers which is easy, intuitive, circumvents language barrier and at the same time provides a relaxed environment to the users. The present day digital cameras make it possible to capture and store good quality graphics and large video clips. The small size and low weight of these digital cameras, which are increasingly becoming affordable, open up the possibilities of providing computer based demonstration clips to educate the farmers.

2. Output devices :

Monitor screens, printers & plotters, data projectors support high resolution and good quality output. The quality of these output devices have the potential of generating renewed interest in the farmers in using IT based services. The light weight portable data projectors can be easily carried by the agricultural extension personnel for serving larger audience. Similarly, speakers can also be attached to the computers to incorporate voice based trainings for farmers.

3. Processors:

The processing speeds of computers have gone up. At present, Intel P-IV based processors @ 1.5 Ghz are available in the PC range which makes it possible to undertake substantial processing of data at the client side.

4. Storage Devices :

40GB and even higher hard disk drives have become common in PC range of computers. This makes it possible to store substantial information at the local level which facilitates faster access. Similarly, high capacity floppy disk drives, CDs make it possible to transfer large volumes of data to locations which can not be connected to networks immediately. These storage devices are also used for backup of crucial data. As a precaution, many corporates store their backups at locations away from the place of work.

5. Software :

Various operating systems are available which act as interface between the user and the machine. The graphic user interface (GUI) has become an accepted prerequisite for end users. Microsoft's 'Windows' continues to be a favourite. Application softwares which can support complex user requirements are available. Of the shelf solutions for office automation packages, groupware applications, complex database solutions, communication products, solutions based on remote sensing & geographical information systems are available. In addition, solutions based on some or all of these are also readily available. The present downward trend in the IT industry provides an opportunity get customised application for any specific task developed at an affordable price. Rapid Application Development and Deployment (RADD) is a popular model for quick development and deployment of applications. Development environment itself is simplified with tools that quicken the pace of software specialists. Project management and monitoring software are available that facilitate efficient execution of large and complex applications that are required for rural

6. Networking devices:

The capacity of modems, used to convert the data from digital to analog and vice versa, which are popularly employed to use telephone lines have increased. Internal modems are available integrated into the computer so that they are not exposed to outside environment. The capacities of other networking devices such as routers have also gone up which makes it possible to create large networks with smooth data transmission.

7. Transmission Media:

The media through which the data transfer takes place has also undergone revolutionary change. Telephone lines are still the popular source although the reliability and low bandwidth are still major issues. High capacity cables, optical fibre, radio, wireless local loops, satellite transmission and various solutions based on a combination of these are already being used in many parts of the country.

8. Other accessesories :

Uninterrupted Power Supply (UPS) devices are crucial to ensure the longetivity of the IT equipment as well as provide backup mechanisms. The potential of solar power packs to provide a feasible solution to shortage of power in the rural areas needs to be exploited.

Role of IT in Agriculture

In the context of agriculture, the potential of information technology (IT) can be assessed broadly under two heads : (a) as a tool for direct contribution to agricultural productivity and (b) as an indirect tool for empowering farmers to take informed and quality decisions which will have positive impact on the way agriculture and allied activities are conducted.

Precision farming, popular in developed countries, extensively uses IT to make direct contribution to agricultural productivity. The

techniques of remote sensing using satellite technologies, geographical information systems, agronomy and soil sciences are used to increase the agricultural output. This approach is capital intensive and useful where large tracts of land are involved. Consequently it is more suitable for farming taken up on corporate lines.

The indirect benefits of IT in empowering farmer are significant and remains to be exploited. The farmer urgently requires timely and reliable sources of information inputs for taking decisions. At present, the farmer depends on trickling down of decision inputs from conventional sources which are slow and unreliable. The changing environment faced by farmers makes information not merely useful, but necessary to remain competitive.

Conclusion

Information and Communication Technologies (ICT) has the potential to change new and old forms of economic activity. This can result in e-literate groups, low skilled or low paid workers, unemployed people, sole parents, and those with disabilities that do not have access to these ICTs. However, it is likely therefore that assisting people to improve their access to and skills in ICT will be an important means for a Government to grow an inclusive, innovative economy for the benefit of a country. Therefore the ICT-Hub model or mechanism for integrated service delivery to rural communities may be applied for this purpose.

The face of the agriculture can be transformed by a well conceived deployment of IT. The potential of IT as yet remains untapped and urgent measures are required to derive maximum benefit. The key players involved in this process such as industry, government and educational institutions and research centres are required to make contributions in this endeavour. The initiative to develop necessary IT based agricultural services need to be developed immediately. Parallel steps to develop necessary IT communication infrastructure are to be taken up along with the utilisation of fiber optic network whereever it is passing through the rural segments.

It is necessary for the industry related to agriculture, in particular fertilizer companies, to review their present I.T. infrastructure with respect to marketing function and undertake measures to strengthen the same. Online integrated systems, well developed executive information systems, applications to enhance the productivity of the field personnel and efficiently serve the requirements of channel partners & consumers are to be taken up at the earliest. Marketing field personnel need to be provided with the necessary hardware, software,

training and brought on to Internet so that smooth integration is possible. Internet based technologies can facilitate creation of applications which can be operated by the field personnel by using simple browsers. Customer support services can also be partially provided over the Internet which will increase the reach of such programmes. The state and central governments should initiate urgent measures to jump in to IT bandwagon for effective e-governance.

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A Review of some Ecto. and Endoprotozoan Parasites Infecting *Sarotherodon galilaeus* and *Tilapia zillii* from Damietta Branch of River Nile, Egypt

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Abstract: The present study was carried out as a general survey searching for the possible protozoan parasites that can infect the Nile fishes *S. galilaeus* and *T. zillii*. A total of 125 live fish specimens were obtained from Damietta branch of River Nile and El-Sahel canal, Nile tributary. Examination of the investigated fish species revealed that, fishes were infected with eleven parasitic protozoan species belonging to eight genera. These species were: *Apiosoma piscicolum*, *A. conica*, *Scopulata epibranchialis*, *Vorticella* sp., *Ambiphrya ameiuri*, *Amphileptus* sp., *Chilodonella hexasticha*, *Tetrahymena corlissi*, *Trypanosoma mansouri*, *T. syanophilum* and *Trypanosoma* sp. Among the obtained parasites, the following were recovered for the first time in Egypt. *Apiosoma conica*, *Vorticella* sp., *Ambiphrya ameiuri*, *Amphileptus* sp., *Tetrahymena corlissi* and *Trypanosoma* sp. While *S. galilaeus* represent a new host for *Chilodonella hexasticha*. The recorded numerous parasites have pathological effects on the host fish with subsequent economic losses were discussed.
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1. Introduction:

In general protozoa are one of the major sectors of fish parasites that have been long neglected because of its inherent difficulty in studying compared to other larger parasites. Among protozoa, ecto and endo-parasitic protozoa occupy a very important sector as one of the hazardous threats to fish health. These parasites attack the fish causing massive destruction of skin and gill epithelium. Even moderate infection of these organisms on small fish may prove a fatal disease, since the infection may cause the fish to stop feeding (Meyer, 1968; Hoffman, 1970). Parasitic ciliates, particularly sessilines protozoa genera as *Apiosoma*, *Scopulata*, *Ambiphrya* and *Epistylis*, which infect skin and gills of fish. They are obligate parasites, which utilize gills and skin merely as a substrate for attachment. Thus, their pathogenicity is attributed to the mechanical interference with gas exchange activity (Paperna, 1980; Lom and Dykova 1992).

On the other hand endoparastic, trypanosomes are the most dangerous group that probably cause more diseases to fish than any other group of parasites, anemia and edema (Lom and Dykova 1992). In Egypt, fish parasitc protozoa gain a lot of attention (Ali, 1992; Ali et al., 2003-2007; Abdel-Meguid, 1995; Abdel-Ghaffar et al., 1998, 2008).

The present study aims to report on the occurrence of eleven protozoans found infecting two different host fish species. In addition, it will offer a review of some new species to egyptian protozoic fauna as well as add more detailed description of morphological characteristics of these parasites and its pathological effects.

2. Material and Methods

A total of 125 fresh water fish were collected from the River Nile at two locations, which are Demiatta branch near El-Mansoura and El-Sahel canal near Sherbeen city. The collected fish were transported to the laboratory in tank with good aeration. They were kept alive until required in aerated glass aquaria. The collected fish represent two species namely *Sarotherodon galilaeus* and *Tilapia zilliie*.

Fish skin, fins and gills were firstly examined by the naked eye for detection of any macroscopically visible lesions. Samples of mucus were scraped gently from the skin, fins and gills, then spread on a clean slide and freshly examined under phase-contrast microscope for the presence of ectoparasitic protozoans. Some of the positive slides were air-dried and stained according to Klein's dry silver impregnation method. Other positive slides were also air-dried, fixed with absolute methanol and stained with 10% Giemsa stain.

Blood samples were collected from the arteria caudalis using heparinized syringes. Thin blood films were made, air-dried, fixed with absolute methanol and then stained with 10% Giemsa stain for (20-30) minutes (Ali, 1992)

Detected protozoa were examined freshly, stained and identified according to (Shulman, 1984; Kazubski and Migala ,1974; Viljoen and Van As ,1985; Lom and Dykova ,1992). All measurements were taken in micrometers (µm) mean ± SD (range). Figures were drawn with aid of camera lucida.

3. Results

Among 125 examined fishes, 118 fishes were infected with different protozoan parasites. The detected protozoan parasites were classified into two main phyla; Ciliophora (Ciliates) and Mastigophora (Flagellates) that are summarized in (Table 1).

I- Genus: *Apiosoma*
A. piscicolum (Figs. 1A-C &4)

This peritrich is a solitary parasite, its body is goblet- like shaped, tapering towards its scopula by narrow stalk (Figs. 1B &4). The body is divided externally by a groove without cilia, to an oral body part measures 13.2 ±3.3 (9.9-16.5) µm and a basal body part measures 17.1±7.2 (9.9-24.2) µm. This groove is found at nearly one third of the body length from the peristome (Fig. 4). The peristomial lip is narrow and the peristomial disc (adoral spiral) is flat, winding counterclockwise and plunges into the buccal infudibulum. Infundibulum is slightly curved and extends to the non-ciliated groove. Contractile vacuole is large and the food vacuoles are distributed in the oral part only. The compact triangular macronucleus is observed at the level of or just below the groove and measures 13.8±2.8 (11-16.5) µm in length X 12.7±2.8 (9.9-15.4) µm in width (Fig. 1B). The micronucleus is oval and is situated above or alongside the macronucleus and measures 2.2X11 µm. Scopula is broader than stalk, sucker-like disc with undulant margin and measures 4.4(3.3-5.5) µm. Body free of cilia except for peristomial disc (three rows) and measures 33 ±1.1 (31.9-34.1) µm in length X 20.9±1.1 (19.8-22) µm in width. Transverse striations of pellicle are conspicuous and ranged from 33-42 in number (mean 37). Fig. 1C.

2- *A. conica* (Figs. 1D,E & 5)

This is a solitary, stalkless parasite with peculiar, conical body shape, gradually tapering to the scopula. The body measures 32.5±8.9 (21.8-43.2) µm in length X23.6±6.0 (13.8-38.1) µm in width. The non-ciliated groove found more than one third of the body length from the peristome and divided it to an oral body part measures 11.5±4.0 (7-16) µm and a basal part measures 12.1±2.0 (6.8-2.2) µm. The contractile vacuole is large (Fig. 1D). The peristomial lip is narrow and peristomial disc is flat and slanted. The epistomial disc is linguiform and elevated over

cilia of peristomial disc (Fig. 5). Infundibulum is short, curved and extends to the non-ciliated groove (Fig .1E). The compact rounded macronucleus is situated below the groove and measures 14.4±3.2 (11.2-17.6) µm in diameter. The micronucleus is rounded, situated below the macronucleus and measures 3.3 µm in diameter. Scopula broad, sucker-like disc with undulant margin and measures 15.6 µm.

II- genus: *Scopulata*
Scopulata epibranchialis (Figs. 1F, G & 6)

This sessile peritrich is solitary and stalkless. The body is barrel-shaped and measures 38.5±4.4 (34.1-42.9) µm in length X 26.4±3.3 (23.1-29.7) µm in width. The body is divided externally by a non-ciliated groove into nearly equal halves, an oral part measures 17.6±1.1(16.5-18.7) µm length and a basal one measures 19.2±7.2(12.1-26.4) µm length. The peristomial disc is broad and flat. The infundibulum is strongly curved and extends to the groove (Fig .1G). Macronucleus is frequently transverse-ellipsoidal, its transverse axis is longer and measures 14.6±3.6(11-17.6) µm in length X 17.1±2.8 (14.3-18.7) µm in width. It situated just below the groove (Fig .1F). Micronucleus is rounded, situated just below the groove and alongside of the macronucleus and measures 3.9±0.6(3.3-5.5) µm in length X 4.0±0.8(3.3-4.7) µm in width. Scopula is broad and flat (Fig.1G) but usually slightly narrower than the body, sometime bilobed (Fig.1F) and measures 4.4 µm in length (Fig. 6).

III- genus: *Vorticella*
Vorticella sp. (Figs. 1H & 7)

This parasite consists of two main parts, solitary zooid and scopula. The zooid is spherical-shaped and measures 72±6.0(66-78)µm in diameter. The peristomial disc is broad. The epistomial disc is vaulted, slightly elevated above the peristomial lips and slanted (Figs. 1H & 7). The peristomial lip is more or less outwardly and encircles the epistomial disc. The infundibulum is curved and lead to narrow cytopharynx. Large number of different sizes of food vacuoles are observed in fresh specimens. The nuclear apparatus consists of ribbon-shaped macronucleus, often sinuous, situated in the zooid center and measures 33 µm. The micronucleus is very small and far away the macronucleus and measures 1.1-2.2 µm. Scopula secretes contractile stalk provided with myonemes used for shortening and coiling the stalk. Stalk measures 20 µm in length and 6 µm in width. Transverse striations of pellicle are conspicuous and ranged from 70-82 (mean 76) (Fig. 1H). Numerous contractile and food vacuoles are present.

IV- Genus: *Ambiphrya*
 A. ameiuri (Figs. 2A,B & 8)

Solitary sessil ciliates with large vase-shaped body. It measures 75.1±14.6(60.5-84.7) µm in length X 45.7±3.9(41.8-50.6) µm in width. The body is divided externally by a permanent equatorial ciliary girdle motionless into an oral part measures 29.7±2.2(27.5-33) µm in length and a basal part measures 17.9±6.9(11-27.8) µm in length (Fig. 8). The peristomial disc represent one turn around the slightly elevated epistomial disc. Infundibulum is triangular in shape (Fig. 2A). Macronucleus is ribbon-like forming an orally situated U-shape sinuous; its limbs descend parallel to each other and ends at the level of the ciliary girdle by hook-like shape. It measures 126.5X4.4 µm. Micronudeus is rounded situated adjacent to one end of macronucleus and measures 4.4X2.2 µm. Food vacuoles are distributed in the oral part. Scopula is in the form of a broad undulate disc but never exceeds the body width (38.6 µm in width). Reproduction in *A. ameiuri* is usually accomplished by binary fission, in which a new organism is pinched off the adult (Fig. 2B).

V- Genus: *Amphileptus*
 ***Amphileptus* sp.** (Figs. 2C & 9)

This ciliate is compressed, long and lanceolate in outline. It measures 53.9±13.8(39.1-70.4) µm in length X 19.5±5.3 (14.2-24.7) µm in width. Longitudinal kineties (7-9) were observed on the right side while the left side bears longitudinal ciliary rows. Along the anterior edge of the body, there is a cytostomial slit which does not exceed one third of the body length. Nuclear apparatus consists of two oval macronuclei which are closely adjacent to each other being separated by only 1.3-1.9 µm distance (Fig. 2C). The macronuclei measure 8.5(8.1-9.0) µm in length X 6.4(5.3-7.5) µm in width. The micronucleus measures 2.7(2.1-3.4) µm in length X 2.2(2.1-3.2) µm in width, is often found in close contact with one of the macronuclei, in the area separating the macronuclei. Large contractile vacuole is always found in the posterior region of the body (Figs. 2C & 9). There are many food vacuoles with various sizes.

VI- Genus: *Chilodonella*
Chilodonella hexastcha (Figs. 2D,E, &10a,b)

The body is typical oval to foliate-shape dorsoventrally compressed and characterized by the presence of a notch at the anterior body margin. It measures 39±6.4(27.9-50.1) µm in length X 28.4±7.2(22.1-40.5) µm in width. The cytoplasm is coarsely granulated ventrally the ciliature of the body composed of right ventral ciliary band, three circumoral kineties and left ventral ciliary band (Fig. 2E). The right ciliary band is arched, long, number of ciliary kineties range from 6-8(mean7) and is meeting with the left one. The three oral kineties; two short circumoral ones in front of the oral opening and a long preoral one extending along the anterior line of contact of the two ciliary bands (Fig. 10a). The left ciliary band is straight, short and number of its kineties ranges from 7-9 (mean 8). There is a non-ciliated zone (naked zone). The cytostome occurs at the anterior part of the naked zone. It leads into a conspicuous cytopharynx (Fig. 2E). Cytopharynx is prominent and reinforced by 8-10 conspicuous nematodesmata (cuticular bands), forming a funnel-shaped tube with curved inner end (Fig. 10b). The cytopharynx may be slightly extruded to serve for boring into and disrupting the epithelial cells. Two contractile vacuoles are present. The macronucleus is rounded and measures 15.4 (13.1-17-8) µm in length X 13.2(12.2-14.3) µm in width. The micronucleus lies closely adjacent to the macronucleus and measures 4.4(3.2-5.1) µm in length X 3.1(2.3-4.1) µm in width.

VII- Genus: *Tetrahymena*
T. corlissi (Figs. 2F,3A &11)

Body is pyriform and measures 38.1±6.3(25.4-46.4) µm in length X 28.6±4.8(21.1-34.7) µm in width. The body is wholly covered with cilia. The number of meridional kineties ranges from 18-32(mean 25). All these kineties converge anteriorly around an apical loop (Fig. 3A). There are many contractile vacuoles with different sizes (Fig. 2F). Macronucleus measures 22.1(20-24.3) µm in length X 14.6(13.2-15.4) µm in width. Micronucleus is far from the macronucleus and measures 4.8(4.4-6.5) µm in length X 3.4(2.2-3.3) µm in width (Fig. 2F). Cytostome is small, oval and is situated at the anterior end (Fig.11).

VIII- Genus: *Trypanosoma*
1- *T. Mansouri* (Figs. 3B-D &12)

This trypanosome is polymorphic, showing three forms: small, intermediate and large (Figs. 3B-D). All morphometric data are shown in Table (2). The intermediate forms were the most abundant.
The general body of three forms is thin elongated and slender in shape and in many times they are curved in S-shape (Fig.12). The anterior end is more acute than the posterior one. The cytoplasm is finely granular and stained light red with Giemsa stain. The nucleus is situated mostly in the anterior half of the body or at least in front of the middle of the body. It is reniform-shaped and occupies the entire width of the body. Kinetoplast is oval. The flagellum originates from the kinetoplast and extends along the border of

undulating membrane. The undulating membrane bends into deep fold in close contact with the body cell and rise above body margin forming 8 to 15 festoons in plicate-shape. Then the flagellum extends beyond the anterior end of the body as a free flagellum. In the small forms, the undulating membrane is narrow, hyaline and produces not more seven folds (Fig. 3D).

2- *T. cyanophilum* (Figs. 3E & 13)

The body is elongated cylindrical, sinuously curved in horseshoe shape (Fig.3E). The anterior end is more acute than the blunt posterior one and both of them are folded back on themselves. The maximum width is at the nucleus level. This species is characterized by its deeply blue stained cytoplasm. The cytoplasm is also finely granular and has scattered vacuoles with different sizes. The nucleus is situated in the posterior half of the body. It is oval, occupies the entire width of the body and stained pink with Giemsa stain. The kinetoplast is oval and close to the posterior end of the body. Highly

conspicuous, sinuous undulating membrane forms strong 8 to 9 folds with about 33-35 festoons (Fig.13). The free flagellum is short. All morphmetric data are shown in Table (3).

3- *Trypanosome* sp. (Figs. 3F & 14)

Body of this species is stout with pointed anterior end and a short snout represent the posterior end. The cytoplasm is finely granular and stained light pink with Giemsa-stain. The nucleus is situated in the anterior half of the body or at least in front of the middle of the body (Fig. 3F). It is rounded and occupies the entire width of the body. The kinetoplast is oval shape lying at some distance from the posterior end. A distinct vacuole is often found in front of the kinetoplast. The undulating membrane is broader, less folded and end with hook-like shape at the level of the nucleus, then it becomes narrow and weakly folded (Fig. 14). The free flagellum is relatively long. Morphometric data are show in Table (3).

Table 1. Protozoan parasites reported in the present study.

Parasites spp.	Host fish species	Site of infection	References
Ph: Ciliophora			
1- Apiosoma piscicolum	Tilapia zillii	gills	Shulman (1984) ; present study
2- A. conica	T. zillii	gills	Shulman (1984) ; present study
3- Scopulata epibranchiails	Sarotherodon galilaeus	skin & gills	(Viljoen and Van As, 1983); present study
4- Vorticella sp.	S. galilaeus	skin	Present study
5- Ambiphrya ameiuri	S. galil	gills	(Lom and Dykova ,1992) ; the present study
6- Amphileptus sp.	S. galilaeus	gills & skin	Shulman (1984); (Lom and Dykova ,1992) ; present study
7- Chilodonella hexasticha	T. zillii S. galilaeus	gills skin	Shulman (1984); (Lom and Dykova ,1992); Ahmed et al., (2000) ; present study
8- Tetrahymena corlissi	S. galilaeus	skin	Shulman (1984); (Lom and Dykova ,1992) ; present study
Phylum: Mastigophora			
9- Trypanosoma mansouri	T. zillii	blood	Mohammed (1978) ; present study
10- T. syanophilum	T. zillii	blood	Mohammed (1978); Ahmed et al., (2000) ; present study
11- Trypanosoma sp.	T. zillii	blood	Present study

Table 2. Measurements (in μm) of various parts for the three forms of *Trypanosoma mansouri* from blood smears of *Tilapia zillii*.

Parameters	Small form	Intermediate form	Large form
Total length of the parasite including free flagellum	40.7(37.4-44)	48.4(46.2-50.6)	61.1(57.2-64.9)
Length of cell body	38(31.9-44)	35.8(33-38.5)	52.3(49.5-55)
Breadth of cell body	3.3(2.2-4.4)	3.3(2.2-4.4)	4.4(3.3-5.5)
Length of free flagellum	5.5(4.4-6.6)	12.7(12.1-13.2)	7.7(6.6-8.8)
Length of nucleus	4.4(3.3-5.5)	3.9(3.3-4.4)	5.0(4.4-5.5)
Breadth of nucleus	2.8(2.2-3.3)	2.8(2.2-3.3)	4.4(3.3-5.5)
Distance from anterior margin of nucleus to anterior end of body.	14.3(12.1-16.5)	12.1(9.9-14.3)	20.9(19.8-22)
Distance from posterior margin of nucleus to kinetoplast	22(18.7-25.3)	20.9(19.8-22)	25.3(24.2-26.4)
Distance from kinetoplast to posterior tip	2.8(1.1-4.4)	2.2(1.1-3.3)	1.1(0.8-1.3)
Length of kinetoplast	1.1(0.5-1.5)	1.7(1.1-2.2)	1.1(0.6-1.5)
Breadth of kinetoplast	0.9(0.8-0.9)	1.0(0.9-1.1)	0.9(0.9-1.1)
Width of undulating membrane	1.1(0.7-1.4)	2.2(1.0-3.4)	1.7(1.1-2.2)

Table 3. Measurements (in μm) of various parts for *Trypanosoma cyanophilum* and *Trypanosoma* sp. from blood smears of *Tilapia zillii*.

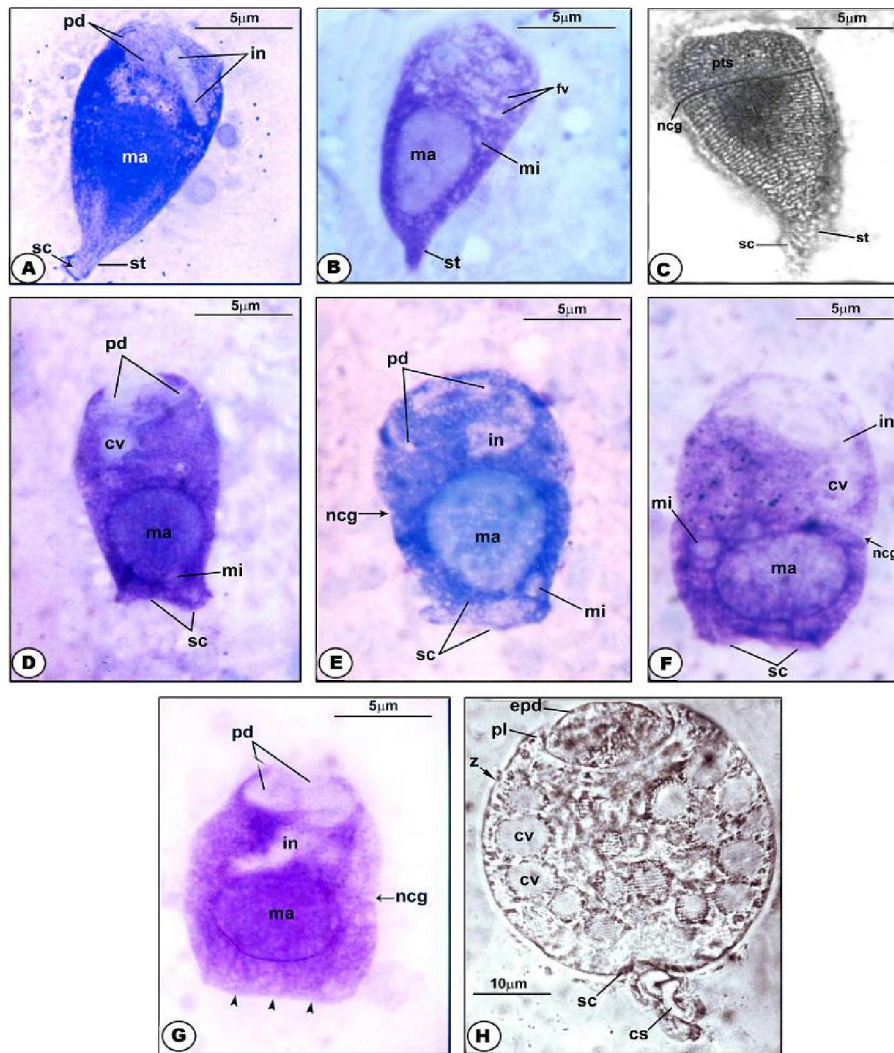| Parameters	*T. cyanophilum*	*Trypanosoma* sp.
Total length of the parasite including free flagellum	42.5(40.1-44.9)	43.3(41.1-45.2)
Length of cell body	38.2(36.7-39.7)	36.1(34.6-37.5)
Breadth of cell body	2.8(2.1-3.4)	4.2(3.2-5.2)
Length of free flagellum	6.3(5.4-7.2)	7.5(6.6-7.8)
Length of nucleus	4.2(3.5-4.9)	2.2(1.9-2.4)
Breadth of nucleus	2.4(2.2-2.6)	2.1(1.8-2.3)
Distance from anterior margin of nucleus to anterior end of body.	21.2(19.8-22.4)	15.2(14.1-16.2)
Distance from posterior margin of nucleus to kinetoplast	11.8(9.8-13.7)	19.1(18.2-20.1)
Distance from kinetoplast to posterior tip	1.4(0.9-1.9)	2.9(2.3-3.5)
Length of kinetoplast	1.3(1.2-1.4)	1.2(1.0-1.4)
Breadth of kinetoplast	0.7(0.3-1.0)	1.0(0.8-1.2)
Width of undulating membrane	1.2(0.9-1.4)	1.4(1.1-1.7)

Figure 1

Fig. 1. Morphology of Giemsa-stained *Apiosoma piscicolum* (A and B), silver-impreganated (C), Giemsa-stained of *A. conica* (D and E), *Scopulata epibranchialis* (F and G). Note broad and flat scopula arrow heads. Phase-contrast microscope of fresh *Vorticella* sp (H). cs, contractile stalk; cv, contractile vacuole; epd epistomial disc; fv, food vacuoles; in, infundubulum; ma, macronucleus, mi, micronucleus; ncg, non-ciliated groove; prestomial disc; pl, presomial lip; pts, pellicle transverse striations; sc, scopula; st, stalk; z, zooid.

Figure 2

Fig. 2. Morphology of Giemsa-stained *Ambiphrya ameiuri* and two small individuals (A and B), *Amphileptus* sp. (C), silver-impreganated of *Chilodonella hexastcha* (D); Giemsa-stained of *C. hexastcha* and *Tetrahymena corlissi* (E and F) al, apical loop; cb, cuticlar bands; ci, cilia; cp, cytopharynx, ct, curved tube; cys, cytostomal slit; L, left ciliary band; ma1 and ma2, macronucleus 1 and 2; mk, meridional kineties; no, notch; nz, naked zone; pk, preoral kinety; R, right ciliary band.
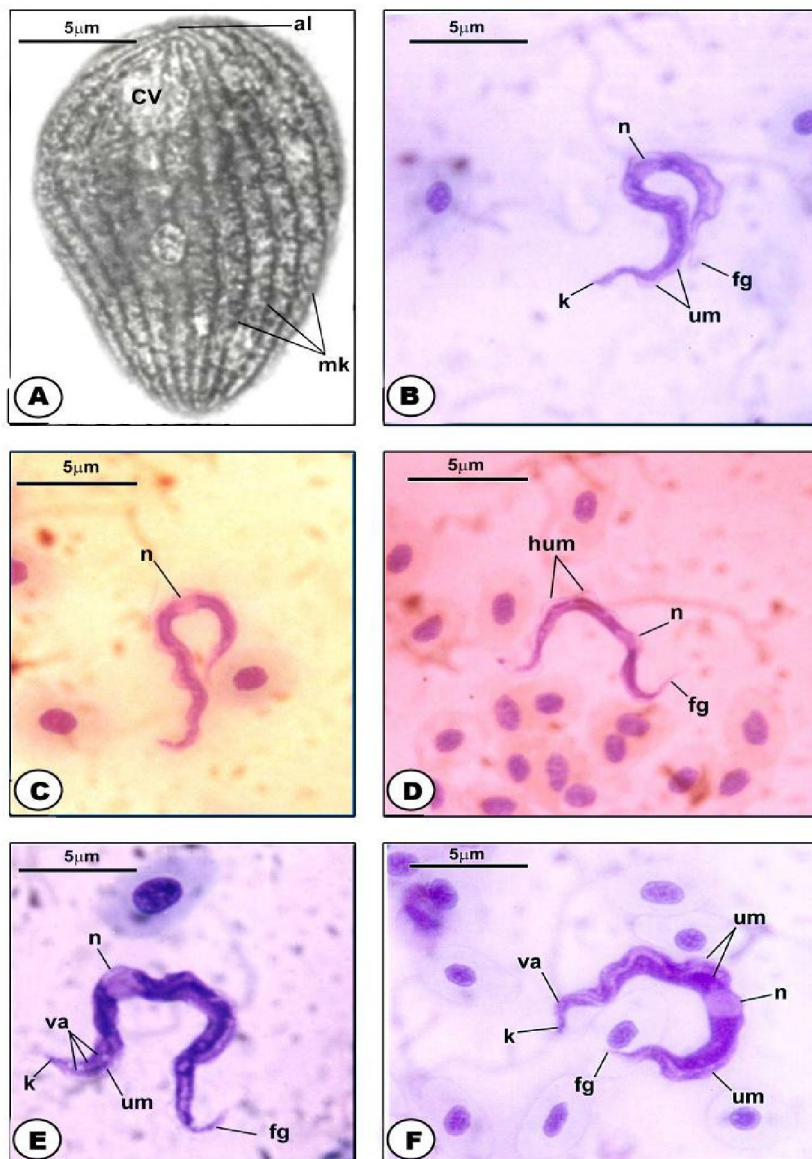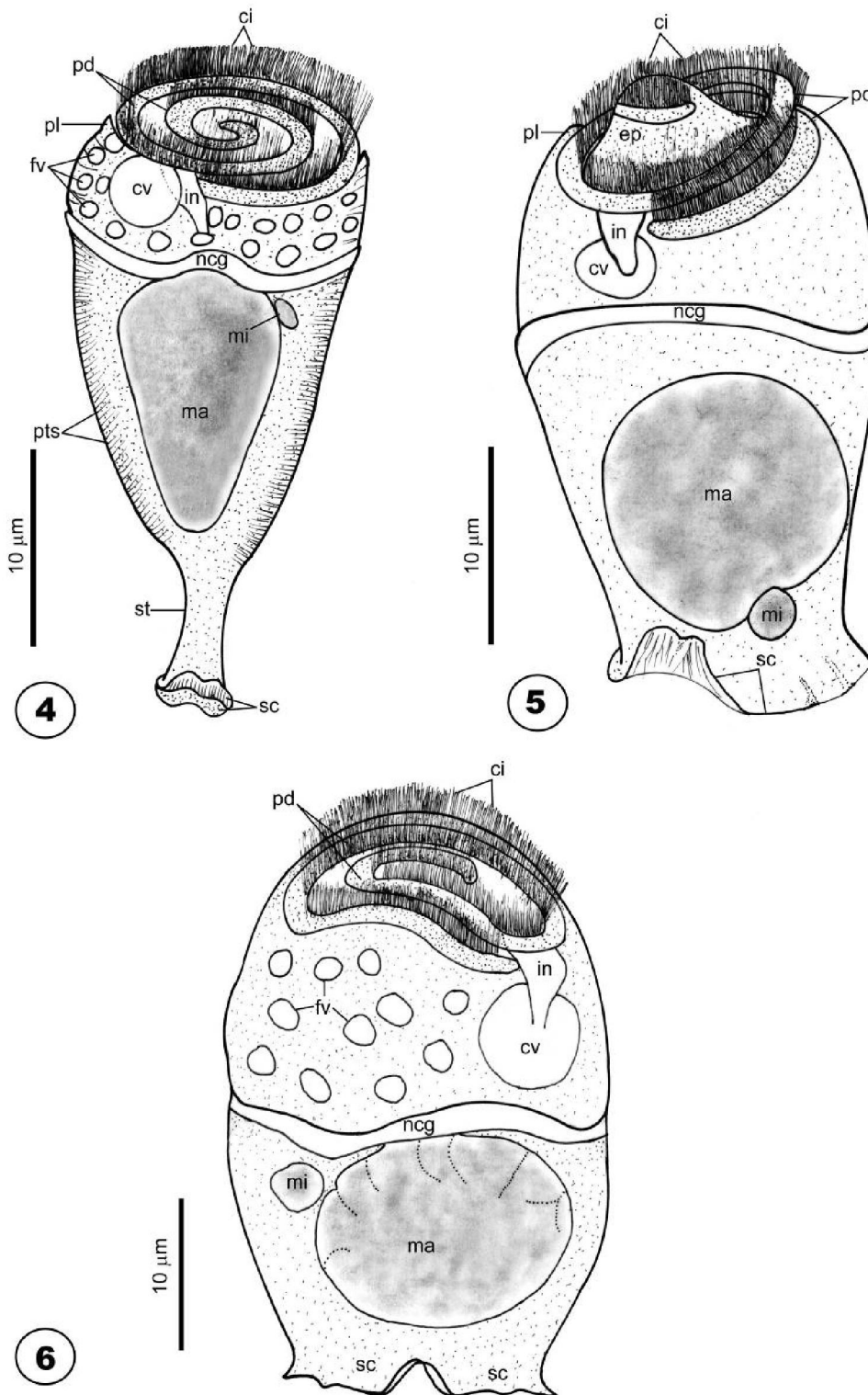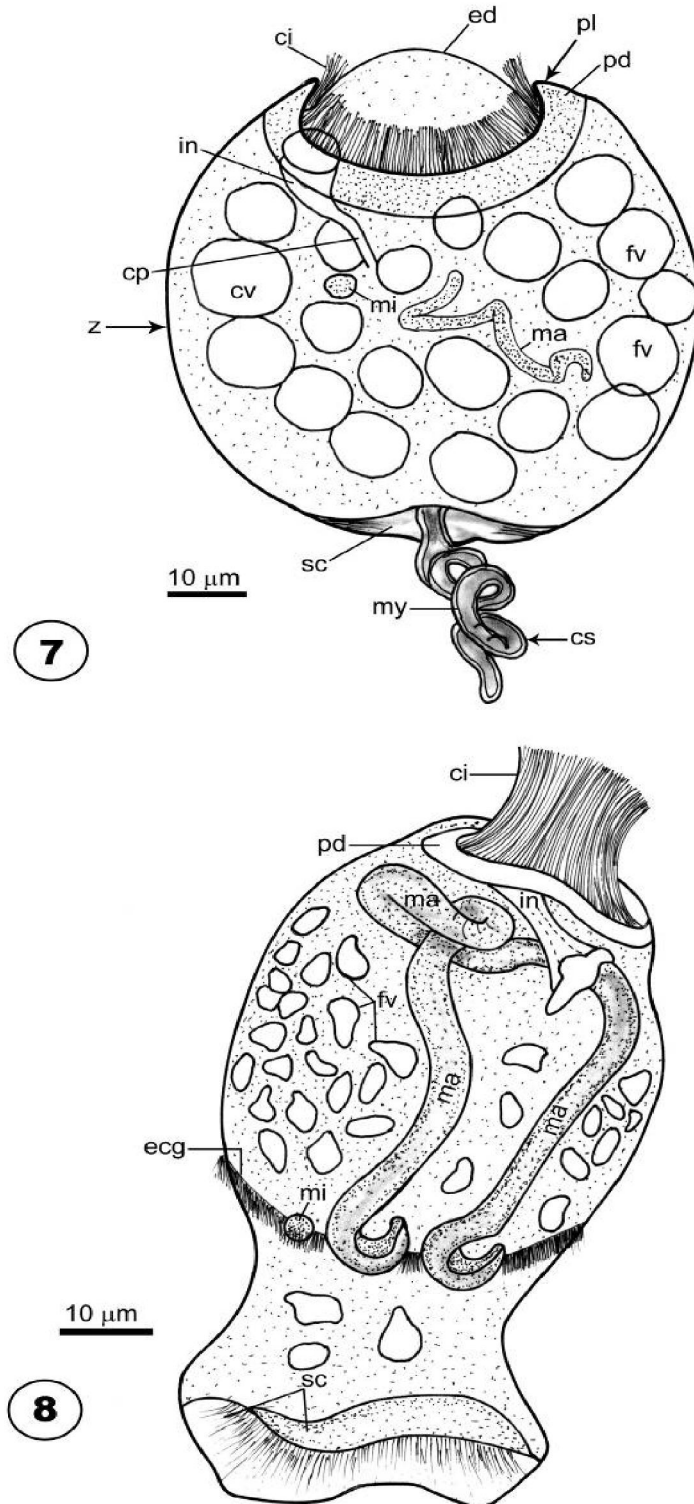
Figure 3

Fig. 3. Morphology of silver-impreganated *Tetrahymena* corlissi (A); Giemsa-stained *Trypanosoma mansouri* forms (B - D), *T. cyanophilum* (E); *Trypanosoma* sp. (F). fg, flagellum; hum, hyaline undulating membrane; k, kinety; n, nucleus; um, undulating membrane; v, vacuole.
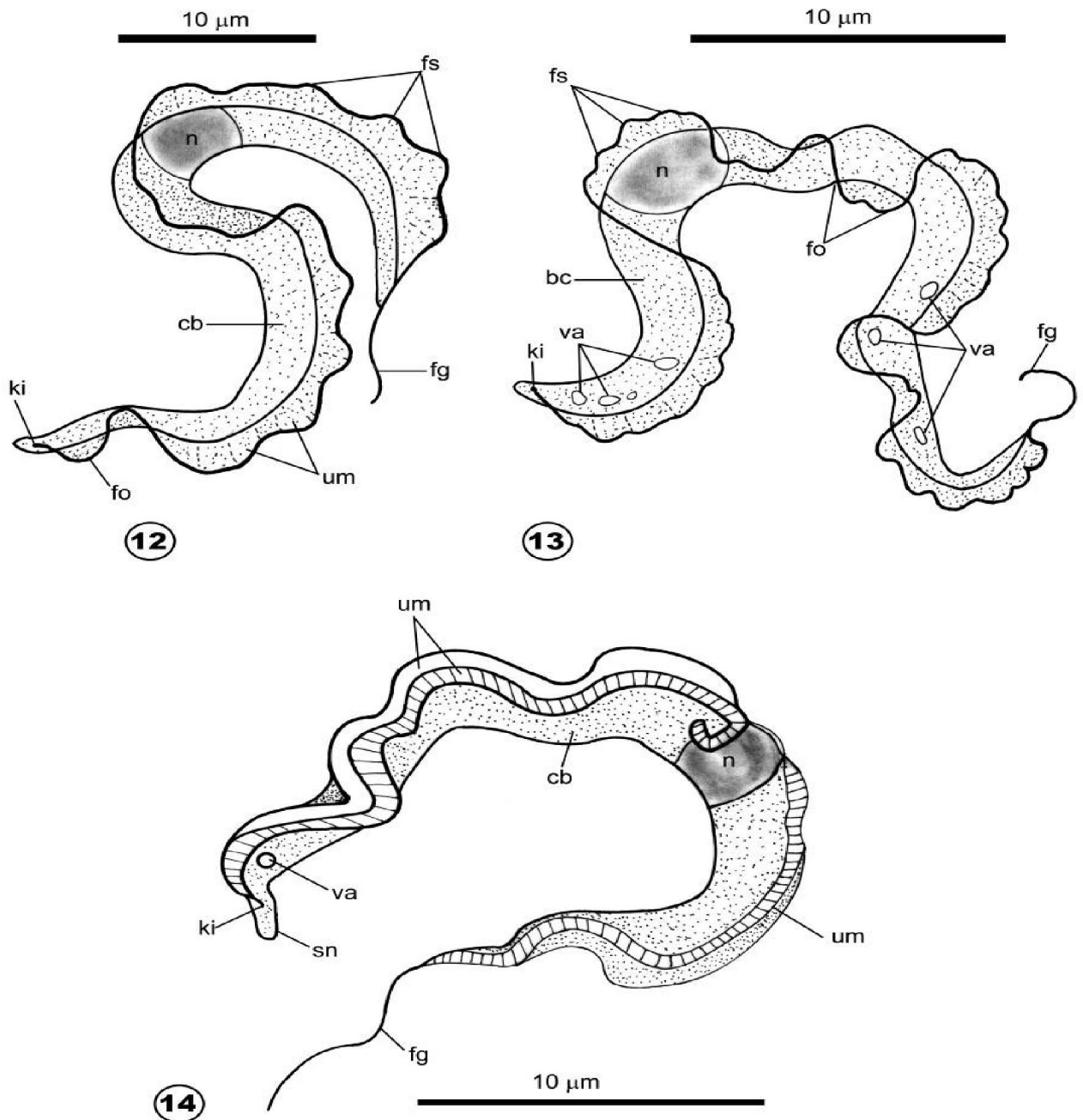
Figs. 4, 5 &6. Line diagram of *Apiosoma piscicolun* (4); *A. conica* (5); Scopulata epibranchialis (6). Abbreviations as in Fig. (1).

Figs. 7 & 8. Line diagram of *Vorticella* sp. (7); *Ambiphrya ameiuri* (8). ecg, equatorial ciliary girdle; my, myonemes. Other abbreviations as in Figs. 1 and 2.
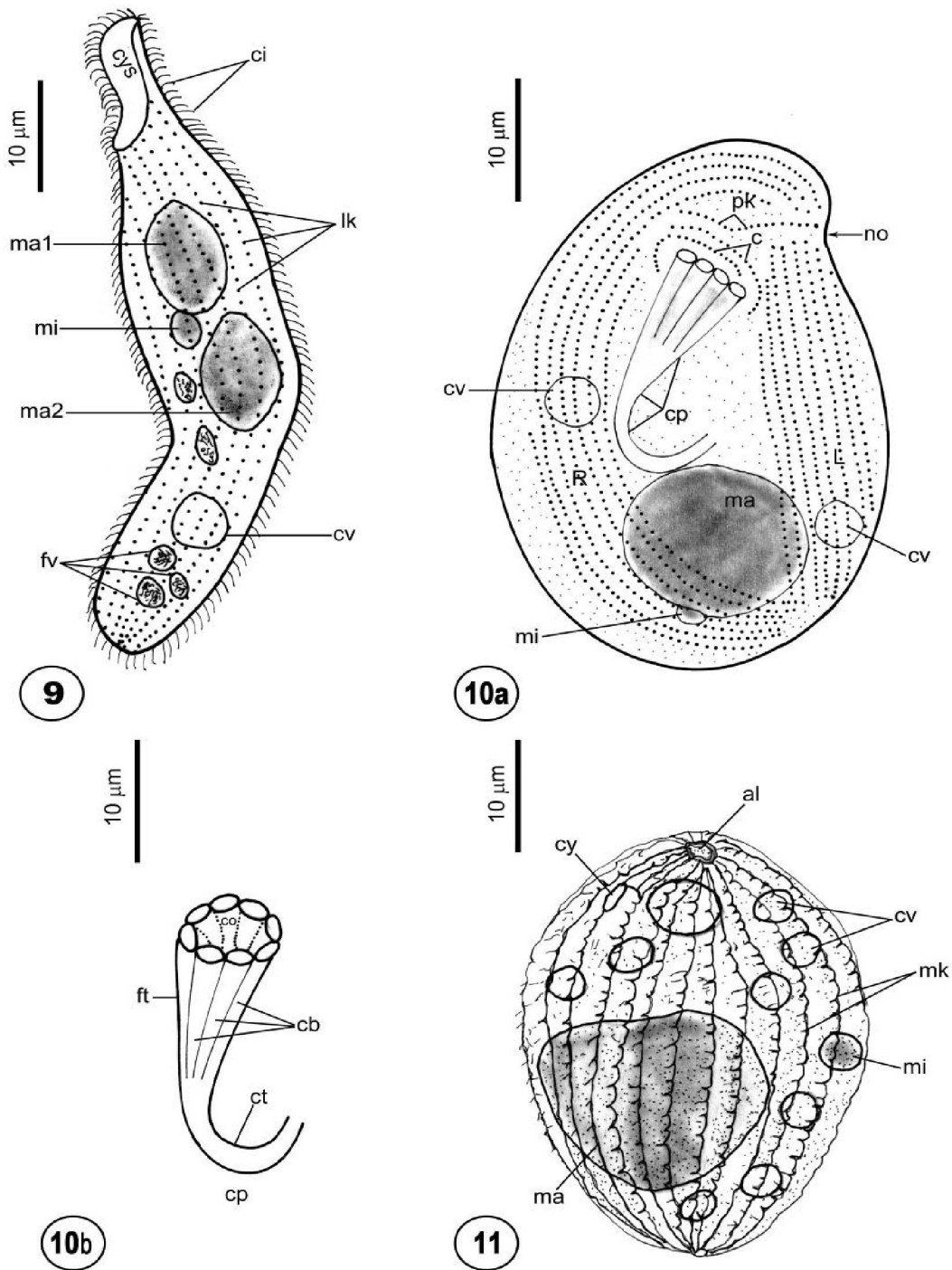
Figs. 9, 10a, b & 11 Line diagram of *Amphileptus* sp. (**9**); *Chilodonella hexasticha* (**10a**); apical view of cytopharynx (**10b**); *Tetrahymena corlissi* (**11**). c, circum oral kineties; co, cytostomal opening; cy: cytostome; lk: longitudinal kineties. Other abbreviations as in Fig. (2).

Figs. 12, 13 & 14. Line diagram of *Trypanosoma mansouri* **(12);** *T. cyanophilum* **(13);** *Trypanosoma* **sp. (14);**
bc, body cell; fo, fold; fs, festoones; ki, kinetiy; sn, snout. Others abbreviations as in Fig. (3).

4. Discussion:

Surveying of some ectoparasitic protozoa and blood parasites from *Sarotherodon galilaeus* and *Tilapia zillii* revealed the parasitism of these host fishes by 11 species representing 8 genera as follows:

Apiosoma piscicolum

This species was characterized by its goblet-like shaped body, narrow stalk, conspicuous transverse striations of pellicle and triangular compact macronucleus. The present parasite showed quite similarity to previously reported apiosomes by (Viljoen and Van As ,1985; Lom and Dykova ,1992).

A. conica

The investigated parasite was characterized by its conical body shape, stalkless and rounded compact macronucleus. This species closely resembles in the general shape and the size of body to *A. conica* reported by Shulman (1984), but the latter differs in site of infection (skin), host (9-spined sticklebacks) and locality (Neva Gulf). So the present parasite represent the first record in Egypt.

Scopulata epibranchialis

(Viljoen and Van As ,1985) created the genus *Scopulata*. Members of this genus are solitary, sessile, stalkless and the body is cylindrical with broad scopula. There are three species in the genus *Scopulata*; *S. constrica*, *S. epibranchialis* and *S. dermata*. However, some distinct features could be revealed between the present *Scopulata* and other three forms. *S. constrica* has a markedly constricted body at the groove. In *S. dermata* has triangular macronucleus. Accordingly, the parasite recorded herein is clearly not *S. constrica* or *S. dermata*. On the other hand the present recorded *Scopulata* conform well to the type specimens of *S. epibranchialis* in the shape of the body, shape and position of macronucleus and micronucleus. Thus the present specimens are comfortably identified as *S. epibranchialis*.

Vorticella sp.

According to (Lom and Dykova ,1992), the members belonging to this genus are free-living organisms and have contractile stalk, single zooid, ribbon macronucleus. The present recorded species conform well to genus characters. As far as our knowledge is concerned, this is the first record of *Vorticella* sp. from skin of *S. galilaeus*. The problem faced the identification of this organism is that it is free-living ciliates and colonize the fishes skin as facultative parasites. Therefore, (Migala and Kazubski, 1972), suggested that a great number of free-living ciliates teem on the skin of debilited, moribund fish which lacking any defence reaction under adverse environmental conditions. The ciliates prey on the body surface of the fishes and feed on the tissues. The stalk coiling is produced by the contraction of myonems that resides in a helical form.

Ambiphrya ameiuri

Members of the genus *Ambiphrya* are characterized by a cylindrical and permanent equatorial ciliary fringe and macronucleus is in the shape of a long, thin and sinuous ribbon. Within this genus there exists two closely related species, *A. ameiuri* and *A. neobolae*. However, according to (Vijoen and Van As ,1985), *A. neobolae* have a deep constriction above scopula and the macronucleus ribbon shaped extends throughout the body. On the other hand, *A. ameiuri*, according to (Lom and Dykova 1992), has a ribbon-like macronucleus forming an orally situated horseshoe, the tips of which descend into the basal part with no deep constriction. Accordingly, *Ambiphrya* recorded in the present study identified as *A. ameiuri*. *A. ameiuri* described by (Thompson et al., 1947) for the first time from the gills of *Ameiurus melas melas* in North America then introduced in Russia and then in Europe and lastly the present study in Africa. This is the first recorded parasite from *S. galilaeus* gills.

Pathogenicity

All the previously mentioned sessilines utilize gills and skin merely as a substrate for attachment with their scopula. The scopula adheres directly to the substrate often being cemented to it with a thin layer of sticky substance. Heavy infection of these parasites can cause ulcers and may cause the fish to be more vulnerable to bacterial infections and lead to "red sore disease" Durborow (2003).

Amphileptus sp.

According to (Shulman, 1962; Lom and Dykova, 1992), members of this genus have lancet-like bodies bearing longitudinal arched ciliary rows on one side only and two oval macronuclei and single micronucleus. The present amphileptid showed some resemblance to *A. branchiarum* in the shape of the body and nuclear apparatus. However, *A. branchiaum* has large dimensions (56-120X35-70) µm and a larger number of kineties (20-25). The present *Amphileptus* sp. showed close resemblance to *A. piger* described by (Sonntag and Foissner, 2004) where the latter has body dimensions (55X13) µm and a single contractile vacuole. Due to the scarce literature about *Amphileptus* and the above mentioned differences, it was found to allocate the present parasite under the generic name only.

Chilodonella hexasticha

Shulman (1966) reported that all the members of genus *Chilodonella* are mostly free-living and two serious pathogenic species infecting freshwater fish. Until the 1970 the two parasitic *Chilodonella* species were usually confused, and it was mostly only *C. piscicola* that was recorded. (Kazubski and Migala, 1974) had redescribed the *Chilodonella* species and confirmed the occurrence of the two parasitic species on fish, based on a morphological analysis of the two ciliates. According to (Lom and Dyková, 1992), *C. hexasticha* rarly speices differs from *C. piscicola* the most dominant species in that it lacks a notch at the posterior body margin, has less numerous and more loosely arranged spaced kineties and smaller body size. The present species satisfy the characters mentioned above, and it is identified as *C. hexasticha*. *S. galilaeus* is recorded as a new host for *C. hexasticha.* The present result agrees with that of (Paperna, 1980; Lom and Dykova, 1992; Ahmed et al., 2000).

Pathogenicity

Since these ciliates are morphologically well adapted to adhesion to body surface and gills of fishes and have a rigid projected cytostomal opening, they will be obligate parasites of fishes and directly injure the fishes by boring and disrupting the epithelial cells. (Paperna and Van As, 1983) reported that the parasitium with *C. hexasticha* produced severe gill damage in the form of epithelial hyperplasia, which shrouded the fine respiratory epithelium and led to the death of the fish. Langdon et al. (1985) reported that heavy *C. hexasticha* infestation causes mass mortality among farmed and wild fish, the cases of death involving gill damage and fusion of adjacent filaments.

Tetrahymena corlissi

According to Jerome et al. (1996), the ciliates of genus Tetrahymena comprise at least 33 species. Most of these ciliates are free living, few are infecting various invertebrates. However, in literature we find tetrahymenids infesting fishes is very scarce. Based on the form of the body and the number of kineties, (Lom and Dykova, 1992) identified three species of genus *Tetrahumena* infected freshwater fishes; *T. pyriformis* has 17-21 kineties, while *T. corrlissi* has 25-31 kineties and *T. rostrata*, has 32-35 kineties. The present tetrahymenid is identified as *T. corrlissi*

Trypanosoma mansouri

Polymorphic trypanosome with three forms (small, intermediate and large) and there is no doubt that these forms belong to one species. The three forms resemble each other in general characters, such as position of the nucleus, structure and staining reaction of the cytoplasm and presence of free flagellum. (Tandon and Joshi, 1973) mentioned polymorphism in *T. maguri* from the blood of *Clarias batrachus* from India. Qadri (1962) reported dimorphism of *T. batrachi* from the blood of the same host *C. batrachus*. In Egypt, Mohamed (1978) described *T. mansouri* from the blood of *Chrysichthys auratus* and *Ch. reuppelli* as a polymorphic trypanosome. Comparing the general features of the present trypanosome with those of other previously described from freshwater fishes, it appears that these features resemble those of *T. mansouri* which was originally described by Mohamed (1978) from *Chrysichthys auratus* and *Ch. reuppelli*. The present trypanosome has greater body measurements where, the body length ranges from 57.2-64.9 µm while in *T. mansouri* Mohamed (1978), it ranges from 34.7-50.8 µm.

T. cyanophilum

Monomorphic trypanosome is characterized by its deeply blue stained cytoplasm, nucleus lies posteriorly and well festooned undulating membrane. This species originally described by Mohamed (1978) as dimorphic trypanosome from *Chrysichthys auratus* and *Ch. reuppelli*. Abu El-Wafa (1988) identified this species as *T. tilapiae* from different species of fishes. Later Negm El-Din (1991) synonymized this species with *T. cyanophilum*. The present investigated trypanosoma was in accordance with trypanosome described by Ahmed et al. (2000).

Trypanosoma sp.

Monotrophic parasite, characterized by stout body, nucleus is situated interiorly and presence of a distinct vacuole in front of the kinetoplast. Comparing the morphological description and morphometric data of this species with *T. mansouri* and T. *syanophilum* described in the present study from the blood of the same host *T. zillii* showed significant differences. Therefore the present parasite is tentatively identified as *Trypanosoma* sp.

Pathogenicity

The pathogenic potential of fishes trypanosomes depends on the intensity of infection. The heavy infection induces series of changes as anemia that induced by hemolysins secreted by live trypanosomes which lyse the RBCs and lead to mortality (Lom and Dykova ,1992).

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Effects of irreversible different parameters on performance of air standard dual-cycle

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Abstract: An irreversible *air standard dual cycle* model is proposed in this paper. The performance of an *air-standard dual cycle* with heat transfer loss and variable specific heats of working fluid is analyzed by using *finite-time thermodynamics*. The objective of this study is to analyze the effects of heat loss characterized by a percentage of the fuel's energy, *friction* and variable specific heats of working fluid on the performance of an *air standard dual cycle* with a restriction of maximum cycle temperature. The relations between the *power output* and the compression ratio, and between the thermal efficiency and the compression ratio of the cycle are derived. Moreover, the effects of heat transfer and global losses lumped in a *friction* like term on the performance of the cycle are shown by detailed numerical examples. [Journal of American Science 2011;7(3):608-613]. (ISSN: 1545-1003).

Keywords: *Air Standard, Finite-time thermodynamics, Dual cycle, Power Output, friction.*

1. Introduction

A series of achievements have been made since finite-time thermodynamics was used to analyze and optimize real heat engines. Several author shave applied finite-time thermodynamics (Andresen and Bejan and Chen et al.) to the analysis and optimization of the internal-combustion engine. Mozurkewich and Berry and Hoffman et al. used mathematical techniques from optimal-control theory to determine the optimal motion of the piston in Otto and Diesel engines. Aizenbud and Band and Chen et al. determined the optimal motion of a piston, fitted to a cylinder containing a gas, pumped with a given heating rate and coupled to a heat bath for a finite time. The heat addition process for an air standard cycle has been widely described as subtraction of an arbitrary heat loss parameter times the average temperature of the heat addition period from the fuel's chemical energy. Orlov and Berry obtained the power and efficiency limits for internal-combustion engines. Angulo-Brown et al. and Chen et al. optimized the powers of the Otto and Diesel engines with friction loss during finite times. Klein studied the effect of heat transfer through a cylinder wall on the work output of the Otto and Diesel cycles. Chen et al. derived the relations between net power output and the efficiency for both the Diesel and Otto cycles, with considerations of heat transfer through the cylinder wall. Blank and Wu and Lin et al. considered the effect of heat transfer through a cylinder wall on the work output of the dual cycle. On the basis of these investigations, the relations governing the net power output and the efficiency for the dual cycle with considerations of heat transfer loss and friction-like terms loss during a finite time

are derived in this paper. heat transfer between the working fluid and the environment through the cylinder wall is considered and characterized by a percentage of the fuel's energy; friction loss of the piston in all the processes of the cycle on the performance is taken into account. Furthermore, we consider the variable specific heats of the working fluid that is significant in practical cycle analysis. The results obtained in the study may offer good guidance for design and operation of the dual cycle engine.

2. Thermodynamic analysis

An ideal air-standard dual cycle is shown in Fig. 1. The compression process ignition is isentropic $1 \rightarrow 2$; the combustion is modelled by a reversible constant volume process $2 \rightarrow 3$ and a constant-pressure process $3 \rightarrow 4$; the expansion process $4 \rightarrow 5$ is isentropic; and the heat rejection is a reversible constant-volume process $5 \rightarrow 1$. As is usual in FTT-heat engine models, we suppose instantaneous adiabats. For the isochoric branches ($2 \rightarrow 3$ and $5 \rightarrow 1$) and the isobaric branch ($3 \rightarrow 4$) in Fig. 1, we propose that heating from states 2 to 4 and cooling from states 5 to 1 proceed according to constant temperatures; i.e.

$$\frac{dT}{dt} = \frac{1}{K_1} \quad (for \quad 2 \rightarrow 3, \ 3 \rightarrow 4)$$

$$\frac{dT}{dt} = \frac{1}{K_2} \quad (for \quad 5 \rightarrow 1) , \tag{1}$$
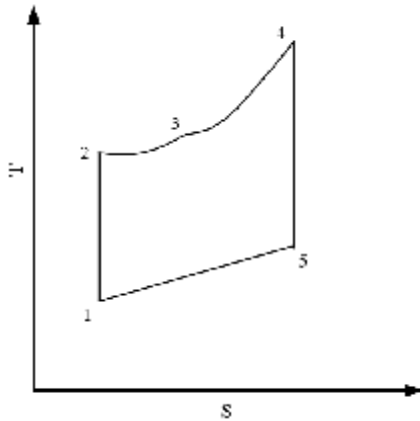
Figure 1. T–s diagram for a dual cycle

where T is the absolute temperature and t is the time. k_1 and k_2 are constants. Integrating Eq. (1) gives

$$t_1 = K_1(T_3 - T_2) \; , \; t_2 = K_2(T_4 - T_1) \; , \qquad (2)$$

where t_1 and t_2 are the heating and cooling times, respectively. Then, the cycle period is

$$t = t_1 + t_2 = K_1(T_3 - T_2) + K_2(T_4 - T_1). \qquad (3)$$

The work output is

$$W = C_v(T_3 - T_2) + C_p(T_4 - T_3) \\ - C_v(T_5 - T_1), \qquad (4)$$

where C_v is the heat capacity at constant volume, and C_p is the heat capacity at constant pressure, i.e., the product of mass flow rate and the specific heat. Thus, the power output is

$$P_1 = \frac{W}{t} \\ = \frac{C_v(T_3 - T_2) + C_p(T_4 - T_3) - C_v(T_5 - T_1)}{K_1(T_4 - T_2) + K_2(T_5 - T_1)}. \qquad (5)$$

Defining the compression ratio, g, the pressure ratio, g_p and the cut-off ratio, g_c as follows:

$$g = V_1/V_2 = (T_2/T_1)^{1/(k-1)} \; , \qquad (6)$$

$$g_p = P_3/P_2 = T_3/T_2 \; , \qquad (7)$$

and

$$g_c = V_4/V_3 = T_4/T_3 \; , \qquad (8)$$

we have

$$T_2 = T_4/(g_p g_c) \; , \qquad (9)$$

$$T_3 = T_4/g_c \; , \qquad (10)$$

$$T_3 = T_4 g_p g_c{}^k \; , \qquad (11)$$

$$g = [T_4/(T_1 g_p g_c)]^{1/(k-1)}. \qquad (12)$$

Substituting Eqs. (6)–(11) into Eq. (5) yields

$$P_1 = \frac{W}{t} \\ = \{C_v \{T_1[1 - g^{k-1} - (k-1)g_p g^{k-1}] \\ + kT_4 - T_1^{1-k} T_4^{k} g_p^{1-k} g^{k(1-k)}\}\} \\ / \{K_1(T_4 - T_1 g^{k-1}) \\ + K_2[T_1^{1-k} T_4^{k} g^{k(1-k)} - T_1]\} \qquad (13)$$

where k is the ratio (C_p/C_v) of specific heats. In special cases, the dual cycle becomes the Diesel cycle or the Otto cycle, i.e. when $g_p = 1$, $g_c = 1$.
The total heat added to the working-fluid during processes $2 \rightarrow 3$ and $3 \rightarrow 4$ is

$$Q_A = Q_{23} + Q_{34} \\ = C_v(T_3 - T_2) + C_p(T_4 - T_3) \\ = C_v[kT_4 + (1 - k - g_p^{-1})g_p g^{k-1} T_1] \qquad (14)$$

For an ideal dual cycle, there are no irreversible losses.

$$Q_A = a - b(T_2 + T_4) \\ = a - b(T_1 g^{k-1} + T_4), \qquad (15)$$

where a and b are two constants related to the combustion and heat transfer.
Combining Eqs. (14) and (15) yields

$$T_4 = \{a - [bg^{k-1} \\ + C_v(1 - k - g_p^{-1})g_p g^{k-1}]T_1\}/(C_p + b). \qquad (16)$$

Substituting Eq. (16) into Eqs. (13) and (14) yields

$$P_1 = \{C_v \{T_1[1 - g^{k-1} - (k-1)g_p g^{k-1}]$$
$$+ [k/(C_p + b)]\{a - [b$$
$$+ C_v (1 - k - g_p^{-1})g_p]g^{k-1}T_1\}\}$$
$$- C_v \{T_1^{1-k} g_p^{1-k} g^{k(1-k)}\{\{a - [b$$
$$+ C_v (1 - k - g_p^{-1})g_p]g^{k-1}T_1\}/(C_p + b)\}^k\}\}$$
$$/\{K_1\{\{a - [bg^{k-1} \qquad\qquad (17)$$
$$+ C_v (1 - k - g_p^{-1})g_p g^{k-1}T_1\}$$
$$/(C_p + b) - T_1 g^{k-1}\}$$
$$+ K_2 \{T_1^{1-k} g^{1-k} g^{k(1-k)}\{\{a - [bg^{k-1}$$
$$+ C_v (1 - k - g_p^{-1})g_p g^{k-1}T_1\}$$
$$/(C_p + b) - T_1 g^{k-1}\}^k - T_1\}\},$$

$$Q_A = C_v \{ak + b[(1 - k - g_p^{-1})g_p - k]g^{k-1}T_1\} \qquad (18)$$
$$/(C_p + b).$$

$$f_m = -m\nu = -m\frac{dx}{dt}, \qquad\qquad (19)$$

where m is a coefficient of friction which takes into account the global losses and x is the piston displacement. Then, the lost power is

$$P_m = \frac{dW_m}{dt} = -m\frac{dx}{dt}\frac{dx}{dt} = -m\nu^2. \qquad (20)$$

If we take the piston mean velocity $\bar{\nu}$ as ν,

$$\bar{\nu} = \frac{x_1 - x_2}{\Delta t_{12}} = \frac{x_2(g-1)}{\Delta t_{12}}, \qquad\qquad (21)$$

where x_2 is the piston position at minimum volume and Δt_{12} is the time spent in the power stroke. Thus, the resulting power output is

$$P_1 = \{C_v \{T_1[1 - g^{k-1} - (k-1)g_p g^{k-1}]$$
$$+ [k/(C_p + b)]\{a - [b + C_v$$
$$\times (1 - k - g_p^{-1})g_p]g^{k-1}T_1\}\}$$
$$- C_v \{T_1^{1-k} g_p^{1-k} g^{k(1-k)}\{\{a - [b + C_v$$
$$\times (1 - k - g_p^{-1})g_p]g^{k-1}T_1\}/(C_p + b)\}^k\}\} \qquad (22)$$
$$/\{K_1\{\{a - [bg^{k-1} + C_v$$
$$\times (1 - k - g_p^{-1})g_p g^{k-1}T_1\}/(C_p + b) - T_1 g^{k-1}\}$$
$$+ K_2 \{T_1^{1-k} g^{1-k} g^{k(1-k)}\{\{a - [bg^{k-1} + C_v$$
$$\times (1 - k - g_p^{-1})g_p g^{k-1}T_1\}$$
$$/(C_p + b) - T_1 g^{k-1}\}^k - T_1\}\} - b(g-1)^2,$$

where

$$b = \frac{mx_2^2}{(\Delta t_{12})^2} \cdot \frac{1}{2} \qquad\qquad (23)$$

The thermal efficiency of the cycle is

$$h = \frac{P}{Q_A} = \{C_v \{T_1[1 - g^{k-1} - (k-1)g_p g^{k-1}]$$
$$+ [k/(C_p + b)]\{a - [b + C_v$$
$$\times (1 - k - g_p^{-1})g_p]g^{k-1}T_1\}\}$$
$$- C_v \{T_1^{1-k} g_p^{1-k} g^{k(1-k)}\{\{a - [b + C_v$$
$$\times (1 - k - g_p^{-1})g_p]g^{k-1}T_1\}/(C_p + b)\}^k\}\}$$
$$/\{C_p (a - 2bg^{k-1}T_1)/(C_p + b)\} \qquad (24)$$
$$- b(g-1)^2\{K_1\{\{a - [bg^{k-1} + C_v$$
$$\times (1 - k - g_p^{-1})g_p g^{k-1}T_1\}/(C_p + b) - T_1 g^{k-1}\}$$
$$+ K_2 \{T_1^{1-k} g^{1-k} g^{k(1-k)}\{\{a - [bg^{k-1} + C_v$$
$$\times (1 - k - g_p^{-1})g_p g^{k-1}T_1\}$$
$$/(C_p + b) - T_1 g^{k-1}\}^k - T_1\}\}$$
$$/\{C_p (a - 2bg^{k-1}T_1)/(C_p + b)\}.$$

Eqs. (22) and (24) are the major results of this paper. They determine the relations between the power output P, efficiency h, compression ratio g, and the pressure ratio g_p. The relation between power output and efficiency may be obtained using numerical calculations.

Two limiting cases are manifested from Eqs. (22) and (24). The first case, when $g_p = 1$, describes the relationship of the power and efficiency of the Diesel cycle

$$P_D = \{C_v \{T_1(1 - k\,g^{k-1}) + [k\,/(C_p + b)][a$$
$$+ (C_p - b)g^{k-1}T_1]\} - C_v \{T_1^{1-k} g^{k(1-k)}\{[a$$
$$+ (C_p - b)g^{k-1}T_1]/(C_p + b)\}^k\}\}$$
$$/\{K_1\{[a + (C_p - b)g^{k-1}T_1]/(C_p + b) \qquad (25)$$
$$- T_1 g^{k-1}\} + K_2\{T_1^{1-k} g^{k(1-k)}\{[a +$$
$$(C_p - b)g^{k-1}T_1]/(C_p + b)$$
$$- T_1 g^{k-1}\}^k - T_1\}\} - b(g-1)^2,$$

$$h_D = \frac{P_D}{\dot{Q}_{DA}}$$
$$= \{C_v \{T_1(1 - k\,g^{k-1})$$
$$+ [k\,/(C_p + b)][a + (C_p - b)$$
$$\times g^{k-1}T_1] - T_1^{1-k} g^{k(1-k)}\{[a$$
$$+ (C_p - b)g^{k-1}T_1]/(C_p + b)\}^k\}\}$$
$$/\{C_p(a - 2bg^{k-1}T_1)/(C_p + b)\} \qquad (26)$$
$$- \{b(g-1)^2\{K_1\{[a + (C_p - b)$$
$$\times g^{k-1}T_1]/(C_p + b) - T_1 g^{k-1}\}$$
$$+ K_2\{T_1^{1-k} g^{k(1-k)}\{[a + (C_p - b)$$
$$\times g^{k-1}T_1]/(C_p + b) - T_1 g^{k-1}\}^k - T_1\}\}\}$$
$$/\{C_p(a - 2bg^{k-1}T_1)/(C_p + b)\}$$

when $b = 0$, the result is the same as that of Chen et al.

The second case, when $g_c = 1$, describes the relationship of the power and efficiency of the Otto cycle

$$P_o = \{\{C_v \{T_1(1 - g^{k-1})$$
$$+ [1/(C_v + b)][a + (C_v - b)g^{k-1}T_1]$$
$$\times (1 - g^{k-1})\}\}/\{(K_1 + K_2 g^{1-k}) \qquad (27)$$
$$\times \{[a + (C_v - b)g^{k-1}T_1]$$
$$/(C_v + b) - g^{k-1}T_1\}\}\} - b(g-1)^2,$$

$$h_o = \frac{P_o}{\dot{Q}_{OA}} = C_v \{T_1(1 - g^{k-1})$$
$$+ [1/(C_v + b)][a + (C_v - b)$$
$$\times g^{k-1}T_1](1 - g^{k-1})\} - b(g-1)^2$$
$$\times (K_1 + K_2 g^{1-k})\{[a + (C_v - b) \qquad (28)$$
$$\times g^{k-1}T_1]/(C_v + b) - g^{k-1}T_1\}\}$$
$$/\{C_v \{[a + (C_v - b)g^{k-1}T_1]$$
$$/(C_v + b) - g^{k-1}T_1\}\},$$

$$g_p = (g_p)_o = T_4 /(T_1 g^{k-1})$$
$$= [a + (C_v - b)g^{k-1}T_1] \qquad (29)$$
$$/[(C_v + b)g^{k-1}T_1],$$

Where $(g_p)_o$ is the pressure ratio for the Otto cycle.

3. Numerical examples

To illustrate the preceding analysis, we have $t = 33.33 ms$, $C_v = 0.7165 kJ/K$ and $C_p = 1.0031 kj/K$. Taking equal heating and cooling times $(t_1 = t_2 = t/2 = 16.6 ms)$, the constant temperature rates K_1 and K_2 are estimated as $K_1 = 8.128 \times 10^{-6} s/K$ and $K_2 = 18.67 \times 10^{-6} s/K$. We consider $m = 7s$ with $s = 12.9 kg/s$ and s is the friction coefficient of the exhaust and compression strokes. Taking the same values of x_2 and Δt_{12} as in Mozurkewich's paper and following the same procedure to determine the losses for the full cycle, we obtain $b = 32.5 W$. The maximum cycle-temperature ratio is $t = 2742/329 = 8.33$. The typical parameter ranges of the four parameters are $g_p = 1 - (g_p)_o$, $a = 2500 - 4000 kJ/kg$, $b = 0.3 - 1.8 kJ/[(kg)K]$ and $T_1 = 300 - 400 K$.

The effects of g_p on the power output vs. efficiency curve for $a = 3500 kJ/kg$, $b = 1 kJ/[(kg)K]$ and $T_1 = 350 K$ are shown in Fig. 2. The $g_p = 1$ characteristic curve corresponds to the Diesel-cycle performance. The $g_p = g_{po}$ characteristic curve corresponds to the Otto-cycle performance. There is a maximum-power output point. When $g_p = g_m = 16.512$ and $g_p = 1.2$, the power output approaches its maximum value at $P_{max} = 29.0214 kW$ and the corresponding efficiency is $h_p = 0.5174$. The effect of b on the

power output vs. efficiency curve for $a = 3500\ kJ/kg$, $T_1 = 350\ K$, $b = 32.5\ W$ and $g_p = 1.2$ is shown in Fig. 3. The effect of a on the power output vs. efficiency curve for $b = 1\ kJ/[(kg)K]$, $b = 32.5\ W$, $T_1 = 350\ K$ and $g_p = 1.2$ is shown in Fig. 4. The effect of T_1 on the power output vs. efficiency curve for $a = 3500\ kJ/kg$, $b = 1\ kJ/[(kg)K]$, $b = 32.5\ W$ and $g_p = 1.2$ is shown in Fig. 5. The effects of b on the power output vs. efficiency curve for $a = 3500\ kJ/kg$, $b = 1\ kJ/[(kg)K]$, $T_1 = 350\ K$ and $g_p = 1.2$ are shown in Figs. 6.
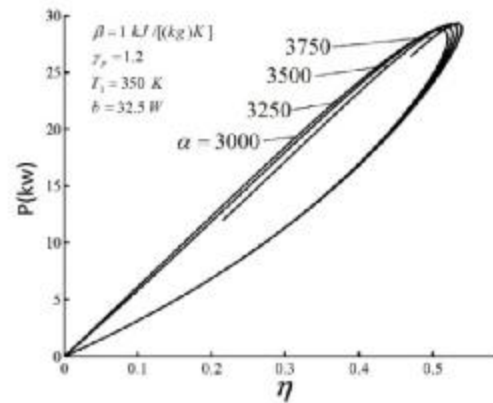

Figure 4. Effect of a on the $P - h$ characteristic.
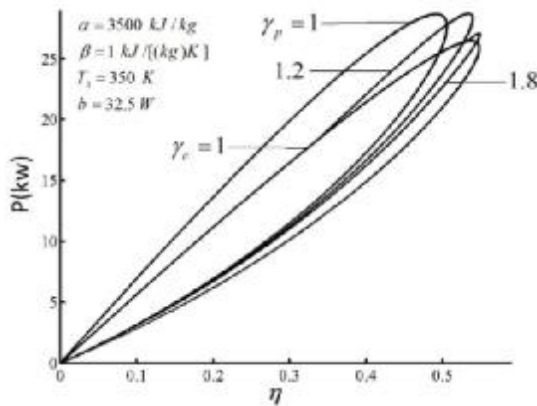

Figure 2. Effect of g_p on the $P - h$ characteristic.
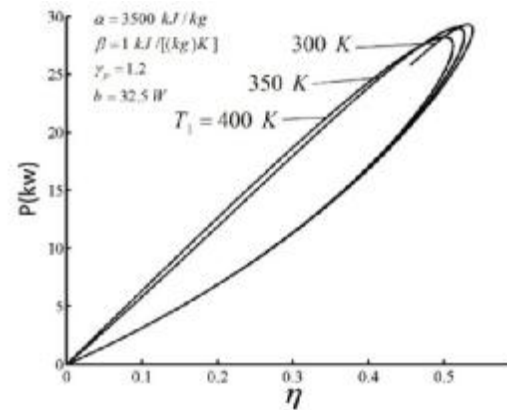
Figure 5. Effect of T_1 on the $P - h$ characteristic.
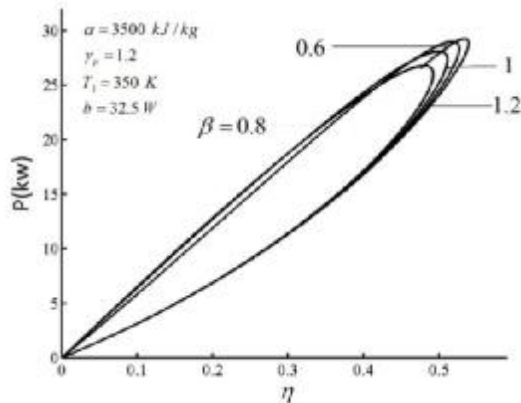
Figure 3. Effect of b on the $P - h$ characteristic.

Figure 6. Effect of b on the $P - h$ characteristic.

4. Conclusions

In this paper, the effects of cylinder wall heat-transfer and global losses lumped in a friction-like term on the performance of a dual cycle during a finite time are investigated. The relations between net power output, efficiency, compression-ratio, and the pressure ratio are derived. The maximum power

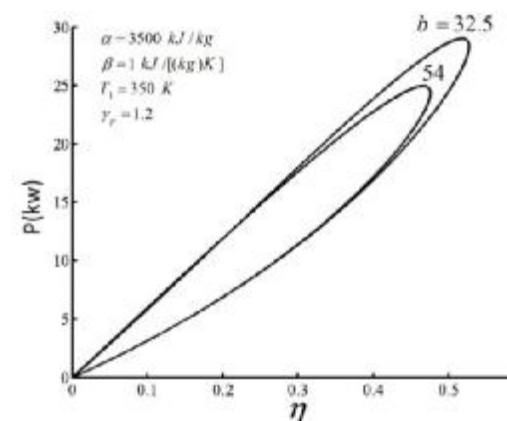output and the corresponding efficiency and the maximum efficiency and the corresponding power output are also calculated. The results can also be applied to the performance analysis of the Diesel and Otto cycles. The detailed effect analyses are shown by numerical examples. The results can provide significant guidance for the performance evaluation and improvement of real dual engines.

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Undergraduate Male Nursing students' Perception about the Image of the Nursing Profession

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Abstract: Gender and sex role stereotyping are recognized as having the potential to limit the professional development of males within the nursing profession. Male nurses have been a minority group within the nursing profession; nevertheless, the nursing community nowadays aims to increase the number of male nursing students and practicing male nurses lately. This study aimed to determine the undergraduate male nursing students' perception about the image of the nursing profession.   The study subjects included 370 male nursing students who were enrolled in the four academic years and internship year within the academic 2008-2009. *Nursing Image –as a profession questionnaire (NIPQ)* was used for data collection. It was developed by the researcher after thorough review of literature. The current study revealed that (62.16%) of the subjects had positive image toward nursing as a profession. The subjects' perceived nursing as women's` profession were 37.8%, while the majority (91.4%) of them agreed that both males and females can be a good nurse. There was significance difference between information received about nursing before joining the faculty of nursing and nursing image .It is concluded that the highest percentage of the male students had a positive image towards nursing as a profession. It was recommended to conduct a longitudinal study to examine male nursing students regarding nursing image.

1. Introduction:

Despite the great advances in the nursing profession, nurses still face considerable challenges related to its image that impact on status, power and the ability to affect changes in health care. Where, a negative image of nursing has a number of negative consequences, it has impacts on the quality and quantity of persons who choose nursing as a profession. The public who are constantly presented with an inaccurate and negative nursing image will view a career in nursing as undesirable. This is especially relevant to the recruitment of men in the field where media portrayals have focused primarily on women while the image of a male nurse is often negative (Kalisch, 2006).

Gender and sex role stereotyping are recognized as having the potential to limit the professional development of males within the nursing profession. Male nurses have been a minority group within the nursing profession; nevertheless, the nursing community nowadays aims to increase the number of male nursing students and practicing male nurses lately. History appears to indicate that men had a place in nursing for as long as records are available, but their contribution has been perceived as negligible, largely because of the dominant influence that the 19th century female nursing movement has had on the occupation's historical ideology (Keogh and O'Lynn, 2007).

Men in nursing face many barriers and obstacles not only from the general public but also from their patients and colleagues (Stott, 2004; Furlong, 2005).

(May, 1991) stated that every feeling a person has about him/herself as a professional will affect his/her way of thought and action in professional relations. Therefore nurses, who have positive professional images, will have more and stronger remedial relations with their patients. (Emiroglu, 2000) found that both doctors and the public have a negative image about nursing as a profession. Doctors see nurses as their handmaiden and therefore negative image of nursing continues to be a problem. Nurses are professionals who are science driven, technically skilled, and caring. The image of nursing has changed and developed throughout the history and the outward image of nursing has changed remarkably within the past 50 years.

Furthermore, (Spouse, 2000) reported that the understanding of what the nurses' image is of their profession and at the same time the fact that they are interacting to get relevant knowledge about their profession, will enable the executors and nursing politicians to enhance nursing preparing activities and on the other hand, nursing students' psychological needs will be recognized and worked on. Also, nursing image continues to progress, however the role of nurse is often defined in relation to physician and is still seen as a handmaiden with nurses often

seen as dependent decision makers, whose only job is to carry out the orders of physicians (Weintraub, 2003).

The image of nursing as a profession is affected by numerous factors and accordingly affecting the choice of nursing as a career. These factors include: media, public image, social prestige, nurses themselves, having a family member, relative or a friend who is a nurse, role models, physician-nurse interaction, nursing educators, nurse preceptors, risk of violence, exposure to health hazards and nursing education programs (Johnson *et al*.,1999)

It may appear that the image of nursing as a profession in the Egyptian community was not improved properly even after the nurse has been qualified a university level. Therefore, for recruiting and retention of nurses, there is a need to promote nursing professional image to enhance its standing in the community especially for males. This study aimed to determine the undergraduate male nursing students' perception about the image of the nursing profession.

2. Material and Methods:
Research design
This is an exploratory descriptive study.

Setting
The study was conducted at the following settings:

The four classes of the four academic years at the Faculty of Nursing, Alexandria University, Egypt.

Five Alexandria University Hospitals, where male nursing students spend their internship year namely Main University Hospital, affiliated to Alexandria University, El-Shatby Maternity Hospital, affiliated to Alexandria University, El-Shatby Pediatric Hospital affiliated to Alexandria University, El-Hadra Orthopedic and Traumatology Hospital, affiliated to Alexandria University, Gamal Abd-El-Naser Hospital ,affiliated to Ministry of Health and Population.

Subjects
The study subjects included 370 male nursing students who were enrolled in the four academic years and internship year within the academic year 2008-2009.

Tool for data collection
Nursing Image –as a profession questionnaire (NIPQ) was used for data collection. It was developed by the researcher after thorough review of literature. It comprised two parts:
Part I:
This part included questions related to Socio-demographic characteristics of the study subjects

such as age, residence, academic year and previous educational experience of the students.

Twelve statements to explore the subject's information about nursing: source of information, factors influencing their image of nursing as a profession (Spouse, 2000; Mooney and Glacken, 2008; Hallett, 2007).
Part II:
It was developed by the researcher and used to explore nursing image among undergraduate male nursing students (Gamel, 2006; Cowen, 2006). It is a Likert scale format that contains 36 statements answered on a 4 point scale of response: strongly agree= 4, agree=3, disagree=2, strongly disagree=1. The total score ranged from 36 to 144.
36-72 indicated negative image.
73-144 indicated positive image.

Methods
The study was executed according to the following steps:

Official Permission to conduct this study was obtained from the dean of the faculty of Nursing, Alexandria University, and heads of each nursing department. The tool used in this study was developed by the researcher after extensive reviewing the relevant and current literature.

Content validity of the tool was tested by a jury of seven experts in the related field and necessary modifications were done. Reliability of the tool was tested using Cronbach Alpha Coefficient test, its value was.87. It was carried out on thirty male nursing students selected randomly from the different academic years and internship year, six students from each academic year.

The purpose of the study was explained to each student and oral consent to participate in the study was obtained from him. A pilot study was carried out on 30 male nursing students selected randomly from the previously mentioned setting in order to test the relevance and applicability of the study tool.

Data was collected through questionnaires that were distributed among the subjects. The data was collected for a period of 2 months started in the beginning of June 2009 and extended to the last of July2009.

Statistical analysis
Data was fed, coded, edited and analyzing using PC with statistical packages for social science (SPSS) version 10.0 for windows. The selected level of significance was P   0.05.Descriptive statistics were done using numbers, percentage, arithmetic mean and standard deviation. Analytical statistics were done using significance test (Mont Carlo Exact

Test), association between categorical variables was tested using Chi-square test.

3. Results:

Table (1) shows nearly two-thirds (67%) of them were in their early twenties. About one-third (29.7%) of them were at third academic year, while 23% were at second academic year. Less than one-fifth (13.5%) of the subjects were at the internship year. The majority of the subjects (81.4%) had a secondary school certificate.

According to data in table (2) less than one-third of the study subjects did join the Faculty of Nursing according to their families' advice (31.3%). Yet, more than one-half (57%) of them did so for a chance to work in other countries. Both the desire to help others and availability of work before graduation were the reasons for 24.4 % and 25.9% of them, respectively. More than one-half (55.9%) of the subjects' family reaction was positive.

Table (3) reveals that, one-half (50.5%) of the study subjects had information about nursing. The main source of such information was family or friends (80.8%). Moreover, 26.7% of them got such information from media. Only 9.1% of them got information from schools. A vast majority (80.7%) of the subjects were influenced by such information. Such influence was positive among 72.8% of them.

Table (4) reveals that the majority (81.9%) of the study subjects perceived nursing as a respectful profession. While, more than one-half (57%) of them perceived nursing as a prestigious profession. Only 28.1% of them perceived nursing as similar to servants' job. Almost equal proportions (47.3 %and 52.7%) of them perceived nursing as independent or dependent profession, respectively. 37.8%, the subjects' perceived nursing as women's` profession. The majority (91.1% and 88.3%) of them stated that nursing is an indispensable profession in any society or nurses are important members in the health team, respectively.

With respect relationship with patients and physician, 41.4% of them stated that physicians preferred to work with male nurses. Only 32.5% of the subjects stated that patients preferred male nurses. The majority (88.4%) of them perceived nursing profession as a hard profession that does not enough appreciation

According to data in table (5) the study subjects ranked the social status of the nursing profession in the society as sixth compared to the profession of medicine which ranked the first. Regarding feeling about nursing 40% of them were defensive about nursing. Almost equal proportions (23.5% and 23.8%) of them were either proud or shy, respectively. Only 2.4% of them attacked the profession.

Table (1): Percent distribution of the study subjects according to their general characteristics

General characteristics	No N=370	%
Age:		
-15years-	117	31.6
-20years-	248	67.0
-25 years or more	5	1.4
Residence:		
-Rural	203	54.9
-Urban	167	45.1
Academic year:		
-First year	64	17.3
-Second year	85	23.0
-Third year	110	29.7
-Fourth year	61	16.5
-Internship year	50	13.5
Academic achievement in previous semester:		
-*Excellent	67	18
-**Very good	144	39
-***Good	91	24.6
-****Satisfactory	59	16
-*****Weak	9	2.4
Pre-university qualifications:		
-Secondary school certificate	301	81.4
-Technical health institution or technical institution	56	15.1
-Faculty of science	13	3.5

-* (A, A-, B+)   -** (B, B-   -*** (C+)
-****(C, C-)   -***** (D)

Table (6) reveals that more than one- half (62.16%) of the subjects had positive image toward nursing as a profession, while (37.84 %) of their image was negative.

Table (7) shows that 54.9% of the subjects` image was improved after enrollment. The image of 35.4% of them got worse. Only 9.7% had no change. According to subjects` image improvement, an equal percent (36.95 %) of the subjects` reasons either relationship with faculty members either relationship with medical staff. In relation to those their image got worse, (69.46%) of subjects got such change related to public view about nursing. Approximately equal proportions (49.61% and 48.85%) of their reasons for such change were working condition or clinical training, respectively.

According to table (8) there was significant difference between age and nursing image as a profession where P= (.018) and pair- wise comparison shows positive correlation between age and image.

Table (9) reveals that there were significance difference between information received about nursing before joining the faculty of nursing and nursing image where P= 0.000 there was a strong correlation. Also there was significance difference between family's` reaction and nursing image.

Table (2):Distribution of the study subjects according to reasons for enrollment to the Faculty of Nursing and family reaction

Reasons*:	No= 370	%
-Secondary school grade	171	46.2
-Desire to help other	94	25.4
-Advice from family members	115	31.1
-Financial reasons	124	33.5
-Availability of national work	171	46.2
-Availability of work in other countries	211	57.0
-Availability of work during undergraduate	96	25.9
Family's reaction to students' enrollment:		
-Positive	207	55.9
-Negative	37	10.0
-Neutral	126	34.1

* More than one answer.

Table (3):   Distribution of the study subjects according to their information about nursing and source of such information

Presence of information about nursing before joining the faculty of nursing:	No 370	%
-Yes	187	50.5
-No	183	49.5
-Source of information*:	**N=187**	**%**
-Family and friends	151	80.8
-School	17	9.1
-Health setting	30	16.0
-Media	50	26.7
Effect of such information on nursing` image:	N=187	%
-Yes	151	80.7
-No	36	19.3
Type of effect:	**N=151**	%
-Negative	41	27.2
-Positive	110	72.8

* More than one answer

Table (4):Distribution of the study subjects according to their perceptions of nursing as a profession

Nursing profession is:	Agree		disagree	
	No	%	No	%
-A respectful profession	303	81.9	67	25.7
-An occupation and not a profession	168	45.5	202	54.6
-A Women's profession	140	37.8	230	62.1
-Similar to that of the servants' job.	104	28.1	266	71.9
-A well appreciated profession in the society	109	*27*	*261*	*73*
-A prestigious profession	*211*	*57*	*159*	*43*
-A dangerous profession	*321*	*86.7*	*49*	*13.3*
-A hard profession that does not receive enough appreciation	*327*	*88.4*	*43*	*11.6*
-An indispensable profession in any society	*337*	*91.1*	*33*	*8.9*
-Nursing is a human profession	333	90	37	10
-An independent profession	175	47.3	195	52.7
-A significant in patient's recovery	315	14.9	55	85.1
-Helping in promotion of health and prevention of diseases	339	91.6	31	8.4
-Provide self actualization	207	56	163	44
Male nurses are:				
-Important members in the health team	327	88.3	43	11.7
-More accepted by patient than female nurses	125	32.5	245	67.5
-More preferred by physicians	153	41.4	217	58.7
-Need much academic preparation	285	77	85	23
-Respected by others as a nurse	173	53.3	197	46.7
-Has autonomy in their jobs	148	40	222	60
-Can be a good nurse as females	338	91.4	32	8.6

Table (5): Distribution of mean and standard deviation of the study subjects according to social rank of job and feeling about nursing as a profession

Students ranking of Professions	Mean ± Std. Deviation	Median (Min.-Max.)
-Physician	2.34 ± 1.828	1.00 (1.00-10.00)
-Pharmacist	4.14 ± 2.183	2.00 (1.00-10.00)
-Officer	4.51 ±2.899	3.00 (1.00-10.00)
-Engineer	4.23 ± 1.773	4.00 (1.00-10.00)
-Teacher	4.92 ± 2.728	5.00 (1.00-10.00)
-Nurse	**5.78 ± 2.906**	**6.00 (1.00-10.00)**
- Journalist	6.84 ± 2.361	7.00 (1.00-10.00)
- Lawyer	7.26 ±1.878	8.00 (1.00-10.00)
- Accountant	6.66 ±1.808	9.00 (1.00-10.00)
-Artist	8.29 ± 2.369	10.00 (1.00-10.00)
Feeling about nursing profession when talking with others*:	**N=370**	**%**
-Proud	87	23.5
-Shy	88	23.8
-Change topic	107	28.9
-Defensive	148	40.0
-Attack nursing profession	20	5.4

* More than one answer

Table (6): Number and percent distribution of the study subjects according to their nursing image as a profession

Score by mean split	N=370	%
-Positive image	230	62.16
-Negative image	140	37.84

Table (7) Distribution of the study subjects according to the change in nursing image after joining Faculty of Nursing

Change in nursing image:	N=370	%
- No	36	9.73
- Yes	334	90.27
-Yes to better*	203	54.9
Factors contributing to the change to better *	**N=203**	**%**
-Working conditions	87	42.86
-Clinical training	77	37.93
-Relationship with faculty members	75	36.94
-Relationship with medical staff	75	36.94
-Relationship with friends	56	27.59
-Family member opinion	32	15.76
-Public view about nursing	27	13.00
-Yes to worst	**N=131**	35.4
Factors contributing to the change to worst *		
-Public view about nursing	91	69.46
-Relationship with faculty members	72	54.96
-Working conditions	65	49.61
-Clinical training	64	48.85
-Relationship with medical staff	55	41.98
-Relationship with friends	25	19.08
-Family member opinion	12	9.16

* More than one- answer

Table (8): Relationship between general characteristics of study subjects and nursing image

General characteristics	Nursing image	Test statistic
	Mean ± SD	(p-value)
Age	Median (Min.-Max.)	
-15-	99.00(55.00-129.00)	MCP
-20-	102.00(59.00-131.00)	0.018*
-25or more	117.00(109.00-117.00)	
Area of residence		MCP
-Rural	100.41 ± 14.70	0.400
-Urban	101.68±14.05	
Academic year		MCP
-First year	99.69±17.63	0.210
-Second year	99.84±14.50	
-Third year	103.52±13.61	
-Fourth year	98.87±13.25	
-Internship year	101.58±12.27	
Academic achievement in previous semester:		
-Excellent and very good	101.08±14.21	
-Good	102.79±12.81	0.143
-Satisfactory to weak	98.25±16.66	
Previous qualifications:	Median (Min.-Max.)	
-Secondary school certificate	101.00(55.00-129.00)	MCP
-Technical health institution or technical institution	102.00(59.00-131.00)	0.335
-Faculty of science	108.00(76.00-126.00)	

MCP= Mote Carlo Significant Test   * Significant (P value 0.05)

Table (9): **Relationship between presence of nurse, knowledge, family reaction and nursing image**

Presence of nurse:	Nursing image Mean ± SD	Test statistic (P-value)
-Yes	99.62±15.31	MCP 0.082
-No	102.23±13.44	
Presence of information about nursing before joining the faculty of nursing:		
-No or yes but did not change my image	100.16±14.39	MCP 0.000*
-Yes and it was negative	93.73±16.80	
-Yes and it was positive	105.31±12.03	
Family's reaction to students' enrollment:		
-Positive	103.93±12.49	MCP 0.000*
-Negative	91.86±16.54	
-Neutral	98.81±15.26	

MCP= Mote Carlo Significant Test   * Significant (P value   0.05)

4. Discussion:

Nursing has been considerably progressing towards professionalism in recent decades and has produced a scientific base by academically preparing programs for it. Now, the time has come when nurses should be aware of the images they draw as professionals for themselves and others.

As the society changes, health care emphasis and lifestyle also change requiring nurses themselves to change (Valizadeh and Ali,2008). Despite this progress, both the profession and mainstream press have given increased attention to the growing nursing shortage. (Gerencher, 2002) reported that if men entered the profession at the same rate as women today, there would be no nursing shortage. (O'lynn and Tranbarger, 2003) added that in order to attract more individuals to the profession, a positive image of nursing needs to be engendered by nurse education and general community . Hence, the purpose of the current study was to determine the *nursing image as a profession among undergraduate male nursing students* at Faculty of Nursing, Alexandria University.

The study revealed that the highest percentage of study subjects joined the faculty of nursing because of the availability of work in and outside country. Streubert, 1994), conducted a study in Pennsylvania about *male nursing students' perception of clinical experience*, his findings revealed that nursing was attractive because of job opportunities, security and availability. This is also in accordance with that of (AbdlKarim *et al*., 2004) had conducted a study in Egypt about *male student nurses: concerns, expectations and fear toward the profession*. They found that around two-thirds of their subjects haven chosen nursing as it represented a good opportunity for them to work. (Buerhaus *et al*., 2005) had conducted a study in the United States of America (U.S.A) about *nursing students' perception of a career in nursing and impact of a national campaign designed to attract people into the nursing profession*. He reported that slightly more than three-quarters of his subjects joined nursing because of availability of work. It is likely that more men will choose nursing as a career because nursing offers stable employment with reasonable wages in an otherwise unstable economy. This may be due to the low economic status in developing countries, which drives students to work outside the country. In addition, it may be due to cultural values in Arab countries where males assume great responsibilities and it was expected to be the main reason for entering the nursing profession since the graduates are automatically hired and have the opportunities to work abroad whether in Arab or western countries.

Yet, this same result is dissimilar to the results of (Gamel, 2006). Her study was conducted in Egypt to determine *the image of nursing as a profession among undergraduate nursing students and interns*. The results revealed that the availability of work and financial reward were the least mentioned reasons among her subjects, as the subjects of such study were female nursing students .

Moreover, findings indicated that the second highest reason mentioned by the study subjects was the secondary school grade. In this respect, (Abu-Gharibeh and Siluman, 1992), stated that the poor image of nursing, combined with university admission policies, in which, students are assigned to various faculties based on their secondary school grade, influence recruitment of high quality students.

More than one-fourth of the study subjects stated that an advice from a family member was the main reason for joining the profession. This may be attributed to the fact that a considerable number of family members, friends or acquaintances who were nurses as the present study revealed. These results are supported by (El Sharkawy and El Hadad, 1996) who studied *"factors affecting students' choice of nursing*

as a career in Egypt and Syria". They found that the family members had the significant impact on the choice of nursing as a career.

(Kelly *et al* .,1996) conducted a study about "*the experience of being a male student nurse"* in Chicago, the study revealed that family members were the most encouraging forces to their entering nursing and was the main source of moral support during the years of schooling. The present study revealed significant relations between the nursing image as a profession and the presence of family members, friends or acquaintances who were nurses. These findings were confirmed, as there is significant relation between nursing image and parents' reaction to joining the faculty of nursing. In this respect, (Heath, 2001) stated that young students may be applying to nursing programs as a response to parents' dream instead of their own.

The results of the present study showed positive changes in the image of nursing as a profession among more than half of the study subjects. The following will explore the factors affecting that positive change; working conditions, clinical training, relationship with faculty members and relationship with friends.

(Moyer, 1996)   had conducted a study at Widener University U.S.A about "*the relationships among sense of coherence, self-esteem and self-perception of clinical competence in junior and senior baccalaureate nursing students"*. This study revealed that most of junior and senior baccalaureate nursing students in the USA had a self-esteem that could be linked positively to improve self-perception of clinical competence and nursing image as a profession. Results of the present study illustrated that more than one-third of the study subjects were positively influenced by clinical training this may be due to the clinical environment increasing interaction between clinical instructors and students, nevertheless, increasing the interaction among students themselves. Moreover, students at hospitals and other clinical training settings have the opportunity to apply what was taught at the labs in real situations and with real patients.

The present study revealed that the highest percent of the study subjects were negatively influenced by public view about nursing.(Gray ,2004) surveyed *"registered nurses in California for their perception of how the public view nursing"*. The study revealed that 23% of nurses perceived that the public portrayed them as handmaidens to physicians.. Furthermore (Shukri ,2005) also reported that nursing is still suffering from negative public image in the Arab world).

The media is one of the most important factors that has influenced, is still influencing, and will most probably continue to influence the public's image of nursing. It always pictures nursing as a low social status, subordinate, unrespected, and female-dominant profession, not to mention nurses, being presented as low social status, careless females who perform housekeeping activities and accept tips from others. According to (Summers ,2004),and (Gamel ,2006) , such image not only influences how consumers view nurses, but also has an impact on the nurse's self image. When nurses are constantly portrayed in negative ways, these images delimit the scope of their work; affect their lives and their aspirations, the quality and quantity of persons choosing nursing as a profession, how nurses see themselves, and unfortunately, the future of nursing as a profession.

The second mentioned reason by the study subjects for the negative change of the nursing image was the relationship with faculty members. This may be due to the interaction with novice faculty members who do not have any experience in dealing with male adult student nurses and possibly cultural factors as male figures totally refusing to take orders from women. In this respect, (Mikelson, 1990) [reported] that the students' perception of being treated differently than females was frustrating and contributed to their feeling of isolation and exclusion. The participants expressed a need for male role models which they believed would diminish the difficulty of nursing being a female dominant profession.

This result was congruent with (Gamel, 2006), who reported that the highest percentage of her subjects was negatively influenced by faculty members due to the negative teacher-student relationship. This was followed by some teachers inhibiting students' self-confidence and discouraging them to ask questions freely. Moreover, students were required to carry out teachers' orders along with their feeling that there is lack of warmth between some teaching staff members and the students, and disrespect to their cultures since large numbers of them were from rural areas.

Working conditions also had a negative influence among less than one-fifth of the study subjects. This could be attributed to uncomfortable working environment, exposure to hazards whether physical or emotional. Moreover, lack of facilities and resources, and the gap between theories taught and the actual practices in governmental hospitals also helped in formulating negative image of nursing.

In relation to the study subjects' perceptions of the nursing image as a profession, more than half of them had positive image of nursing. Results showed that *"nursing as an appreciated profession in the society"* was perceived by about one-fourth of the study subjects. This finding is dissimilar to (Brodi et

al., 2004), who concluded that nursing as an appreciated profession was perceived by a few nursing students. On the other hand, nursing as "*men's work*" was perceived by a high percentage of the study subjects. This finding was not congruent with the researcher's expectation because of the stereotype image of nursing in the society as a female-dominant work. This result is congruent with those of (Rob, 1991), who concluded that the number of male nurses tends to increase obviously and both males and females can be good nurses .Yet, this result opposes that of, (Frizzell, 2004), and (Gamel, 2006) who found that the nursing profession was perceived as "*women's profession*" by more than one-half of the study subjects. This supports the idea that both men and women can be good nurses.

Nevertheless, findings of the current study indicated that the majority of the male nurses expected to work at other countries, while few of them expected to work as nurses in hospitals. This contradicts with the mission of nursing, as the main aim of nursing programs is to graduate a qualified professional nurse to overcome the nursing shortage in Egypt. It seems that the male nursing students considered the nursing to be a transitional period until they build themselves and make families, since Egypt has a male-dominant culture and nursing is primarily a female-dominant profession which is practiced mainly by women and males who are practicing it are very few or nearly not present.

According to the students' academic year, it was found that there was no significant correlation between nursing image and the students' academic year. This finding was not congruent with the researcher's expectations, because the students were expected to have more positive image as they progress from year to year during the undergraduate education and it contradicted with the significant correlation found between age and nursing image. This negative change as the students' progress could be due to their exposure to reality shock, and due to gaps between theory and practice. This finding contradicts that of (Heyman, 1983); (Harrison and Katz, 1998), and (Gamel,2006) , who found that nursing students became more attracted to their profession and more closely identified with it as they progressed in their nursing education

Moreover, as regards receiving information about nursing, a significant correlation was detected between presence of information and its effect on one hand and image of nursing as a profession on the other. It seems that receiving information about nursing before joining the faculty- whether from family, friends or from schools of nursing- plays an important role in influencing the students' image of nursing and their career choice. The previous finding

matches with that of (Steven and Walker, 1991), and (Gamel, 2006) who indicated that high school educators must develop strategies that target recruitment of high school students to professional careers in nursing.

5. Conclusion and Recommendations

The highest percentage of the male students had a positive image towards nursing as a profession. Working conditions, clinical training, relationship with faculty members, and relationship with medical staff, and public image about nursing had either positive or negative influence on the male students` nursing image. Also, the majority of the study subjects expected to work at other countries.

I-Recommendations for nurse educators and nursing programs:

1- Conducting conference for newly admitted students to orient them about the nursing profession, history of nursing , nursing education programs and different clinical experiences
2- Workshops for newly appointed instructors to teach them how to deal with male students.
3- Regular visits to secondary school and provision talks of a role model nurse about nursing and role of nurse

II- Recommendations for further research:

1- Longitudinal study to examine male nursing students regarding nursing image
2- A study to examine role strains in nursing education and nurse educator's perspective.

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Serum Levels of Vaspin and Osteoprotegerin in Premenopausal Women with the Polycystic Ovary Syndrome

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Abstract: Polycystic ovary syndrome (PCOS) is a common endocrine disorder that affects 5-10 % of reproductive-age women. It is characterized by menstrual dysfunction and hyperandrogenism and is associated with insulin resistance, impaired glucose tolerance, dyslipidemia and visceral obesity. Vaspin (visceral adipose tissue-derived serine protease inhibitor) levels increase with hyperinsulimenia and obesity. Osteoprotegerin (OPG) is a member of the tumour necrosis factor receptor superfamily. Recent data showed that obesity and insulin resistance result in decrease in serum OPG concentrations. The present study aimed to measure serum vaspin and osteoprotegerin levels in women with PCOS to show possible involvement in the pathogenesis of PCOS. Forty eight women with PCOS, 25 non-obese [body mass index (BMI) less than 30 kg/m^2] and 23 obese (BMI >30 kg/m^2) were enrolled for the study. Each group of them is compared to apparently healthy women as a control group matched for each in age and BMI. Clinical history, anthropometric measurements and biochemical and hormonal analysis were determined. The mean serum level of fasting blood sugar (FBS), insulin, homeostasis model assessment (HOMA-IR), triglyceride (TRIG) and high density lipoprotein-cholesterol (HDL-C) showed statistically significant difference between PCOS patients (non-obese and obese) when compared to control women (non-obese and obese) respectively. In both PCOS non-obese and obese patients groups as compared to the non-obese and obese control groups, the mean serum level of vaspin showed a statistically significant increase (P<0.001) in both PCOS groups, while the mean serum level of OPG showed a statistically significant decrease (P<0.001) in the same PCOS groups. Also, the levels of both previous two parameters (vaspin and OPG) showed significant differences between PCOS obese patients and PCOS non-obese ones. It is concluded that serum vaspin level increased in PCOS women particularly the obese, whereas serum OPG concentration reduced in the same patients group. These data suggest their involvement in the pathogenesis of PCOS.
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1. Introduction

Polycystic ovary syndrome (PCOS) is the commonest endocrinopathy of premenopausal women characterized by both reproductive and metabolic abnormalities. It affects 5-10 % of reproductive-age women (Economou *et al*., 2009). Women with PCOS are characterized by chronic anovulation and hyperandrogenism (Toulis *et al*., 2009). They commonly display a clustering of metabolic abnormalities, including impaired glucose tolerance, insulin resistance, dyslipidemia, increased prevalence of obesity, low-grade chronic inflammation and increased oxidative stress (Aroda *et al*., 2009). Obesity is present in approximately 44% of women with PCOS and it is characterized by central distribution of fat (Oris *et al*., 2003).

Insulin resistance (IR) is the most important pathophysiological factor in PCOS (Salley *et al*. 2007). It has been demonstrated in both obese and non-obese women with PCOS (Sepilian and Nagamani, 2005). The cellular and molecular mechanisms of insulin resistance in PCOS have not yet been elucidated, but they are considered to be distinct from those of other diseases associated with insulin resistance. Therefore, they have an increased prevalence of hypertension, diabetes and cardiovascular disease (Oh *et al*., 2009).

Osteoprotegerin (OPG) is a glycoprotein first described in 1997 by Simonet *et al., 1997*. It is produced in many different tissues, including bone, vasculature , heart, lung, kidney, and placenta , and also circulates in plasma, although the concentration

here is much lower than in bone and arterial tissue (Mand and Rasmussen, 2008).

Osteoprotegerin (OPG) is a member of the tumor necrosis factor receptor superfamily and is a secretory basic glycoprotein that exists in a 60 kDa monomeric form and a disulfide-linked homodimeric form of 120 kDa and, by binding and neutralizing the receptor activator of nuclear factor-κB ligand (RANKL), exerts an inhibitory effect on osteoclastic bone resorption (Escobar-Morreale *et al.*, 2008). So, OPG-mediated pathways might have a role in osteoporosis because estrogen increases OPG gene expression. OPG maintains the structure of healthy bone and inhibits osteoclast activation and differentiation. In the vascular system, OPG inhibits pathological calcification in the media intima (Yi and Lin, 2007). OPG has been proposed for therapy of osteopenic disorders, such as postmenopausal osteoporosis, Paget disease, rheumatoid arthritis, hypercalcemia, and lytic bone metastases (Chen *et al.*, 2001).

Besides, OPG has other biological functions, including anti-inflammatory actions, such as an anti-apoptotic effect resulting from the binding of TNF-related apoptosis-inducing ligand (TRAIL) with a consequent inhibition of the apoptosis process of susceptible cells (Escobar-Morreale *et al.*, 2008). Interestingly, endothelial cells are one of the sites in which the anti-apoptotic effects of OPG have been demonstrated, suggesting a protective vascular role of the latter. Therefore, OPG has, apart from the aforementioned effects on osteoclastogenesis, other functions in vascular processes and immune responses that may be relevant to the pathogenesis of PCOS and its associated co-morbidities (Malyankar *et al.*, 2000; Schoppet *et al.,* 2002).

Recently published data show that obesity and insulin resistance result in a decrease in serum OPG concentrations (Holecki *et al.*, 2007).

The adipose tissue, predominantly visceral adipose tissue (VAT), produces and secretes a variety of bioactive adipocytokines. However, VAT accumulation induces adipocytes dysfunction, including oversecretion of interleukin-6, tumor necrosis factor-α, plasminogen activator inhibitor-1 and visfatin, and hyposecretion of adiponectin, which were supposed to be involved in the pathogenesis of insulin resistance and abnormal glucose metabolism (Yin *et al.,* 2009).

Recently Hida *et al.,* 2005 characterized vaspin as an interesting novel adipokine with insulin-sensitizing effects. Vaspin (visceral adipose tissue-derived serine protease inhibitor) belongs to the serine protease inhibitor (serpine) superfamily and is produced in the visceral adipose tissue depot of Otsuka Long-Evans Tokushima Fatty (OLETF) rats,

an animal model of obesity with type 2 diabetes mellites (T2DM) (Hida *et al.*, 2005; Tan *et al.*, 2008). It is demonstrated convincingly in the initial report that administration of vaspin to obese mice improved glucose tolerance and insulin sensitivity (Gulcelik *et al.*, 2009). Furthermore, dysregulated expression of insulin sensitivity-modulating genes in adipose tissue including adiponectin and leptin was reversed after vaspin treatment (Seeger *et al.,* 2008; Glintborg *et al.,* 2006). Moreover, vaspin production was down-regulated with worsening of T2DM in OLETF rats (Hida *et al.,* 2000). In addition, it has recently shown that induction of vaspin mRNA expression in human adipose tissue is regulated in a fat depot-specific manner and could be associated with parameters of obesity, insulin resistance, and glucose metabolism (Kloting *et al.,* 2006). A few studies were reported about serum vaspin levels in humans and the correlation between vaspin serum levels and markers of insulin sensitivity, and glucose metabolism are unclear (Gulcelik *et al.*, 2009).

The aim of the present study was to measure serum vaspin and osteoprotegerin levels in women with PCOS and asses possible correlations between each of them and clinical, biochemical and hormonal parameters of the syndrome as serum levels of vaspin and osteoprotegerin may show possible involvement in the pathogenesis of PCOS.

2. Subjects and Methods
Subjects:

Forty eight women with PCOS, 25 non-obese [body mass index (BMI) less than 30 kg/m2] and 23 obese (BMI >30 kg/m2) were enrolled for the study , all of whom were outpatients at Obstetrics and Gynecology Department, Faculty of Medicine, Minoufiya University. The diagnosis of PCOS was made according to the National Institute of Child Health and Human Development criteria (Ferriman and Gallwey, 1961; Zawadski and Dunaif, 1992): 1) evidence of chronic anovulation or oligoamenorrhea (menstrual intervals > 35 days and or less than nine menstrual cycles per year); 2) clinical or biochemical evidence of hyperandrogenism [presence of acne, hirsutism or Ferriman-Gallwey (FG) score >8] and or elevated serum level of total testosterone more than 2.5 nmol/L and 3) exclusion of pregnancy, thyroid disease, prolactinoma, Cushing's syndrome and late onset nonclassic congenital hyperplasia.

Forty six healthy women (employees from Minoufiya University) matched for age, 24 non-obese (BMI <30 kg/m^2) and 22 obese (BMI > 30) participate to this study as controls. All controls were evaluated by a medical history and physical and pelvic examination. Women with a menstrual cycle less than 26 days or more than 30 days were

excluded. The normal ovulatory state was confirmed by TV-USG and plasma progesterone (P) assay during the luteal phase of the cycle. Exclusion criteria were pregnancy, hypothyroidism, hyperprolactiemia, Cushing's syndrome, current or previous (within the last 6 months) use of oral contraceptives, glucocorticoids, ovulation induction agents, antidiabetic and antiobesity drugs or other hormonal drugs. None of the patients or controls women were affected by neoplastic, metabolic or cardiovascular disorder. All the procedures used in this study were approved by the Research Ethics Committee of Faculty of Medicine, Minoufiya University, Egypt. An informed consent was obtained from all subjects in this study.

All subjects were studied during the early follicular phase (second to fifth day) of the spontaneous or progesterone-induced menstrual cycle. 10 ml of venous blood were withdrawn after an overnight fasting from all subjects and allowed to clot in a plain sterile tube and then centrifuged. The separated serum was stored into aliquots at -80^0C for biochemical and hormonal determinations.

Anthropometric measurements:
Height and weight were measured with every subject. BMI was calculated as weight (kg)/ square height (m^2). Waist /hip ratio (WHR) was calculated by dividing the circumferences of the waist and hip.

Biochemical and hormonal analysis:
- Fasting blood sugar (FBS) and lipid profile including total cholesterol (T.Chol), triglyceride (TRIG) and high density lipoprotein-cholesterol (HDL-C) measurement using Beckman Coulter Automated Analyzer (Synchron CX9 ALX) clinical system, USA. Low density lipoprotein-cholesterol (LDL-C) was calculated by using Friedewald's formula as T.Chol (mg/dl) - HDL-C (mg/dl) – TRIG (mg/dl)/5 (Rifai *et al*., 1992).
- Luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin (PRL), progesterone (PRG), Estradiol (E2) and total testosterone (TT) were measured by an automated quantitative enzyme immunoassay on the VIDAS instrument, BioMerieux, France using the Enzyme Linked Fluorescent Assay (ELFA).
- Fasting insulin, free testosterone (FT), 17-OH progesterone (17-OH PRG), androstendione, sex hormone-binding globulin (SHBG) and dehydroepiandrosterone sulfate (DHEA-S) were performed using a solid phase automated competitive chemluminescence's immunoassay (IMMULITE 2000, DPC, Germany).
- Serum OPG concentrations were measured using a commercial ELISA (BioVendor- GmbH-Laboratorni

medicina, European Union) (Hofbauer, 1999). According to the manufacturer's directions , the amount of OPG present in the serum samples was determined from a calibration curve as pmol/ l.
- Serum level of vaspin (visceral adipose tissue-derived serine protease inhibitor) in sera were measured using a commercially available enzyme-linked immunosorbent assay (BioVender- GmbH-Laboratorni medicina, European Union). It is sandwich enzyme immunoassay for the qnuatitative measurement of human vaspin (Wada, 2008).
- Free androgen index (FAI) was defined as 100 times the molar ratio of total testosterone to SHBG [FAI= 100 × total testosterone (ng/ml) / SHBG (nmol/l)] (Fenkci *et al*., 2008).
- Insulin resistance was calculated by using homeostasis model assessment (HOMA-IR) score that employs the formula: fasting insulin concentration (uIU/ml) × glucose (mmol/l) / 22.5 (Matthews *et al*., 1985). Measurement of glucose level by mg/dl was multiplied by 0.555 to get result by mmol/l to calculate HOMA-IR.

Statistical Analysis:
Data were collected and entered to the computer using SPSS (Statistical Package for Social Science) program for statistical analysis. The Pearson`s correlation coefficients were calculated for the normally distributed values. However, Spearman`s correlation coefficients were done for the not normally distributed values. Continuous normally distributed variables were tested for association by student's t-test. On the other hand, Mann–Whitney *U* test or z test is a non-parametric test for assessing whether two independent samples of observations have equally large values. It is one of the best-known non-parametric significance test used for the not normally distributed values. Data were presented as mean ± SD. p value <0.05 and <0.01 were considered statistically significant.

3. Results
Table 1 showed clinical and biochemical data in PCOS patients and control women according to BMI. As expected from appropriate matching, no differences were observed among PCOS patients and control women (non-obese or obese) in age, BMI and WHR. While, the mean serum levels of FBS, insulin, HOMA-IR, TRIG and HDL-C showed statistically significant difference between PCOS patients (non-obese and obese) when compared to control women (non-obese and obese) respectively. Regarding the mean serum level of T.CHOL and LDL-C, a statistically significant increase was observed only in PCOS obese patients when compared to the obese controls.

There was statistically significant increase in the mean of serum levels of FT, TT, DHEA-S, prolactin, androstenedione and in FAI, FG score in patients with PCOS (non-obese and obese) when compared to the control women (non-obese and obese) who matched in BMI. While, there was no statistically significant difference in the mean serum level of LH, PRG and 17-OH-PRG in the same groups (Table 2).

In both PCOS non-obese and obese patients groups as compared to the non-obese and obese control groups, the mean serum level of vaspin showed a statistically (P<0.001) significant increase in both PCOS groups while the mean serum level of OPG showed a statistically (P<0.001) significant decrease in the same PCOS groups (Table 2).

Table 3 showed a statistically significant increase in the mean serum level of vaspin, BMI and HOMA-IR in the PCOS obese patients when compared to PCOS non-obese patients while, the mean serum level of OPG showed a significant decrease in the PCOS obese patients when compared to PCOS non-obese patients.

Serum vaspin level was negatively correlated with FT (r=-0.354, P=<0.05) but positively correlated with DHEA-S (r=0.812 & P=<0.001), FG-score (r=0.569 & p=<0.001), HOMA-IR (r=0.429 & p=<0.05), insulin (r=0.377 & p=<0.05) and BMI (r=0.736 &p=<0.001) as shown in table 4.

Considering the serum level of OPG as in table 5, it showed inverse correlation relationship with DHEA-S (r= -0.719 & P=<0.001), FG-score (r=-0.621 & P=<0.001), HOMA-IR (r=-0.399 & P=<0.05) and BMI (r=-0.721 & P=<0.001). While, it was positively correlated with FT (r=0.388 & P=<0.001) and insulin (r=0.346 & P=<0.05).

Table (1): Clinical and biochemical features of PCOS patients and controls according to BMI.

	PCOS (Non-obese) N=25 Mean±SD	Control (Non-obese) N=24 Mean±SD	P-value	PCOS (Obese) N=23 Mean±SD	Control (Obese) N=22 Mean±SD	P-value
Age (years)	29.5±0.9	29.3±2.0	NS	30.5±1.8	30.8±3.9	NS
BMI (Kg/m^2)	22.6±2.6	22.1±2.8	NS	43.0±4.0	42.1±5.9	NS
WHR	0.89±0.0	0.89±0.01	NS	0.89±0.03	0.89±0.01	NS
FBS (mg/dl)	93.8±8.3	89.4±7.3	<0.05*	99.8±13.6	92.7±5.9	<0.05*
Insulin (uIU/ml)	19.5±1.4	13.2±3.1	<0.001*	20.3±1.7	14.1±3.7	<0.001*
HOMA-IR	2.5±0.2	1.7±0.3	<0.001*	2.6±0.2	1.8±0.4	<0.001*
T.CHOL (mg/dl)	168.4±21.3	167.5±28.1	NS	197.0±41.8	177.0±19.6	<0.05*
TRIG (mg/dl)	208.8±29.5	108.0±12.7	<0.001*	247.9±43.0	143.2±9.4	<0.001*
HDL-C (mg/dl)	33.7±3.5	44.6±6.4	<0.001*	29.8±3.3	35.3±4.7	<0.001*
LDL-C (mg/dl)	96.1±21.5	93.9±34.5	NS	157.2±20.4	133.1±42.6	<0.05*

Values are given as mean ± SD.   NS= non significant (P-value >0.05)
 P-value <0.05 & P<0.001: statistically significant.
In this table, student's t-test was done for all the variables as they were normally distributed.

Table (2): Hormonal features of PCOS patients and controls according to BMI.

	PCOS (Non-obese) N=25 Mean±SD	Control (Non-obese) N=24 Mean±SD	P-value	PCOS (Obese) N=23 Mean±SD	Control (Obese) N=22 Mean±SD	P-value
FT (pg/ml)	7.1±1.4	0.6±1.1	<0.001*	6.2±1.2	0.2±0.1	<0.001*
TT (ng/ml)	4.7±0.7	2.4±0.6	<0.001*	4.8±0.9	2.6±0.4	<0.001*
SHBG (nmol/L)	28.2±2.3	89.9±27.1	<0.001*	24.6±4.2	85.1±21.4	<0.001*
FAI	16.8±2.2	2.8±0.7	<0.001*	19.5±2.2	3.4±1.3	<0.001*
FG score	10.9±0.6	3.9±0.1	<0.001*	12.5±0.5	4.9±0.3	<0.001*
LH (mIU/ml)	4.5±1.7	4.2±1.6	NS	4.1±1.4	3.9±1.7	NS
FSH (mIU/ml)	8.2±1.1	8.9±0.3	<0.05*	7.5±0.5	8.6±0.1	<0.001*
PRL (ng/ml)	15.2±0.8	11.5±0.5	<0.001*	13.5±0.5	12.6±0.5	<0.001*
E2 (pg/ml)	35.7±1.1	94.1±20.9	<0.001*	30.8±1.0	81.9±17.2	<0.001*
PRG (ng/ml)	0.99±0.2	0.90±0.3	NS	0.99±0.2	0.95±0.2	NS
17-OH-PRG (ng/ml)	1.8±0.7	1.4±0.5	NS	1.8±0.9	1.7±0.8	NS
Androstenedione (ng/ml)	4.8±0.7	2.3±0.8	<0.001*	5.2±0.7	1.8±0.6	<0.001*
DHEA-S (ug/dl)	522.2±62.4	209.6±94.9	<0.001*	548.1±41.2	156.2±82.7	<0.001*
OPG (pmol/l)	1.15±0.8	4.67±1.26	<0.001*	0.83±0.1	2.77±0.6	<0.001*
Vaspin (ng/ml)	1.78±0.1	1.11±0.1	<0.001*	1.92±0.0	1.30±0.08	<0.001*

Values are given as mean ± SD.   NS= non significant (P-value >0.05)
 P-value <0.05 & P<0.001: statistically significant.
In this table, student's t- test was done for all the variables except for DHEA-S (z-test was used because this variable isn't normally distributed).

Table (3): Clinical, biochemical and hormonal features of PCOS patients according to BMI.

	PCOS (Non-obese) N= 25 Mean±SD	PCOS (Obese) N= 23 Mean±SD	P-VALUE
Insulin (uIU/ml)	19.5 ± 1.4	20.3 ± 1.7	**NS**
HOMA-IR	2.5 ± 0.2	2.6 ± 0.2	**0.05* <**
BMI (kg/m^2)	22.6 ± 2.6	43.0 ± 4.0	**<0.001***
Vaspin (ng/ml)	1.7 ± 0.1	1.9 ± 0.1	**<0.001***
OPG (pmol/l)	1.1 ± 0.1	0.8 ± 0.1	**<0.001***

Values are given as mean ± SD.   NS= non significant (P-value >0.05)
 P-value <0.05 & P<0.001: statistically significant.
 In this table, student̓s t-test was done for all the variables as they were normally distributed.

Table (4): Correlation analysis with serum vaspin level as the dependant variable.

	r	p value
DHEA-S (ug/dl)	0.812	<0.001*
FT (pg/ml)	-0.354	<0.05*
FG score	0.569	<0.001*
HOMA-IR	0.429	<0.05*
Insulin (uIU/ml)	0.377	<0.05*
BMI (kg/m^2)	0.736	<0.001*

 P-value <0.05 & P<0.001: statistically significant.
In this table, Pearson̓s correlation coefficients was used for all the variables except for DHEA-S (Spearman̓s correlation was done as this variable isn't normally distributed).

Table (5): Correlation analysis with serum osteoprotegerin level as the dependant variable

	r	P value
DHEA-S (ug/dl)	-0.719	<0.001*
FT (pg/ml)	0.388	<0.001*
FG score	-0.621	<0.001*
HOMA-IR	-0.399	<0.05*
Insulin (uIU/ml)	0.346	<0.05*
BMI (kg/m^2)	-0.721	<0.001*

P-value <0.05 & P<0.001: statistically significant.

In this table, Pearson's correlation coefficients was used for all the variables except for DHEA-S (Spearman's correlation was done as this variable isn't normally distributed).

4. Discussion:

Polycystic ovary syndrome (PCOS) is the commonest endocrinopathy of premenopausal women that represents a major cause of infertility (Azziz *et al*., 2004). It is characterized by both reproductive and metabolic abnormalities including menstrual dysfunction, hyperandrogenism and is associated with insulin resistance, pancreatic β-cell dysfunction, impaired glucose tolerance, type 2 diabetes, dyslipidemia and visceral obesity (Azziz *et al*., 2004; Azziz, 2006; Tan *et al*., 2008).

The aim of the current study was to evaluate the levels of serum vaspin and osteoprotegerin in normal weight and obese PCOS women to find their possible involvement in the pathogenesis of this syndrome.

This study showed a statistically significant higher levels of FBS, insulin and HOMA-IR in (non obese) PCOS patients when compared to the (non obese) control subjects although BMI was in normal range in each group. The same statistically significant relationship in the previous parameters was observed when compared the PCOS obese patients with the obese control subjects. When compared PCOS non-obese women with PCOS obese women, a statistically significant decrease in HOMA-IR was found in non-obese group while, no significant difference was observed in the serum level of insulin in the same two groups when compared to each other. These results agreed with Economou and his colleagues, 2009 as they stated that insulin resistance was present in 41.6% of the overweight/obese PCOS women whereas it was present in 13.6% of the lean PCOS women. Also, Aigner *et al.,* 2009 stated that although the precise pathogenesis of PCOS remains unclear, a close link to insulin resistance (IR) and consecutive hyperinsulinemia, impaired glucose tolerance, type 2 diabetes mellitus, atherogenic dyslipidemia and visceral obesity has been well established. Dunaif, 1997 suggested that hyperinsulinemia occurs as a result of insulin resistance & accelerates ovarian androgen overproduction and it may also contribute to the development of diabetes and dyslipidemia in PCOS patients.

Regarding lipid profile, this study showed a statistically significant increase in the mean serum levels of T.CHOL, TRIG and LDL-C in PCOS-obese patients when compared to the obese controls and in the serum level of TRIG only in PCOS non-obese patients when compared to the non-obese controls. While, a statistically significant decrease was detected in the serum level of HDL-C in both groups of PCOS (non-obese & obese) when compared to the controls (non-obese & obese) respectively.

Fenkci *et al.,* 2008 agreed with the previous results as they reported that dyslipidemia is frequently accompanied by decreased HDL and increased T.chol, LDL-C, TRIG and can be observed in insulin resistance conditions as in PCOS.

In this study, like others (Aigner *et al.,* 2009; Alvarez-Blasco *et al.,* 2009; Ciaraldi *et al*., 2009), the mean levels of FT, TT, FAI, FG-score, androstenedione and DHEA-S were significantly higher in both obese and non obese PCOS women when compared to both obese and non obese control women respectively while, significant decrease in the levels of SHBG and E2 in the same groups were observed.

In the current study, the mean serum vaspin level showed a statistically significant increase in PCOS obese women when compared to PCOS non-obese women. In addition, significantly higher mean serum vaspin levels were detected in both the same previous groups [PCOS (obese & non-obese)] when compared to the control women (obese & non-obese) respectively. Furthermore, a statistically significant positive correlation was observed between serum vaspin level and each of DHEA-S, FG-score, HOMA-IR, insulin and BMI. Tan and his coworkers, 2008 agreed with these results as they found a higher serum and adipose tissue vaspin levels in women with PCOS and they reported that obese insulin-resistant subjects had higher serum vaspin levels and it showed a significant positive associations with BMI & insulin sensitivity. Also, Kloting *et al*., 2006 reported significant positive associations between

serum and omental adipose tissue vaspin levels with glucose and HOMA-IR and after metformin therapy given to the overweight PCOS women for 6 months, a significant decrease in circulating vaspin & glucose levels with a concomitant improvement in insulin sensitivity and a decrease in insulin resistance indexes. While, Escobar-Morreale and his colleagues, 2009 disagreed with this study as they found the serum vaspin concentration in premenopausal women are not affected by PCOS, obesity or glucose tolerance. Also, Ye *et al.*, 2009 stated that no significant correlations between vaspin and body fat indexes were detected.

From the previous data, this study hypothesize that the increased circulating vaspin levels may be a compensatory mechanism for insulin resistance and/or glucose metabolism in the PCOS subjects, with glucose playing a pivotal role as explained by Tan and his colleagues, 2008.

The present study showed that mean serum OPG concentration was statistically significant reduced in PCOS women (obese & non-obese) when compared to the control women (obese & non-obese) respectively and this finding is dependent from obesity as it showed a statistically significant decrease in obese PCOS women when compared to non-obese PCOS. Furthermore, OPG showed a statistically significant positive correlation with FT and insulin and a statistically inverse correlation with DHEA-S, FG-score, HOMA-IR and BMI.

Sandberg *et al.*, 2006 and Kiech *et al.*, 2007 explained the previous results as they stated that the reduction of serum OPG in PCOS and obesity might be related to the increased cardiovascular risk associated with these disorders, given that OPG may play a protective role in the vasculature by both inhibition of serum receptor activator of nuclear-κβ ligand (RANKL) and TNF-related apoptosis-inducing ligand (TRAIL), preventing plaque complication and apoptosis of endothelial cells. On one hand, by failing to neutralize RANKL, reduced OPG levels might favor cardiovascular disease, because RANKL overexpression is a prominent feature prone to rupture vulnerable atherosclerotic lesions, RANKL may also contribute to the transition from a stable to an unstable plaque and the serum level of RANKL is a highly significant predictor of cardiovascular disease (Kiech *et al.*, 2007).

Also, Avignon *et al.*, 2007 reported that increased circulating OPG levels have also found in association with the presence and severity of coronary artery disease, peripheral artery disease and stroke, suggesting that OPG may serve as a biomarker of established atherosclerosis in humans.

Saika and his coworkers, 2001 speculated that the lower levels of estradiol observed in PCOS women when compared with the controls in their study (as in this study) could have one of the pathophysiological factors involved because estradiol have been shown to upregulate OPG gene expression and production. Also, Chen *et al.*, 2004 agreed with this study as they found that testosterone may indeed increase OPG expression and it correlates positively with serum OPG concentrations in men.

In conclusion, serum vaspin level increased in PCOS women particularly the obese and it showed a positive correlation with DHEA-S, FG-score, insulin, BMI and HOMA-IR. Whereas, serum OPG concentrations reduced in the same patients depending on obesity with an inverse correlation with DHEA-S, FG-score, BMI and HOMA-IR. These data suggest their involvement in the pathogenesis of PCOS. Further studies are needed to explain the pathophysiological roles of the increased serum vaspin and decreased serum OPG observed in PCOS.

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Evaluation of immediately loaded dental implants placed in healed bony sites with or without addition of autologous platelet-rich plasma

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Abstract: The concept of immediate loading by using titanium one-piece implant can be preferred to the two stage technique due to the ability of the immediate loading to eliminate the need for the healing period to restore the implant. The aim of the present study was to evaluate the effect of adding   platelet-rich plasma with immediately loaded self-tapping dental implant (OsteoCare™ Maxi-Z one piece) placed in healed bony sites (posterior maxillary area) on accelerating the rate of osseointegration or reducing the crestal bone resorption around these implants through the first three months follow-up period. **Materials and Methods:** The present study was conducted on 12 patients; 9 males (75%) and 3 females (25%) with a mean age of 37.5 years (28-55). Twenty four Maxi Z implants were used; each patient received two implants placed bilaterally in healed bony sites in the posterior maxillary area after the addition of platelet-rich plasma in one side while the other side was used as a positive control. All implants were immediately loaded after implant placement. **Results:** Complete soft tissue healing had occurred in all patients and all the implants were successfully osseointegrated over the twelve months follow-up period with a success rate of 100%. The results of the present study showed that there was no statistical difference between the two sides (test + control) regarding PD, MBI, MPI, implant mobility, crestal bone resorption and bone density through the twelve months. **Conclusion:** The Osteocare's Maxi Z one-piece, self-tapping self-drilling implant has shown high success rate regarding initial stability and successful osseointegration. However, within the limitations of the present study, local application of autologous platelet-rich plasma into the prepared drill holes immediately before implant placement didn't accelerate the rate of osseointegration or decrease the crestal bone resorption "through first three months period" in immediately loaded dental implant placed in posterior maxillary area.

1. Introduction:

Through a history of nearly 50 years, dental implants have gained a high reputation due to their high success rate, (**Buser et al 1997**). It was proposed according to **Branemark et al (1985)** that having successful tissue integration needs a two-stage surgical protocol and an undisturbed healing period of at least three to six months. The immediate loading protocol is being recognized as an alternative technique to the classical delayed loading approach. Immediate nonfunctional prosthetic restoration which does   not   seem   to   affect   the   process   of osseointegration has proven to be more effective **(Pillar et al 1986; Brunski 1992 and Nkenke et al 2005).**

PRP gel is an autologous modification of fibrin glue. It is formed by mixing PRP derived from centrifugation of autologous whole blood with thrombin and calcium chloride. PRP gel includes a high concentration of platelets and a native concentration of fibrinogen. When platelet concentrate is activated by the addition of thrombin and calcium chloride this results in the release of a cascade of growth factors from the platelet alpha granules, **(Whitman et al 1997 and El-Sharqawy et al 2007).** The osseointegration of dental implants arises from cell migration, differentiation, bone formation and bone remodeling along the implant surface, each of these processes is platelet-and blood clot- dependent. Therefore, PRP can be used to enhance   osseointegration   in   patients   whose osseointegration may be less predictable such as the elderly, individuals with osteoporosis, diabetics or

other forms of compromised regeneration, as well as, in the posterior maxillary area *(Monov 2005).*

Kim et al 2002 assessed the efficacy of demineralized bone powder (DBP) alone or combined in a mixture with platelet-rich plasma to enhance osseointegration of dental implants in dogs. Histo-morphometric results revealed a higher percentage of bone contact with DBP and PRP compared with control and DBP alone. *Zechner et al (2003)* evaluated histologically and histo-morphometrically the effect of topical application of PRP during implant placement on local bone formation in 12 adult mini-pigs. Un-decalcified ground sections were prepared at 3, 6 and 12 weeks after PRP and implants placement and histo-morphometric results showed a significantly more bone-to-implant contact at the sites with topical PRP application during the early healing phase.

Duka et al (2008) tested the influence of PRP combined with bovine deproteinized bone BDB and resorptive membrane of bovine origin RBDM on the bone defect filling and level of bone resorption during early implant insertion in 10 dogs. Radiologic analyses were done immediately and 10 weeks after the insertion. Bone defect filling was measured by a graduated probe 10 weeks after the implant insertion. The results showed that the use of PRP combined with BDB and RBDM have a positive effect on increasing the bone defect filling and decreasing the level of bone resorption around early inserted dental implants. On the other hand, *Nikolidakis et al (2006)* evaluated the effect of local application of autologous platelet-rich plasma on bone healing around titanium implants with two different surface configurations. PRP fractions were obtained from venous blood sample of 6 goats and applied via dipping of the implant in PRP liquid before insertion. The evaluation of the bone mass close to implant surface indicated that all the groups induced a significant increase of the bone mass except the PRP gel groups. So, the additional use of PRP did not offer any significant effect on the bone response to the implants. The same was observed with *Gurgel et al (2007)* in experimental study on ten male adult mongrel dogs, who evaluated histometrically bone healing in surgically created dehiscence-type defects around titanium implants treated with an association of platelet-rich plasma and guided bone regeneration. Within the limit of this study it was concluded that PRP does not exert additional effects on the bone healing.

In a human study by *Mannai (2006)* who studied the re-construction of the maxilla in ninety-seven consecutive patients with the simultaneous placement of implants combined with a small amount of intraoral autogenous bone providing the necessary viable stem cells and a larger amount of xenogenic bone used as a scaffold and a purely autologous platelet concentrate for optimal bone formation. A total of 314 implants, sand blasted acid-etched type were placed simultaneously in the anterior, posterior or both parts of the maxilla. 97.8% of all cases healed uneventfully with excellent hard and soft tissues healing. Bone maturation was excellent at 3 months as seen on x-rays and CAT scans. The same with *Lee et al (2008)* who evaluated dental implant survival rates using the concept of a nonfunctional, immediate loading protocol with non splinted dental implants in the grafted maxillary sinus during a 52-week period. Random histo-morphological analysis was completed to evaluate the early healing effect of platelet-rich plasma and 50% autogenous bone combined with three different substitute graft materials in a 50:50 composite ratio. During the 52-weeks observation period, no implants were lost. Between 4 to 8 months of graft healing time, histological and histo-morphometrical analysis revealed formation of new vital bone in different graft specimens ranging from 77% to 100%. The results of this clinical study indicate that immediate nonfunctional loading with the use of PRP combined with bone graft is a predictable protocol.

On the other hand, a randomized prospective controlled study was done by *Schaaf et al (2008)* to verify the effect of adding platelet-rich plasma on bone density in the maxilla after sinus floor elevation in combination with autologous bone. Bone biopsy was performed 4 months after augmentation. The results of histo-morphometric evaluation of the biopsies showed that the topical use of PRP did not improve the maxillary bone volume either clinically or statistically when compared with the conventionally treated patients.

2. MATERIALS AND METHODS:
2.1. Materials:
2.1.1. Subjects:

The present study was conducted on 12 patients; 9 males (75%) and 3 females (25%) with a mean age of 37.5 years (28-55). All patients were in acceptable general health having no systemic disease that contraindicates surgery. patients had favorable oral hygiene and motivation to provide a reasonable

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prognosis for long-term benefit from the implant and each patient had bilateral posterior (premolar or molar) maxillary edentulous area suitable for implant placement.

The study was performed according to the split-mouth design. The posterior area of the upper arch of each patient was divided into:

Experimental side(A): Implant placed in healed bony site with the addition of PRP.

Control side (B): Implant placed in healed bony site without PRP. One side was randomized to be the control side while the contralateral site served as the experimental one. The implants were non-functionally immediately loaded.

2.1.2. Preparation of platelet-rich plasma PRP:

Adequate amount of blood was drawn from each patient by veni-puncture of the anticubital vein. Blood was collected into glass test tubes that contained 10% trisodium citrate solution as an anticoagulant. The blood containing glass tubes were centrifuged at 2,500 rpm for 10 minutes, which resulted in the separation of three basic components: Platelet poor plasma PPP in the top, PRP in the middle and then RBC layer in the base of the test tube. The PPP layer was aspirated and discarded. Then the remaining plasma was collected and a second centrifugation (15 minutes at 3600 rpm) was performed to concentrate the platelets. At the time of the application, a sterile syringe was used to aspirate 1cm3 of PRP, 1cm3 of sterile saline solution containing 10% calcium chloride (a citrate inhibitor that allows the plasma to coagulate) and 100 U/mL of sterile human thrombin and 1cm3 of air. Adequate mixing was done to initiate the coagulation process. Then the needle was removed and the PRP gel was slowly injected. Cell count for the platelet cells was done before and after centrifugation

2.1.3. Implant System:

Twenty four Osteo Care™ Maxi Z one-piece implants (Osteo Care™ Implant System, London, United Kingdom) were placed in twelve patients who participated in this study. They were tapered self-tapping self-drilling implants and available with diameters of 3.30, 3.75, 4.50 and 5.50 mm and lengths of 11, 13, 15 and 17mm. Maxi Z one-piece implant was designed to allow immediate loading in healed bone sites and allows simultaneous expansion and compression of the bone in a process thus called

"Comp-Ex procedure" which in turn improves bone quality and overall width. The Maxi Z one-piece were placed with a minimally invasive trans-mucosal flapless procedure and had a tapered pointed tip. The Maxi Z one-piece implant was machined from titanium alloy of 6AL-4V ELI (Extra Low Interstitial) to provide maximum strength and incorporates both the implant body and the abutment in a single component. The surface treatment of the implant is GBA (Grit-Blasted and Acid Etching) to create macro and micro roughness enhancing the osseo integration and improving the bone to implant contact. Also presented with a unique "Buttress" thread design that allows a maximum bone to implant contact and achieving higher initial stability even in poor bone quality. The Maxi-Z one piece also had an anatomical abutment design with double flat facets to minimize the time needed for the preparation and to improve the retention of the provisional as well as the final restoration.

2.2. Methods:
2.2.1. Preoperative measures:

All patients received instructions in self-performed plaque control measures and were subjected to a series of full mouth scaling and root planning using curettes and ultrasonic instrumentation. Pre-surgical radiographic evaluation was performed by obtaining panoramic radiographs and periapical radiograph on the area of interest. The preoperative radiographs were used to confirm the diagnosis and estimate the implant length using the radiographic stents. The ridge width was evaluated using the diagnostic casts, ridge mapping or directly in the patient's mouth using bone calipers.

2.2.2. Surgical Technique:
(Flapless Trans-mucosal Technique)

Osteotomy preparation: Implant surgery were performed under local anesthesia Ultra-caine D-S forte containing 1:200000(0.5mg/ml) epinephrine. In each patient site preparation and implant placement was done bilaterally in two healed bony sites. No incisions or flap was made. Osteotomy preparation was carried out through free hand flapless trans-mucosal drilling. Osteotomy preparation was done sequentially using 1.3mm profile pilot drill, 2.2mm drill and 2.75mm drill. The drills were mounted on a low speed reducing hand piece and saline was used for irrigation. One piece implant was used in both test sites (implant with PRP) and control sites (implant without PRP). In the test sites, the prepared PRP was slowly injected at low pressure into the drill holes

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immediately before implant placement. In addition, the implant was dipped in PRP before seating.

Implant placement: The implant was removed from its protective pouch and held using the attached plastic carrier then placed into the prepared site. The implant was rotated clock wise for several revolutions until a resistance was met. The plastic carrier was removed and the ratchet wrench with the 2.4mm overhex driver was used for complete seating of the implant into its final position (the coronal part of the collar of the implant was flush or below the cestal bone of the alveolar ridge). After that, the initial stability was checked using the 30N/cm torque wrench. Attaining primary stability of over 30N/cm was considered crucial with all the placed implants to allow for the immediate loading protocol. Finally, a periapical radiograph was taken to check the final implant position and to estimate the initial bone level around the implant.

2.2.3. Abutment preparation and provisional restoration: Immediately after implant placement, the abutment was prepared using carbide burs with copious water irrigation to avoid overheating. Then, a temporary crown of either acrylic resin, composite or readymade acrylic temporary crown was fabricated. The provisional crown was cemented to the prepared abutment of the Implant and adjusted to be completely out of functional occlusion in centric and eccentric position. The patients were instructed to eat soft food for 2 months and to avoid direct biting on the provisional restoration.

2.2.4. Post-operative care: Oral antibiotic regimen of Augmentin 1 gm (Glaxo Wellcome Smith Kline Beecham)was given every 12 hours for five days following the procedure. NSAIDs were prescribed for one week to prevent post-surgical pain.

Final restoration: The provisional acrylic resin restorations were removed after a healing period of 6 months. The prepared abutments were treated as a normal crown and bridge case; full-arch rubber base impressions were made using the conventional impression technique. The final porcelain-fused-to-metal restorations were constructed and then delivered after few days and cemented permanently using zinc phosphate cement after being checked for shade matching, marginal fitness and occlusion.

2.2.5. Post operative measures and evaluations:

2.2.5.1. Clinical records:

- Peri-implant soft tissue health according to *Mombelli et al 1987* was measured by the following parameters which were recorded for each implant in each patient at 6 months and 12 months post operatively: Modified bleeding index (MBI), Modified plaque index (MPI) and Peri-implant probing depth (PD).

- Implant mobility was tested using the Periotest M (Medizintechnik Gulden, Bensheim, Germany), records were taken immediately post operatively then at three, six and twelve months post operatively.

2.2.5.2. Radiographic evaluation: Stadardized periapical x-rays films were taken immediately after implant insertion, three, six and twelve months post operatively to detect: 1) Marginal bone level. 2) Change in bone density around the implant.

2.2.5.3. Statistical evaluation: Analysis of data was performed using SPSS 17 [®] (Statistical Package for Scientific Studies) for Windows. Description of variables was presented as follows: - Description of quantitative variables was in the form of mean, standard deviation (SD). Data were explored for normality using Kolmogorov-Smirnov test of normality. The results of Kolmogorov-Smirnov test indicated that most of data were normally distributed (parametric data) so parametric tests were used for the comparisons. - Comparison between quantitative variables was carried out by student T-test of two independent samples. As well as paired T-test for dependent samples. Results were expressed in the form P-values. The significance of the results was in the form of P-value that was differentiated into: * Non-significant when P-value > 0.05 *, significant when P-value 0.05 * and highly significant when P-value 0.01.

3. Results:

3.1. Complete soft tissue healing had occurred in all patients without any postoperative inconvenience during the study period.

3.2. Six patients experienced no postoperative pain, five patients had mild pain while only one patient had moderate pain. Minimum need for analgesics was reported for all patients.

[®] SPSS, Inc., Chicago, IL, USA.

3.3. The 24 Maxi Z implants were successfully osseointegrated as revealed by the clinical and radiographic examinations.

3.4. Clinically:

The statistical analysis comparing test and control sites at 6 and 12 months revealed no significant differences between them regarding any of the following clinical parameters : Modified bleeding index, Modified plaque index and Peri-implant probing depth.

Fig. (1): Pre-operative clinical photograph

Fig. (2): PRP gel.

Fig. (3): Manual placement of the implant (test side).

Fig. (4): Clinical photograph showing the implants at both sides immediately after placement.

Immediately post-operative, the mean and standard deviation values of PTMV were -2.23±0.62 at the PRP site and -2.14±0.52 at the control site. There was no statistically significant difference between the means of PTMV at the two sites (P-value = 0.699). *At 3 months follow-up period,* the mean and standard deviation values of PTMV were -2.91±0.62 at PRP site and -2.80±0.60 at the control site. There was no statistical significant difference between the means of PTMV at the two sites (P-value = 0.669). *At 6 months follow-up period,* the mean and standard deviation values of PTMV were -3.51±0.57 at PRP site and -3.44±0.38 at the control site. There was no statistically significant difference between the means of PTMV at the two sites (P-value = 0.740). *At 12 months follow-up period,* the mean and standard deviation values of PTMV were -4.04±0.74 at PRP site and -3.89±0.55 at the control site. There was no statistically significant difference between the means of PTMV at the two sites (P-value = 0.580).
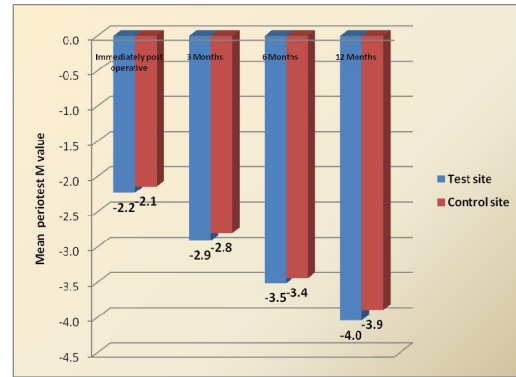
Figure (5): Mean periotest M value of both sites

3.5. Radiographic evaluation:

Results revealed that *at 3 months follow-up period,* the mean and standard deviation values of crestal bone resorption were 0.57±0.29 mm at the PRP site and 0.60±0.30 at the control site. There was no statistically significant difference between the means of 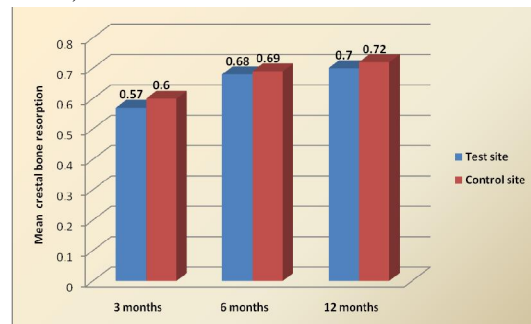crestal bone resorption at the two sites (P-value = 0.839). *At 6 months follow-up period,* the mean and standard deviation values of crestal bone resorption were 0.68±0.29 mm at PRP site and 0.69+_0.30 mm at the control site. There was no statistically significant difference between the means of crestal bone resorption at the two sites (P-value = 0.913). *At 12 months follow-up period,* the means and standard deviation values of crestal bone resorption were 0.70±0.30 mm at PRP site and 0.72±0.30 mm at the control site. There was no statistically significant difference between the means of crestal bone resorption at the two sites (P-value = 0.866).

Figure (6): Mean crestal bone resorption scores of both sites
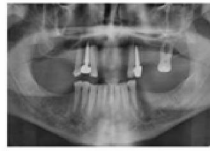
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Fig. (7): Final ceramo metal restoration.

Fig. (8): Immediate post-operative panoramic x-ray.
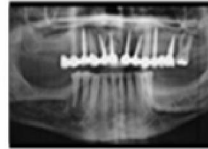
Fig. (9): 6 months post-operative panoramic x-ray.

Fig. (10): 12 months post-operative panoramic radiograph

Immediately post-operative, the mean and standard deviation values of bone density were 88.36±5.02 at the PRP site and 88.42±4.92 at the control site. There was no statistically significant difference between the means of bone density at the two sites (P-value = 0.978). ***At 3 months follow-up period,*** the mean and standard deviation values of bone density were 75.61±4.26 at PRP site and 75.95±4.32 at the control site. There was no statistically significant difference between the means of bone density at the two sites (P-value = 0.847). ***At 6 months follow-up period,*** the mean and standard deviation values of bone density were 78.44±3.76 at the PRP site and 77.35±4.12 at the control site. There was no statistically significant difference between the means of bone density at the two sites (P-value = 0.507). ***At 12 months follow-up period,*** the mean and standard deviation values of bone density were 81.84±4.56 at PRP site and 80.73±4.21 at the control site. There was no statistically significant difference between the means of bone density at the two sites (P-value = 0.544).

Discussion:

In implant dentistry it was proposed according to ***Branemark et al (1985)*** that having successful tissue integration needs a two-stage surgical protocol and an undisturbed healing period of at least three to six months. The rationale for this recommendation was that premature loading may prevent direct bone apposition and may lead to fibrous tissue encapsulation and clinical failure. The immediate

loading protocol is being recognized as an alternative technique to the classical delayed loading approach. Immediate nonfunctional prosthetic restoration which does not seem to affect the process of osseointegration has proven to be more effective ***(Pillar et al(1986); Brunski (1992) ; Nkenke et al(2005).***Immediate loading protocol has obvious advantages for the patient by decreasing the treatment time and reducing the number of surgical interventions. Both function and esthetics can be immediately restored with the temporary restoration ***(Fischer 2008).***
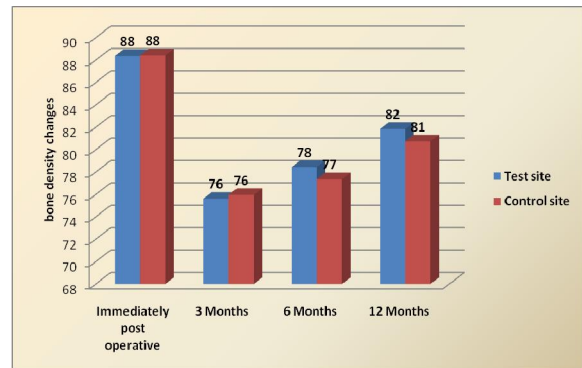

Figure (11): Mean bone density changes of both sites

The drawbacks of the two-piece implants system that was reported by ***Hahn (2005)*** included the structural weakness at the implant abutment junction and the need to remove the healing abutment to replace it with the final one which adds complexity to the procedure and affect the already healed gingival tissues. All these were avoided in the present study by using the one-piece implant. No complications were reported with the use of one-stage surgical technique which corresponds well with other reports on similar treatments as ***Ericsson et al (1994)***.

Platelet-rich plasma (PRP) is an autologous source of high platelet concentrate and native fibrinogen to support bone and soft- tissue healing. It is obtained by sequestering and concentrating platelets using gradient density centrifugation. A blood clot is the centre focus of initiating any soft tissue healing and bone regeneration. In all natural wound, a blood clot forms and starts the healing process. PRP is a simple strategy to concentrate platelets or enrich natural blood clot which forms in normal surgical wounds to initiate a more rapid and complete healing process

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Raja and Naidu (2008). Activation of the PRP is done by mixing it with thrombin and calcium chloride which results in the release of a cascade of growth factors from the platelet alpha granules. The growth factors are a diverse group of polypeptides that have important roles in the regulation of growth and development of a variety of tissues. They seem to signal the local mesenchymal and epithelial cells to migrate, divide and increase collagen and matrix formation (*Kim et al 2002 ; Sanchez et al 2003)*.

The present study was conducted to evaluate the effect of injecting platelet-rich plasma into the prepared drill holes "immediately before implant placement" on accelerating the rate of osseointegration or   reducing the crestal bone resorption in immediately loaded dental implants placed in healed bony sites (posterior maxillary area). Twenty four implants were placed in twelve patients. Each patient received two implants (the test side with PRP and the control without) immediately loaded with a non-functional fixed temporary restoration for 6 months and left to integrate with the surrounding bone then replaced with Porcelain Fused to Metal final restorations. All the implants were successfully osseointegrated over the twelve months follow-up period with a success rate of 100%. Implants used in this study were Maxi Z one-piece (Osteo Care implant system, London, United Kingdom) self tapping, self drilling tapered dental implants with GBA (Grit-Blasted and Acid-etched) surface treatment to create macro and micro irregularities to enhance and   fasten the process of osseointegration and to improve the bone to implant contact. Also the implant is presented with a unique "Buttress" thread design that allows a maximum bone to implant contact to achieve higher initial stability even in poor bone quality. The Maxi Z one-piece implant is designed to allow for immediate loading in healed bone sites as well as in immediate post extraction cases. It is placed in under-sized osteotomy and modifies the quality of bone by condensation thus enhancing the process of osseointegration *(Zahran et al 2010).*

In the present study Soft tissue healing was generally uneventful in all patients included in this study. At six and twelve months follow-up period none of the patients suffered from pain or peri-implant infection, all implants were found to be successfully osseointegrated without any signs of peri-implantitis. Clinical parameters were measured at 6 months and 12 months at both sides in each patient. There was no statistically significant difference between means of (MBI), (MPI) and (PD) at test and control sides. The mobility of all implants in this study was measured using the Periotest M. It proved to be a valuable gadget for the numerical evaluation of the initial stability of the implants at the time of insertion as well as for assessment of osseointegration at the follow-up times. It could replace the torque wrench as a tool of measuring the initial stability which is not available in the surgical kits of many implant systems. The Periotest M gave more precise readings than the classical Periotest as it measures in a decimal number. This apparatus is widely used to assess implant outcome and provides a measurement of the implant reaction to a defined impact load which is used for the assessment of primary stability, *(Tricia et al 1995;Fischer 2008 ; Alsaadi 2008).* During the study period none of the implants showed any signs of clinical mobility and Periotest measurements were taken immediately after placement (baseline) and then at 3, 6 and 12 months postoperatively. Periotest mean values for the test sides (PRP) were immediately post-operatively -2.23, -2.91 at 3 months, 3.51 at 6 months and -4.04 at 12 months. At the control sides the Periotest mean values were -2.14 immediately post-operative, -2.80 at 3 months, -3.44 at 6 months and -3.89 at 12 months. There was no significant difference between the mean Periotest values for both the test and control sides at the base line, 3 months, 6 months and at 12 months follow up period.

The radiographic parameters in this study included measuring the crestal bone loss and bone density changes around the implants. Regarding the bone loss, on initial examination at the third month postoperatively (baseline readings) the mean radiographic bone loss for the test sites was 0.57 then at the sixth month 0.68 and at the twelfth month 0.70. While in the control sites the mean radiographic bone loss at the third month postoperatively was 0.60 and then at the sixth month 0.69 and at the twelfth month the mean radiographic bone loss was 0.72. There was no statistically significant difference between mean crestal bone resorption scores around implants in both test and control sides at 3 months or at 6 and 12 months postoperatively. However, the increase in the crestal bone resorption from the baseline till twelve months in both the test and control sides of all implants throughout the study was considered normal and met with the results of many studies done by *Drago and Lazzara (2004) ;and Jaffin et al (2007); Andersen et al(2002)* reported 100% success of immediately loaded implants with mean crestal bone level 0.53 mm over 12 months period. The results of

this study also coincide with the results of *Thor et al (2005)* as they found no significant difference in the marginal bone level measurements and clinical function of dental implants after one year of loading their implants in the maxilla whether or not PRP is added. An overall survival rates of 98.7% was obtained in this study. According to *Olsson et al (1995)* the self-tapping implant is a design modification that is specifically designed to be used in poor bone quality (as the posterior maxillary area). When the self-tapping implant is inserted the denser cortical bone is compressed leading to the increase in primary stability. Primary stability is a prerequisite and indicator for having adequate osseointegration *(Adell et al 1981; Meredith 1998 ;Friberg et al 1999a)*.

In the current study, all implants attained high initial stability over 30N/cm which may be due to their tapered design, buttress threads and under-dimensioned drilling. It was reported by *O'Sullivan et al(2000) ; Sakoh et al (2006)* that tapered implant design in combination with undersized drilling could lead to higher initial stability than conventional implants. *Monov et al (2005)* concluded that if the primary stability is high, it seems that the healing process has only little influence on future implant stability, so even the instillation of PRP into the drill holes of the fixtures will not show any additional effect. This was in agreement with our present study as there was no significant difference in the bone densities changes around the implants at both sides (the test and the control sides). Immediately post-operatively the mean bone density measurement around implants at the test sites measured 88.36 then at three months post-operatively it was 75.61 then at six months post-operatively it was 78.44 and finally at twelve months post-operatively it was 81.84. At the same time the mean bone density measurement around implants at the control sites measured 88.42 immediately postoperatively, then it was 75.95 at three months postoperatively. At six months postoperatively the mean bone density measured 77.35 and finally at twelve months postoperatively it was 80.73. The addition of PRP into the drill holes immediately before implant placement did not fasten the rate of osseointegration during the first three months period. At the same time the level of crestal bone resorption was the same in the test and control sides.These results were in agreement with *Froum et al (2002) ; Thor et al(2005)* as they evaluated the efficacy of platelet-rich plasma on bone growth and osseointegration and found no positive effect after adding PRP.

On the other hand, the results of our study were in contrast to the finding of *Lynch et al (1991) ; Zechner et al (2003)* who found that the growth factors presented in PRP have a statistically significant positive effect on bone regeneration and implant-to-bone contacts. Also the clinical studies by *Marx et al (1998); Anitua (1999) ;Sykaras et al (2001)* have shown that the local application of PRP increased the amount of peri-implant newly formed bone and its density.The same positive effect of using PRP occurred in a clinical study by *Lee et al (2008)* using immediate nonfunctional loading implants in the maxilla during a 52-weeks period. There high implant survival rate 77% to 100% was due to the early formation of large percentages of new vital bone with the help of PRP addition as confirmed by using histologic and histo-morphometric analysis. The appropriate concentration of platelets in PRP is an important issue that must be analyzed. The platelet counts of PRP in the present study were in the expected range and correspond to the values described by *Weibrich et al (2004)* who added- PRP in peri-implant bone defects and concluded that a concentration of 1 million platelets/ ML is the most productive concentration for bone healing. This concentration was confirmed by other studies who found that the most regenerative concentration of platelets in PRP in both humans and animals is 1 million platelets/ ML and a viable platelet concentrate levels of 400% to 600% above baseline is necessary to achieve optimal bone and soft tissue healing, *(Marx et al 2002; Kim et al 2002b).* In the current study, the mean donor platelet counts from whole blood had a value of 224.000-+ 35.653 / ML, this value was in comparable with the mean value of 260.300 / ML from the *Weibrich et al (2002)* study. Platelet counts in the PRPs had a mean value of 1025.000 -+ 58.547/ ML, which is comparable with the mean value of 908.500/ML found by the same study. At the same time the mean platelet count increased in this study by 4.58 folds after centrifugation which was within the range obtained by other several studies *(Marx 2001; Marx et al 2002 ; Rodriguez et al 2003).* It was found that if the platelet concentration in the PRP is lower than 4-6 folds of the baseline, the effect of PRP is suboptimal, while higher concentration might have inhibitory effect *(Floege et al 1991 ; Pollard 2001).* However, recently *Smith et al (2009)* found that using the triple spin technique to gain platelets concentrate of 23 times the baseline is more effective to increase the bone height and quality for optimum endosseous implant placement.

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The explanation for our results and for the absence of significant changes in bone density or crestal bone resorption after the addition of PRP may be attributed to the simultaneous effect of bone trabeclua compression that occurred around both the test and control sides   implants with the use of self-tapping, self-drilling implants or due to the fact concluded by *Nazaroglou et al (2009)* who found it truly difficult to predict the final concentration of platelets into the surgical region because of the bleeding from the bone. Bleeding from the gap might dilute the PRP and consequently the actual concentration of platelets would be less than what was expected. Furthermore, as the regenerative potency of PRP undoubtedly depends on its growth factors levels, it must be taken into consideration that the appropriate GF levels are not known yet. Recent studies observed differences in GF levels even in PRP samples with the same concentration of platelets, *(Lartineau et al 2004 ; Frechette et al 2005).* At the same time, the addition of PRP might have positive effect in bone with compromised healing capacity as in patients with diabetes or osteoporosis *,Schaaf et al (2008)*. The strict case selection and using simple not complicated cases might hide the benefit effect from adding PRP. Another characteristic of PRP is the influential time in which its growth factors would exert their effect. Some scientists found that this influential time is up to 2 months, while others concluded that the effect of PRP lasts only for a few days. Taking into consideration that the main part of osteogenesis takes place in the first month after surgical procedure and bone is removed and formed with more fast rate for the first 6-7 months, it is obvious that if the effect of PRP can be prolonged, its use can be considered as serviceable. Reversely, if PRP action lasts for only a few days, it can be concluded that this technique is not as beneficial as many believe *(Marx et al 1998; Butterfield et al 2005;Raghoebar et al 2005; Gerard et al 2006 ;Thor et al 2007).* The potential of PRP to promote soft and hard tissue healing is accepted without doubt due to the biological characteristics of the released growth factors and their effect on human tissues (haemostasis, creation of new vascularity, production of collagen matrix, migration, proliferation and differentiation of epithelial and osteoproductive cells) *,Schaaf et al (2007).* However, it is obvious that more studies are needed on the physical, biological and biochemical features of platelets, optimal concentration of PRP and growth factors levels, interactions between the growth factors and to find the actual duration of PRP's effect which simultaneously indicates the duration of the regenerative potency of platelet's growth factors necessary to enhance bone formation around endosseous dental implants.

Conclusion

Within its limits, the present study has shown that the local application of autologous platelet-rich plasma into the prepared drill holes immediately before implant placement didn't accelerate the rate of osseointegration or decrease the crestal bone resorption "through first three months period" in immediately loaded dental implant placed in posterior maxillary area.

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Adjectival Phrases as the Sentiment Carriers in the Urdu Text

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Abstract. In this paper we present a comprehensive overview of the structures of the adjectival phrases in the Urdu language with respect to the task of sentiment analysis. Urdu is a widely spoken but one of the least explored languages by the computational linguistics community. After a detailed analysis of adjectival phrases in Urdu text we conclude that this language is orthographically, morphologically and grammatically different from other well established languages, like English and hence, it requires updated or different approaches and algorithms for the task of sentiment analysis. We present our approach in which the adjectival phrases are combined with polarity shifters, and conjunctions to make sentiment expressions in the opinionated sentences. We label these sentiment expressions as the SentiUnits. We apply shallow parsing based chunking to extract the SentiUnits. The overall polarity of a sentence in a given review can be determined by computing the polarity of these expressions. Adjectives are the head words, which appear with modifiers and postpositions. The experimentation based evaluation of the model with a sentiment-annotated lexicon of Urdu words and two corpuses of reviews as test-beds, shows encouraging achievement in terms of sentimental analysis and accuracy.
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1. INTRODUCTION

An adjective is a fundamental part of speech (POS) that expresses an attribute of a noun (place, thing or, person). Generally in the sentence structure adjectives appear in two ways, whether they are directly linked with the noun within the noun phrase or they associate with the noun through some other part of speech, e.g., verb. In both cases they describe the characteristic features of the noun they qualify. This point suggests that any opinion, sentiment, or judgment about a noun can be determined by analyzing its adjectives. Due to this characteristic the first effort for the automatic sentiment analysis (SA) of the English text employ adjectives as the main feature of the given text (Hatzivassiloglou & McKeown, 1993). Therefore, in sentiment analysis community, adjectives remain center of attention (Turney, 2002), (Riloff et al., 2003), (Riloff & Wiebe, 2003) and (Bloom & Argamon, 2010).

As with all parts of speech, in every language the use, type, and structure of the adjectives differ. Urdu is morphologically rich and hence its adjectives and adjectival phrases tend to be more complex, due to the frequent inflections and derivations. In addition to the morphological complexity the variability in vocabulary and grammar rules in Urdu text is regular and is considered normal. This is due to the fact that this language is strongly influenced by many other languages like, Persian, Arabic, Sanskrit and English. For example, the adjective "   " (tazah, fresh) remain unmarked because it is Persian loan word and follow Persian grammar, whereas, most of the Sanskrit based adjectives show inflection to agree with the noun they qualify. For example, the demonstrative adjective "   " (jaisa, such as), becomes "   " (jaisee, such as) and "   " (jaisay, such as) for gender and number, respectively. Moreover, the use of post positions as independent lexemes involves more specific patterns and rules.

These aspects suggest that Urdu have distinct characteristics and features. Particularly, it is far more different from the well established languages in the field of sentiment analysis or other NLP applications. The SA research community requires a complete understanding of the computational as well as linguistic aspects of the language. We therefore, present in this paper a comprehensive overview of the structures of the adjectival phrases in the Urdu text with respect to the task of sentiment analysis. For Urdu based NLP research this is the very first effort. So far, syntactic and morphological aspects of the language are considered related to verbs, nouns and other parts of speech (Muaz et al., 2009), (Riaz, 2007), and (Durrani & Hussain, 2010). There is no endeavor about analyzing the sentiments in the given text. Also,

we find no contribution which investigates Urdu adjectival phrases independently.

The given analysis covers almost all the aspects of adjectives and adjectival phrases. We describe their morphological structures, as marked and unmarked through the types of the agreement with the noun they qualify. This agreement is more frequent for gender, number, and case. Also, we discuss their structure when used with a sequence of nouns and for the formations of reduplications. Moreover, we define and illustrate with examples different adjective classes, i.e., descriptive, predicative, attributive, possessive, demonstrative, and reflexive possessive. For each class we describe the morphological structure of the adjectives and their inflected forms. We take most commonly used adjectives as examples and clearly describe their modifications.

Given an inclusive overview of the adjectival phrases, we present our proposal for the sentiment analysis of Urdu text. We use a grammatically motivated approach, which employs a sentiment-annotated lexicon. In this scheme, adjectives and adjectival phrases are combined with polarity shifters, and conjunctions to make sentiment expressions in the opinionated sentences. We label these sentiment expressions as, SentiUnits (Syed et al. 2010). For the identification and extraction of the SentiUnits from the given review we use shallow parsing based chunking. The classification algorithm works in combination with a sentiment-annotated lexicon of Urdu words. We evaluate the system using two corpuses of reviews from the domains of movies and electronic appliances.

The rest of the paper proceeds as follows: Section 2 gives a comprehensive overview of Urdu adjectival phrases in terms of morphology, classes and sentence structure. Section 3 describes the SentiUnits based sentiment classification model. Section 4 briefly presents the state of the art research in the field of sentiment analysis. Section 5 gives our experimentation and its results. Section 6 concludes our effort and suggests potential future endeavors.

2. URDU ADJECTIVAL PHRASES

In linguistics, for understanding the parts of speech (POS) of a language, we need to recognize their morphological structures and the processes through which these structures are made. Another significant aspect is to look at their different forms or classes. Therefore, we explore in this Section these two features of Urdu adjectival phrases. We first describe their morphological structures and then the classes.

2.1. Morphological Structure of Adjectives

The morphological structure of the Urdu adjectives is complex and exhibit frequent inflections and derivations with the agreement of the noun they qualify. Morphologically Urdu adjectives are categorized as unmarked and marked (Schmidt, 2000), (Omkar, 2008).

Unmarked adjectives: The unmarked adjectives do not show any inflection according to the nouns they qualify. In other words, they do not alter to show agreement with nouns through suffixes. Most of the Persian loan adjectives remain unmarked. For example the unmarked adjective; "   " (*dilchasp,* interesting) remains unmarked with the nouns *(a)* masculine-singular, " " (*kaam*, task) as "   " (*dilchasp kaam*, interesting task), *(b)* feminine-singular, "   " (*khani*, story) as "   " (*dilchasp khani*, interesting story) and (c) feminine-plural "   " (*khanian*, stories) as "   " (*dilchasp khaniian*, interesting stories).

Adjective marking: agreement in gender and number: The adjective marking is done through the suffixes for gender; masculine *(m)* and feminine *(f)* and for number; singular *(s)* and plural *(p)*. For example, the masculine adjective, "   " (*acha*, good) is inflected for gender as "   " (*achi*, good) and for number as "   " (*achay*, good). These suffixes are attached to agree with the noun or nouns, which the adjective qualifies. Therefore, there are three suffixes, i.e., singular-masculine *(a)*, singular-feminine *(ee)* and plural-masculine *(ay)*. Only one feminine suffix *(ee)* is used for singular and plural both.

Some examples of marked adjectives are given in Table 1, in this table, we have considered three nouns; *(a)* masculine-singular, "   " (*bacha*, kid), *(b)* feminine-singular, "   " (*car*, car) and masculine-plural " " (*din*, days). These noun cause inflection in the respective adjectives; "   " (*acha*, good), "   " (*lamba*, long), and "   " (*bura*, bad).

Table 1. Adjective marking with gender and number

Adjective *(m, s)*	For gender *(f)*	For number *(m, p)*
(*acha bacha*, good kid)	(*achee car*, good car)	(*achay din*, good days)
(*lamba bacha*, tall kid)	(*lambee car*, long car)	(*lambay din*, long days)
(*bura bacha*, bad kid)	(buree *car*, bad car)	(*buray din*, bad days)

Agreement in case: Urdu nouns have three cases; oblique, nominative and vocative (Schmidt, 2000). All these cases cause an inflection in the adjectives. It means that the adjectives that qualify an oblique noun also become oblique. The masculine-singular suffixes *(a)* and *(an)* are replaced by, *(ay)* and *(ayn)*, respectively. The feminine adjectives remain the same as shown in Table 2. The adjectives " " (*chota*, little) and " " (*satwan*, seventh) inflect with case.

Table 2. Adjective marking with gender and number

	Masculine	**Feminine**
Nominative	(*chota*) (*satwan*)	(*chotee*) (*satween*)
Oblique	(*chotay*) (*satwayn*)	(*chotee*) (*satween*)
Vocative	(*chotay*) (*satwayn*)	(*chotee*) (*satween*)

Adjectives with noun sequences: Some times adjectives appear in a sentence with more than one noun or multiple nouns making a sequence. In this case, the nouns may differ in gender and number. The adjective agrees with the noun, which is nearest to it. Examples are given in Table 3, in which, " " (*bara*, big) inflects for " " (*palang*, bed) and " " (*choti,* younger) inflects for " " (*khala,* aunt).

Table 3. Adjective agrees with the nearest noun

Adjective	With the sequence of nouns
(*bara*, big)	(*bara palang aur almarian*, big bed and cupboards)
(*choti,*younger)	(*choti khala, mamoon aur bachay,* younger aunt, uncle and kids)

Table 4. Adjective with partial and full reduplication

Partial Reduplication	Full Reduplication
(*dheela dhala libas,* loose dress)	(*baray baray kaam,* great tasks)
(*choti moti baat,* minute matter)	(*choti choti batain,* minute matters)

Reduplication of Adjectives: Urdu adjectives show reduplication either fully or partially. In full reduplication the whole word is repeated as it is, whereas, in partial reduplication some syllables of the word are reduplicated with different spellings. Examples of full and partial reduplication are given in Table 4.

2.2. Classes of Adjective

Urdu adjectives can be categorized as descriptive, predicative, attributive, possessive, demonstrative, and reflexive possessive, explained in following paragraphs:

Descriptive Adjectives: These are the most frequent and important type of adjectives. They describe attributes of the noun they qualify in terms of its size, dimensions, sound, color, shade, shape, quality, personal trait, or time, etc. Some examples of descriptive adjectives in Urdu are given in Table 5, where, " " (*chota*, little) and " " (*lamba*, long) describe the size of a noun, and " " (*peela*, yellow) and " " (*surkh*, red) express the color.

Table 5. Descriptive adjectives in Urdu

Category	Examples
Size	(*chota*, little) (*lamba*, long)
Color	(*peela*, yellow) (*surkh*, red)
Shape	(*muraba*, square) (*tikona*, triangular)
Personal trait	(*udaas*, sad) (*majboor*, helpless)
Qualities	(*mehrbaan*, kind) (*acha*, good)

Attributive Adjectives: If the descriptive adjectives directly precede a nominal head as modifiers then they are called attributive adjectives, because, they attributively modify or restrict the meaning of the noun. For example, the adjective " " (*peela*, yellow) modify the noun " " (*ghubara*, balloon), to make it " " (*peela ghubara*, yellow balloon). In this way the attributive adjective becomes part of the noun phrase. Some more examples are given in Table 6.

Table 6. Attributive adjectives modify the nouns

Nouns	Modified attributively
(*ghubara*, balloon)	(*laal ghubara*, red balloon)
(*chiria*, sparrow)	(*udaas chiria*, sad sparrow)
(*badshah*, king)	(*naik badshah*, kind king)

Predicative Adjectives: When the adjectives are used predicatively, they bring in new information about the noun instead of modifying it. These are not the component of the noun phrase, but are the complements of a copulative function, which links them to the noun, e.g., in Table 7, " " (*ghubara laal hay*, the balloon is red). In this case, the adjective " " (*laal*, red) identify the color of the noun " "(*ghubara*, balloon). Only a specific feature of the noun is described both parts of speech, i.e., adjective and noun remain in their individual role. Some more examples are given in Table 7.

Table 7. Examples of predicative adjectives

Nouns	With predicative adjectives
(*ghubara*, balloon)	(*ghubara laal hay*, balloon is red)
(*chiria*, sparrow)	(*chiria udaas thee*, sparrow was sad)
(*badshah*, king)	(*badshah naik hay*, king is kind)

Possessive Adjective: Possessive adjectives are used to indicate the possession. This possession relation is realized in two ways; whether, adjectives precede the head noun as modifiers in noun phrases like the attributive adjectives or they may be preceded by a suitable form of the genitive postposition " " (ka, of), " " (kee, of), and " " (kay, of). These genitive postpositions are lexically independent like "*of*" in English, but they agree in number and gender with the object noun. Consider the first example from Table 8, " " (*Irtaza ka peela ghubara*, Itraza's yellow balloon). In this example the genitive postposition " " (ka, of) is used with a singular masculine noun, i.e., " " (*peela ghubara*, yellow balloon). In the second example, " " (*meri*, my) is a possessive adjective which is used for the first person and in this case is inflected for gender.

Third example also contains the genitive postposition " " (ka, of) with a singular masculine noun.

Table 8. Examples of possessive adjectives

Examples
(*Irtaza ka peela ghubara*, Itraza's yellow balloon)
(*meri udaas chiria*, my sad sparrow)
(*Iran ka mehrbaan badshah*, kind king of Persia)

Demonstrative Adjective: The demonstrative pronouns act as the adjectives to indicate or demonstrate the specific inherent features of noun/ nouns of a particular type. As shown in Table 9, the Urdu demonstrative pronouns are different for *near* " " (*aisa,* like this), *far* " " (*waisa*, like that), *relative* " " (*jaisa*, such as) and *interrogative* " " (*kaisa*, how) demonstrations.

Table 9. Examples of demonstrative adjectives

Adjectives	Examples
(*aisa,* like this)	(*aisa libas,* dress like this)
(*waisa*, like that)	(*wasisa libas*, dress like that)
(*jaisa*, such as)	(*jaisa libas*, such dress)
(*kaisa*, how)	(*kaisa libas*, what kind of dress)

Reflexive possessive adjective: The reflexive possessive adjectives are very frequently used in agreement with the noun they qualify, i.e., they inflect for gender, number and case. For example, " " (*apna*, own), " " (*uska*, someone else's) and " " (*iska*, someone else's) are used to indicate *one's own*, *someone else's far*, and *someone else's near*. The examples of the reflexive possessive adjective " " (*apna*, own) are given in Table 10, it is inflected for gender as " " (*apni chabee*, one's own key) and for number as " " (*apnay loag*, one's own people).

Table 10. Examples of reflexive possessive adjectives

Nouns	With predicative adjectives
(*ghar*, house)	(apna *ghar*, one's own house)
(*chabee*, kay)	(apni *chabee*, one's own key)
(*loag*, people)	(apnay *loag*, one's own people)

Given this analysis we conclude that Urdu adjectival phrases are morphologically complex. In Section 2.1, we have discussed both marked and unmarked adjectives, which are borrowed from many languages, like Persian, Arabic, Hindi, Sanskrit, and English. This diversity results into flexibility and variety in the morphological and grammatical rules. For example, the adjectives which are Persian loan follow Persian grammar and usually remain unmarked, likewise, the Sanskrit based adjectives show inflections for gender and number, etc.

Similarly, some other linguistic phenomena are specific to Urdu language, e.g., frequent reduplication (partial as well as full) of adjectives, their inflection rules when used with a sequence of nouns. Almost all types of adjectives, descriptive, attributive, predicative, demonstrative, etc. show agreement in case, gender and number with the noun they qualify.

This linguistic behavior entails the need for the exact and well defined Urdu language specific grammar rules for the appropriate implementation and hence for getting better accuracy levels. We therefore, present a grammatically motivated approach for sentiment analysis of Urdu text.

3. SENTIMENT CLASSIFICATION USING ADJECTIVES AS HEAD WORDS

In our approach for sentiment classification based on adjectival phrases, we emphasize on the identification and extraction of the subjective expressions from the given text and coin a term "SentiUnits" for such expressions (Syed et al. 2010). The SentiUnits are the expressions made by single or multiple words, which are solely responsible for the whole sentimental orientation of a subjective sentence, i.e., a comment or an opinion. Our model is grammatically motivated and uses a sentiment-annotated lexicon for the identification of such expressions.

3.1. Examples of Adjective Based Subjective Expressions:

We take some examples of different types of adjectives discussed in Section 2, and discuss them in terms of SentiUnits, see Table 11. In the first sentence, a single predicative adjective " " (*umdah*, good) represents the SentiUnit. The other possessive adjective " " (*naee*, new) in the sentence is a part of the noun phrase. In sentence *b)* two predicative adjectives along with a conjunction in between, " " (*thanda aur badmazah*, cold and tasteless) are recognized as the SentiUnit. In this case, the presence of conjunction " " (*aur*, and) augment the negative orientation of the sentence. The sentence *c)* expresses a strong positive orientation by a single attributive adjective in superlative structure " " (*sab se umdah*, the finest). The positive adjective in the last sentence *d)*, " " (*khoosh-rang*, of good color) joins with a polarity shifter " " (*naheen*, not) to make a SentiUnit with negative polarity.

Table 11. Examples of sentences from Urdu

Example sentence	
- ____ (*tmhari naee kitab aur qalam umdah hain,* Your new book and the pen are good.)	(a
- ____ (*khana thanda aur badmazah hay,* The meal is cold and tasteless.)	(b
- ____ (*yeh sab se umdah novel hay,* This is the finest novel.)	(c
- ____ (*libaas khoosh-rang naheen hay,* The dress is not of good color.)	(d

a) Predicative adjective with sequence of nouns. Possessive adjective as part of noun phrase, *(positive)*
b) Multiple predicative adjectives with conjunction, *(negative)*
c) Single attributive adjective in superlative structure *(positive)*
d) Single predicative adjective with polarity shifter *(positive to negative)*

In most cases, the polarity shifters are the negation particles. In Urdu, both sentential and constituent negations exist (Omkar, 2008). The sentential negations are represented by the particles " " (*mat*, don't), " " (*na*, no) and " " (*naheen*, not). Some frequently used constituent negations are " " (*naheen*, not), " " (*bina*, without), and " " (*baghair*, without).

3.2. The Sentiment Analyzer Diagram:

Figure 1 shows the classification process of a given review. Each sentence in the review is analyzed one by one and for each sentence the polarity is calculated independently. Then these sentence polarities are combined to give review polarity.
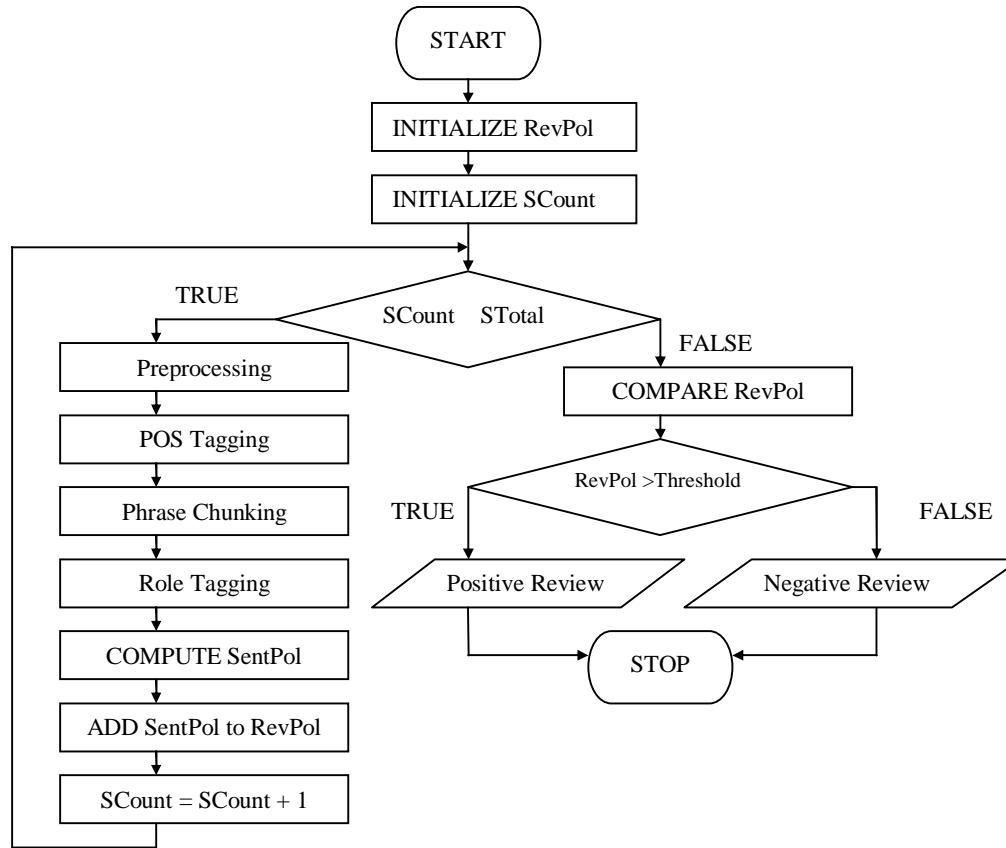

Figure 1. Sentiment classification of a review as positive or negative.

The algorithm initializes the review polarity *RevPol* and sentence count *SCount*. The sentiment analysis begins with the preprocessing of the given text. This step includes normalization, tokenization and finally the word segmentation. Urdu uses context sensitive script and therefore the tokenization and word boundary identification are handled separately (Syed et al. 2010). The preprocessed words are then assigned parts of speech tags, e.g., nouns, verbs, adjectives, conjunctions, and negations etc. These tagged words are converted into phrases by phrase chunking. Consequently, we get noun phrases, verb phrases, and adjective phrases etc.

The algorithm takes the adjectives as the head words and looks for the polarity shifters, and conjunctions to assemble them as SentiUnits in the opinionated sentences. As a result role tagging is done and the complete SentiUnits with attached polarity shifters and conjunctions are identified. The algorithm compares and then computes the polarity values for each SentiUnit, whereas, the sentence polarity *SentPol* is computed by the total polarities of its constituent SentiUnits. To calculate the review polarity *RevPol*, all the known sentence polarities *SentPol* are added and compared to the threshold value. If the result is greater than threshold then, the review is positive and vise versa.

3.3. Example:

As shown in table 12, we take an example of Urdu sentence and apply shallow parsing based chunking to extract the SentiUnits. As the Figure 1, depicts, this extraction is achieved in three steps, *a)* assign parts of speech tags to all preprocessed words, *b)* identify phrases through phrase chunking, and *c)* extract SentiUnits through role tagging. The given sentence contains a complex SentiUnit made by a positive descriptive/predicative adjective and a polarity shifter.

"-   "

(*Irtaza aur fatima ka skool kushadah naheen,* Irtaza and fatima's school is not spacious)

Table 12. Shallow parsing based chunking of the given sentence.

Parse of the complete sentence	[N CJC N PM N] [ADJ NEG] à NP SU	
Parse of the Noun phrase with possession marker (PM) and conjunction (CJC)	N CJC N PM N à NP	
Parse of the SentiUnit with negation (NEG)	ADJ NEG à SU (SentiUnit)	

The SentiUnit " " (*kushadah naheen,* not spacious) contains an adjective head and a negation word. The noun phrase in the opinion is even more complex, i.e., " " (*Irtaza aur fatima ka school,* Irtaza and fatima's school).

We give the adjectives based sentiment classification analyzer in Figure 1, the system identify and extract the adjective based subjective expressions called SentiUnits and on the basis of their polarity it classy each sentence one by one. The final classification of the review is an accumulation of all computed scores. The working of the analyzer along with an example clearly explains our approach. Next, we evaluate this approach using a lexicon and two corpora of the reviews.

4. SENTIMENT ANALYSIS RESEARCH

The part of speech based features of the given text, particularly of adjectives, can help a lot in sentiment analysis. That is why one of the earliest works in this domain (Hatzivassiloglou & McKeown, 1997) uses adjectives as subjectivity indicators. They employ a log-linear regression model for identification and validation of the positive or negative semantic orientation of the conjoined adjectives. A clustering algorithm divides the adjectives into groups with respect to orientations, and labels them as positive or negative. Before that (Hatzivassiloglou & McKeown, 1993), present an approach for automatic recognition of adjectival scales this approach group or cluster the adjectives carrying same semantics, but this was not with the perspective of sentiment analysis. (Bruce & Wiebe, 2000) recognize subjectivity within the text by manual tagging. They take a case study of sentence level categorization and categorize clauses from the "Wall Street Journal" as objective or subjective. Each clause is given a final classification on the basis of an agreed decision by four judges.

(Hatzivassiloglou & Wiebe, 2000) analyze two main features of adjectives for subjectivity prediction, i.e., gradability and semantic orientation. They extract reliability of

gradability values using an automatic method for extracting. (Turney, 2002), suggest that the proverbs are also carriers of sentiments in a sentence and should be considered in combination with adjectives. In their work, the sentences are divided into pre-structured grammatical patterns, which include adjectives and adverbs as the core word. (Riloff et al., 2003) emphasize on the identification of the subjective nouns, which are modified by the use of adjectives. They compute the orientation of the phrases in the sentence that contained them. (Riloff & Wiebe, 2003), use unsupervised learning method for automatic extraction and learning of the patterns for subjective expressions in the given text.

(Whitelaw et al., 2005) propose the use of appraisal theory for sentiment analysis. They work on appraisal expressions extraction. These appraisal expressions are the sentiment oriented phrases which contain adjectives as head words. (Bloom & Argamon, 2010) extended this model and propose an approach for automatic learning of these appraisal expressions. Research contributions related to adjective based sentiment analysis are shown in Table 13.

Table 13. Research contributions related to adjective based sentiment analysis

Feature	Example Contributions
Adjectives	Hatzivassiloglou, McKeown (1993)
	Hatzivassiloglou, McKeown (1997)
	Bruce and Wiebe (2000)
	Hatzivassiloglou, Wiebe (2000)
Adjectives and proverbs	Turney (2002)
Subjective Nouns	Riloff et al. 2003
Expressions	Riloff and Wiebe (2003) Whitelaw et al. (2005) Bloom and Argamon (2010)

5. EXPERIMENTATION

The performance evaluation of the NLP based classifiers is done through experimentation. Experiments are performed with the help of lexicons applied on the test-beds (corpuses). In sentiment classification, the domain specific lexicons and corpuses exhibit variations in results. Thus, the task of evaluation of the sentiment classifier itself becomes a major concern. Our lexicon and corpuses are briefly described below:

5.1. Sentiment- Annotated Lexicon and Corpus:

Lexicon construction with appropriate coverage is a challenging and time consuming task. There are a number of efforts (Hatzivassiloglou & Wiebe (2000), Turney (2002), (Riloff et al., 2003) and (Higashinaka et al., 2007) which have tried to develop algorithms and techniques for automatic lexicon construction using unsupervised learning methods. Some other contributions have tried to use or extend the existing lexicons, e.g. the extension of WordNet is SentiWordNet. (Hu & Liu, 2005), (Andreevskaia & Bergler, 2005), and (Annett & Kondrak (2008) use WordNet or its extension for sentiment analysis.

Most of these efforts are for English language and use already prepared linguistic recourses like corpuses for automatic extraction of required lexicons. Therefore, for English language this facet of research is no more an unresolved issue. But for a recourse poor language (Muscand & Ghosh, 2010) like Urdu this task poses many challenges. Therefore, for experimentation we use our manually constructed lexicon of Urdu words. All the entries are marked with sentiment polarity values.

We have collected two corpuses as the test-beds from the domains of movies and electronic appliances. The movie reviews based corpus *C1* contains 450 reviews, among which 226 are positive and 224 are negative. For obtaining diversity in opinions we present 20 different movies to the reviewers, from three categories, i.e., action, comedy, and horror. The corpus containing reviews of electronic appliances *C2* includes 328 reviews, among which 177 are positive and 151 are negative. The electronic appliances presented for review are refrigerators, air-conditioners and televisions, taken from three different brands.

5.2. Results

For result generation we have applied accuracy *A* as the classification performance metric. Two experiments are performed on corpora *C1* and *C2*. We also analyze the behaviors of positive and negative reviews separately. Table 14, shows the results, with accuracy of 66-74% for *C1* and 77-79% for *C2*.

Table 14. Experimental results in terms of accuracy

Orientation	Corpora	A
Negative	*C1*	66%
	C2	77%
Positive	*C1*	74%
	C2	79%

Comparison between the results from both corpora is given in Table 15. The total accuracy of the model is 74%.

Table 15. Comparison of accuracy from both corpora *C1* and *C2*

	Accuracy				
C1 Neg	66%	8%	70%		
Pos	74%			8%	74%
C2 Neg	77%	2%	78%		
Pos	79%				

From the above results it is clear that the accuracy of a sentiment classifier is domain dependent. The movie reviews show lower accuracy (accuracy difference = 8%) than the electronic appliances. This variation is due to the difference in the complexity levels of both domains. This is noted that the appliances reviews are simpler and to the point as compared to those of movies. In movie reviews the target of opinion or comment is not always the movie but it can be the characters, story, or the direction etc. Another observation from the results is that the rate of misclassification for negative reviews is more as compared to positive. The main reason for this misclassification is the use of polarity shifters which sometimes can cause erroneous results.

6. CONCLUSION

This research work gives a comprehensive analysis of morphology, grammar and structure of Urdu adjectives and adjectival phrases, and concludes that these are morphologically complex and follow flexible grammar rules due to the extendable vocabulary of the language. We take more perceptible features of adjectival phrases and then write the rules for accurate extraction of these phrases. These features include, sentence structures containing these phrases, their position with respect to nouns, use of postpositions, morphological changes, reduplication, etc. As the next step we

extract the SentiUnits which, are made by adjectives/ adjectival phrases, conjunctions and polarity shifters. This extraction is achieved by implementing shallow parsing based chunking. Despite of inherent complexities of the language we achieve excellent results of this effort (74%).

Our future focus is on the same SentiUnits based approach. We plan to attach these expressions with the candidate targets. These targets are the noun phrases. For this purpose we need to extend the classification model for noun phrase identification as well as the lexicon.

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Isolation and Biotyping of *Brucella melitensis* from Upper Egypt

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Abstract: A total 106 seropositive samples from sheep, cattle and goats were collected from May 2009 to May 2010. Species of *Brucella* were isolated from, 9 (28.13%) of 32 in cattle, 25 (36.23%) out of 69 in sheep and of 5(100%) out of 5 in goats, from lymph nodes and spleen tissues. the south province of Egypt. The species examined by biochemical characteristics and had identical reactions with the standard strain. Oxidative metabolic tests performed, by substrate specific tetrazolium reduction (SSTR) test on the species, confirmed them as *B. melitensis*. Based on the biochemical, oxidative metabolic, and biotyping tests (CO_2 requirement, H_2S production, growth in the presence of thionin and basic fuchsin dyes, and agglutination test with monospecific A and M anti-sera) the strains were determined as *B. melitensis* biotype 3.

1. Introduction

The most reliable and the only unique method for diagnosing animal brucellosis is isolation of *Brucella* species (Alton *et al.*, 1988). A definite diagnosis requires the isolation of *Brucella* sp. from blood, bone marrow or other tissues. However, cultural examinations are time-consuming, hazardous and not sensitive. Thus, clinicians often rely on the indirect proof of infection (Al-Dahouk *et al.*, 2003; Bounaadja *et al.*, 2009), bacteriological isolation and identification of the etiological agent are necessary steps in the design of epidemiological and eradication programs (Refai, 2002, Zinstag et al., 2005).

Brucella melitensis is the main etiological agent of brucellosis in sheep and goats, and is also the main agent responsible for human brucellosis, a predominantly occupational disease related to professions in direct contact with livestock (Blasco and Molina-Flores, 2011).

Brucella species are highly monomorphic, with minimal genetic variation among species (Tiller et al., 2009) and maintain a close taxonomic relationship and can only be distinguished by rigorous metabolic, immunologic, and biochemical analyses.

The similarities among the *Brucella* species extend to the genetic level at which all species share greater than 90 % DNA homology (Hoyer and McCullough, 1968 (a), (b)). Species of *Brucella* were differentiated in the laboratory by colonial morphology, growth requirement, various biochemical tests and lysis by bacteriophage (Christina, 1998). The accurate distinction between *Brucella* species and their biovars is performed by differential tests based on phenotypic characterization of lipopolysaccharide antigen, phage typing, dye-sensitivity, CO_2 requirement, H_2S production and metabolic properties (Alton et al., 1988).

The comprehensive testing of metabolic activity allows cluster analysis within the genus *Brucella*. The biotyping system developed for the identification of *Brucella* and differentiation of its species and biovars may replace or at least complement time-consuming tube testing especially in case of atypical strains (Al-Dahouk et al., 2010).

The present study aimed to isolate *Brucella* sp. from sheep, cattle and goats by using standard cultural methods, and to biotype these isolates in order to establish a epidemiological base for studies on the control and prevention of brucellosis in Assuit governorate.

2. Materials and Methods:

This study was conducted during the years 2009 and 2010 in the south province of Egypt (Assuit governorate) and the tests were performed on all field and standard strains (*B. abortus* 544, *B. melitensis* 16M and *B. suis* 1330 originally provided by AHRI).

2.1. *Brucella* isolation

The isolates discussed in this study are described in Table 1. *Brucella*s from seropositive animal cultures were isolated in Animal Health Research Institute (AHRI) laboratory by the methods of Alton et al. (1988).

2.2. Bacteriological examinations.

All obtained tissues cultured on *Brucella* agar selective media (Oxoid) at 37°C in presence of 10% CO_2 for up to 2 weeks. The suspected colonies were examined for *Brucella* sp. *Brucella*-suspected colonies were characterised by the morphology, Gram stain, oxidase, catalase, urease production, and nitrate reduction tests (Sahin, et al., 2008). Colonial phase and staining were studied by, agglutination in acriflavine, crystal violet, and Zehil-Neelson staining. In addition, motility and serum requirements.

2.3. Metabolic characteristics.

Oxidative metabolic studies were conducted by using substrate specific tetrazolum reduction (SSTR) test (Broughton and Jahans, 1997, Ewalt et al., 2001), and the substrates used were previously reported in Ewalt and Forbes (1987) in addition to uroconic acid.

2.4. Biotyping tests.

The CO_2 requirement, H_2S production, growth in the presence of thionin (1: 25,000, 1:50,000, and 1:100,000 dilutions) and basic fuchsin (1:50,000, and 1:100,000 dilutions) dyes, and agglutination with monospecific A, M and R anti-sera, were performed as the methods of Alton et al. (1988).

3. Results

Brucella isolation.

Brucella sp. was isolated from different lymph nodes and spleen tissues was of 9 (28.13%) out of 32 in cattle, 25 (36.23%) out of 69 in sheep and of 5(100%) out of 5 in goats, while the overall rate of isolation was 36.8% of the total number of examined animals.

Species identification and biotyping

The results obtained in Table 2 revealed identification at the *Brucella* genus of 39 field isolates compared to reference strains by their colonial morphology, staining, serum requirement, motility and biochemical reactions. Suspected resultant colonies were further identified as *Brucella* sp. by the morphological appearance of each colony and microscopic appearance according to Alton et al. (1988) where, all cultures isolated from different animal species were characterized. The culture smears showed Gram-negative coccobacili in Gram's staining. The colonies were round, convex, smooth margin, translucent, hony-coloured, glistenining, and bulish on *Brucella* selective media. There was no agglutination with acriflavine, and not stain with crystal violet staining.

The cultures were positive for biochemical reactions (catalase, oxidase, nitrate reduction, and urease tests). There are some variation in urease activities shown between reference strains, rabid, slow, and moderate in *Br. suis*, *Br. abortus*, and *Br. melitensis*, respectively. Moreover, positive urease activity was observed on Christensen's medium.

In oxidative metabolic studies (Table 3), both field and standard *Brucella* strains utilized the substrates, amino acids (D-alanine, L-alanine, L-asparagine, and L-glutamic acid), carbohydrates (L-arabinose, D-galactose, D-ribose, D-glucose, and Meso-erythritol), and didn't utilize, urea cycle amino acids, uroconic acid and L-arabinose.

From the growth pattern on basic fuchsin, thionin, the dominant M and A antigen, non requirement of carbon dioxide and non production of H_2S in Table (4), the *Brucella* strains identified as *B. melitensis*. Based on the results in Table 2, 3 and 4, biochemical tests, morphology and agglutination test with monospecific A and M antisera, all the *Brucella* field isolates were determined as *B. melitensis* biovare 3.This finding is consistent with reports of *B. melitensis*, particularly biovar 3, being the main cause of brucellosis in animals among Assiut governorate.

4. Discussions

Brucellosis is a worldwide zoonotic disease that is recognized as a major cause of heavy economic losses to the livestock industry and poses serious human health hazard (Ocholi et al., 2005). *B. melitensis* is the main aetiologic agent of brucellosis in small ruminants. Ewes' and nanny-goats' aborted foetuses and products derived from sheep and goats remain the main source of infections. Ovine and caprine brucellosis were reported as a most common epidemic infection in Mediterranean and Middle Eastern countries, Asia, Latin America, and South Europe (Minas, 2006, Refai, 2002).

The studies in various parts of Egypt indicate that the *Br. melitensis* biovar 3 isolated from sheep, goats (Sayour et al., 1970 and El-Bayoumy, 1989), and cattle (El-Gibaly, 1969, Sayour et al., 1970, Montasser, 1991, and Helmy et al., 2007). Confirmatory diagnosis must be provided by the isolation of aetiological agents. Therefore, the isolation of *B. melitensis* is important to study the epidemiology of brucellosis.

The isolation of 39 *B. melitensis* strains from 106 (32 in cattle, 25 in sheep and 5 in goats) may indicate very high prevalence of *B. melitensis* infection among these animals in this region and due to that, the disease may threat human and animal health which was coincide (Esmaeil et al., 2008, Sahin et al., 2008, Aras and Ate , 2011).

Limited reports are available on the identification and biotyping of *B. melitensis* in Assiut. Salem, et al. (1987) and Ali et al. (1993) indicate that the disease is widespread among cows,

ewes and goats and isolated *B. melitensis* biovar 3. The overall rate of isolation was 36.8%. Cvetni et al. (2009) isolated *Brucella* from 88 out of 151 serologically positive pigs (58.3%) and 7 of 93 (7.5%) wild boar, Al-Farwachi et al., (2010) isolated from 4 (33.3%) of 12 samples, and Muñoz et al., (2010) recovered 104 isolates (19.3%) were obtained from seropositive animal cultures.

The application of biochemical tests are used for the identification of *Brucella* sp. The isolates in the present study identification at the *Brucella* genus of 39 field isolates compared to reference strains by their colonial morphology, staining, serum requirement, motility and biochemical reactions. Suspected resultant colonies were further identified as *Brucella* sp. by the morphological appearance of each colony and microscopic appearance according to Alton et al. (1988) which was similar to the biochemical test reported (Leyla et al., 2003, Mantur et al., 2004, Songer and Post, 2005, Unver et al., 2006, Helmy et al., 2007).

Although *Brucella* is a monophyletic genus, apparent differences between its species do exist e.g. host specificity and pathogenicity. Nowadays, *Brucella* species and biovars are distinguished by a limited number of microbiological tests measuring quantitative or qualitative differences of dye bacteriostasis, hydrogen sulfide production, urea hydrolysis, carbon dioxide requirement, bacteriophage sensitivity and agglutinin absorption as carried reported (Broughton and Jahans, 1997, and Ewalt et al., 2001) by substrate specific tetrazolium reduction (SSTR).

For at least half a century these microbiological procedures have not changed, although various new *Brucella* species showing variable phenotypic traits have been detected and new diagnostic methods have been developed. Neither the classical biochemical tests nor antigenic properties and phage-sensitivity can be considered a reliable guide to the identification of *Brucella* species. Contradictory results were often reported (Meyer and Morgan, 1962). However, variations in H_2S production, CO_2 requirement, a change in dye tolerance or atypical surface antigens i.e. inconsistent A and M antigens usually do not affect the oxidative metabolic pattern of a strain (Cameron and Meyer, 1958, Wundt, 1963).

Metabolic activities have proven to be stable parameters allowing unambiguous species identification, particularly in strains which show conflicting identities by conventional determinative methods (Meyer, 1961a, Meyer 1961b, Meyer 1962). Using the most discriminating carbon substrates i.e. D-glucose, D-trehalose, D-ribose, palatinose, L-fucose, L-malate, and DL-lactate more than 80% of

the *B. melitensis* and *B. abortus* strains could be correctly identified (Al-Dahouk et al., 2010).

In our series, all field *Brucella* species identified as *Br. melitensis,* displayed an atypical metabolic pattern could be identified. Oxidative metabolic profiles remain qualitatively stable for long periods of time and usually show no change in characteristic patterns after in vivo and in vitro passages (Jensen et al., 1996).

Comprehensive metabolic studies including all currently known species and biovars are rare. Using 13 differentially oxidized substrates, *Brucella* spp., could be grouped into *Br. melitensis*, their behavior on the substrates are identical with the presently recognized species (Broughton and Jahans (1997), Ewalt et al. (2001), Álvarez et al., 2011). However, *Br. melitensis* strains were tested and biovars were differentiated as biovar 3, which included all of the thirty nine *Brucella.* which was coincide with those reported by (Buyukcangaz and Sen, 2007, and Sahin et al., 2008, Aras and Ate , 2011).

The limited number of field isolates tested per species may have produced inconclusive results, particularly when only reference strains were available which are well known for atypical phenotypic traits. Future studies on larger strain collections may reveal more unique metabolic profiles suitable for species and biovar differentiation.

5. Conclusion:

In conclusion, the comprehensive testing of biochemical, metabolic activity allows cluster analysis within the genus *Brucella*. The biotyping system developed for the identification of *Brucella* and differentiation of its species and biovars may replace or at least complement time-consuming tube testing especially in case of atypical strains.

The isolation and biotyping of *Br. melitensis* particularly biovar 3, the most pathogenic strain and the main cause of brucellosis in some animal species among Assiut governorate, is a very dangerous alarm and gives spot light for application of preventive hygienic measures and control program of *Brucella* not only in upper but in all Egypt.

Table 1: *Brucella* sources and isolation percentages

Brucella source	Sample number		Isolate number	Percentage (%)
Animal species/ Number	Lymph nodes*	Spleen		
Cattle/32	150	32	9	28.13
Sheep/ 69	336	69	25	36.23
Goats/5	20	5	5	100
Total/106	506	106	39	36.8

* Five lymph nodes for each carcass including tetropharyngial, prescapular, ptefemural, internal iliac, and supramammary.

Table 2. Morphological and Bacteriological examinations of *Brucella* isolates.

Culture		Colnial morphology		Colonial phase & staining				Motility	Serum requierments	Biochemical reactions **			
		Indirect inspection	Direct inspection	Acriflavin test	Crystal violet	Gram's staining	Modified ZN*			Catalase	Oxidase	Urease	Nitrate reduction
Total number of isolates /host	9/cattles	Round, convex, 1-2mm. in diameter, smooth margin, translucent and honey-coloured	Round, glistenining, and bulish	No agglutination	No staining	Gram negative coccobacilli	Weak acid fast	Non motile	-	+	+	+	+
	25/sheep								-	+	+	+	+
	5/goats								-	+	+	+	+
Reference strains	*Br. melitensis* 16M								-	+	+	+	+
	Br. abortus 544								-	+	+	-	+
	Br. suis 1330								-	+	+	++	+

*Ziehl-Neelsen stain, ** Results: - negative, + positive, ++ strong positive

Table 3. Oxidative metabolic profiles[*] of *Brucella* spp.

Culture		Substrate** groups												Urocanic acid
		Amino acid			Carbohydrate						Urea cycle amino acid			
		A	B	C	D	E	F	G	H	I	J	K	L	
Number of *Brucella* spp. /host	9/Cattle	3	1	2	-	1	2	3	2	3	-	-	-	-
	25/Sheep	3	2	2	-	2	1	2	2	2	-	-	-	-
	5/Goats	1	2	1	-	3	2	3	2	3	-	-	-	-
Reference strains	*Br. abortus* 544	1	1	2	1	2	3	2	1	3	-	-	-	1
	Br. melitensis 16M	2	2	2	-	2	1	2	1	2	-	-	-	-
	Br. suis 1330	1	1	2	3	2	3	2	3	1	2	2	2	2

*Optical density with substrate/Optical density with no substrate = 1-3, 1 = 3-5; 2 = 6-8; 3 = 9-12.**Substrates: A-L-alanine; B-L-asparagine; C-L-glutamic acid; D-L-arabinose; E-D-galactose; F-D-ribose; G-D-glucose; H-D-xylose; I-Mesoerythritol; J-L-arginine; K-DL-ornithine; and L-L-lysine.

Table 4. Biotyping tests of *Brucella melitensis* strains.

Culture		CO_2 requirements	H_2S production	Growth on dye					Nonspecific antisera			Biovare metabolic
				Thionin			Fuchsin		A	M	R	
				A	b	C	a	b				
Number of *B. melitensis* field strains/	9/Cattle	-	-	-	+	+	+	+	+	+	-	*Br. melitensis* bv3
	25/Sheep	-	-	-	+	+	+	+	+	+	-	
	5/Goats	-	-	-	+	+	+	+	+	+	-	
Reference strains	*Br. melitensis* 16M	-	-	+	+	+	+	+	-	+	-	1
	Br. abortus 544	+	+	-	-	+	+	+	+	-	-	1
	Br. suis 1330	-	-	-	+	+	-	-	+	-	-	1

Abbreviations: a-Dye concentration 1:25,000(40ug/ml); b-Dye concentration 1:50,000(20ug/ml); c-Dye concentration 1:100,000(10ug/ml); A-Monospecific antisera; M-Monospecific antisera; R-Rough Brucella antisera.

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Effects of Some Cations on Dissolution Rate of Calcium Phosphate.

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Abstract: Dissolution of HAP crystals studied at   =0.6, temperature 37^oc and at I= 0.15 mal dm-3 using NaCl as electrolyte at pH= 7.4 using constant composition method. The HAP crystals prepared and confirmed using XRD, SEM, IR and chemical analysis .it was found that n 2 suggesting surface mechanism. The rate of dissolution increased with increasing PH and ionic strength of medicine. The Mg^{2+} , Zn^{2+} ,and Cd^{2+} rates of dissolution were studied . The order of inhibited the dissolution of HAP at the same conditions. the order of inhibition was; Mg^2+ > Cu^{2+} > Zn^{2+} >Mn^{2+} >Cd^{2+} the cations inhibited the dissolution by blocking the active sites on the surface of HAP crystal .From langmuir isotherm , K_L were found $12x10^5$, $4.7x10^5$, $4.38 x10^5$, $3.85 x10^5$ and $1.8 x10^5$ for Mg^{2+} , Cu^{2+} ,Zn^{2+} , Mn^{2+} and Cd^{2+} respectively inhibition of the dissolution rates of HAP in the presence of Cd^{2+} was found increase with increasing ionic strength and PH of the medium . The inhibition was found to change the morphology of HAP crystals depending on the order of mixing the reagents at the begging of the dissolution process.
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Keywords: Cations, calcium phosphate, HAP crystals, ionic strength.

1. Introduction

Hydroxy apatite (Ca_5 $(PO_4)_3OH$ HAP) is the most stable calcium phosphate salt under physiological conditions. This model compound has been used to study hard tissue calcification such as bone and teeth and in many undesirable cases of pathological mineralization of articulor cartilage [1& 2] cardiac valves [3] and kidney stones [4]. The precipitation of HAP is of great importance in a number of industrial purposes ranging from the water purification and fertilizer production.

Since hydroxy apatite is the most widely applied biomaterials various techniques have been developed to synthesize hydroxy apatite powders [5]. Alkaline earth cations present in biological fluids and in natural waters may play an important role in regulating the formation of calcium phosphate solid phases

Metallic materials have found wide application in restorative surgery as basic biomaterials for manufacturing implant pro stheses for skeletal replacement and fixtures [6]. The release rate of Ca and P from Fe – SHA (iron –synthetic hydroxyapatite) and Mn –SHA were in some cases greater than the result rates of Ca and P from pure SHA in distilled water Investigation was aimed to study the effect of Mg^{2+}, $Ca2^+$, Zn^{2+}, $Mn2^+$, and Cd^{2+} on the rate of dissolution and morphology of HAP crystals and the effects of changing the experimental conditions in the presence of additive on the rates and morphology of the HAP crystals.

2. Material and Methods:

Solutions were prepared from analytical grade chemicals (El- Naser Pharmaceutical Chemical Company) and distilled demonized water. Calcium chloride were analyzed by passing aliquots through ion exchange resin (Dewix – 50) in the hydrogen from and titrating the eluted acids with standardized sodium hydroxide solution of suitable concentration using phenolphthalein as indicator. All solutions prepared and stored in Pyrex vessels. Calcium phosphate seed was prepared in details by adding 1 L of (0.02 M) Calcium chloride solution to I L of sodium monohydrogene phosphate (0.012 M) after advanced the pH to 7.4 by using conc. HCL and the addition happen at room temperature and suddenly in 2 second. The seed solution was constantly stirred for one weak and was then filtered and the seed crystals washed with deionized distilled water to remove surface contamination due to chloride and sodium ions. The seed crystals were aged for one month then refiltered and carefully washed with deionized distilled water and this process was repeated several times. The seed was then filtered and dried. The seed crystal was characterized by X-ray powder diffraction, practical sizes, measured by single point BET nitrogen adsorption at 77 k was 0.36 m^2g^{-1}.

3. Results and discussion:

Dissolution of HAP has already been performed to characterize the dissolution behavior of it. The term apatite describes a family of compounds having similar structures but not necessary having identical composition. HAP is the most stable phase of calcium phosphates having definite composition and definite composition and definite crystallographic structure. It is often as a proto type for bone and tooth mineral [7].

In the present work the dissolution of HAP crystals at σ = 0 – 6, t = 37°C, I = 0.15 mol dm^{-3}, using NaCl as background electrolyte, and pH = 7.4 using constant composition method which ensures that the factors which can influence, the under saturation such temperature, ionic strength and solution speciation remain constant during the entire experiments. So the rate reduction of dissolution of HAP crystals can not be attributed to alteration in HAP under saturation during the reaction.

HAP crystals are prepared; x-ray, IR, and SEM fig (1) analysis confirm the HAP structure. Chemical analysis showed the molar ratio of HAP of 1.69 using EDTA and atomic absorption for calcium and phosphate was determined spectro-photomatrically.
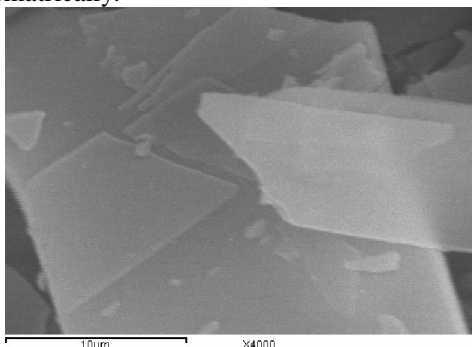
The rates of dissolution of HAP crystals, determined from the slops of lines from the plots of volume of titrant added to the cell with the time, are summarized in table (1) . Since the extent of the dissolution reaction was very small (less than 5% of the total surface area of seed crystals), so the change in the crystals surface area accompanying dissolution could be ignored. During the reaction the HAP crystals maintained their morphology as observed in SEM (fig. (1)). during experiments aliquots were drawn from time for (Ca^{2+}) and PO_4^{3-} . Each experimental were made triplicate for certainty.

Previous studies have noted that the dissolution rates of some sparingly soluble salts, such as DCPD, OCP and HAP microcrystals, decrease with the time at constant undersaturations. This was attributed to decrease in the density of active sites on the crystal surface or the smoothing of crystal surfaces [8] .

The rates of dissolution of HAP crystals are plotting against the degree of undersaturation, (σ = 0.316 – 0.891) as shown in fig. (2).

The effective order of reaction was determined. Table (1) and fig (2). It can be seen that the dissolution reaction rates follow a parabolic rate law with n ≅ 2, which suggest surface- controlled mechanisms. For n ≤ 2 the suggested mechanism is transport and spiral mechanism. Previous studies showed that in dissolution of HAP, (n) = value between 4 and 6 at pH= 6.75, 6.3 and 7.15[9]suggesting poly nuclear mechanism, surface nucleation process[10, 11], surface processes combined with transport processes[12], both surface and diffusion mechanism[13,14,15] at higher temperature of 60°C, is mass transport while at lower HAP has generally been found tobe surface controlled with n>1, n>2[22-31),32,33,(34-41)]

The surface and transport mechanism over a range of relative under saturation can be supported as can be seen from table (1). From the table the rates of dissolution of HAP are independent on the changes in the rate of stirring (fluid dynamics)(exp (11) and (12)). This evidence may be inconclusive on such small particles for which changes in the stirring rate may have little influence on the fluids shear forces at crystal surfaces. The particles will tend to move with fluid flow. The rates of dissolution were also proportional to the weight of inoculating seed used to initiate the dissolution process which confirm the surface mechanism.

Table (4): Effect of under saturation on the rate of dissolution of calcium phosphate at 37°C.

Exp No		- log	R x 10^{-8}	- log R
1	0.891	0.05	35.481339	6.45
2	0.794	0.1	28.840315	6.54
3	0.70	0.155	22.6464	6.645
4	0.650	0.187	19.40886	6.712
5	0.603	0.22	17.37801	6.760
6	0.550	0.26	14.79108	6.830
7	0.501	0.3	11.29796	6.949
8	0.4	0.4	8.70964	7.06
9	0.355	0.45	6.76083	7.170
10	0.339	0.47	6.23735	7.205
11	0.316	0.5	5.55904	7.255
12	0.603			

a = stirring note = 500 rpm

The rate of dissolution of crystals in aqueous suspension is in general either controlled by surface processes, or by the transport of substance between the volume adjacent to the dissolving surface and the bulk

solution or by combination of such processes. In all cases the transport is taking with the rate controlled by diffusion (in a stagnant medium) or by convective-diffusion.
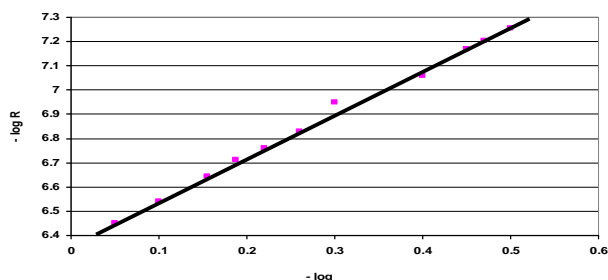
There is a change over from diffusion to convective- diffusion at about a certain crystal size, this depending on the statements is true:

(1) If surface processes are rate- controlling, the linear rate of dissolution is independent of the size of the crystals. The rate is also independent on the fluid dynamics. The concentration near the crystal surface is the same as in the bulk.

(2) If the rate is controlled by diffusion in stagnant medium, the linear rate of dissolution is inversely. propotional to the crystal radius. The rate independent of the fluid dynamics, if the mean distance between crystals is larger than about 20r. The concentration near the crystal surface is not the same as in the bulk.

(3) If convective diffusion is the determining process, the linear rate is inversely proportional to the square root of angular velocity.

White and Nancollas[13]. Found, the rate of dissolution of bovine enamel to be transport- controlled but with rate faster than expected for convective-diffusion. This was explain as a result of a couple transport of hydrogen ions inwards and dissolution product \s outwards. The rate of dissolution was found to be described by the empirical expression

$$R_d = \left[(HAP)_o - (HAP) \right] \left[a(H^+) + b \right] S D_{HAP} / \delta_N \quad \ldots(1)$$

Where:

R_d : is the rate of dissolution.,

S: area of enamel exposed.,

D_{HAP}: The overall diffusion coefficient.,

δ_N : The thickness of diffusion layer.,

(H^+): The activity of hydrogen ions

(HAP) is given by: $HAP = \left(\left[a^{2+} \right] f_2 / 5 \right)^{5/9} \left(\left[PO_4^{3-} \right] f_3 \right)^{3/9} \left(\left[OH^- \right] f_1 \right)^{1/9}$

$(HAP)_o$ in saturated solution, it is independent on pH and composition of the saturated solution.

The rate of transport process is normally proportional to $C - C_o$, where "C" is bulk concentration of the dissolving substance and C_o is the concentration near the dissolving surface. If transport of dissolved substance from the region near tooth surface into the bulk solution is the rate controlling process, the rate should be proportion to "C" whereas the rate given by:

Rd: $(HAP)_6$ [a (H^+) + b] SD_{HAP}/ δ_N ....(2)

Assuming the solution next to the surface to be saturated and Ca/P = 1.67 and considering the transport of OH^- ions $\left(PO_4^{3-} \right)$ ions from the surface and the transport of H^+ ions to the surface as a coupled diffusion phenomenon, H^+ ions are consumed in the boundary layer and H_2O, HPO_4^{2-} ions and $H_2 PO_4^-$ ions are formed. The result of this coupling is the effective distance that OH^- ions and PO_4^{3-} ions have to diffuse are shorted, because some the ions are transformed into other species. The effective distance the Ca^{2+} ions have to diffuse is independent of the coupling as the effective distance phosphate ions have to be diffuse if all the photolytic forms are considered as a whole. The bottle neck is the transport of H^+ ions to the crystalsurface. This bottle- neck is removed by using buffered solutions or by working with microcrystal [12].

Rotating disc method provided in case of dissolution of HAP about surface processes combined with transport processes [13]. For pH in the physiological pH controlled by surface processes [13].

The rate of dissolution of HAP may be depend on the rate which H^+ ions can be delivered to the dissolving surface. H^+ ions as shown by Gray[21], be transported from the bulk. Solution to the crystal surface, either in the form of free ions or in the form of a weak acid which adissociates at the crystal surface. The rate-determining process for a rotating of HAP, as well as for suspensions of HAP may be:

(i)   transport of H$^+$ ions from the bulk to the crystal surface;
(ii) Surface processes;
(iii) Transport of dissolved substance from the volume or a combination of these processes [13].

Around pH = 7 about half the phosphate groups in the crystal surface should thus be in the form of hydrogen phosphate [9].

The rate controlled process can be significantly influenced by factors such as temperature, crystal size, fluid hydrodynamics and concentrations of the ionic species [22]. Surface charge is also an important property of solid. Since it can determine such processes as adsorption and surface properties. Indeed, processes such as adsorption, particularly of surfactants or macromolcules, can change the interfacial behavior of solids. Marisdly.

The affect of change of ionic strength on the dissolution Rates of HAP crystals was studied. It was found that the rates of dissolution increase with increasing ionic strength of mediums table (2) fig (3).
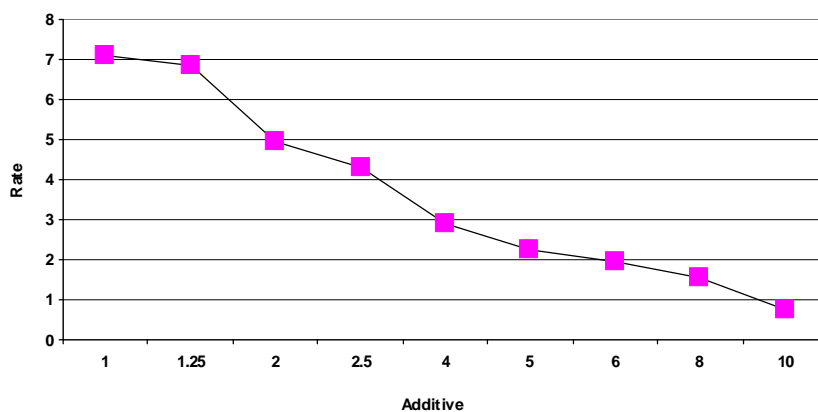
The equilibrium stability of HAP is greatly increased by lowering the PH of the medium. The dissolution rates of HAP crystals were found to increase with increasing PH of the medium fig(4)
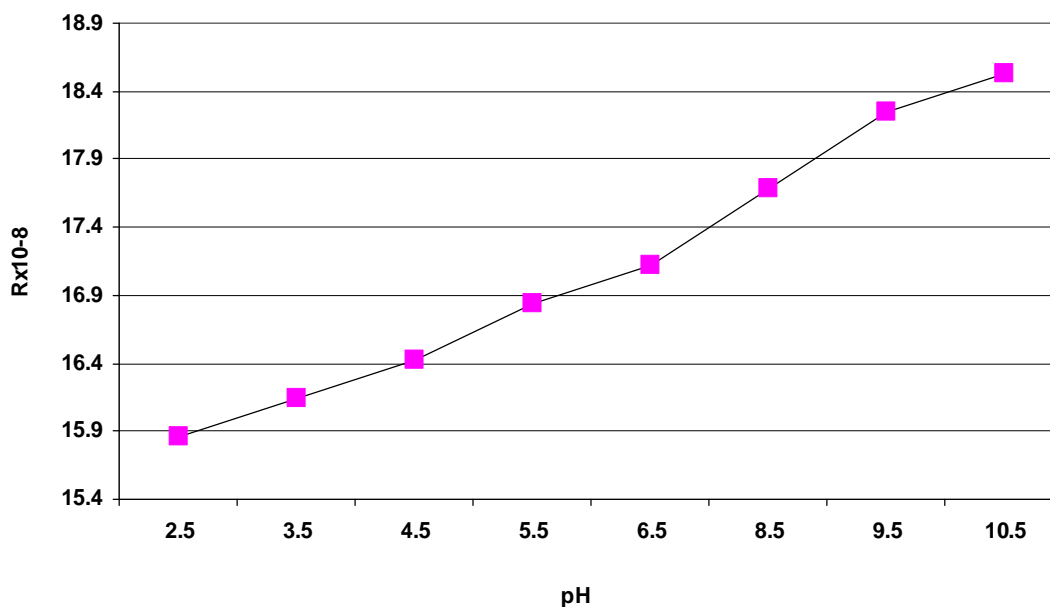
Dissolution of HAP may depend on the rate by which hydrogen ions can be delivered to the dissolving surface. Hydrogen ions may, as shown by Gray, be transported from the bulk solution to the crystal surface, either in the form of free ions or in the form of a weak acid which dissociates at the crystal surface. The rate controlling Process for a rotating disc of HAP may be

(I) trans port of hydrogen ions from the bulk to the crystal surface
(II) surface processes

Transport of dissolved substance from the volume adjacent to the crystal surface to the bulk solution. Or a combination of these processes [22] in the case of HAP , information a bout surface processes combined with transport processes .as shown later , for PH in the physiological PH range , the rate of dissolution of HAP microcrystal sin on aqueous suspension is controls by surface processes. These processes can therefore be studied without interference from transport processes by investigation such suspensions. [22]

Inhibition

The rate of dissolution of HAP crystals are studied in the presence of some cations like Mg^{2+} ,Cu^{2+} ,Zn^{2+} ,Mn^{2+} and Cd^{2+} .

The dissolution inhibition in the presence of these metal ions, the order of inhibition of dissolution of HAP crystals are in the order

$Mg^{2+} > Cu^{2+} > Zn^{2+} > Mn^{2+} > Cd^{2+}$ fig (5)
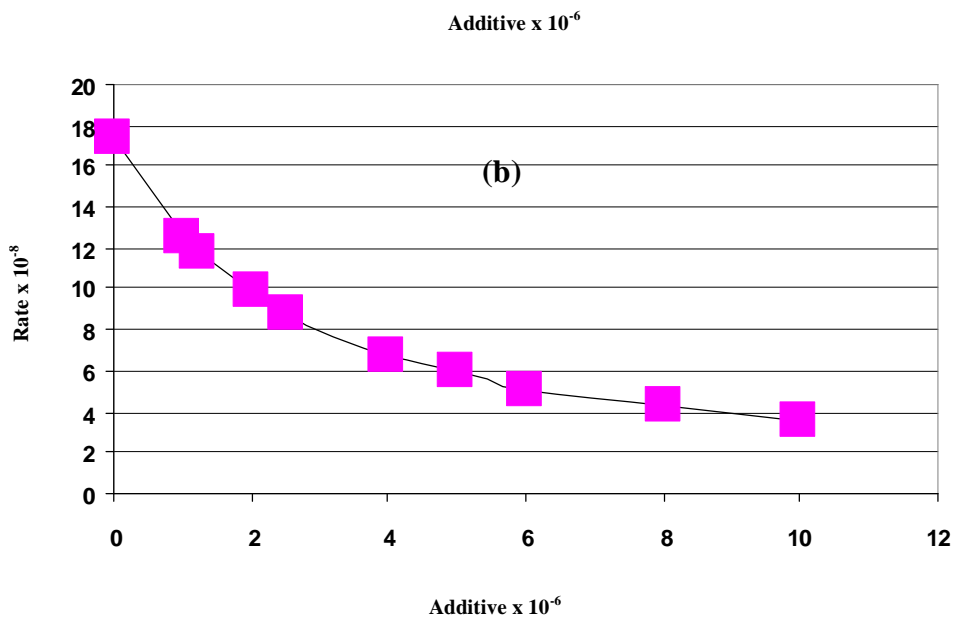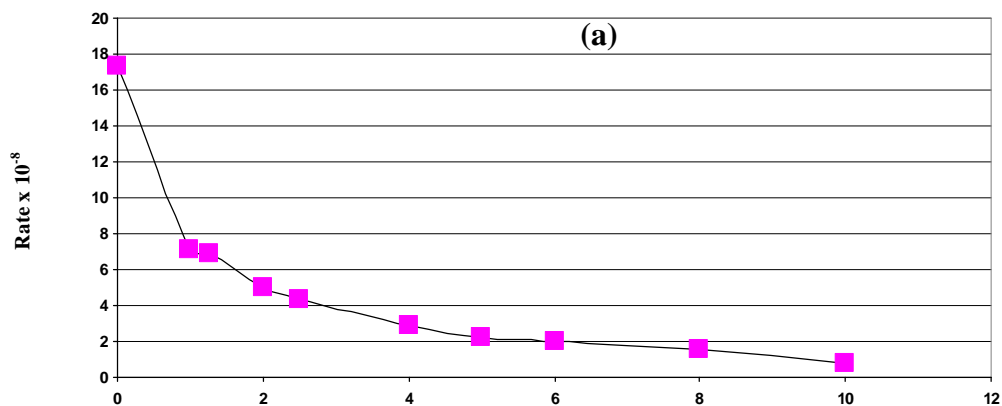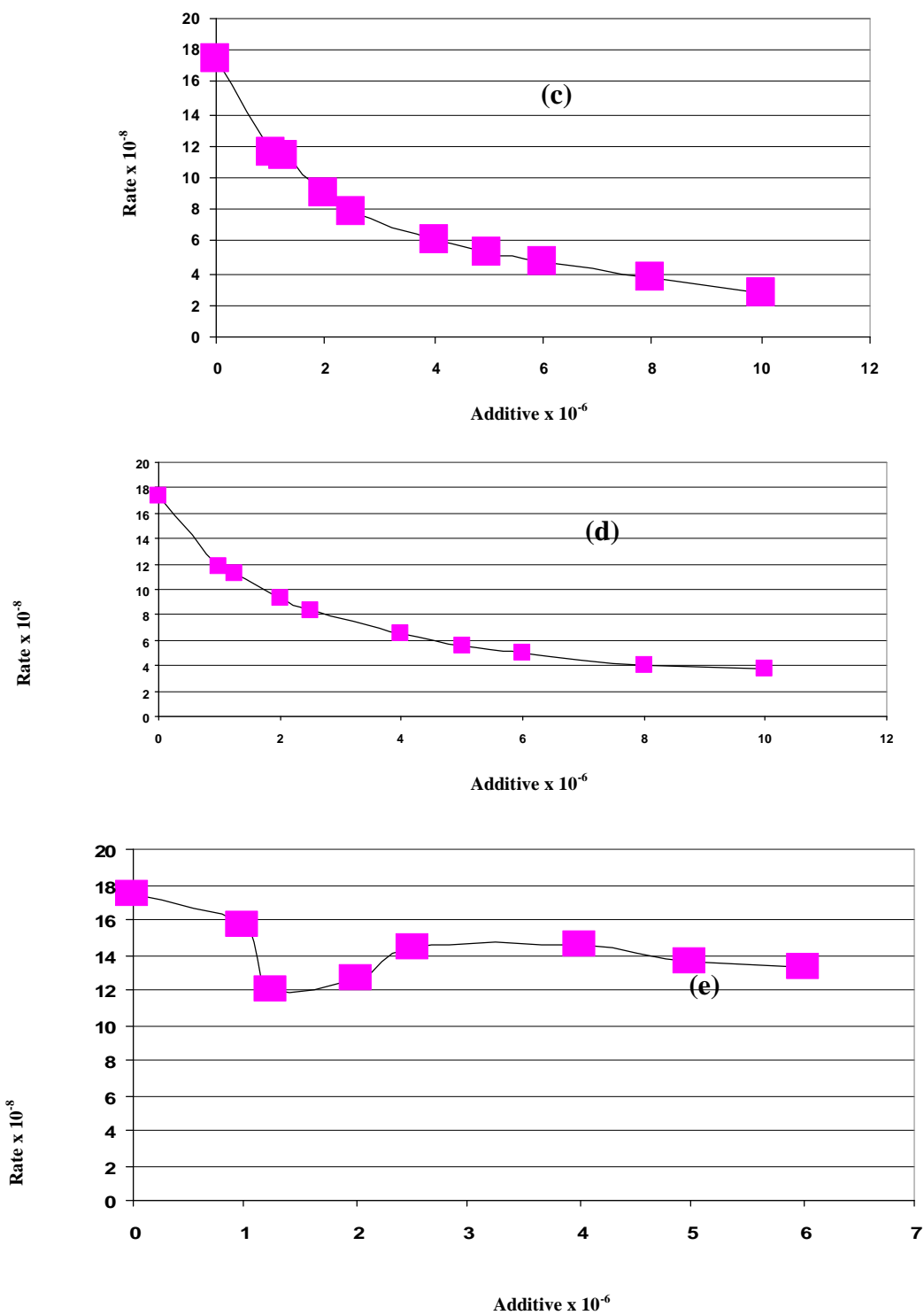
Figure 5 (a,b,c,d, e): Plot of Rate x 10^{-8} against Additive x 10^{-6} in the presence of Mg^{2+}, Mn^{2+}, Cu^{2+}, Zn^{2+},Cd^{2+}

The decrease of rate of dissolution of HAP crystals can be interpreted in terms of blocking of active sites through adsorption of the metal ions at crystal surface during dissolution this can b interpreted in terms of Langmuir isotherm. Fig (6).
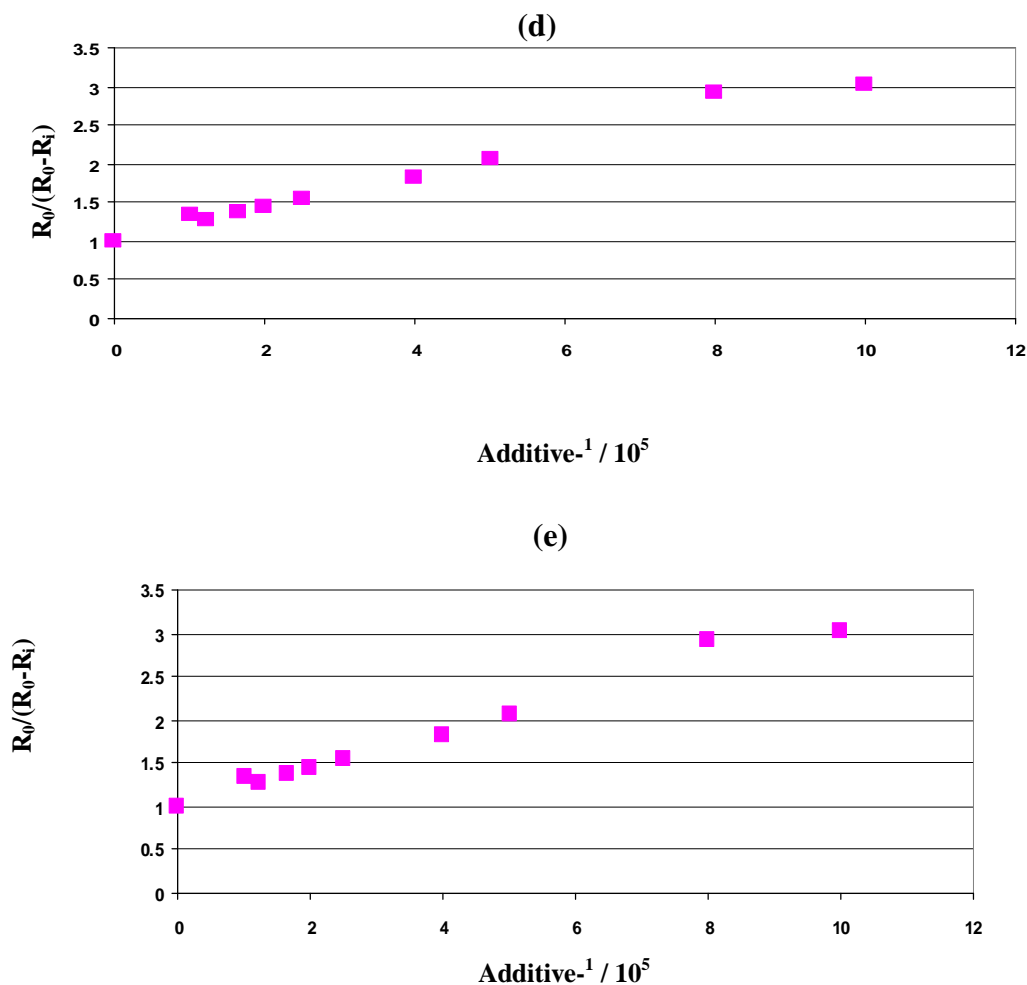

(A)

(b)

(c)

(d)

Additive^{-1} / 10^5

(e)
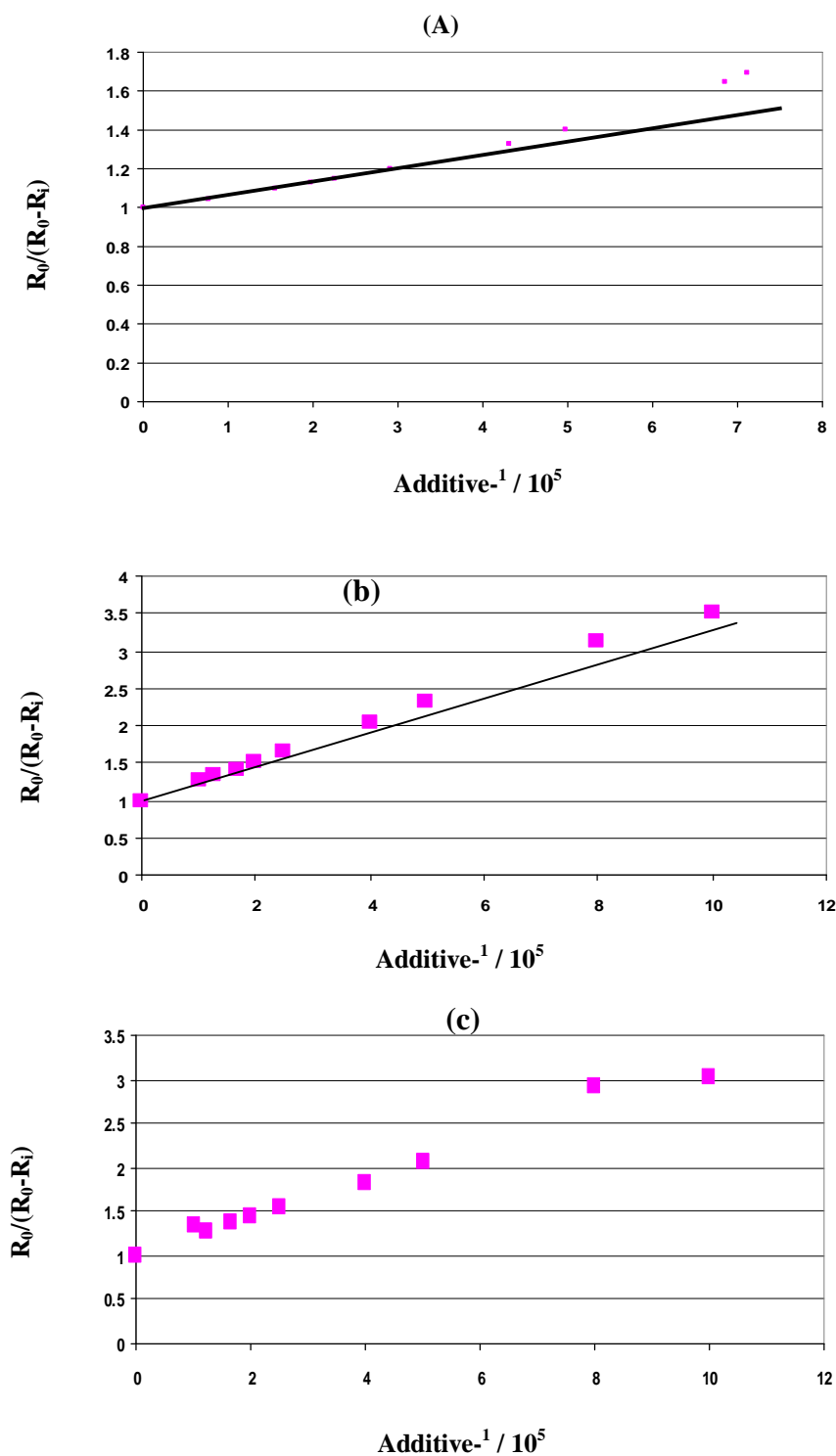
Additive^{-1} / 10^5

Figure 6 (a,b,c,d, e): Plot of $R_0/(R_0-R_i)$ against Additive^{-1} / 10^5 in the presence of Mg^{2+}, Mn^{2+}, Cu^{2+}, Zn^{2+},Cd^{2+}

From the fig (5) K_L= adsorption affinity constant are $12x10^5$, $3.85x10^5$, $4.38x10^5$, $4.7x10^5$, and $1.8x10^5$ in the case of the presence of Mg^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+} and Cd^{2+} respectively. The radii of these metal ions are smaller than that of Ca ions and from K_{so} values of MgPo$_4$, CuPo$_4$, ZnPo$_4$, MnPo$_4$ and CdPo$_4$ are less than that of HAP so the validity of formation of their phosphate salt is big. Also the bond between Cu^{2+}, Zn^{2+}, Mn^{2+}, and phosphate ions are strong than that between Ca^{2+} and phosphate . so these metal ions are adsorbed on to phosphate active sites leading decrease the number of active sites, leading decrease the dissolution rates of HAP crystals . Cd^{2+} make strong bond with phosphate ions, but the K_{so} of CdPo$_4$ nearly equal to that of HAP and ionic radii OF Ca^{2+} and Cd^{2+} nearly the same so the affect of Cd^{2+} as inhibitor is less than the other additives.

To discus the effect of experimental condition on the inhibition activity of additive Cd^{2+} was taken as example. The effect of change of   on the rates of dissolution of HAP crystals in the presence of Cd^{2+} was studied. From fig (7)

The order was found   2 , suggesting surface controlled mechanism at active sites on the surface .
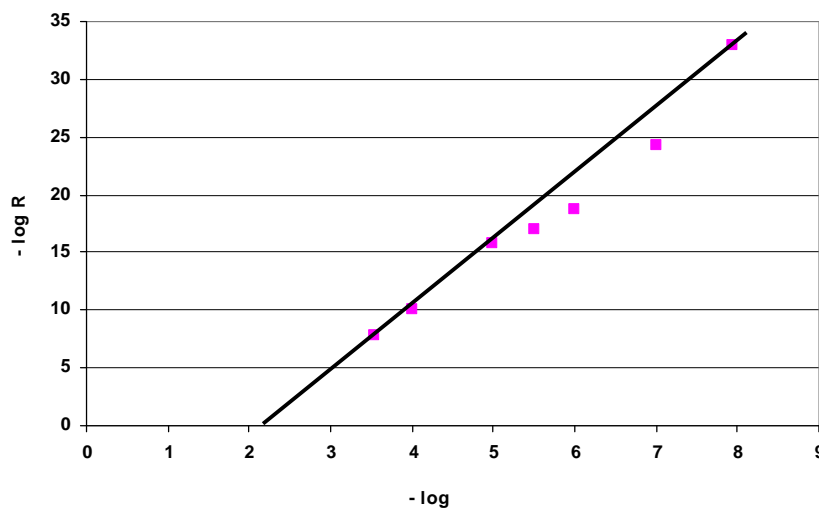
The effect of change of pH on the rates of dissolution of HAP crystals in the presence of Cd^{2+} was studied. From fig (8) was found that rate of dissolution of HAP crystals decrease with increasing the pH of the medium.
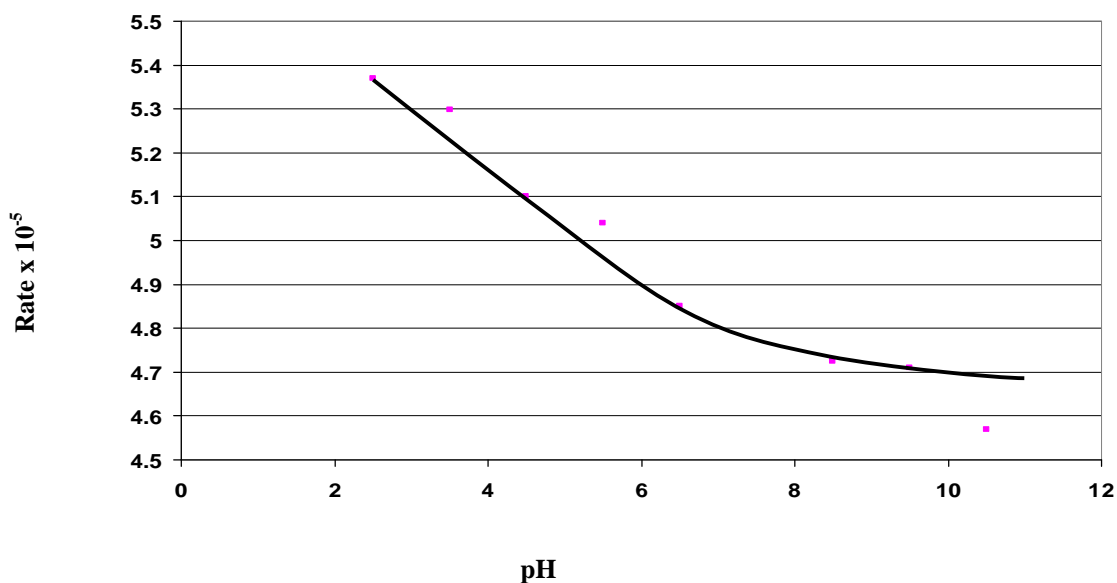
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3/3/3011

Assessing Major obstacles to rural women's participation

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Abstract Development along with economic growth and income increase is an important goal for most countries. Recently the growth of awareness about destructive effects of poverty has made countries believe that the best way to achieve sustainable development is to eradicate poverty; therefore most development programs have been oriented towards poverty eradication by micro-credit services. Supporting poor to raise their life standards should be based on the belief that the poor are able to help themselves. Explicitly, this proves that among a variety of deprivation they do consider their survival. Overall, the goal of all credit plans is to increase the poor's income through creating self-employment opportunities and providing educational services to make the best use of resources.
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Keywords rural women , participation

Introduction:

Participation means women's presence in all stages of development, including: needs evaluation, identification of problems, planning, management, implementation and evaluation. It's not easy to get equal participation in a patriarchal society, such a matter requires participation of women and especially rural women in particular projects that they are somewhat beneficiary(Saadi and Arab Mazar, 2005).

In all communities, rural women are considered as an important factor in achieving rural development goals and in fact are half of the manpower needed for rural development. However, in the rural community of Iran, there are gaps between the ruling class (capital owners) and villagers, between literate and illiterate, and between men and women. Especially in villages women have fewer possibilities in terms of investment and less power and credit. Role of rural women, over of men, is more influenced with different economic, social, cultural and ecologic factors. Rural women are considered as a noticeable potential in the community either directly (crops production, livestock, handicrafts, cottage industries) or indirectly by helping the agricultural sector (as labor). About 5.6 million women are involved in agricultural production, and activities related to planting... harvesting, preparation of animal food, and taking care of livestock and poultry and some certain activities related to trading and marketing are all different fields of rural women's role and participation. Based on current statistics, women in rural area participate about 50% in conversion industries, 22% in producing crops and livestock, 75% in handicrafts and in areas related to planting…harvesting , respectively, 25, 24 and 4.26. And also in activities related to livestock, they handle 23% of livestock grazing, 42% of animal care and 100 percent of total poultry in the village. Therefore their

role in achieving food security is undeniable. But, like most developing countries, this crucial role in society and in process of rural development, is not obvious. In Iranian rural community, about 80% of women work, but they are mostly considered as housewives, unpaid employment, domestic workers, family workers, or independent employers. The statistics often do not take into account seasonal, part-time, unpaid employment, and housekeeping activities. In economics and social sciences, those of women's activities that have emerged out of house and affected national economy, are the ones to be noticed. In most research and statistics men are known as the heads of household and they are also the owners of lands and fields. That only 1% of the rural lands are belonging to women does confirm such matter (Samadi Afshar, 2004).

Development is a multidimensional process and has various economic, social, political and cultural dimensions. Rural women's participation has not been active and effective; because this participation's most important aspect, namely economics, is for rural women. However the value of their work in agricultural products is rarely considered as income and they are not independent either (Amiri, 2000).

For an active participation of women in development, first we need to give a definition for their role in development and then barriers related to their role will be discussed. Although apparently there is no difference of gender in development programs but reality is that women are less considered in participatory programs and most of these plans are planned for men. Finally, planner's optimistic look toward women's participation will be greatly helpful improve rural family budget and will increase the difference between urban and rural families. If, by credit, loans and other financial facilities, rural families are able to build up their own business and make a living through the income and become financially self-reliance or

independent, no doubt we will witness some social, economic and cultural changes in villages (Varzgar and Azizi, 2001).

2- Rural women's participation:

Women, being half the population, play an effective role in the economic welfare of family and society. In Iran's economy, women are one of productive factors, but, so far, researchers and writers have ignored the issue of women's participation in economic activities. While in present situation considering the role of women's participation seems to be obligatory (Balali, 2005).

Participation in its broader sense means to motivate people and thus increase the sensitivity to understand and become responsive of development programs and it also carries the concept of local initiatives.

In fact, participation is to guide people caught by disability, to help them realize their potentials and to empower them to make the best use of life.

According to preceding definition of participation, and the ability of participation to turn potential into imminence power, women should participate more actively in economic affairs. Statistics regarding women and girls' activities, especially in rural areas, are always presented much lower than the real numbers (Ghaffari, 2000).

Village with the word "woman" removed will lose its literally in production and economic activities, their everyday activities in different fields all are evidence of woman being required in rural production. Rural girls and women are responsible for a variety of roles and duties including wife, mother, producing crops, livestock and agricultural activities, making and marketing handicrafts which are common in each area, and food preparation.

Daily activities of girls and women in different fields all are evidence of woman being required in rural production sector. Women are the major potential for developing the rural economy which leads to further growth of rural production. Increasing awareness towards the role of this class in production and towards necessity of their broader participation in economical and social development, have forced the countries to consider and support their activities while making new rural, Local and national policies (Rahmani Andalibi, 2001).

Being aware of women and girls' important role in rural activities, many countries have established institutes and organizations to advance women's progress. These organizations try to remove legal barriers that prevent women to participate in development activities as much as men. And finally improve their social, political and economic status in society. With continues evaluation and analysis of current development plans, we can provide especial conditions to ease women's access to production recourses and social services, and considering women's social situation in every society, we can provide the opportunity for them to increase their participation, share income like men, and take part in decision-making(FAO, 1998).

Rural women's participation:

In fact, participation is to guide people caught by disability, to help them realize their potentials and to empower them to make the best use of life.

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Importance of women's participation:

Studies by FAO show that more than half of the world's crops are collected by women. According to estimates, 1.3 billion of world's poor are women, thus the slogan "poverty has a feminine face" is spread worldwide.

Given that in many parts of the world, the production potential of women is not used properly, a cost-benefit study by the World Bank shows that investing on women in developing countries will be more profitable than any other investment. In addition to financial benefits of this huge force, its ancillary results will also be useful. The ancillary benefits of women's employment include: lower population growth and children mortality rates (Navab Akbar, 1997).

Rural women are a big part of productive force and in developing countries third to half of them are supervising households; as a result they face numerous problems, such as:

-Lack of access to social and health facilities
-Various daily chores inside and outside the home
-Men's skill and increase of women's responsibility
-Lack of professional to educate women

Around the world and in Iran, the issue related to female employment, especially in rural areas (which is a manifestation of participation), is not unemployment but unpaid employment; because all the unpaid work done by women at home, such as cleaning, washing, nursing, social affairs, agriculture and livestock,… are encountered as non-economical activities; While visible economic sector cannot continue to exist without this invisible sector's goods and services. On the other hand, rise of industrial system and expansion of factory job all over the world, attracted men to these economic systems and this has given men an objective vision; Whereas, the majority of women, due to working alone at home have got a subjective vision. Now, as women enter labor market and start to participate, they'll become objectified; because the work system will encourage them to think like men. Being more around the house and their local area will help both men and women in terms of subjectivity and objectivity (Arab-Mazar and Jamshidi, 2005).

Thus the issue of women's participation has important effects, including:

-Acceleration of plans implementation
-Realization of people's every day needs with cooperation and consultation
-Increasing efficiency and reducing functional expanses of projects
-Creating opportunities for talent realization and scientific activities
-Creating sense of solidarity and cooperation
-Increase social and personal awareness
-Women, sharing ideas in decision-making and determining their own destiny
-Participation of women as an important factor and a major power to achieve development

Therefore, according to preceding discussions and importance of women's participation in future plans, it's of great importance to study and recognize the factors affecting their participation in social activities of rural area (Fakhraee, 2002).

Major obstacles to women's participation:

Considering society's current conditions and the issues mentioned above, major obstacles which result in women's less participation can be classified as follows.

1- Educational barriers

Apparently, one essential factor for development is education. Studies indicate that compared with men and boys women and girls do not have sufficient access to education. Some of the factors effecting women's access to education are:

1. Great need of parents to their daughters as labor force
2. Lack of access to educational experts and planners
3. Lack of schools or proper places for girl's education
4. Mixed classes for boys and girls and rural bias on this issue
5. Education expenses
6. Lack of attention to the importance of girls' roles
7. Social, cultural and traditional beliefs about girls
8. Early marriage

Report by UNICEF, claims that literacy rate of women in developing countries is two third of men's, and of about 860 million illiterate adult worldwide, 640 million are women who never had the possibility to go to school or have left school unfinished(Bakhshoodeh and Salami, 2005).

2- Social and cultural barriers

Sociologically, women in third world countries-especially in rural areas- believe to be dependent on men.

The thought is deeply attached to their historical beliefs. Thereupon they never share ideas while decision-making or planning. As some sociology and politic experts stated, it's the reason they have developed "the silence culture" and they never let themselves to comment on, or participate in planning.

In addition, customs and prejudices that they have been trained with, indirectly affects women's participation. Such ideology of knowing a sex to be lower than the other is a crippling disease that causes a big part of mental and power sources of community remain disadvantaged. These are all prejudice emphasizing on men's value and denying those of women (Changizi Ashtiani, 2003).

3- Structural barriers

In fact, in most countries, governing power, marketing and production conditions and some values related to them, create serious structural barriers to women's participation. According to United Nations' research institute of second development program, these structures are anti-participation; because they lead to unequal access to the control of wealth and social status. They cause failure of many national-regional innovations encouraging participation, and finally make a small group be responsible for everything and we won't have the beneficial results associated with women's participation. The structure determines the conditions of participation and reacts strongly to any renovation. Its objective is to keep women in their position as a labor. Labor market divides the jobs in workshops and factories in a way that some

occupational fields are only for women and some other only for men. Men are chosen to be the administrator in all professions and it's assumed that women are not interested in or not able to handle these positions. Thus, in practice the world of production and work is subject to gender discrimination.

4- Political and organizational barriers

In third world countries, women face with many obstacles for participation in decision making, planning, implementation, and evaluation of projects related to country's developing plans. Although, the structure of the country play an important role in making suitable conditions for participation in different areas, but because they have focused plans and such decisions are made by public organizations and official systems, usually the potential force of participation in society will be palled and in practice, participation will face serious obstacles and problems. A focused government always encourages focused official structures. Such a structure is a major barrier to women's participation. They control structures and systems resource allocation and information and knowledge people need to participate in social activities, besides they never let people and especially women control all these. So it's apparent that such programs are either not comprehensive or it's facing problems because designers are not aware of the realities in their community.

5- Barriers related to wife-mother role

UNICEF reports indicate that women's work hours is 25% longer than men's; because a large number of them work at home to produce livelihood products without payment.

The main role of all women in each society is the role of mother and wife; therefore every other matter such as their employment is subject to these roles. Possibility of finding a job (as administrator or in a lower rank) for a girl is affected by various factors including educational level and their socialization method as a child. They have always been thought that they are not identical to boys in terms of social privileges or social status. Emphasize on the roles of mother and wife may make women think there is no need to promote their social status and in the other hand society will not provide necessary facilities for their development. In this situation they are prevented from studying and schooling. This issue will still be a problem after they are married.

It should be noted that with women getting paid, total household's welfare improves; because field studies claim that all women earning money, spend their income on their family and particularly children's needs.

So we should mention that not only participation is a woman's civil right but also it will make her more autonomic, and she'll become more creative and innovative.

4-6- Economical barriers

One of the factors indicating development progress is how and how much different classes of society participate in vital activities. Although importance of women's participation has always been completely apparent, the appropriate balance between men and women in different fields is not yet established in our country. As women can only posses a limited sort of jobs and also they always have the smaller share of each job opportunity, they are not able to compete in labor market. What's more, mostly they do not own the capital needed for economical participation, so providing personal credits can solve their problem in some extent.

Discussion and results:

In the new system of advanced agricultural economy, the value of women's work that previously was unpaid labor now must be paid in cash. Expect for agriculture which is rural women's main work field they have rarely participated in tow other fields of economy. The most important issue of women's social and political participation is to take part in planning, decision making, implementation of decisions, and evaluation of results. Generally they have had a little share in such processes. Although in recent years rural women have participated more in villages' management, social and cultural organizations, and cooperative institutions' management; but having a lower level of literacy, education, income and social status than urban women they still have the smaller share of administrative and official jobs. Some barriers to women's participation which can be categorized in 3 groups of personal, familial, and social include: law literacy level, large volume of work both inside and outside of home for many reasons including seasonal migration of men and the great diversity of rural women's activities(nursing, housekeeping, agriculture, handicrafts, livestock,…), malnutrition, law health indicator, Patriarchal structure of society, father or husbands disagreement with a woman's participation in social and economic activities for various reasons like cultural reasons or unwilling to lose the labor force at home, negative attitudes towards women's abilities, gender discrimination, family's poverty, superstitious beliefs, misleading customs like fatalism, law access of women to credit and facilities, inaccessibility of extension services, men-orientated social activities and participation plans, deficiency of professionals needed to educate rural women, problems of access to health services and social facilities, low income of rural women compared with men, lack of non-governmental

organizations dealing with rural women's problems, few women managers in rural area. (Rahimi, 2001)

Nowadays, micro-credit and micro-financing have changed people's lives; it has brought back life to poorest and richest communities of the world. So we can easily observe a great increase in people's access to general financial services. Facilitating the access of families to financial services, they begin to invest on educational expenses, healthcare, healthy nourishment, trading, and housing based on their priorities. Overall in many countries financial plans mostly focus on women. Women, provided with financial facilities, will receive a loan, guarantee to pay it back, keep their saving account and also they'll have insurance coverage. Micro-financial plans have an important message for families and communities. Many studies have proven that women's access to mentioned facilities may improve their conditions in family and society; it also helps them feel more self-confident and makes them aware of their own abilities. Thus providing micro-credit services for the poor in society is a powerful tool to reduce poverty and so that they are able to create assets, earn more money and become less vulnerable against the economic pressure. Of about 1.3 billion poor in the world there are 900 million poor women, this obviously shows that poverty has a feminine face. According to UN's development fund, 10% of world's income and less than 10% of world's assets belongs to women. While a majority of them never posses the capital needed for their activities, women still play an important role in the economic development of country. Therefore women draw the micro-credit policy maker's attention more than others. Choosing women as the main target of micro-credit plans is an effective strategy to eradicate poverty; because their income will upgrade the family welfare; furthermore earning money improves their social status. In some countries this choice is influenced by society's attitude and culture (Araghzadeh, 2002).

For instance founder of Grumman Bank of Bangladesh, Mohammad Yunes, has stated that: "women have plans for themselves, their children, and their family life; they always have an overlook while men just look for fun" to explain why 94% of their clients are women.

Women's access to micro-credits have shown that their income benefit to improve their family and provide livelihood. In addition to all these another reason of women being the target of micro-credit plans is that women have higher loan recovery rates. Totally, expanding women's access to micro-credits may lead to many useful results which in economy is mentioned as "virtuous spiral"; because their access to micro-credits results in family welfare and in a broader point it'll improve community's welfare and shall be increased welfare this process is repeated.

In researches that conducted by Nanda (2004) became clear that women participation in credits programs had positive effects on their demand about health care. Fiona Steele and et al (2008) in researches that conducted as called "

influences of credits programs on empowering women at Bangladesh , found that women who joined to credits programs , have participated in more educational programs and have married with more educated men and also they have saved more and they had more cash .

Shahnaj and Chaudhury(2009) in research as "credits and its role on empowering women " concluded that there is meaningful relation between attending in credits programs and empowering women , at economical dimensions .

Maybe the main challenges that threaten credits associations , is lack of necessary emphasizes on social dimensions and on reinforcing their basics , that practically cause that this social foundations lose its efficiency soon and practically changed to unsuccessful institution .

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Ellen and her Colleagues (2009) used approach called it "credits and education at Bolivia, Ghana, Honduras, Mali and Thailand". This approach looks for empowering women through financial services with education. In this approach, women get familiar with importance of credits through education and extension and also familiar with ways to access it through establishing different groups.

Ruhal Amin and others (2010) found that those who joined credit funds had more ability rather than those who didn't.

Jameela (2010) presented that credit programs has shown lot of affects on empowering women so that has increased their social, politic and economic ability.

Thus it is obvious that credits programs and its educational and empowering programs can be affective on social, humane and economic development or rural society, if it be associated with proper and gradual practices and base on reciprocal communications principles and apply opinion of local society.

A study conducted by Chabokru et al (1384) shows the crucial importance of micro-credits for farmers who do not possess physical financial assets (land, building, livestock, well…) and work in agricultural sector because of environmental conditions (such as living in a village) or because it's their ancestral occupation.

So today, women's participation in sustainable economic, social, and cultural development in rural areas is not optional but an essential matter. Those communities that have not seriously considered the necessity of participation faced failures and delayed community's development, welfare and security process. In any community, village, or social group, broad participation of every women in decision-making and any other matter related to national or local development programs, is a key variable in social sciences and in the last few decades, it has interested many scholars of socio-economic and especially cultural issues, and is considered as one of the most fundamental

democratic rights of women in a society. As we know in a popular participation, all people are given the opportunity to participate in planning and decision making for their society and for their own future. When in practice women feel that they can be involved in planning, policy making and deciding or solving problems in the society certainly they'll feel more solidarity and become more interested in social, economic, and cultural development programs.

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Improving empowerment of rural women through micro- credit

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Abstract: One of the raised strategy , in order to accelerate investment process and reinforcing financial foundations , and saving , at deprived and rural areas , has been empowering and eradicating poverty of rural societies through efficiency with emphasize on applying micro-credits. Micro-loans as useful tool to fight against poverty and starvation, has proven its capabilities and values to develop these areas. These tools have ability to change and improve human's life, especially poor peoples**.** Supplying credits and analyzing credits approaches cause opportunity to activate poor men's working power , establishing field for sustainable production and income , prevent usurers and pre shoppers of agriculture productions to plunder poor rural men and finally empowering poor people especially women who can work but were deprived to have capital and work tools , and extension accordance to their activities such as needs assessment , identifying target group , organizing poor people , giving needed specialized and public training and … have important role on effectiveness and make effective activities of these credits.

Introduction:

Aforementioned plan, due to containing special advantage such as giving accessible loan with low commission fee and no interest rate and also long-term repayment, could provide chances for many farmers to release from dealers and broker jobbers. In this approach, giving micro-credits to rural women seems more effective. because alongside agricultures activities that needed more investments , women by enjoying of very micro-credits not only could create remarkable creativities in rural productions but also gained worthy economic and social abilities , and even improved their field of social presence , well .(Lahsaeizadeh, 2000).

If rural women can work through receiving credits , loan and others finance facilities at favorite jobs and live through earned income (as it called "self-reliance and independence") , so undoubtedly we would see changes in social, economic and cultural relations of village.

Here, Basic issue is that if changes happened following of these events in villages, have positive aspects or negative? Naturally, every change in institutions and social phenomena has both positive and negative dimensions. (Farghdan, 2001)

Being high and low of each one is depended on various conditions and terms so it is varied from one society to another society. In Iranian rural societies, cultural and social context is such that, consequences

of these phenomena maybe being different and sometimes contradictory. However these actions caused that women stand in good economic condition and also gain self reliance and rely themselves with no help from husbands, but dominant cultural space on villages may create some disorders. At most of villages in Iran, patriarchal with all features dominate and women's financial self reliance may not being pleasant for some human and rural groups. When women gain financial independence in villages, impacts and social and cultural consequences would emerge. (Chabokru and etal, 2005)

Increasing Suffrage, lack of relying on vast patriarchal families, increasing cultural acknowledgment, relation with newer institutions, having intellectual independence, making decision for marrying, occupation, emigration and etc are those rights that they gain. gaining aforementioned rights by women in context of cultural and social framework followed some changes that maybe lead to disfunctions and even create disorders and abnormalities at traditional , familial and kinship relations that dominated on villages (Fakhraee 2002).

What that performing credits programs, has made in recent years, was on broad outlook with purpose to access to same results as above findings.

Thus, in one inclusive outlook , it is possible to use micro-credits programs to solve those issues which involved with rural women's economic

limitations , so that lead them toward social empowerment, in the context of economic growth(Rahmani andalibi, 2001) .

Micro-credits:

Micro-loans as useful tool to fight against poverty and starvation, has proven its capabilities and values to develop these areas. These tools have ability to change and improve human's life, especially poor peoples. Micro loans , saving accounts , and giving various bank services , cause this belief in low income and poor family that , by accessing to these services , their income will increase ,so they can protect themselves against barriers of unexpected problems and their current level of life and also invest on nutrition , housing and their children's education.(Varzgar and azizi, 2001)

Accessing to these conditions is among main goals of third millennium program (i.e. eradicating absolute poverty of human societies).

Nowadays micro-credits and supplying micro financial resources, has changed human's life and cause to revive different societies at poorest and richest countries of world, so that we can see growth in human's power to access to common financial services. By accessing to wide range of financial tools, families according to their priorities, invest on cases such as costs of education, healthcare, healthy and good nutrition or housing.

Applicants for Microfinance resources mostly involved family supervisor women, pensioners, homeless people, frugal workers, small farmers and micro entrepreneurs. These people are divided into four groups: Poor, very poor, relatively poor and vulnerable poor.

Whenever repayment afford , bond terms and accessing to data , in this classification will change , in order to supply sustainable financial needs of various clients , procedures and operation structures will be develop .(Fami, 2001)

Generally, in most countries, micro finance sources are considered for poor women. By women's access possibility to finance services, they committed to loan and ensure its repayment and preserve their saving accounts and also enjoy insurance cover. Supplying programs for micro financial resources have strong message for families and societies. Most of qualitative and quantitative studies and researches have proven that accessing to financial services; will improve women's conditions in family and society. Women's confidence has increased and they are aware of their abilities. (Banihashem, 1999)

In micro-credits programs other than offering and distributing micro loans, there are also small savings and deposits so that they are designed as form of saving-credit programs. The existing term in

phrase "micro-credits" points to two basic concepts that is due to dominant perspective on this approach. First term (i.e. credits) points to rural areas and lack of access for many villagers to formal resources that are one of their major problems. And at system of micro-credits, are tried to decrease poor families' access barriers to credit sources and also to increase effectiveness of these markets. Second term (i.e. micro) emphasize on deficiency of development, according to classic economist's method. Emphasizing on concept of "micro" means revising recommendations of market economy at rural society's development.

Empowering rural women:

Empowerment is capacity that woman can obtain in cultural and social environment, for economic independency and self reliance, by controlling over emotional decision making and far from violation. Empowering means, evolution and developing activities through non governmental organizations (NGOS) that lead empowerment to improve economic dimensions. (Amiri, 2000)

Enabling is process that, during it, people of society do activities to overcome barriers of advancement that finally cause their domination to determine their own density. The term "enabling" means overcoming fundamental inequalities. So it is different from self-reliance. (UNICEF, 1997)

Enabling, enables individual to overcome any problematic condition and consider barriers and problems as part of life and positive campaign. Finally, enabling provides energy to overcome most intellectual barriers and external problems at private life.

Thus, among all what have been said, it is possible to present suitable definition of enabling women, as follows:

"Process of explaining women about themselves (and also men about them) for instances that they must or want to do, and growth of their willingness and courage until they reach to needed competency "(management of rural and tribal women).

it should be noted here , that major factor which should be considered about women's ability , is eliminating individual and social barriers , and finally preparing field of economic and social participation for women at all fields . purpose of women's participation , is because of their dominance on all affairs of village including decision making process , organizations , forums , enterprising posts and … that involve , participation at all social and economic dimensions .

Criteria of empowering women:

Enabling as a theory of policy making for women, in it present five criteria:

Welfare, access, Concientisation, participation and control.

1- welfare criteria :

In this criteria, men and women as human resources of development should enjoy of desirable welfare conditions and equality (Paknazar, 2000).

Most of timing developmental programs, have worked on base of women's welfare. They have considered and provided some services for women who were passive recipient of these services. But these services were limited to physical needs and mostly were considered to revive their role of productivity, again. sometimes , it has been said that this approach has begun at colonial era and has considered women from poor country and intended services for them that dose not exceed from that poverty level . Agricultural and industrial projects were designed for men and social programs for women and children. Most of welfare programs were inadequate or its success was limited. Considerable point in this criteria is that men and women as human resources of development should enjoy equality and desirable welfare conditions. At this stage, women's material welfare and their enjoyment of welfare programs, compared to men (nutrition, death rate and …) were considered. And women's role as producer to supply their own needs isn't very important.

2. access criteria :

Lack of access or limited access for women to sources including (fields, job, capital and training) cause that their functions at production is less than men (Paknazar 2000). Access to facilities, sources, designed program and projects for women and access to schools and … are in this part. Just whenever most of other legal, cultural and social issues being solved, men and women would equally access to sources and facilities. Concept of enabling at this stage is that women have equal right to access to sources at family and greater society.

3- Concientisation criteria

Women should know that their problems aren't due to their individual inefficiency and shortage but it has emerged by social system in which discriminations has become formal and acceptable issue. (Araghzadeh, 2002). This stage is more critical and important than other stages. Because women can participate at development activities not just be passive users. Women have real equality at development, just when be aware. Concientisation will help to increase women's ability to equality at participation at society. At this stage, women face

with critical analysis with society and will find that what has been considered natural and unchangeable reality, is changeable. (Bakhshoodeh, 2005).

4- Participation criteria

One the most important items that this criteria has considered , is men and women's equal participation at decision making process of affairs of family at society (Paknazar 2000) . Men and women both should participate at process of assessment needs, designing, performing and evaluation of projects and development programs (UNICEF, 1998). In summary, this criterion means women's participation at all stages of surveying needs, detecting problems, planning, management, performing and valuation.

5- Control criteria

This criterion emphasize on this point that in addition to equal access of men and women to development sources , they must have adequate control on these sources that this issue is balance criterion , between men and women so that no one exceed other one (Paknazar 2000) . Women should have opportunities for decision making at workplace and home. If woman is producer, should be shared with part of her interest and wage. Women like men, should be able to choose her individual and social field and able to make decision and also development activities should be facilitator of these processes.

FAO (food and agricultural organization) addresses these three purposes as strategic goals while enabling women:

1- equality between men and women to access production sources
2- women's participation at policy and decision making
3- decreasing rural women's workload and increasing job opportunity and income for them (Paknazar 2000)

within theoretical framework of enabling women , having control on sources is presented as highest stage at women's participation process on development , but existing data at most developing countries , indicates that not only rural women haven't any control on financial resources of family but even they were deprived to access to sources and credits , specially through formal credits system (Shaditalab, 2002).

The question that arises here is that what relation is there between enabling women and micro-credits programs? Nowadays, micro-credits are considered as effective mechanism to eradicate poverty for women. Interests of micro-credits further increasing women's income, include:

- improving women's role in family

- Increasing women's confidence, not only through obtain financial success through business activity, but through increasing women's access to social services and communication with other women.
- Changing at social level (social class) at perspective of women's role.

Discussion and conclusion:

Supplying credits and analyzing credits approaches cause opportunity to activate poor men's working power , establishing field for sustainable production and income , prevent usurers and pre shoppers of agriculture productions to plunder poor rural men and finally empowering poor people especially women who can work but were deprived to have capital and work tools , and extension accordance to their activities such as needs assessment , identifying target group , organizing poor people , giving needed specialized and public training and … have important role on effectiveness and make effective activities of these credits .

Woroniuk Schalkwyk (1998) at their conducted research believe that now, micro credits, micro finance sources and small business unites are most effective mechanism to decrease poverty.

Plitt and others, conducted research as they called it "do credits programs, can empower women "? Results showed that corporation at credits programs helps empowering women.

Goetz Sengupta (2003), presented negative image of credits effects on empowering women. They concluded that most women have minimum control on their loans. And when repayment period is short, this shortage of control has devastating effects on women welfare.

Hashemi and others (2004) found that joining to Gramin Bank, has meaningful positive affects on controlling women, and helps to family income.

In researches that conducted by Nanda (2004) became clear that women participation in credits programs had positive affects on their demand about health care.

Fiona Steele and etal (2008) in researches that conducted as called " influences of credits programs on empowering women at Bangladesh , found that women who joined to credits programs , have participated in more educational programs and have married with more educated men and also they have saved more and they had more cash .

Ellen and her colleagues (2009) used approach called it "credits and education at Bolivia, Ghana, Honduras, Mali and Thailand". This approach looks for empowering women through financial services with education. In this approach, women get familiar with importance of credits through education and extension and also familiar with ways to access it through establishing different groups.

Shahnaj and chaudhury(2009) in research as "credits and its role on empowering women " concluded that there is meaningful relation between attending in credits programs and empowering women , at economical dimensions .

Ruhal amin and others (2010) found that those who joined credit funds had more ability rather than those who didn't.

Jameela (2010) presented that credit programs has shown lot of affects on empowering women so that has increased their social, politic and economic ability.

Thus it is obvious that credits programs and its educational and empowering programs can be affective on social, humane and economic development or rural society, if it be associated with proper and gradual practices and base on reciprocal communications principles and apply opinion of local society.

Maybe the main challenges that threaten credits associations , is lack of necessary emphasizes on social dimensions and on reinforcing their basics , that practically cause that this social foundations lose its efficiency soon and practically changed to unsuccessful institution .

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Preparation and Characterization of Sulphated Zirconia Catalyst Precipitated in Acidic Medium

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Abstract: Recently, sulphated zirconia has been widely studied, particularly its potential application as solid catalysts in acid catalyzed reactions. In addition, sulphated zirconia has oxidizing properties which may play a role in synthesis of organic compounds. The aim of this work is to prepare a series of SO_4/ZrO_2 catalysts precipitated by different sulphuric acid concentrations using sol gel preparation method. The prepared catalysts were calcined at 450, 550, 650, 800 ^{o}C. The surface acidity of the prepared catalysts was measured by n-butylamine method, and by pyridine adsorption. Sulphated zirconia tetragonal phase was successfully prepared in acidic medium using 2N H_2SO_4. It was observed that increasing of the calcination temperatures was associated with transformation of amorphous phase to crystalline phase. The incorporation of sulphate ions into ZrO_2, increased the surface acidity of the catalysts. Moreover, the acidity was found to increase with increasing the calcination temperature from 450 to 650 ^{o}C and then decreased. The surface area was found to increase with increasing of SO_4/ZrO_2 ratio upto 15% and then decreased. Also, the surface area was found to increase upto 550°C and then decreased. The precipitation of hydrous zirconia in acidic medium followed by calcination, produces solid materials with useful properties that favor their application in catalysis. The activity of these catalysts were tested for synthesis of 7-hydroxy 4- methyl cumarin. Usually the higher activity of these catalysts was attributed to its higher acidity.

1. Introduction:

Pechman condensation of phenols with keto esters to prepare cumarin and its derivatives has been known to be an important process, during which sulphuric acid is most frequently employed as catalyst (Potrdar et al., 2001). Cumarin belongs to the benzopyrone class of compounds that are present in many natural products, and have a great biological importance, many cumarin derivatives act as antibiotics.

This process is associated with problems of corrosion, large catalysts amounts, difficulties in the separation of the catalyst, solid acid catalysts such as, zeolites, clays, and sulfonic acid resins (delaude et al., 1993; Holderich and Bekkum, 1991).

Recently, zirconium oxide and compounds containing zirconium are increasingly being recognized as useful catalytic materials(Luo et al., 2005). Particularly, zirconium oxide (zirconia) is an important supporting material for catalysts having both acidic and basic properties(Chuah et al., 1996). Zirconia has an additional advantage that the nature of the active sites is known and may be defined by the generating Bronested and Lewis acid sites. In light of recent studies, many preparation methods have been explored to obtain the superfine ZrO_2 powders, such as hydrothermal process, vapor phase hydrolysis, sol gel process, and combustion methods (Ray et al., 2002).

It has also reported that sulfated zirconia has attracted considerable interest and was intensively studied in the last 20 years (Yamaguchi et al., 1990). So far, it is recognized as a very strong acid and possesses all the advantages of heterogeneous catalysts, such as easy separation, recovery and reutilization. Moreover, various recent works confirm that the acidity of sulphated zirconia is not stronger than the acidity of pure sulphuric acid (Babou et al., 1994; Babou et al., 1995).

This material exhibits high catalytic activity in a number of industrially important reactions (Zhao et al., 2008).

Sulphate-doped zirconia samples have also attracted great interest, due to their strong acid characteristics, and their potential as solid acid catalysts for selective hydrocarbon isomerisation and several other acid-promoted reactions. In this way sulphated zirconia was classically synthesized by precipitation of zirconium hydroxide by hydrolysis of $ZrOCl_2$ aqueous salt solutions (Morterra et al., 1994; Melada et al., 2004; Li et al., 2006).

It's now well known that, sol gel template method is a popular one because of its distinctive advantages such as the high purity, the

homogeneous multi component and the easy chemical doping of the prepared materials (Yang et al., 2005, 2006).   So far, this method typically entails hydrolysis of a solution to obtain a colloidal particles (sol) and then a gel is formed. The latter point   is of a particular importance for surface area of zirconia as usually rather low, in order 20 - 50 m^2/g. Different routes to obtain zirconia with high

surface area have been reported (Ozawa et al., 1990; Ciesla et al., 1996).

However, most of the reported catalysts suffer from various drawbacks such as long reaction times, and some of the reagents used are expensive. In this study, we employed the promoted ZrO$_2$ catalysts for the synthesis of substituted cumarin from resorcinol and substituted resorcinol with ethyl acetoacetate (Scheme 1) (Reddy et al.,2001; Reddy et al., 2005).
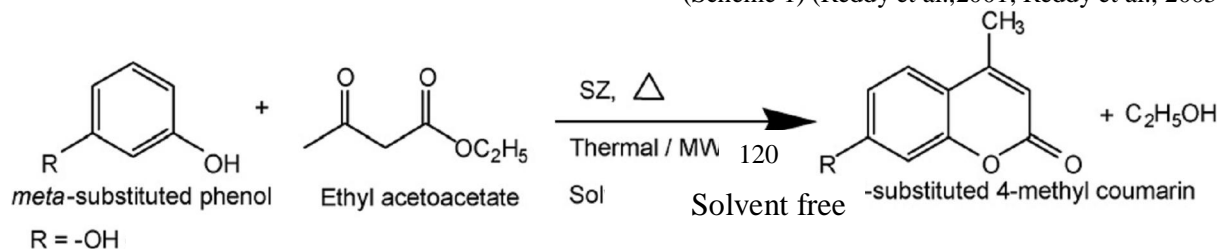
Scheme (1). Schematic synthesis of 7-substituted 4-methyl cumarin.

We conducted a test: A solvent free Pechman reaction mixture was irradiated with microwaves of low power. The temperature of the reaction mixture started to rise.   After 20 min of irradiation, the microwave oven was switched off. The reaction mixture continued to rise in temperature after addition the catalyst (Manhas et al., 2006). The reaction was completed in less than 10 min (monitored by TLC). The target cumarin was isolated. This new energy-saving procedure was found to be useful for the efficient preparation of several cumarins.

The aim of the present work is to bring new evidences concerning the role played by the sol gel preparative variables, and surface features of sulphated zirconia catalysts, as well as the effect of the type of starting salts on the sol gel method. Additionally trying to establish the correlation between the catalytic properties of sulphated zirconia samples calcined between 450 - 800°C and their acidic properties.

2. Material and Methods

Zirconium hydroxide was prepared by sol gel method from zirconium oxy chloride salt dissolved in ethanol (Dominguez al., 2000) and precipitated

by adding a drop wise of H$_2$SO$_4$ (analar) with vigorous stirring. These gels were dried at 120 ^{o}C for 24 hrs, to obtain the powder from sulphated zirconia, followed by the calcination at different temperatures (450, 550, 650, 800°C) for 3 hrs.

In the designation of these samples, the letters S, Z denotes to SO$_4$ and ZrO$_2$ respectively. The roman numbers I, II, III, and IV referred to the calcination temperature. While the Arabic numbers 1, 2, 3 and 4 represents SO$_4$ concentration. The designation 2SZI indicates, the sample precipitate by 2N H$_2$SO$_4$, and calcined at 450 ^{o}C.

Thermal analysis (TGA) of the uncalcined samples was carried out using a Shimadzu thermal analyzer, type 50-H. The samples under examination being heated in N$_2$ stream at a rate of 10 ^{o}C/min throughout.

X-ray diffraction patterns of the calcined samples were recorded using a Philips PW 105 diffractometer using Ni-filtered Cu Ka radiation (=1.540 A °) at 40 kV, 30 mA, and a scanning range 2hrs of 18-80°. The percentage of zirconia tetragonal phase was estimated with the following formula (Devassy et al., 2005)

$$\% \text{ Tetragonal} = \left[\frac{IT(2\theta = 30.15)}{IT(2\theta = 30.15) + \left(\frac{IM(2\theta = 28.16) + IM(2\theta = 31.44)}{2} \right)} \right] \times 100$$

The crystallite size (nm) was calculated from the reflection of tetragonal zirconia phase at 2hrs of

30.15, using the Scherer relationship (patterson, 1939)

$$D = \frac{k\lambda}{\beta \cos\theta}$$

where k is the crystallite shape constant (1),   is the radiation wavelength (A˚),   is the line breadth (radians) and   is the Bragg angle.

The specific surface areas of the calcined samples were determined from nitrogen adsorption studies conducted at -196°C using the high vacuum conventional volumetric glass system (BET). Prior to any adsorption measurement, the sample was degassed at 250 ^{o}C for 3 hrs under a reduced pressure of 10^{-5} Torr.

The total acidity of all catalysts was determined by means of the n-butyl amine titration method (Suryanarayana et al., 2006; Ahmed et al., 2007) , using an orion 420 digital model using a double junction electrode. In this method, 0.2 gm catalyst was heated under vacuum, then 10 ml of acetonitrile was added. After agitation for 2 hrs, the suspension was titrated by 0.01 N. n-butyl amine in acetonitrile. The addition is continued till no further change of mv recorded.

The FTIR spectra of the samples were carried out at room temperature. 0.05 gram of each sample was degassed for about 2 hrs at 250 C to reduce the amount of adsorbed molecular water. The samples were maintained in contact with pyridine vapor, at room temperature, for one week, after that 0.05gram of the sample is mixed with 0.1 gram of KBr in a 30 mm diameter self supporting discs.   The FTIR spectra of the 0.05 gram of samples were recorded at room temperature in region 1700-1400 cm^{-1}. (Mohamed et al., 2000)

The catalytic activity of the investigated samples towards peckman condensation reaction was studied. In a typical experiment, a mixture of resorcinol (1.1 gram, 10 mmol) and ethyl acetoacetate (2.5 ml, 20 mmol) was added in a 50 ml round flask. This reaction mixture was placed in oil bath and refluxed for 2hrs at 120˚C (Sethna et al., 1953; shockravib et al., 2005). At the same time, (0.1 gram) of catalyst was activated at 120˚C in oven, then added to the reaction mixture and refluxed continuously for another 2 hrs. Finally, the reaction mixture was allowed to cool down to room temperature by pouring the reaction mixture at 50ml beaker containing crushed ice. The product 7-hydroxy-4-methyl cumarin was separated, tested and characterized by its melting point and FT-IR spectroscopy.   The yield (wt%) was obtained as follows (Tyagi et al., 2008) :

$$yield\ (wt\ \%) = \frac{obtained\ weight\ of\ product}{theoritical\ weight\ of\ product} \times 100$$

3. Results:

X-ray diffraction: Zirconia precipitated by sulphuric acid samples were investigated to confirm the effect of sulphate group presence on the surface area and on the tetragonal phase of zirconia. The phase composition and the crystallites sizes were determined for all the precursors and calcined samples. Figures 1, 2 and table 1 show the effect of the variation of the concentration of sulphuric acid, and the calcination temperature on the crystal size and the percentage of tetragonal phase respectively. The results show that the tetragonal phase increases by increasing the sulphate content upto 15% and then decreases. While by increasing the calcination temperature the phase changes from amorphous to crystalline (tetragonal and monoclinic) phases.

Thermo gravimetrical analysis: Figure 3 shows three main weight loss regions. The first centered at about 100°C, due to the physisorbed water.   The second in the range 150–350°C, suggesting that beside the dehydration and dehydroxylation processes, elimination of carbonaceous species from the surface occurred.   The third at 570 - 800°C, associated to the amount of sulphate introduced during the sol gel synthesis.

Surface characterization by nitrogen physisorption: The BET analyses of all the samples give isotherms of type II, with hysteresis loop of type H3 or H4 of the IUPAC classification, which is characteristic of slit-shaped pores. The first remark concerning the morphology deals with the surface area of the samples. Figures 4 , 5 and table 2 show the effect of both the concentration of sulphate and the calcination temperature on the surface area, respectively.   Figure 6 shows the pore size distribution in order to determine the types and the distribution of the pores. The results show that the surface area increased by increasing the sulphate content up to 15% and then decreases. While by increasing the calcination temperature the surface area increases upto 550 ^{o}C and then decreases.

Surface acidity: The surface acidity of the investigated catalysts was determined by non aqueous titration of n-butyl amine (pKa=10.73), which is a basic molecule suitable for titrating the medium and strong acid sites on the surface of the investigated catalysts (Pecchi and cid, 1985). Figure 7 illustrates the variation of the electrode potential for the investigated catalysts calcined at 650 ˚C with volume added from n-butyl amine.This figure shows that, as the acid sites of the solid become neutralized, a buffer behavior becomes more apparent.   The trend of the titration curve is asymptotic, leading to a characteristic value on the potential (mV) axis. This is related to the volume added from n-butyl amine / g needed for neutralization of the surface acidity (Dominguez. et al., 2000). The magnitude of

change of the electrode potential in this method is related to the surface acidity of the catalyst (Barrett et al., 1951). Table 3 shows the volume of n-butyl amine /g needed for the neutralization of the surface acidity of catalysts and the total number of acid sites /g. This technique was carried out by measuring the electrode potential (mV) as a function of the progressive increase of the n-butyl amine concentration expressed as (mmol n-butyl amine / g catalyst). The total number of acid sites /g of the catalysts were calculated from the following relation:

Total number of acid sites / g = (ml equiv./g) × N × 1000 (Where N is Avogadro's number).

Figure 8 shows that sulfated zirconia samples contain mixture of Bronested and Lewis acid sites. The number of Bronested and Lewis acid sites were calculated from adsorption of pyridine. The Bronested and Lewis acidity were calculated and quantified from integrated areas of the absorbance bands at 1541 and 1446 cm^{-1}, respectively. Advantages of this technique are : Bronested (B) and Lewis (L) acid sites can be distinguished because the IR spectra of adsorbed pyridine shows characteristic difference (Tanabe, 1970). The fact that the spectrum of pyridine coordinately bonded to the surface permits the differentiation between acid types on the surface of a solid acid (Parry, 1963). It is well known that the shift in the band wave number to higher values indicates that the strength of the acid sites increases, in contrast, the shift to lower wave number indicates that, the strength of the acid site decreases.

Catalytic activity for synthesis of cumarin: Table 4 shows that, Packman reaction depends mainly on the acidity of the catalysts (number of bronested and Lewis acid sites) which characterized using (FT-IR).

Figures 9 and 10 show the correlation between number of acid sites and the cumarin % with the variation of the sulphuric acid concentration, and the cumarin % with the calcination temperature. The results show that by increasing the sulphate content the acidity and cumarin % increases. While by increasing the calcination temperature the acidity and cumarin % increased up to 650°C and then decreased.

Table 1: Phases identified by XRD, as a function of SZ calcined at 650°C and as a function of different calcination Temperatures for 2SZ samples

Samples	Crystal size nm	Tetragonal %	Samples	Crystal size	Tetragonal%
1SZIII	17	53.62	2SZI	Amorphous	-
2SZIII	25.8	82.11	2SZII	Amorphous	-
3SZIII	23.3	76.69	2SZIII	25.8	82.11
4SZIII	16.5	63.39	2SZIV	47.7	51.34

Table 2: Textural parameters of the catalysts investigated as evaluated from N$_2$ adsorption at -196 ^{o}C

Samples	S_{BET} m^2/g	S_s m^2/g	S_T m^2/g	r$^-$ A^o	V_T ml/g
1N SZ 450	41.14	41.05	41.21	36.9	0.069
2N SZ 450	60.36	58.20	61.1	38.70	0.117
3N SZ 450	51.01	50.80	51.10	34.90	0.085
4N SZ 450	38.09	38.11	37.89	36.0	0.057
1N SZ 550	73.87	73.76	74.60	37.94	0.140
2N SZ 550	97.54	94.82	90.50	38.64	0.188
3N SZ 550	89.02	89.52	83.3	34.98	0.156
4N SZ 550	71.71	68.89	69.1	35.62	0.128
1N SZ 650	64.12	63.99	64.08	43.20	0.130
2N SZ 650	81.38	79.41	80.10	44.0	0.179
3N SZ 650	73.12	73.01	73.15	40.20	0.147
4N SZ 650	55.00	54.91	54.98	41.80	0.119
1N SZ 800	30.1	30.05	30.08	17.89	0.0170
2N SZ 800	51.11	50.99	51.01	18.28	0.047
3N SZ 800	44.31	44.20	44.25	14.50	0.015
4N SZ 800	24.30	24.22	24.29	15.10	0.008

Table 3 :Acidity parameters of the investigated catalysts:

Samples	No^o. of Acid sites x 10^{-18}	No^o. of lewis acid sites x 10^{-18}	No^o. of Bronested acid sites x 10^{-18}
1SZIII	2.41	0.96	1.36
2SZIII	3.01	1.05	1.70
3SZIII	3.14	1.35	2.00
4SZIII	3.207	1.45	2.22
2SZI	2.28	0.89	1.38
2SZII	2.67	0.99	1.56
2SZIII	3.01	1.05	2.00
2SZIV	2.35	2.20	-

Table 4: Effect of concentration and calcination temperature on the cumarin conversion %

Samples	Cumarin % by microwave	Cumarin % by simple method
1SZIII	10.89	11.12
2SZIII	20.50	21.15
3SZIII	26.79	27.20
4SZIII	39.88	40.87
2SZI	11.01	11.38
2SZII	16.85	17.43
2SZIII	20.83	21.15
2SZIV	11.42	11.60
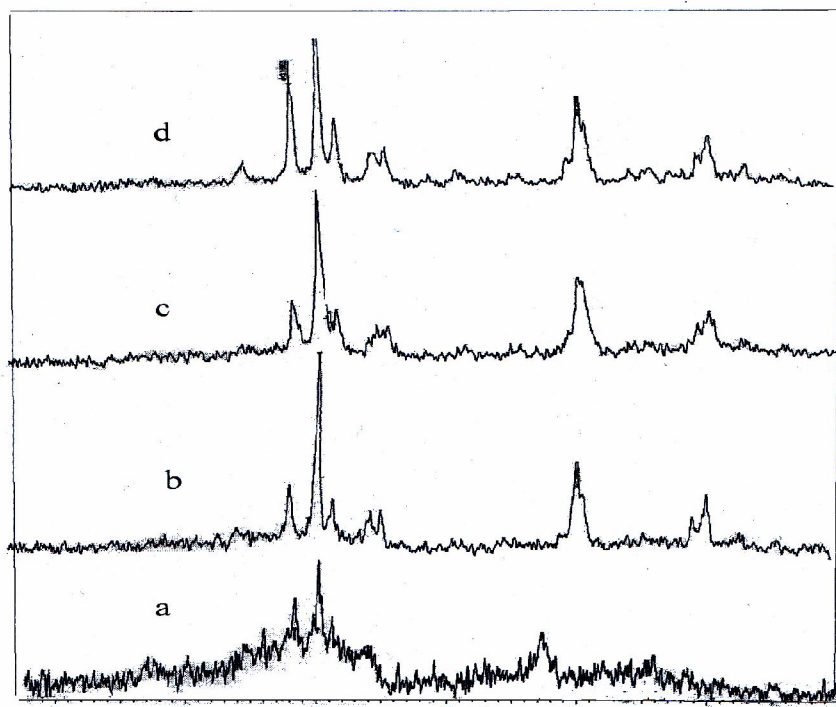
Fig. 1: X-ray diffraction patterns of the different SZIII concentration, (a)1SZ III, (b)2SZ III, (c) 3SZ III, (d) 4SZ III
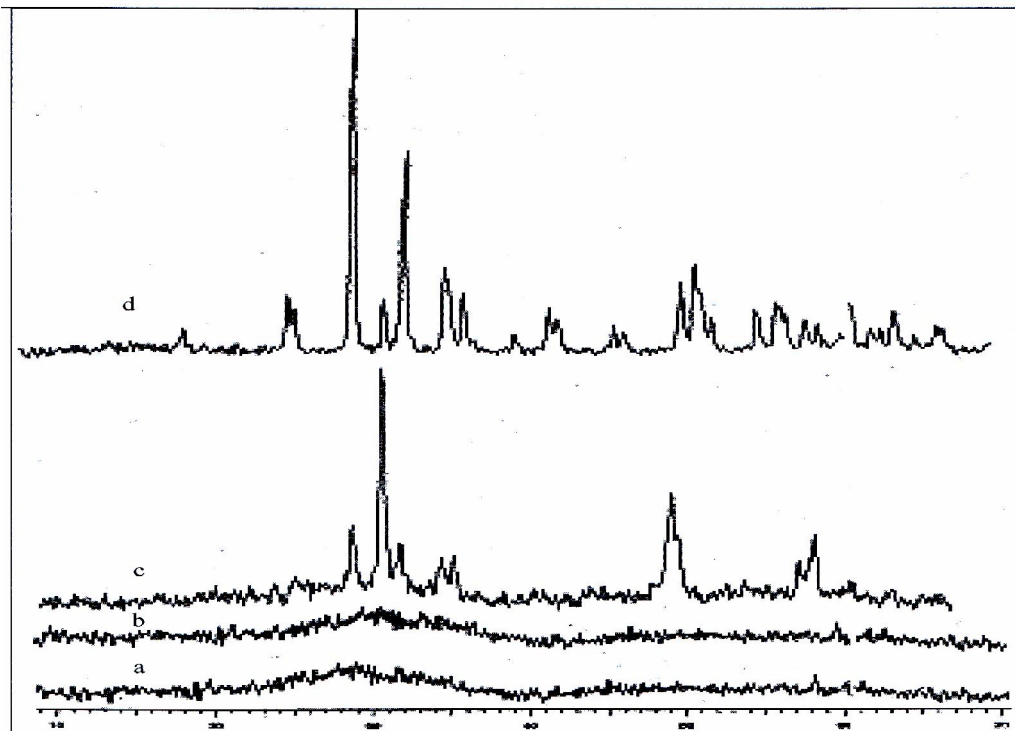
Fig. 2: X-ray diffraction patterns of 2SZ samples calcined at (a) 450°C,(b) 550°C, (c) 650°C, (d) 800°C
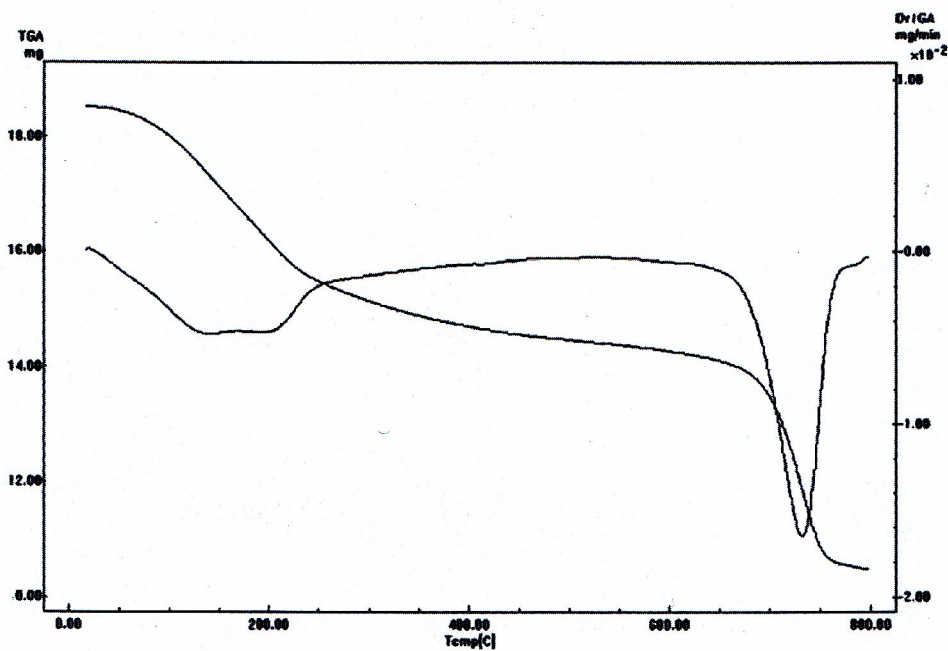
Fig. 3 : Thermogravimetric and Derivative Thermogravemetric Curves for 2NSZ
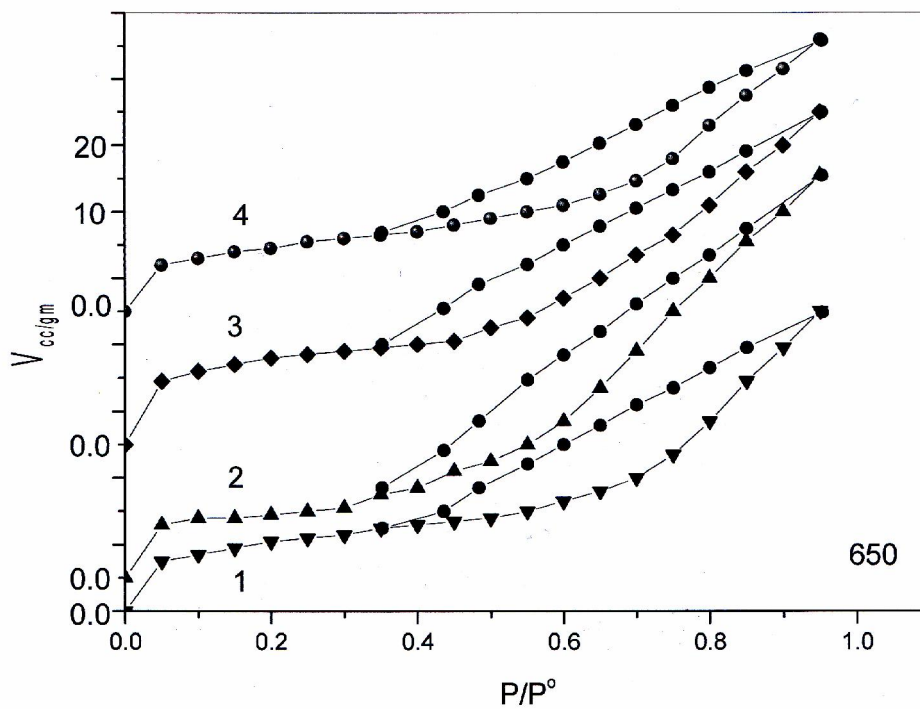
Fig. 4 : **Adsorption – desorption Isotherms of Nitrogen at -196°C, on different SO$_4$ concentration for samples calcined at 650°C**
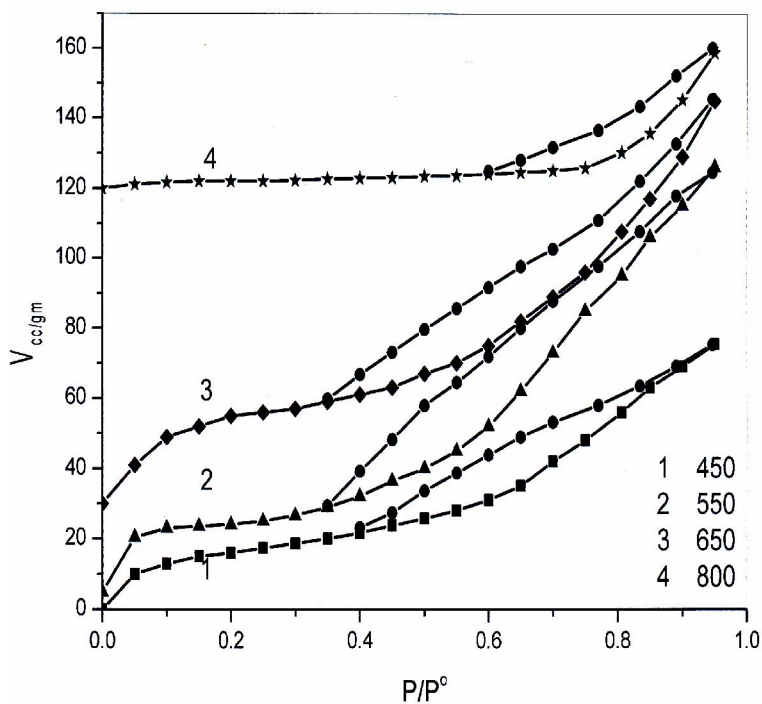
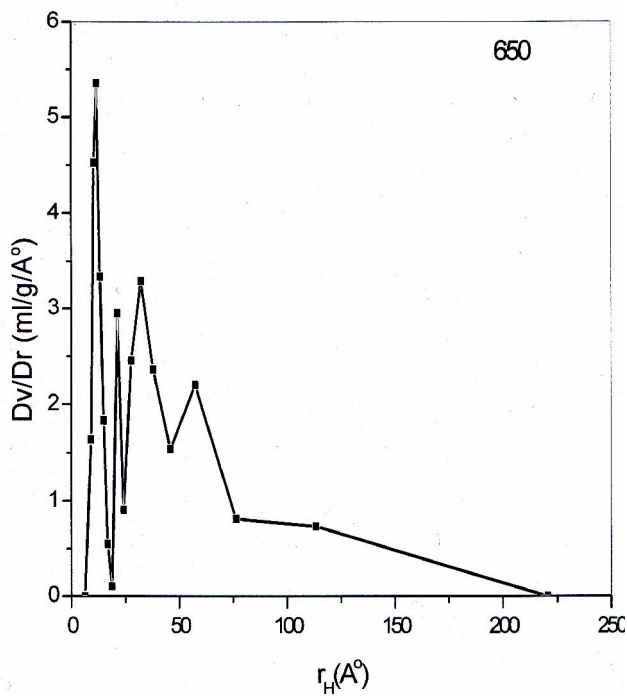**Fig. 5:** **Adsorption – desorption Isotherms of Nitrogen at -196°C, on different Calcination temperature for samples at concentration 2N**

Fig 6 : Pore volume distribution for sample calcined at 650 ^{o}C
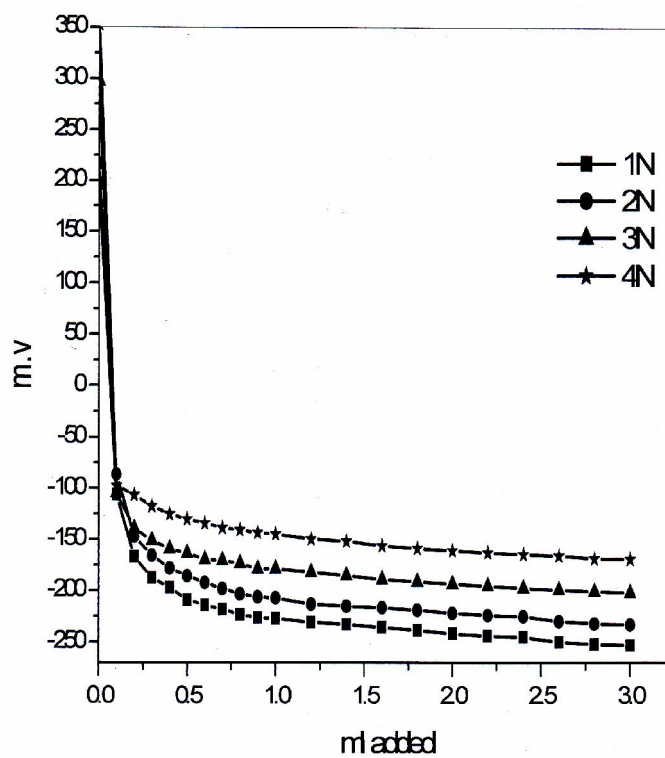
Fig. 7 : potentiometric titration curve for sample calcined at 650 ^{o}C
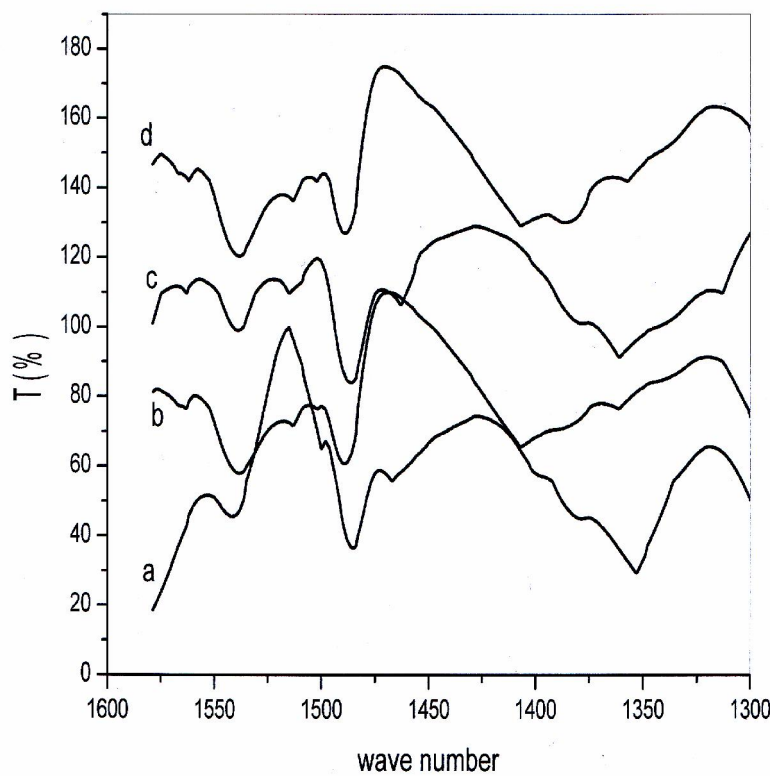
Fig. 8. FT-IR spectra of pyridine adsorbed of the different SZIII concentration, (a) 1N,(b) 2N, (c) 3N, (d) 4N.
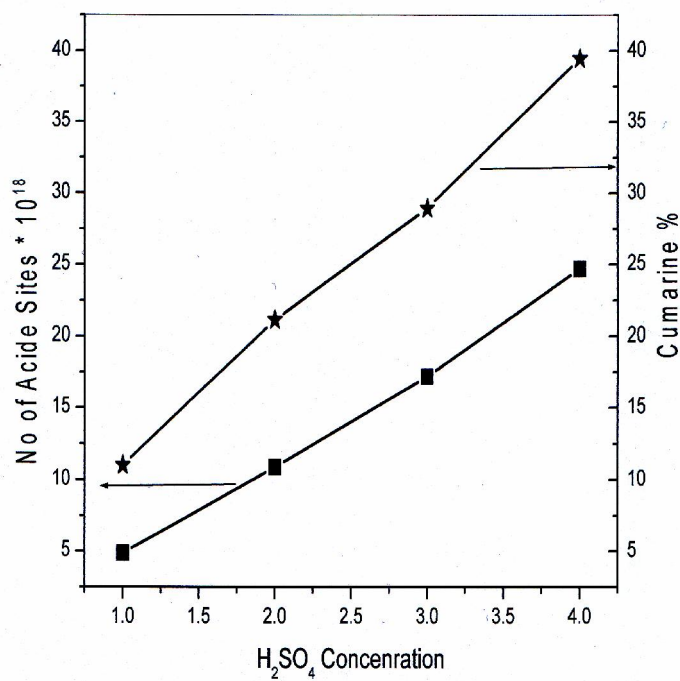
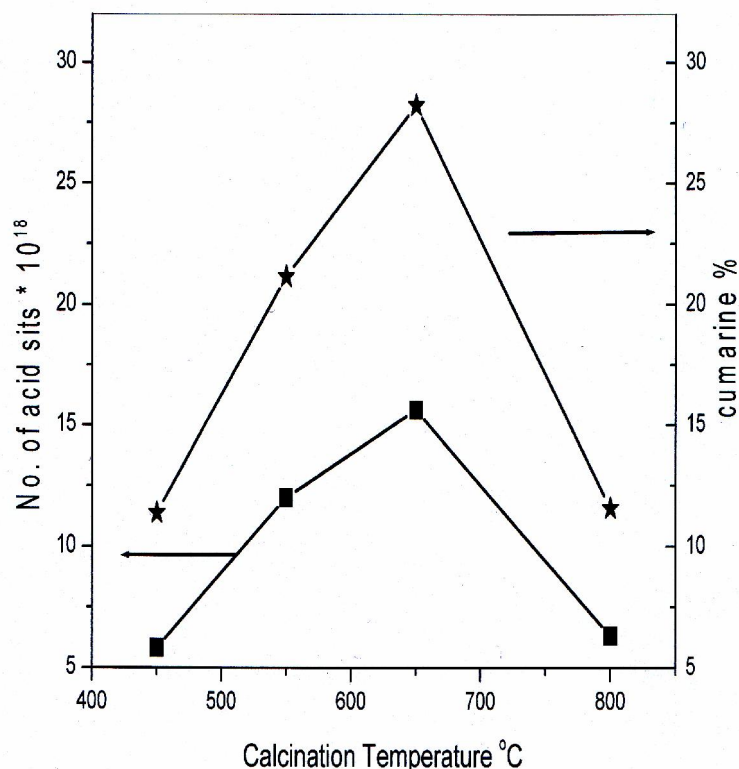**Fig. 9: Correlation between No. of acid sites and Cumarine%**

Fig. 10 : Correlation between No. of acid sites and Cumarine%

4. Discussion:

The obtained x-ray data shows the importance of the sulphate introduction step in the crystal phase development of sulphated zirconia. This feature could be explained on the basis of sulphate in stabilizing the tetragonal polymorph (Babou et al., 1994) and delaying the growth of zirconia crystallites (Babou et al., 1995). Increasing the concentration of SO_4^{2-} upto15%, the tetragonal phase increases.

All the sample precursors calcined below 650°C are amorphous. While the samples calcined above 650°C begin to crystallize, and the monoclinic (2 = 28° and 31 °) and tetragonal (2 = 30 °) phases appear.

It is clear from the nitrogen adsorption desorption isotherms that, by the increase of the concentration of sulphuric acid surface area increases upto 15%, and then decreased. This can be attributed to the increase in sulphate concentration which blocks the active sites on the surface. This was interpreted by the X-ray where, the tetragonal phase increases with increase of the sulphuric acid up to 15% and then decreases. On the other hand, the surface area was found to increase by the increase of the calcination temperature upto 550 °C and then decreased. This is

illustrated also by the X- Ray data. The samples calcined at 450 and 550 °C are amorphous, which show relatively higher surface area. While by increasing the calcinations temperature over 650 °C, the surface area decreases, which may be attributed to the sintering process and the grain growth of the pores.

It was clear from the potentiometric titration curves that the number of acid sites increases with the increase of sulphuric acid concentration. This could be attributed to the number of SO_4^{2-} groups bonded to the zirconia surface. So SO_4^{2-} / ZrO_2 interaction could be responsible for the acidity of the catalysts.

The results of calcination temperature on the surface acidity indicate that, the optimum temperature is 650˚C. Further increase in calcination temperature to 800˚C leads to notable decrease in the total acidity. This may be due to evolution of SO_3 gas as a result of decomposition of the sulfate groups bonded to the surface of zirconia .

The pyridine adsorption technique, enables us to evaluated the surface and the bulk acidity, because of the high absorptivity of sulfated acid catalyst to polar molecules as pyridine (Devassy, 2005; De Castro et. Al., 1998; Sun et al., 2002). In addition to the reaction with the surface protons, pyridine also

penetrates and reacts with the bulk acid sites of sulphated zirconia. The integrated area of pyridine bands is a measure of number of Bronested and Lewis acid sites. The intensity of Bronested band (1541 cm^{-1}) and that for Lewis (1446 cm^{-1}) changed as the sulphate concentration on zirconia and the calcination temperatures changed.

Sulfated groups generate strong Lewis and Bronested acidity when adsorbed on the surface of sulphated zirconia. Sulfate species are itself Lewis acid sites or by attracting electrons, they generated Lewis acid centers on the oxide surface. Moreover, the chemical state of the sulfate groups sometimes determines the acidity of the oxide surface (Das et al., 2003). These results indicate that, the number of sulfate groups distributed on the surface of zirconia is maximum and responsible for the maximum acidity reaches at $650°$C. At calcination temperatures higher than $650°$C, the sulfate groups on the surface of zirconia decompose and thus the surface acidity decreases.

It's clear that from the activity measurement by cumarin conversion, and acidity measurements, the increase of H$_2$SO$_4$ concentration, increases both the acidity and the catalytic activity. Moreover, the activity increases by increasing the calcinations temperature upto 650 °C and then decreases. This may be attributed to the decomposition of the sulphate group after $650°$C and its evolution as SO$_x$.

5. Conclusion:

On the basis of the above finding, the following are the main points that can be summarized: The increase in sulphate concentration in zirconia samples, the tetragonal phase increases by increasing the concentration upto 15% and then decreases. The samples calcined at 450-$550°$C are totally amorphous or poorly crystalline. The rise of calcination temperature resulted in appearance of tetragonal and monoclinic phases. The increase in sulphate concentration leads to a continuous increase of S$_{BET}$ upto 15%. Further increase of H$_2$SO$_4$ concentration decreases the S$_{BET}$. One can attribute the change of surface area of samples investigated to its capability to control zirconia phase transition and sintering of support. SO$_4^{2-}$ / ZrO$_2$ interaction as well as the calcination temperatures will be responsible for acidity. As the rise of H$_2$SO$_4$ concentration the acidity increases, this is due to the increase of SO$_4$ group. Also as calcination temperature increases the acidity increases up to 650 °C and then decreases. This due to above 650 °C the SO$_4$ decompose and went as SO$_x$. The increase of surface acidity was associated with an increase of cumarin conversion up to 650 °C and then decreased.

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Stabilization of Sand Dunes in North Sinai Using Some Economical Plants

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Abstract: Three plant species; *Acasia saligna, Prosopis jullflora* and *Morus alba* were cultivated in sand deposited at El-Maghara station, North Sinai. Every kind of plants was planted in three perpendiculars to wind dominant directions to control the sand encroachment wards to the economic cultivation. Sand collectors (traps) were set up at the four wind directions to study the transportation of sand and its accumulation. The transportation of sand in an open area and in front of three kinds of plants was collected during two annuals. The analysis of sand trapped by the sand collectors reveals Acasia plants were superiors in minimizing of sand encroachment than the two kinds of plants, i.e. Prosopis jullflora and Morus alba. The growth behaviours of *Acasia saligna* and *Prosopis jullflora* were superior to *Morus* species. Physical and chemical analysis showed differentiation in edaphic factors in both areas, i.e., unstabilized and stabilized as well as immerge the three kinds of plants.

Key Words: Meteorological – Edaphic – Sand dunes – Stabilization - movement - growth behavior - *Acasia saligna - Prosopis jullflora - Morus alba* – Migration – accumulation – traps.

Introduction:

Sand dune fixation is designed to prevent the movement of sand long enough to enable either natural or planted vegetation to become established. In the arid and semi arid regions various species of trees, shrubs and grasses can be used for dune fixation (Kaul, 1985). *Atriplex spp.* and *Acacia spp.,* are among the effective plant species used for the control of shifting sand dunes (Draz *et al.*, 1992). In El-Shaikh Zuweid Gad (2004) used biological fixation of coastal sand dunes (Moghat, Liquorice, Sisal and *Opuntia*). Ndiaya *et al.* (1993), showed the growth and yield of *Casuarina eqisetifolia* plants cultivated for the control of the coastal sand dunes of Senegal. Moreover, the monitoring of four plant species grown for sand drift control in India showed that, the growth and the survival of such plants were best on the dune crest and lee ward slopes (Kumar and Shankaranarayan, 1988).

Furthermore, the most popular cultivated plants tolerated the stress conditions under sand dunes in Egypt are: *Acasia saligna, Prosopis pallide* and *Atriplex nummularia* (Draz and El-Maghrabi, 1997 and Gad 1999).

This study is dealing with the prevailing environmental factors and mean activities under El-Maghara sand dunes conditions in North Sinai to clarify the ecological factors affected fixation of mobile of sand dunes in such area.

Materials and Methods:

El-Maghara Research Station is located at 70 Km Southwest El-Arish city, North Sinai peninsula, the elevation is 895 foots above the sea level. The prevailing wind directions are NNW (North- North-West) and WNW (West-North-West), where the resultant sand drift direction was SSE (South-South-East).

This study was carried out to get some clue information about the growth behavior and the effect of the plant fences of three suggested plant species cultivated under El-Maghara on sand dune stress conditions. The center is located at 30º 52' 07" N and 32º 55' 09" Et

The economical plants of *Acasia saligna, Prosopis jullflora* and *Morus alba* were cultivated in three plots (20 x 80m =1600m²) each, and arranged in grid system 4 x 4m (alternated row). The cultivations were carried out at the North to West direction perpendicular with the dominant wind directions.

These plots were located at the windward side of the mobile sand dunes and perpendicular to the effective wind directions. The plants were irrigated by drip irrigation technique.

Acasia saligna and *Prosopis jullflora*, seeds were obtained from private form at El-Arish and sown after H_2SO_4 treatments in polyethylene bag 20 × 30cm, left to germinate and grown under polyethylene greenhouse conditions for one year then retrains planting under permanent conditions at 30/11/2007

Morus alba, Stem cutting were collected from the experimental farm of the faculty of Agricultural (Moshtohor), sown after indol buteric acid (I. B. A. 1 ppm concentrate) treatments in polyethylene bag 20 × 30 cm and left to germinate

and grown under polyethylene greenhouse conditions for one year then retrains planting under permanent conditions at 30/11/2007

Ecological studies

Table (1) Average seasonal changes in recorded metrological data at El-Maghara research stations during the period extended from 2005/2008.

Seasons	Wind speed (Km/h)	Air temperature (ºC)	Air relative humidity (%)	Total rainfall (mm)	Sun shine (Kw/m²)	Evapo-transpiration (mm/day)
Winter	7.503	20.430	74.163	6.27	3.557	1.890
Spring	8.460	24.933	76.400	0.00	5.083	3.903
Summer	8.130	31.000	76.613	0.00	6.493	5.790
Autumn	7.947	25.273	76.470	8.31	4.250	2.523
Total	-	-	-	14.58	-	-
Mean	8.010	25.409	75.912	-	4.846	3.527

Source: El-Maghara station (Desert Research Center).

2- The irrigation water of El-Maghara research station. The chemical analysis of irrigation water was carried out according to (Richards, 1954) and indicates that water is highly saline with medium sodium content, (Table, 2)

Table (2) - Chemical analysis of the irrigation water of El-Maghara research station

Seasons	Soil pH	E.C		Soluble cations (ppm)				Soluble anions (ppm)			
		ppm	Mmhos/cm	Ca^{++}	Mg^{++}	Na^+	K^+	CO_3^{--}	HCO_3^{--}	SO_4^{--}	Cl^-
Winter	7.7	2961.44	4.63	142.7	232.8	541.2	9.38	12.9	203.2	682.5	113.7
Summer	7.5	3195.65	4.99	366.3	155.7	469.4	85.6	10.5	78.4	629.7	795.5

Soures: - Seidhom;S.H. and Evon,K. Rizk (2006)

3- Edaphic data:-
These edaphic factors were evaluated for soil samples collected from the El-Maghara sand dunes.
Analysis of soil samples

The collected soil samples were subjected to the following analysis:-
a) - Soil moisture content was determined for the soil samples of 0-10, 10-20 and 20-30 cm depth at 105 °C constant weight.

$$\text{Soil moisture content \%} = \frac{\text{Fresh weight – dry weight}}{\text{Dry weight}} \times 100$$

b) - The collected soil samples were air dried, grind and passed through a 2mm sieve. The fine soil samples were subjected to physical and chemical analysis as described by Page *et al.* (1982).
c) - Particle size distribution was carried out for all soil samples using the standard sieving technique (Folk and Ward 1973).
d) - Soil reaction (pH) was measured in soil water suspension (1:2.5) using Beckman bench type pH meter, KM 7001digital pH meter
e) – Soil salinity (EC) of soil extracts measured by electric conductivity in soil water suspension (1:2.5) after Jackson method (1973) using EC meter (digi meter L21).

f) - Organic Matter (OM) content was determined by use of ferrous sulphate (Jackson, 1958).
g) - Soluble cations and anions were determined in the soil saturation extract according to Jackson (1973) as follows:-
- Sodium and potassium were determined by flame photometer (Perken – Elmer, model 149).
- Calcium and magnesium were determined titrimetrically by versenate (EDTA) method.
- Soluble carbonates and bicarbonates were determined by titration with standard hydrochloric acid.
- Chloride was determined by titration with silver nitrate using potassium chromate indicator.
- Sulphate was determined by the turbidity method using spectrophotometer.

Migration and soil accumulation

The amount of migrated soil collected by soil traps (kg) measures soil accumulation, while measuring stands were used to calculate soil erosion. The distribution of soil traps and measuring stands were arranged in different directions on various sand dunes unstabilized and stabilized by cultivated plants in four directions (North, East, South and West). Soil particle size and the depth (cm) of soil burial accumulations on cultivated plants were also determined.

4- Phytological data:
The economical plants cultivated on stabilized El-Maghara sand dunes were analyzed as follows:-

4-1- The growth parameter
Dominant plant height (cm), shoot diameter (cm), number of shoots, shoot length (cm), number of leaves on shoot, and leaf area (cm²). Moreover, the plant crown cover (m²) and crown volume (m³) were calculated according to the formula of Thalen (1979) as follows:-
Crown cover (m²) = ¼ M. D1. D2
Crown volume (m³) = 1/6 M. D1. D2 .H
Where:
D1 = the largest diameter of the crown.
D2 = the smallest diameter of the crown.
M = 22/7 and,
H = height of the tree.

4-2- Chemical constituents of the plant organs (leaves and stems) were detected as follows:-
Crude dry weight was measured according to official Agricultural Chemical (A. O. A. C., 1970). Sodium and potassium were determined according to the method of Johonson and Ulrich (1959), by the use of flame photometer (Perken – Elmer, model 149). Data were presented as gram per 100 grams dry weight. Phosphorus was colorimetrically determined by ascorbic acid method as described by John (1970). Total nitrogen was detected by Kjeldahl method (A. O. A. C. 1970).

Method of tabulated data and Statistical analysis
The tabulated data were presented as the probable relations between three economical plants. i.e. {*Acasia saligna, Prosopis jullflora* and *Morus alba*}. Statistical analyses were conducted as randomized complete blocks design. New L.S.D. at 5% level and simple correlation coefficients (r) between certain environmental factors and collected data were calculated using "Computer, Co-Stat program" according to the method described by Snedecor and Cochran (1980).

Results and discussion:

Meteorological factors: The climatologic data were collected from El-Maghara station (Table 1).These data show:
i)- There is no doubt that the climatic data are quite different from one season to another and from month to another.
ii)- Wind speed is considered the main factor affecting the formation of sand dunes and their activity of mobilization. Its direction may affect the shape type of sand dunes.
iii)- The total rainfall and evapo-transpiration in relation to air temperature and sunshine affect distribution of plants and their growth.
iv)- The total rainfall is generally below 80cm/ year, there fore the area may be considered under semi arid conditions and most of rainfall is found in autumn and winter seasons, while others seasons are dry.

Edaphic data:
Soil moisture percentage to unstabilized and stabilized sand dunes (Table 3), Soil moisture percentage is considered as one of the most soil properties affecting sand dune stabilized through its direct effect as adhesive agent of soil particles or indirect through vegetation stabilization. As a general, soil moisture level was higher under sand dune stabilization than unstabilized during different seasons of both annuals.

Soil chemical analysis (Table 4)
Soil (pH). The pH values are generally high in the soil of the unstabilized plot compared to the cultivated plots. The average recorded values vary from 9.5 to 9.6 for the former and from 8.30 to 8.47 for the latter. The lower pH values in the cultivated plots can be explained on the basis of the higher content of organic matter which affects the soil reaction, (Aggarwal and Lahiri, 1977 and Draz and El-Maghraby, 1997). In stabilized dunes, the pH values showed significant difference related to the soils of the different plant species.
Organic matter content (O.M). The average percentage of organic matter content of soils is generally low, not exceeding 0.27% has recorded its maximum amount in the stabilized plot (0.21%), while being at minimum in the unstabilized one (0.18%). In stabilizes plots, the O.M values are some what higher at depth (0-10cm). The variation of the O.M. content with respect to the different dunes is presumably due to the variable amount of leaf litter and root decay in such locations.
Soil salinity (E.C). Table (4) shows the values of the E.C, anions and cations in the unstabilized and stabilized dunes. Generally, the E.C

values of the stabilized plot are higher compared to the unstabilized ones. Such increment of the E.C values in the stabilized plot is essentially due to the irrigation water (Draz and El-Maghraby, 1997). In the stabilized plot, the E.C values are higher under *Prosopis jullflora* (0.96dS/m³) compared to the other plants. In general, E.C values indicate non-saline soil condition. In most cases, the concentration of the cations and anions, especially Ca++, Mg++ and Na+

are higher under *Prosopis jullflora* and (Cl⁻ and SO4⁻⁻) under *Morus alba* compared to another. Such trend may be explained on the bass of different growth behavior of the plant as well as the variation in particle size distribution of soil which affect the evapo-transpiration rate and salt accumulation, (Keng *et al.,* 1979).

Table (3) Mean values of seasonal changes of soil moisture percentage (%) in relation to: -plant species, seasons and sand samples depths of unstabilized and stabilized sand dunes of El-Maghara in North Sinai during two annuals.

Dunes	Scientific name	Depths (cm)	First annual				Mean	Second annual				Mean
			Winter	Spring	Summer	Autumn		Winter	Spring	Summer	Autumn	
Un stabili-zed		0-10	0.407	0.036	0.495	0.418	0.339	1.749	1.955	0.346	1.143	1.298
		10-20	0.510	0.117	0.584	0.485	0.424	1.242	1.71	0.710	0.219	0.970
		20-30	0.392	0.768	0.549	1.469	0.795	1.432	1.713	0.862	0.311	1.080
		Mean	0.436	0.307	0.543	0.791	0.519	1.474	1.793	0.639	0.558	1.116
Stabilized	*Acasia saligna*	0-10	1.053	1.053	0.542	1.273	0.980	3.097	2.001	0.337	1.197	1.658
		10-20	1.346	0.710	1.049	1.208	1.078	3.911	2.116	2.581	1.209	2.454
		20-30	1.993	1.001	1.576	1.226	1.449	4.001	2.749	2.660	2.258	2.917
		Mean	1.464	0.921	1.056	1.236	1.169	3.670	2.289	1.859	1.555	2.343
	Prosopis jullflora	0-10	2.061	0.713	0.399	1.549	1.181	3.046	2.053	1.537	2.239	2.219
		10-20	2.100	1.116	1.841	1.542	1.650	3.735	2.346	1.731	1.274	2.272
		20-30	2.034	1.955	1.798	2.584	2.093	4.215	2.710	2.011	2.283	2.805
		Mean	2.065	1.261	1.346	1.892	1.641	3.665	2.370	1.760	1.932	2.432
	Morus alba	0-10	2.036	1.346	0.397	1.601	1.345	3.061	2.242	0.997	1.219	1.880
		10-20	2.097	1.576	1.901	1.532	1.777	3.991	2.049	2.110	1.278	2.357
		20-30	2.046	1.901	1.599	2.489	2.009	3.993	2.576	2.984	2.253	2.952
		Mean	2.060	1.608	1.299	1.874	1.710	3.682	2.289	2.030	1.583	2.396
	Average		1.863	1.263	1.234	1.667	1.507	3.672	2.316	1.883	1.690	2.390
	New L.S.D. at 5%:-Species		0.483	0.061	0.034	0.501	-	N.S.	N.S.	N.S.	N.S.	-
	Depths		N.S.	0.053	0.043	0.449	-	0.741	0.393	N.S.	0.501	-

Table (4) – Chemical analysis of soils stabilized sand dunes under plant species and unslabilized dunes of El-Maghara in North Sinai during two annuals.

Dunes	Scientific name	Depth (cm)	Soil pH	E.C ds/m³	O.M (%)	Soluble cations (p.p.m)				Soluble anions (p.p.m)			
						Ca++	Mg++	Na+	K++	CO3⁻⁻	HCO3⁻⁻	Cl⁻⁻	SO4⁻⁻
Unstabiliz-Ed		0-10	9.51	0.37	0.09	1.20	2.00	4.95	43.93	-	3.37	4.49	3.77
		10-20	9.65	0.33	0.09	1.11	1.98	4.98	44.85	-	3.69	4.51	3.72
		20-30	9.60	0.35	0.08	1.11	1.99	5.01	42.99	-	3.92	4.43	3.75
		Mean	9.62	0.35	0.09	1.14	1.99	4.98	43.92	-	3.77	4.48	3.73
		L.S.D. at 5%	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-	N.S.	N.S.	N.S.
Stabilized	*Acasia saligna*	0 - 10	8.30	0.94	0.27	0.47	8.39	4.24	113.59	-	3.99	3.20	3.30
		10 - 20	8.22	0.86	0.24	0.37	8.44	4.80	112.89	-	4.27	3.00	3.07
		20 - 30	8.47	0.75	0.24	0.33	8.28	4.51	114.53	-	2.72	3.01	3.08
		Mean	8.33	0.85	0.25	0.39	8.37	4.64	113.67	-	3.66	3.07	3.15
	Prosopis jullflora	0 - 10	8.40	0.94	0.26	0.62	13.11	5.15	96.00	-	3.10	3.75	3.79
		10 - 20	8.29	0.95	0.23	0.61	15.05	3.95	98.01	-	3.03	3.59	3.99
		20 - 30	8.21	0.99	0.23	0.66	14.11	4.65	89.00	-	3.20	3.84	3.09
		Mean	8.30	0.96	0.24	0.63	14.09	4.65	94.33	-	3.11	3.66	3.49
	Morus alba	0 - 10	8.46	0.92	0.20	0.60	4.43	4.80	65.71	-	3.80	3.96	3.50
		10 - 20	8.38	0.89	0.14	0.55	4.43	4.70	67.15	-	3.11	3.59	3.71
		20 - 30	8.57	0.99	0.14	0.56	4.19	4.01	69.13	-	3.30	3.49	3.32
		Mean	8.47	0.44	0.16	0.57	4.35	4.50	66.33	-	3.40	3.68	3.51
	Average		8.42	0.65	0.18	0.43	7.23	4.70	79.67	-	3.66	3.07	3.15
	New L.S.D. at 5%:- Species		0.10	0.01	0.02	0.01	0.06	N.S.	0.97	-	N.S.	N.S.	N.S.
	Depths		0.08	0.03	0.01	0.11	0.03	N.S.	0.84	-	N.S.	N.S.	N.S.
	Species × Depths		N.S.	0.43	0.10	0.21	3.22	0.06	N.S.	-	0.05	0.59	0.11

Migration of sand dunes: Migration of soil particles under unstabilized and stabilized of sand dunes, (Table 5) differed greatly according to differed factors, i. e. active dunes, directions, seasons and annuals as follows.

Unstablized sand dunes: The highest soil migration is associated with South and North directions (14.89 and 15.86 kg) during the first annual and second ones, respectively. The least ones were recorded at North directions (13.81) in spring of the first annual. Therefore, it may be concluded that soil migration varied at different directions.

Stabilized sand dunes: Soil migration (Table 6) is differed greatly according to several factors, i. e., plant species, directions, seasons and years. The highest values of soil migration were recorded under *Morus alba* (4.745 and 6.612 kg) during the first and second years, respectively. The highest values of soil migration (4.472 and 6.456) are shown at North and East directions during the first and second years respectively. On the contrary, the lowest values of soil migration (2.681 and 4.594) are shown during winter in both annuals.

Table (5) - Mean values of seasonal changes of migrated soil in sand collectors (traps, kg.) at different directions in relation to: - plant species and seasons of stabilized of El-Maghara sand dunes in North Sinai during two annuals.

Dunes	Seasons	Scientific name	First annual					Second annual				
			N.	E.	S.	W.	Mean	N.	E.	S.	W.	Mean
Unstabilized	Winter		10.89	13.15	11.30	12.42	11.940	13.40	14.26	13.47	11.63	13.190
	Spring		9.93	10.31	12.77	11.81	11.205	19.29	18.78	17.73	19.15	18.738
	Summer		22.32	22.24	23.22	21.85	22.408	16.77	15.43	15.56	15.19	15.738
	Autumn		12.09	11.65	12.26	11.50	11.875	13.98	13.68	13.64	13.72	13.755
	Mean		13.81	14.34	14.89	14.40	14.36	15.86	15.54	15.10	14.92	15.36
Stabilized	Winter	*Acasia saligna*	2.19	2.34	2.34	1.89	2.19	4.30	4.88	4.13	3.94	4.31
		Prosopis jullflora	2.87	3.06	2.85	3.44	3.06	4.53	5.07	4.37	4.23	4.55
		Morus alba	2.59	3.01	2.82	2.77	2.79	4.63	5.30	4.99	4.75	4.92
	Mean		2.55	2.81	2.67	2.70	2.68	4.49	5.08	4.50	4.31	4.59
	Spring	*Acasia saligna*	5.68	2.38	3.84	3.80	3.93	7.40	8.53	7.70	7.35	7.75
		Prosopis jullflora	4.10	3.82	3.96	5.81	4.42	8.45	8.53	8.37	8.22	8.39
		Morus alba	5.05	3.92	3.04	4.77	4.20	9.14	8.56	8.43	8.52	8.66
	Mean		4.93	3.37	3.61	4.79	4.18	8.33	8.54	8.17	8.03	8.27
	Summer	*Acasia saligna*	5.55	5.03	5.28	5.27	5.15	6.04	6.12	6.11	6.21	6.12
		Prosopis jullflora	6.20	6.15	5.74	6.25	6.09	6.75	6.83	6.76	6.58	6.73
		Morus alba	6.58	6.46	6.10	6.44	6.40	7.16	7.27	7.20	6.93	7.14
	Mean		5.93	5.88	5.71	5.99	5.88	9.18	8.91	8.91	8.73	8.93
	Autumn	*Acasia saligna*	2.65	1.92	2.63	2.70	2.48	5.03	5.01	4.950	4.96	4.99
		Prosopis jullflora	4.65	4.5	4.5	4.46	4.53	5.73	5.65	5.560	5.63	5.64
		Morus alba	5.55	5.63	5.62	5.57	5.60	5.69	5.72	5.750	5.75	5.73
	Mean		4.28	4.02	4.25	4.24	4.20	7.61	7.52	7.48	7.52	7.53
	Average		4.472	4.018	4.060	4.431	4.234	6.238	6.456	6.193	6.089	6.244

N. = North   E. = East   S. =S south   W. = West

Migrated soil percentage under unstabilized and stabilized (Table 6). Migrated soil percentage differed greatly according to differed factors, i. e., active dunes, directions, seasons and years.

Unstablized dunes. The soil migration percentage is shown to be generally high at the different directions (100%) during four seasons in the first and second years.

Stablized dunes. Migration soil percentage (%) differed greatly according to different factors, i. e., plant species, directions, seasons and years. The highest values of soil migration were recorded under *Morus alba* (34.361% and 42.681%kg) during the first and second annuals, respectively (Table 6). Moreover, the highest values of soil migration percentage may be shown at North and East (33.795% and 41.179%) directions during the first and second annuals, respectively. On the contrary the least values of soil migration percentage (22.526%

and 34.886%) are recorded during winter in both annuals.

Percentage of soil particles size of migrated sand under unstabilizated and stabilized sand dunes. (Table 7)

Unstabilized sand dunes: The soil particle size (mm) of migrated sand differed according to the directions of such migration. It must be mentioned here, that the soil particle size seemed to be mostly from those of 0.5 - 0.063 mm, the highest proportion of soil particle size is mainly concerned with 0.5 - 0.25mm, 19.94%, 0.25 - 0.125 mm, 49.62% and 0.125 - 0.063mm 23.72%, while migration of other soil particle sizes seemed to be negligible.

Stabilized sand dunes: The soil particle size (mm) of migrated sand differed according to the directions of such migration. It must be mentioned here, that the soil particle size seemed to be from those of 0.5 -

0.063 mm, the highest proportion of soil particle size is mainly concerned with 0.25 - 1.25mm, 52.17%, 0.125 - 0.063 mm, 25.12% and 0.5 - 0.025mm

15.65%, while other soil particle sizes seemed to be negligible.

Table (6) - Mean values of seasonal changes of migrated soil percentage (%) in sand collectors (traps) different directions in unstabilized and stabilized of El-Maghara sand dunes in relation to plant species and seasons in North Sinai during two annuals

Dunes	Seasons	Scientific name	First annual					Second annual				
			N.	E.	S.	W.	Mean	N.	E.	S.	W.	Mean
Barren			100%	100%	100%	100%	-	100%	100%	100%	100%	-
Stabilisation	Winter	*Acasia saligna*	20.110	17.795	20.708	15.217	18.458	32.090	34.222	30.661	33.878	32.712
		Prosopis	26.354	23.270	25.221	27.697	25.636	33.806	35.554	32.442	36.371	34.543
		jullflora	23.783	22.890	24.956	22.303	23.483	34.552	37.167	37.045	40.843	37.402
		Morus alba										
		Mean	23.416	21.318	23.628	21.739	22.526	33.483	35.648	33.383	37.031	34.886
	Spring	*Acasia saligna*	57.200	23.084	30.070	32.176	35.633	38.362	45.421	43.429	38.381	41.398
		Prosopis	41.289	37.051	31.010	49.196	39.637	43.805	45.421	47.208	42.924	44.840
		jullflora	50.856	38.021	23.806	40.390	38.268	47.382	45.580	47.547	44.491	46.250
		Morus alba										
		Mean	49.782	32.719	28.295	40.587	37.846	43.183	45.474	46.061	41.932	44.163
	Summer	*Acasia saligna*	22.401	22.617	22.739	24.119	22.969	36.017	39.663	39.267	40.882	38.957
		Prosopis	27.778	27.653	24.720	28.604	27.189	40.25	44.264	43.445	43.318	42.819
		jullflora	29.480	29.047	26.270	29.474	28.568	42.695	47.116	46.272	45.622	45.426
		Morus										
		Mean	26.553	26.439	24.576	27.399	26.242	39.654	43.681	42.995	43.274	42.401
	Autumn	*Acasia saligna*	21.919	16.481	21.452	23.478	20.832	35.980	36.623	36.290	36.152	36.261
		Prosopis	38.462	38.627	36.705	38.783	38.144	40.987	41.301	40.762	41.035	41.021
		jullflora	45.906	48.326	45.84	48.435	47.127	40.701	41.813	42.155	41.910	41.645
		Morus alba										
		Mean	35.429	34.478	34.666	36.899	35.368	39.223	39.912	39.736	39.699	39.642
		Average	33.795	28.739	27.791	31.656	30.495	38.886	41.179	40.544	40.484	40.273

Table (7) Average changes in soil particles size (%) of unstabilized and stabilized sand dunes at collection from the different directions on El-Maghara sand dunes in relation to: - trap directions and plant species

Dunes	Scientific name	Trap directions	Percentage of particle size distribution (mm)							Texture class
			> 2	2 - 1	1 - 0.5	0.5 - 0.25	0.25 - 0.125	0.125 - 0.063	< 0.063	
Unstab-ilized		N.	0.00	0.06	2.34	27.85	48.10	18.27	3.38	Sand
		E.	0.00	0.08	2.64	22.69	53.79	18.06	2.74	Sand
		S.	0.22	0.14	4.04	15.34	45.26	30.94	4.06	Sand
		W.	0.43	0.12	3.12	13.87	51.34	27.59	3.53	Sand
		Mean	0.16	0.10	3.04	19.94	49.62	23.72	3.43	Sand
Stabilized	*Acasia saligna*	N.	0.00	0.00	0.32	3.80	57.86	34.05	3.97	Sand
		E.	0.00	0.38	1.94	16.84	55.81	22.05	2.98	Sand
		S.	0.00	0.06	1.40	15.74	53.56	25.9	3.34	Sand
		W.	0.00	0.17	2.89	19.76	52.3	21.18	3.7	Sand
		Mean	0.00	0.15	1.64	14.04	54.88	25.80	3.50	Sand
	Prosopis jullflora	N.	0.11	0.10	3.19	21.60	46.68	24.61	3.72	Sand
		E.	0.23	0.11	2.87	18.27	52.56	22.84	3.14	Sand
		S.	0.11	0.07	2.18	9.57	51.56	32.50	4.02	Sand
		W.	0.20	0.26	2.55	15.34	53.58	24.82	3.26	Sand
		Mean	0.16	0.14	2.70	16.20	51.10	26.19	3.54	Sand
	Morus alba	N.	0.08	0.03	0.86	9.77	55.71	29.90	3.66	Sand
		E.	0.00	0.28	2.42	18.30	54.06	21.62	3.34	Sand
		S.	0.06	0.08	2.30	18.67	50.12	25.25	3.53	Sand
		W.	0.10	0.14	2.89	19.01	52.43	22.31	3.10	Sand
		Mean	0.06	0.13	2.12	16.44	53.08	24.77	3.41	Sand
		Average	0.10	0.13	2.37	16.65	52.17	25.12	3.47	Sand

Depth of erosion under unstabilized sand dunes. Data in (Table 8) indicated that the tested factors, i.e. directions, seasons and years affected the depth of erosions. The highest erosion seemed to occur during autumn season of the two annuals (7.577 and 9.532cm).

Table (8) Mean seasonal changes in soil depth of erosion (cm) on unstabilized sand dunes of El-Maghara, North Sinai during two annuals

Seasons	First annual	Second annual
Winter	6.565	7.642
Spring	6.783	7.917
Summer	7.558	9.023
Autumn	7.577	9.532
Mean	6.995	8.529

- Sand accumulation on cultivated plants (cm) (Table 9). The amount of sand accumuled on cultivated plant, is shown to be plant morphogenesis and its architecture must have a role on the amounts of accumulated sand under sand dune system. The highest amounts were accumulated on *Acasia saligna* followed by those accumulated on *Prosopis jullflora* and finally the least amounts were shown on *Morus alba.*

Table (9) Mean values of seasonal changes in soil accumulation on cultivated plants (cm) on stabilized sand dunes of El-Maghara in North Sinai during two annuals

Scientific name	First annual					Second annual				
	Winter	Spring	Summer	Autumn	Mean	Winter	Spring	Summer	Autumn	Mean
Acasia saligna	14.565	20.915	22.684	22.177	22.177	40.054	42.782	46.045	48.217	44.195
Prosopis jullflora	14.856	18.845	22.246	20.509	20.509	38.936	40.963	43.127	44.051	41.824
Morus alba	13.451	17.987	18.927	18.189	18.189	31.534	32.963	36.639	38.483	35.553
Mean	14.291	19.249	21.286	26.340	20.291	36.841	38.903	41.435	41.937	40.191
New L.S.D. at 5%	N.S.	1.640	2.666	0.577	-	3.503	2.521	2.564	2.639	-

Ecophsiological behaviuor of cultivated plants, (Table 10):- The results obtained concerning the growth behaviour of *Acasia saligna, Prosopis jullflora* and *Morus alba* are affected by the seasons and years are displayed in the following manner:-

The plant height (cm), in both study seasons and annuals showed variable effective differences among the three plants species. In general, the trees was grown ascending with age. The ultimate recorded values vary from 146cm to 420cm in *Acasia saligna,* 151cm to 326cm in *Prosopis jullflora* and 119cm to 199cm *Morus alba,* respectively.

The diameter of shoot (cm), for the trees grown during the two annuals displayed the highest values for the trees grown in summer of the second annual. Generally, the highest values of shoot diameter were recorded in *Acasia saligna* followed by *Prosopis jullflora* while the least values were attained for *Morus alba.*

The number of lateral shoots, the maximum and the minimum values of the number of lateral shoots are 54 and 25.667 in *Acasia saligna* and *Morus alba* for the trees grown in summer of the second annual.

The Shoot length (cm), varied significantly between three cultivated plants *Acasia saligna, Prosopis jullflora* and *Morus alba* during winter at the first annual and during winter, spring and summer of the second annual.

The number of the leaves/ shoot, varied significantly among the trees grown during all seasons except spring of the second annual.

The leaf area (cm²), the leaf area of different tested plants increased gradually during the spring season of the first annual and during the summer season of the second annual. In general, the plants grown in the different seasons during the two annuals displayed significant variations in leaf area.

The crown cover (C.C. m²) and the crown volume (C.V. m³), were found to be significantly variable in the plants grown in the different seasons and during the two annuals. In general, *Prosopis jullflora* have the highest values of both crown cover (C.C. m²) and the crown volume (C.V. m³) followed by *Acasia saligna* while *Morus alba* attains the least values of both parameters.

Dry matter percentage of the different cultivated trees, Table (11), both stems and leaves of the cultivated trees showed slight differences during the different seasons. In winter season, the extreme values vary from 48.01 to 38.82% for the stem (*Acasia saligna* and *Morus alba*) and from 40.80 to 23.34% for the leaves (*Acasia saligna* and *Morus alba*).

In summer season, the extreme values vary from 48.86 to 44.26% for the stem (*Acasia saligna* and *Morus alba*) and from 41.84 to 32.77% for the leaves (*Acasia saligna* and *Morus alba*), i. e., being somewhat higher, on average, during summer relative to winter. Noteworthy to mention that dry matter percentage in both stem and leaves followed the descending order *Acasia saligna, Prosopis jullflora* and *Morus alba*. Morover, dry matter content of stem of all plant species is considerably higher than that of leaves.

Table (10) Mean seasonal changes in some growth parameters of the different tree species cultivated during growth seasons on stabilized sand dunes of El-Maghara in North Sinai during two annuals

Annuals	Seasons	Scientific name	Plant height (cm)	Shoot diameter (cm)	No. of shoots	Shoot length (cm)	No. of leaves on shoot	Leaf area (cm²)	C.C (m²)	C.V (m³)
First	Autumn	*Acasia saligna*	146.000	1.400	3.330	119.500	259.333	99.500	1.386	4.351
		Prosopis jullflora	151.670	0.750	4.330	117.380	263.000	101.767	5.282	8.504
		Morus alba	119.000	0.660	5.670	87.500	100.000	0.000	0.634	0.828
		Mean	138.890	0.937	4.443	108.127	207.444	67.089	2.434	4.561
		New L.S.D. at 5%	6.115	N.S	1.630	3.463	93.889	1.277	0.485	0.795
	Winter	*Acasia saligna*	149.670	1.680	8.330	120.500	246.330	93.610	1.448	4.481
		Prosopis jullflora	150.000	0.820	10.000	117.540	441.000	85.993	4.948	8.491
		Morus alba	118.000	0.620	9.330	88.000	48.670	34.457	0.614	0.897
		Mean	139.223	1.040	9.220	108.680	245.333	71.353	2.337	4.623
		New L.S.D. at 5%	N.S.	1.040	N.S.	N.S.	159.480	3.900	1.150	0.513
	Spring	*Acasia saligna*	161.333	1.840	12.333	129.250	274.330	137.757	1.525	4.468
		Prosopis jullflora	156.667	0.950	12.333	117.210	421.670	104.000	5.434	8.499
		Morus alba	121.670	0.900	8.333	94.200	231.000	46.700	0.632	0.852
		Mean	146.557	1.230	11.000	113.553	309.000	96.152	2.530	4.606
		New L.S.D. at 5%	N.S.	1.213	N.S.	N.S.	139.900	9.811	0.950	0.811
	Summer	*Acasia saligna*	178.670	1.990	20.000	141.750	296.000	71.433	1.332	4.281
		Prosopis jullflora	168.330	1.320	21.667	126.370	458.000	101.167	4.596	8.665
		Morus alba	124.330	1.060	13.667	97.060	276.000	60.100	0.650	0.877
		Mean	157.110	1.457	18.445	121.727	343.333	77.567	2.193	4.608
		New L.S.D. at 5%	N.S.	N.S.	N.S	N.S.	164.070	3.888	0.487	0.829
Second	Autumn	*Acasia saligna*	171.000	1.975	23.333	138.167	316.667	123.457	1.251	4.380
		Prosopis jullflora	161.667	1.082	35.000	136.250	457.000	94.710	4.223	9.418
		Morus alba	125.667	1.356	18.667	102.422	228.330	0.000	0.652	0.879
		Mean	152.778	1.471	25.667	125.613	333.999	72.722	2.042	4.892
		New L.S.D. at 5%	N.S.	N.S.	7.632	N.S.	169.879	7.462	0.554	0.676
	Winter	*Acasia saligna*	398.333	3.387	33.333	375.778	631.333	131.543	2.653	10.472
		Prosopis jullflora	307.000	3.347	26.333	292.028	494.667	104.000	7.748	16.669
		Morus alba	186.667	1.472	21.667	141.111	286.667	48.023	0.975	1.300
		Mean	297.333	2.735	27.111	269.639	470.889	94.522	3.792	9.480
		New L.S.D. at 5%	32.188	1.099	7.997	44.511	1.309	19.455	0.510	0.398
	Spring	*Acasia saligna*	277.000	3.713	50.000	393.833	694.000	126.670	2.021	7.835
		Prosopis jullflora	235.333	3.323	43.000	336.361	579.333	107.623	6.117	13.203
		Morus alba	152.667	1.518	21.667	152.656	417.333	59.357	0.793	1.067
		Mean	221.667	2.851	38.222	294.283	563.555	97.883	2.977	7.368
		New L.S.D. at 5%	78.477	0.695	20.375	71.035	N.S.	11.262	3.628	7.033
	Summer	*Acasia saligna*	420.000	3.737	54.000	402.639	208.944	110.290	3.151	11.666
		Prosopis jullflora	326.000	3.362	46.667	213.528	198.361	96.520	9.545	17.667
		Morus alba	199.667	1.541	25.667	164.406	91.389	63.857	1.378	1.392
		Mean	315.222	2.880	42.111	260.191	166.231	90.222	4.691	10.242
		New L.S.D. at 5%	11.547	0.896	21.299	2.267	56.257	9.837	2.329	0.590

Table (11) Mean values of seasonal changes in dry weight (%) of the different tree species cultivated on stabilized sand dunes of El-Maghara in North Sinai during two annuals

Scientific name	Stem			Leaves		
	Winter	Summer	Mean	Winter	Summer	Mean
Acasia saligna	48.01	48.86	48.44	40.80	41.84	41.32
Prosopis jullflora	41.58	44.97	43.28	25.69	33.92	29.81
Morus alba	38.82	44.26	41.54	23.34	32.77	28.06
Mean	42.80	46.03	44.42	29.94	36.18	33.06
New L.S.D. at 5%						

Concentrations of some nutrients in shoots and leaves of different cultivated plants (Table 12)

i) In shoots:

Total Nitrogen (N): The highest concentration of N was found in *Acasia saligna* stem, followed by *Prosopis jullflora*, while the lowest values were found in *Morus alba*. It must be mentioned here that *Acasia saligna* is one of "fabaceae" family members.

Accordingly, its stem must contain higher concentration of nitrogenous compounds. There were significant differences between the concentrations of N due to the kind of plants.

Phosphorus (P) concentration in shoots of *Prosopis jullflora* seemed to be the highest one followed by *Acasia saligna*, while the least one was found in *Morus alba* shoots.

Potassium (k). Concentration of K in shoots of *Acasia saligna* seemed to be the highest followed by *Morus alba*, whereas the least one was found in *Prosopis jullflora* shoots.

Sodium (Na). The least concentration of Na was found in *Acasia saligna* and increased in *Prosopis jullflora* while the highest one was found in *Morus alba.*

From (Table 14) the highest proportion of the studied elements in shoots of the concerned plant species is as follows.

Acasia saligna Na, K, N and P

Prosopis jullflora Na, N, K and P

Morus alba Na, N, K and P

ii) In leaves:

Total nitrogen (N). The highest concentration of N was found in *Prosopis jullflora* leaves, followed by *Acasia saligna*, while the lowest values were found in *Morus alba*.

Phosphorus (P). P concentrations in leaves of *Prosopis jullflora* seemed to be highest one followed by *Acasia saligna* and *Morus alba* leaves in a descending order.

Potassium (k). Concentration of K in leaves of *Prosopis jullflora* seemed to be highest one followed by *Morus alba*, while the least content is that of in *Acasia saligna* leaves.

Sodium (Na). The lowest concentration of Na was found in *Acasia saligna* followed by *Prosopis jullflora* whereas the highest one is that of *Acasia saligna* leaves.

In brief, the highest proportion of the studied elements in leaves of plant species seemed to be as follows in descending order:-

Acasia saligna: N%, K%, P%, Na p.p.m

Prosopis jullflora: N%, K%, P%, Na p.p.m

Morus alba: N%, K%, P%, Na p.p.m

Table (12) - Nutrient contents of the different plant species

Scientific name	Shoots				Leaves			
	(%)			Na (p.p.m)	(%)			Na (p.p.m)
	N	P	K		N	P	K	
Acasia saligna	1.13	0.32	1.25	24.0	1.55	0.35	0.90	34.0
Prosopis jullflora	1.04	0.34	0.65	33.0	2.04	0.37	1.40	50.0
Morus alba	0.99	0.31	0.84	41.0	1.06	0.30	1.00	64.0
Mean	1.05	0.32	0.92	32.67	1.55	0.34	1.10	49.33
New L.S.D. at 5%	0.08	0.02	0.03	2.99	0.14	0.02	0.12	1.89

Simple correlation coefficients (r) between some factors interrelationships

Significant (*) simple correlation coefficients (r) is those of 0.622 to 0.707, while the highly significant (**) simple correlation coefficients (r) is those of 0.707 and over.

1- Simple correlation coefficients (r) between some meteorological factors under the variable conditions of El-Maghara sand dune (Table 13).

Significant and highly significant simple correlation coefficients are eight. The positive relationships are four and the negative relationships are four simple correlation coefficients. On the other hand, no significant simple correlation coefficients are seven. The highest simple correlation coefficients (r= -0.956) is found between total rainfall (mm) and sun shine (Kw/m²) indicates highly significant negative correlation. The least simple correlation coefficients (r= 0.635) is found between sun shine (Kw/m²) and evapo-transpiration (mm/day), indicates positive significant correlation.

- Wind speed (Km/h) is highly significant negatively correlated with air temperature (ºC) and evapo-transpiration (mm/day) (r= -0.737 and -0.861) respectively, highly significant positively correlated with air relative humidity (%).

- Air temperature (ºC) is highly significant positively correlated with sun shine (Kw/m²) and evapo-transpiration (mm/day) (r= 0.802 and 0.894) respectively.

- Air relative humidity (%) is significant negatively correlated with evapo-transpiration (mm/day) (r= -0.700).

- Total rainfall (mm) is highly significant negatively correlated with sun shine (Kw/m²) (r= -0.956).

- Sun shine (Kw/m²) is significant positively correlated with evapo-transpiration (mm/day) (r= 0.635).

2- Simple correlation coefficients (r) between some edaphic factors under the variable conditions of El-Maghara sand dune (Table 14).

Significant and highly significant simple correlation coefficients are eight. The positive relationships are five and the negative relationships are three simple correlation coefficients. On the other hand, no significant simple correlation coefficients are twelve. The highest correlations (r= 0.989) is found between soil migration (traps kg) in unstabilized dunes and soil migration (traps kg) of stabilized dunes during the second annual, indicates positive highly significant correlation. The least simple correlation coefficients (r= 0.772) is found

between soil migration (traps kg) in sand dunes stabilized and erosion depth (cm) in un stabilized during first annual, indicates positive highly significant correlation.

-Soil moisture (%) 2007 is highly significant negatively correlated with soil migrated (traps kg.) in sand dunes stabilized (-0.821).

- Soil moisture (%) 2008 is highly significant negatively correlated with erosion depth (cm) in sand dunes un stabilized and soil accumulation in sand dunes stabilized (r= -0.851 and -0.953) respectively.

- Soil migrated (traps kg) in sand dunes un stabilized 2007 and 2008 is highly significant positively correlated with soil migration (traps kg.) in sand dunes stabilized (r= 0.818 and 0.989) respectively.
- Soil migration (traps kg) in sand dunes stabilized 2007 is highly significant positively correlated with erosion depth (cm) (r= 0.772).
- Erosion depth (cm) 2007 and 2008 is highly significant positively correlated with erosion depth (cm) and soil accumulation in sand dunes stabilized (r= 0.878 and 0.963) respectively.

Table (13) - Simple correlation coefficients (r) between some meteorological factors under the variable conditions of El-Maghara

Meteorological factors	Wind speed Km/h	Air temperature (°C)	Air relative humidity (%)	Total rainfall (mm)	Sun shine (Kw/m²)
Air temperature (°C)	- 0.737 **	-	-	-	-
Air relative humidity (%)	0.916 **	- 0.426	-	-	-
Total rainfall (mm)	- 0.069	- 0.590	- 0.365	-	-
Sun shine (Kw/m²)	- 0.222	0.802 **	0.106	- 0.956 **	-
Evapo-transpiration (mm/day)	- 0.861 **	0.894 **	- 0.700 *	- 0.407	0.635 *

Table (14) - Simple correlation coefficients (r) between some edaphic factors under the variable conditions of El-Maghara sand dunes under two annuals

Edaphic factors	Sand dune	Soil moisture (%)		Soil migration (traps kg)				Erosion depth (cm)	
				Un stabilized		Stabilized			
		2007	2008	2007	2008	2007	2008	2007	2008
Soil migrated (traps kg.)	Un stabilized	- 0.539	- 0.312	-	-	-	-	-	-
	Stabilized	- 0.821**	- 0.450	0.818**	0.989**	-	-	-	-
Erosion depth (cm)	Un stabilized	- 0.333	-0.851**	0.568	- 0.233	0.772**	- 0.084	-	-
Soil accumulation	Stabilized	- 0.256	-0.953**	0.134	0.030	0.553	0.180	0.878**	0.963**

3- Simple correlation coefficients (r) between some growth behaviour factors under the variable conditions of El-Maghara stabilized sand dune (Table 15).

Significant and highly significant simple correlation coefficients are eighteen all that are positive relationships. On the other hand, no significant simple correlation coefficients are teen. The highest simple correlation coefficients (r= 0.998) between plant height (cm) and plant crown cover (C.v³). The least simple correlation coefficients (r= 0.670) is found between No. of shoots and C.V (m³).
- Plant height (cm) is highly significant positively correlated with shoot diameter (cm), shoot length (cm), C.C (m²) and C.V (m³) (r= 0.852, 0.748, 0.992 and 0.998) respectively and significant positively correlated with No. of shoots (r= 0.695).

- Shoot diameter (cm) is highly significant positively correlated with No. of shoots, shoot length (cm), No. of leaves/shoot, C.C (m²) and C.V (m³) (r= 0.854, 0.958, 0.803, 0.877 and 0.860) respectively.
- No. of shoots is highly significant positively correlated with C.C (m²) (r=0.779) and significant positively correlated with, shoot length (cm) and C.V (m³) (r=0.693 and 0.670) respectively.
- Shoot length (cm) is highly significant positively correlated with leaf area (cm²) C.C (m²) and C.V (m³) (r= 0.900, 0.751 and 0.774) respectively.
- No. of leaves/ shoot is highly significant positively correlated with leaf area (cm²) (r= 0.786)
- C.C (m²) is highly significant positively correlated with C.V (m³) (r= 0.983).

4- Simple correlation coefficients (r) between some meteorological factors and some edaphic factors

under the variable conditions of El-Maghara cultivated sand dune (Table 16).

Significant and highly significant simple correlation coefficients are thirty. The positive relationships are sixteen and the negative relationships are fourteen simple correlation coefficients. On the other hand, no significant simple correlation coefficients are thirty. The highest simple correlation coefficients (r= 0.998) is found between air temperature (ºC) and soil migration (traps kg.) stabilized dunes during first annual, indicates highly significant positive correlation. The least simple correlation coefficients (r= - 0.633) is found between total rainfall (mm) and sand migration (traps kg.) in sand dunes stabilized during first annual, indicates negative significant correlation.

In the first annual

- Wind speed (Km/h) is highly significant negatively correlated with sand migrated (traps kg.) in sand dunes unstabilized, erosion depth (r= - 0.724 and - 0.926) and significant negatively with migrated (traps kg.) in sand dunes stabilized and soil accumulation (r= - 0.670 and - 0.655).
- Air temperature (ºC) is highly significant negatively correlated with soil moisture (%) (r= -0.790) and positively with sand migration (traps kg.) in sand dunes unstabilized and stabilized and erosion depth (r= 0.822, 0.998 and 0.804) respectively.
- Air relative humidity (%)) is highly significant negatively correlated with erosion depth (r= -0.730).
- Total rainfall (mm) is highly significant positively correlated with soil moisture (%) (r= 0.922) and significant negatively with sand migrated (traps kg.) in sand dunes stabilized (r= -0.633).

- Sun shine (Kw/m²) is highly significant negatively correlated with soil moisture (%) (r= -0.966), positively with sand migrated (traps kg.) in sand dunes stabilized (r= 0.833) and significant positively with sand migrated (traps kg.) in sand dunes unstabilized (r= 0.696).
- Evapo-transpiration (mm/day) is highly significant positively correlated with sand migrated (traps kg.) in sand dunes unstabilized, stabilized and erosion depth (r= 0.968, 0.880 and 0.755) respectively.

In the second annual

- Wind speed (Km/h) is highly significant negatively correlated with erosion depth and soil accumulation (r= - 0.877 and - 0.931).
- Air temperature (ºC) is highly significant negatively correlated with soil moisture (%) (r= -0.810), positively with soil accumulation (r= 0.807) and significant positively with and erosion depth (r= 0.660).
- Air relative humidity (%)) is highly significant positively correlated with sand migrated (traps kg.) in sand dunes unstabilized (r= 0.702) and negatively with erosion depth (r= -0.721).
- Total rainfall (mm) is highly significant negatively correlated with sand migrated (traps kg.) in sand dunes unstabilized and stabilized (r= -0.847 and - 0.848).
- Sun shine (Kw/m²) is highly significant positively correlated with sand migrated (traps kg.) in sand dunes unstabilized and stabilized (r= 0.744 and 0.788).
- Evapotranspiration (mm/day) is significant positively correlated with soil accumulation (r= 0.667).

Table (15) - Simple correlation coefficients (r) between some growth behaviour factors under the variable conditions of El-Maghara sand dunes during two annuals

Factors	Plant height (cm)	Shoot diameter (cm)	No. of shoots	Shoot length (cm)	No. of leaves/ shoot	Leaf area (cm²)	C.C (m²)
Shoot diameter (cm)	0.852**	-	-	-	-	-	-
No. of shoots	0.695*	0.854**	-	-	-	-	-
Shoot length (cm)	0.748**	0.958**	0.693*	-	-	-	-
No. of leaves/shoot	- 0.068	0.329	- 0.024	0.574	-	-	-
Leaf area (cm²)	0.396	0.803**	0.599	0.900**	0.786**	-	-
C.C (m²)	0.992**	0.877**	0.779**	0.751**	- 0.097	0.422	-
C.V (m³)	0.998**	0.860**	0.670*	0.774**	- 0.011	0.427	0.983**

Table (16) - Simple correlation coefficients (r) between some meteorological factors with some edaphic factors under the variable conditions of El-Maghara

Annuals	Factors	Sand dunes	Wind speed (Km/h)	Air temperature (ºC)	Air relative humidity (%)	Total rainfall (mm)	Sun shine (Kw/m²)	Evapotrans-piration (mm/day)
First	Soil moisture (%)		0.167	- 0.790**	- 0.212	0.922**	- 0.966**	- 0.520
	Sand migration (traps kg.)	Un stabilized	- 0.724**	0.822**	- 0.591	- 0.522	0.696*	0.968**
		Stabilized	- 0.670*	0.998**	- 0.379	- 0.633*	0.833**	0.880**
	Erosion depth		- 0.926**	0.804**	- 0.730**	- 0.004	0.294	0.755**
	Soil accumulation		- 0.655*	0.580	- 0.430	0.137	0.100	0.371
Second	Soil moisture (%)		0.639*	- 0.810**	0.309	0.252	- 0.473	- 0.548
	Sand migration (traps kg.)	Un stabilized	0.371	0.346	0.702*	-0.847**	0.744**	- 0.020
		Stabilized	0.237	0.465	0.601	-0.848**	0.788**	0.086
	Erosion depth		- 0.877**	0.660*	- 0.721**	0.189	0.097	0.603
	Soil accumulation		- 0.931**	0.807**	- 0.580	- 0.070	0.341	0.667*

5- Simple correlation coefficients (r) between some meteorological factors and some growth behaviour factors under the variable conditions of El-Maghara cultivated sand dune (Table 17).

Significant and highly significant simple correlation coefficients are thirteen. The positive relationships are nine simple correlation coefficients. The negative relationships are four simple correlation coefficients. On the other hand, no significant simple correlation coefficients are thirty five. The highest simple correlation coefficients (r= 0.999) of air temperature (ºC) and leaf area (cm²). The least simple correlation coefficients (r = -0.642) is found between air relative humidity (%) and the No. of shoots.
- Wind speed (Km/h) is highly significant negatively correlated with No. of shoots and leaf area (cm²)

(r = - 0.807 and - 0.711).
- Air temperature (ºC) is highly significant positively correlated with shoot diameter (cm), shoot length (cm), No. of leaves/shoot and leaf area (cm²) (r = 0.785, 0.880, 0.785 and 0.999) respectively.
- Air relative humidity (%)) is significant negatively correlated with No. of shoots (r = -0.642).
- Total rainfall (mm) is highly significant negatively correlated with No. of leaves/shoot (r = -0.924).
- Sun shine (Kw/m²) is highly significant positively correlated with No. of leaves/shoot and leaf area (cm²) (r = 0.974 and 0.813) and significant with shoot length (cm) (r = 0.695).
- Evapo-transpiration (mm/day) is highly significant positively correlated with No. of leaves/shoot and leaf area (cm²) (r = 0.718 and 0.871).

Table (17) - Simple correlation coefficients (r) between some meteorological factors with some growth behaviour factors under the variable conditions of El-Maghara sand dunes during two annuals

	Wind speed (Km/h)	Air temperature (ºC)	Air relative humidity (%)	Total rainfall (mm)	Sun shine (Kw/m²)	Evapotranspir-ation (mm/day)
Plant height (cm	- 0.182	0.362	0.104	- 0.023	0.133	- 0.023
Shoot diameter (cm)	- 0.600	0.785**	- 0.265	- 0.249	0.461	0.504
No. of shoots	- 0.807**	0.600	- 0.642*	0.229	0.043	0.500
Shoot length (cm)	- 0.529	0.880**	- 0.147	- 0.516	0.695*	0.586
No. of leaves/shoot	- 0.289	0.785**	- 0.015	- 0.924**	0.974**	0.718**
Leaf area (cm²)	- 0.711**	0.999**	- 0.387	- 0.607	0.813**	0.871**
C.C (m²)	- 0.289	0.393	- 0.021	0.051	0.091	0.043
C.V (m³)	- 0.166	0.391	0.138	- 0.089	0.192	- 0.006

6- Simple correlation coefficients (r) between some edaphic factors and some growth behaviour factors under the variable conditions of El-Maghara cultivated sand dune (Table 18).

Significant and highly significant simple correlation coefficients are forty. The positive relationships are thirty simple correlation coefficients. The negative relationships are teen correlations. On the other hand, no significant simple correlation coefficients are forty. The highest simple correlation correlations (r = - 0.999) between soil migration (traps kg.) in stabilized dunes and leaf area (cm²) during first annual and between soil moisture (%) and

shoot diameter (cm) during second annual. The least simple correlation coefficients (r = -0.643) is found between soil migrated (traps kg) in dunes stabilized and No. of leaves/shoot during second annual.
In the first annual
- Soil moisture (%) is highly significant negatively correlated with shoot length (cm), No. of leaves/shoot and leaf area (cm²) (r = -0.806, -0.884 and -0.811) respectively.
- Soil migrated (traps kg) in dunes unstabilized is highly significant positively correlated with No. of leaves/shoot and leaf area (cm²) (r = 0.808 and 0.797).

- Soil migrated (traps kg) in dunes stabilized is highly significant positively correlated with shoot diameter (cm), shoot length (cm), No. of leaves/shoot and leaf area (cm²) (r = 0.775, 0.883, 0.812 and 0.999) respectively.

- Erosion depth (cm) is highly significant positively correlated with shoot diameter (cm) and No. of shoots (r = 0.833, 0.991) and significant with shoot length (cm), leaf area (cm²) and C.C (m²) (r = 0.690, 0.654 and 0.670) respectively.

- Soil accumulation (kg) is highly significant positively correlated with plant height (cm), shoot diameter (cm), No. of shoots, shoot length (cm), C.C (m²) and C.V (m³) (r = 0.853, 0.925, 0.968, 0.778, 0.910 and 0.835) respectively.

In the second annual

- Soil moisture (%) is highly significant negatively correlated with shoot diameter (cm), shoot length

(cm), No. of shoots, leaf area (cm²), C.C (m²) and C.V (m³) (r = -0.824, -0.999, -0.865, -0.959, -0.826, -0.854 and -0.832) respectively.

- Soil migrated (traps kg) in dunes stabilized is highly significant positively correlated with shoot length (cm) and No. of leaves/shoot (r = 0.658 and 0.643).

- Erosion depth (cm) is highly significant positively correlated with shoot length (cm), No. of shoots, leaf area (cm²), C.C (m²) and C.V (m³) (r = 0.852, 0.945, 0.766 and 0.795) respectively.

- Soil accumulation (kg) is highly significant positively correlated with shoot diameter (cm), No. of shoots, No. of leaves/shoot, leaf area (cm²) and C.C (m²) (r = 0.939, 0.954, 0.858, 0.809 and 0.754) respectively and significant with plant height (cm) and C.V (m³) (r = 0.687 and 0.681).

Table (18) - Simple correlation coefficients (r) between some edaphic factors with some growth behaviour factors under the variable conditions of El-Maghara sand dunes during two annuals

Annuals	Growth behaviour factors	Soil moisture (%)	Soil migrated (traps kg)		Erosion depth (cm)	Soil accumulation (kg)
			Dunes un stabilized	Dunes stabilized		
First	Plant height (cm)	- 0.369	- 0.224	0.358	0.535	0.853**
	Shoot diameter (cm)	- 0.604	0.316	0.775**	0.852**	0.925**
	No. of shoots	- 0.147	0.268	0.564	0.945**	0.968**
	Shoot length (cm)	- 0.806**	0.454	0.883**	0.766**	0.778**
	No. of leaves/shoot	- 0.884**	0.808**	0.812**	0.269	- 0.018
	Leaf area (cm²)	- 0.811**	0.797**	0.999**	0.795**	0.591
	C.C (m²)	- 0.316	- 0.175	0.382	0.620	0.910**
	C.V (m³)	- 0.426	- 0.199	0.390	0.524	0.835**
Second	Plant height (cm)	- 0.824**	0.384	0.478	0.609	0.687*
	Shoot diameter (cm)	- 0.999**	0.338	0.473	0.833**	0.939**
	No. of shoots	- 0.865**	- 0.200	- 0.054	0.991**	0.954**
	Shoot length (cm)	- 0.959**	0.537	0.658*	0.690*	0.858**
	No. of leaves/shoot	- 0.359	0.607	0.643*	0.055	0.272
	Leaf area (cm²)	- 0.826**	0.384	0.503	0.654*	0.809**
	C.C (m²)	- 0.854**	0.278	0.384	0.670*	0.754**
	C.V (m³)	- 0.832**	0.443	0.535	0.586	0.681*

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New Device for Controlled Resection of Nasopharyngeal Swellings

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Abstract: Presently otorhinolaringological surgeons face a problem of lacking of the control in adenoidectomy, which means excision of   a swelling that are located between the nasal airway and the back of the throat (nasopharynx). That is why it's called blind operation in otorhinolaringology, leading to uncertain removal of the bad and good tissues. This research removes this uncertainty, eliminates a major problem, and risks during the surgery. This research introduces a new tool to enable the surgeon to view surgical area, to be able to control the certainty of the operation. This developed tool consists of three main parts; DC motor connected to blade, visualization sensor connected to a monitor and suction tube. The motor is connected to special shaped blade (rotational knife in a window) suits the volume and shape of the swelling, and also follows the international dimensions standards. This device enables the surgeon to see the target area and directing the blade towards the desired tissue to be removed, frees surgeon's hand used to carry the endoscope. Moreover, the device gets rid off the unseen region under the endoscope as it avoids the surgeon to use the nasal opening for the endoscope. The cut tissue is drawn via suction tube. The amount of removed tissue can be easily visualized online and perfectly controlled, which increases the safety factors for adjacent structures/tissues such as; The Eustachian tube (orifice) and pre-vertebral muscles. Hence, the adenoidectomy operation becomes more accurate. Theoretical model was made by calculating the tool parts which was obtained through the existent experimental work, to get the optimum blade velocity for removing the target tissue. For a better visualization and precise control a built-in suction orifice added to the tool to clean the bloody field during the operation. A small lamp is located backward of visualization sensor to allow perfect vision during the operation. Also aggregation cavity was added to the new tool which is used to aggregate fluid during adenoidectomy. As a result, the adenoidectomy performance becomes faster and safer than the available traditional devices, besides freeing the surgeon's hand used to carry the endoscope.

1. Introduction

Adenoid removal surgery is to take out the adenoid glands, which are located between the nasal airway and the back of the throat (nasopharynx).

This procedure is also called an adenoidectomy. It is frequently done at the same time as a tonsillectomy [1, 2]. While the patient is under general anesthesia, the ENT surgeon inserts a small instrument into the mouth to prop it open. The adenoid tissue can be removed with an instrument such as a curette or a microdebrider. Some surgeons may cauterize the adenoids instead of removing the tissue.

Bleeding is controlled with packing and cauterization [3, 4, 5]. The adenoid is a mass of lymphoid tissue in the roof of the nasopharynx, located just inferior to the sphenoid sinus and anterior to the basi-occiput. Laterally, the adenoids blend with the lymphoid tissue of the fossa of Rosenmuller near the opening of the Eustachian tube. The adenoids are present in all infants and children, and start to regress just before puberty. They are usually absent in adults [6, 7]. Large adenoids produce nasal obstruction, mouth breathing, nasal voice, snoring and restless sleep. Chronic mouth breathing during the age when the facial bones are changing toward the adult configuration often produces a high arched palate, the pinching in of the nose and a shortened upper lip, with a staring expression of the eyes. The face becomes slightly elongated and the upper teeth may be prominent [8, 9].

These changes are called adenoid faces [10]. In addition, hypertrophy of the adenoids may also produce obstruction of the Eustachian tubes and contribute to the formation of middle ear effusions.

Adenoidectomy is a common procedure for treating pediatric ear, nose, and throat patients. Nowadays, it is used to treat Enlarged adenoids that have not responded to conservative treatments which are indicated in obstructive sleep apnea, recurrent serious otitis media, and resistant rhino sinusitis [11, 12].Typically, the adenoids might not have been visualized during clinical examination in ENT clinics because the pediatric patient may not cooperate during a mirror or endoscopic examination [13]. Furthermore, lateral radiographs of adenoids are not routinely investigated before an adenoidectomy. In many cases, the size of the adenoids is assessed by a transoral finger palpation or a mirror examination following the anterior retraction of soft palate by a nasal catheter [14, 15]. Most surgical procedures performed today with classic tool until now [8]. The surgeon cannot view the desired tissue well so that the surgical process becomes difficult and more complex [2, 8].

Partial adenoidectomy with the microdebrider was performed with the Xomed Adenoid microresector blade at 3000 rpm in oscillate mode without Irrigation. After tissue removal via either technique, electrocautery was used for hemostasis [14] . This technique involves the removal of the superior 50% to 80% of the adenoid pad, which leaves an

inferior tissue remnant undisturbed to ensure adequate velopharyngeal closure [10] .We have shown that partial adenoidectomy with the microdebrider is faster remove speed, but there is no tissue remove matching with microdebrider speed, and so may be injury to adjacent structures [10,13,15] . Also adenoidectomy with endoscopy is particularly useful for pediatric age group patients who have small oral cavities. The assessment and excision of the adenoids in these cases are often difficult. Because the size assessment of the adenoids by conventional palpation and mirror examination is challenging, and the placement of the adenoid curette guided by the indirect mirror is difficult. Moreover, it uses the classic adenoidectomy curette, so the operator uses his both hands during the operation, as a result difficult management and decreased accuracy of the procedure [3,4,7,8]. One of the main problems of this operation is that the surgeon cannot view the desired tissue well, so that the surgical process becomes difficult and more complex.

This new tool designed to perform good removing tissue by obtaining the desired velocity of blade and motor by choosing the best result from our work. and also, This tool supplied with control unit that allows a perfect control during adenoidectomy, Safety divider to prevent remove the undesired tissue during adenoidectomy operation , and aggregation cavity and etc. as shown tool design, the tool presented in this paper solved this problem, where adenoidectomy operation could be performed in a safe and accurate way due to the added facility of proper visualization of the nasopharynx which enables the surgeon to completely remove adenoids. In addition, Adenoidectomy with this presented tool is faster than adenoidectomy with curettes or a microdebrider, because of the perfect matching between rotational blade and bad tissue (adenoid) shape and size during tissue removal.

2. Tool design

This paper tool which is faster and more accurate for adenoidectomy is designed to have important advantages over the conventional adenoidectomy like improvements in curette or a microdebrider accuracy and shorter duration of operation.

This technique involves the removal up to 100% - depending on the surgeon's own skills - of the superior adenoid pad, which leaves an inferior tissue remnant undisturbed to ensure adequate velopharyngeal closure.

This tool is equipped with visualization sensor which is connected to a monitor allowing perfect vision during the operation. Consequently enabling the surgeon to view surgery area and becomes able to fully control the certainty of the operation.

3. Design

The design considers the four principal factors to control this research tool performance; the distance of blade movement, accuracy of cutting tissue, safety of surrounding tissue and time of tissue removal. According to the international standards the distance of cutting blade is chosen to match the available classic tool (curettes) which is 26 mm. This distance also matches the optimum speed and time of cutting unwanted tissues. Experienced surgeons approved that the slower cutting velocity, the safer operation. But on the other hand, the increase of velocity

reduces operation time which is appreciated by surgeons and patients. A comparison between those two crucial factors is considered in this paper tool design. Hence the blade velocity V could be related to the tissue under removal size and elasticity as given $V=Wr = 2\pi Nr/60$ where N is the DC motor speed, W is the angular velocity and r is the radius of gearbox. The two gears having the parameters r1 and N1 for the first and r2 and N2 for the second. Considering linear velocity $v = x/t$ where x distance of blade movement and t time of tissue removal. Since gear B and blade are tightly contacted, they rotate with the same velocity Therefore, $v2=x2/t$. For x equal the standard distance 26 mm then; $v2 =26/t$. From results shown in table 1 page 6 and according to the experienced surgeon's recommendations we select operation time 60 sec and its corresponding velocity 0.43 mm/sec. Hence according to the equation $v2 = 2\Pi N2 r2/60$, N2 = 50. The speed ratio of the gears I = N1/N2 = the speed ratio, which is selected as 2:1 therefore N1 = 100 r.p.m. where N1= 2N2. The diameters of two gears D1 and D2 respectively, are related to each other with the equation: $\Pi D1 N1= \Pi D2 N2$ OR $1/N2=D2/D1$. So we can get D1 = N2 D2 /N1 =50 *10/100 =5 mm and r1 =2.5 mm. Finally the blade velocity v could be figured out as $v = 2\Pi N1 r1/60=2\Pi*100*2.5/60=26.16 mm/s$.

This obtained velocity would be considered the best match between movement and adenoid tissue. This velocity support higher cutting torque for the tool to ensure better safety.

4. The tool

The tool as shown in figure (1) consists of three main parts, DC motor connected with blade, visualization sensor connected to a monitor and suction tube connected to external suction device.
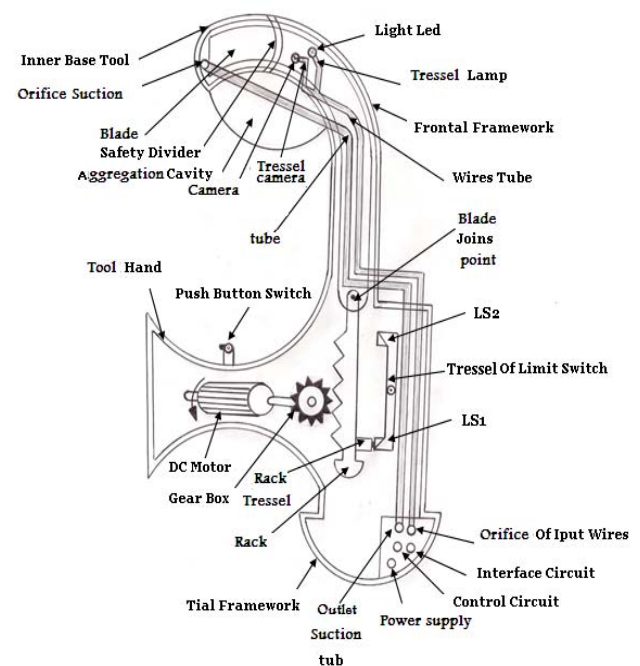
Fig. 1. Tool Construction.

The tool could be summarized as follows:

DC motor: permanent magnet type, with rated load speed 100 r.p.m, and rated load current 300 mA. Two limit switches are used to control the speed by connection gear box with motor shuft.

Gear box: located between the DC motor shaft and rack to control the speed of motor, and the cutting torque. Also matches the blade motion and motor speed.

Rack: located between gearbox and blade to transfer rotational motion of gear box into linear motion of blade. The rack is a rectangular prism with gear teeth machined along one side, could be considered a gear wheel with an infinite pitch circle radius.

Rack tressel (Rack arm contact): metal contact plate located on the rack that connect limit switches and the rack. The rack motion could be controlled by metal plate (Rack arm contact) connected to limit switches.

Blade: stainless steel metal anti-corrosion. It is connected with rack by bolt. Hence, start and stop motion of blade depend on the connection of rack arm contact with limit switch.

Limit switches: this new tool consists of two limit switches to perform perfect control during operation. First limit switch, It is normally closed (N.C) that is located towards the rack, the reverse direction is stopped when limit switch LS1is moved from its normal position, the normally closed contact opens and the drops out coil L. Second limit switch, it is has two sets of contact, one normally open (N.O) and the other normally closed (N.C), that is located towards the rack; it controls the direction of blade. Also Pushbuttons are used to initiate the motion control of the motor with second (N.C) limit switches so that blade then shaves the desired tissue. The auxiliary contact of the forward starter is connected in parallel with the pushbutton contact to maintain the circuit during the running of the motor in the forward direction. And terminate the forward motion control of the motor when rack arm contact connected with terminal second limit switch the (N.C) and (N.O) at the same time the motor reverses direction so blade motion direction is reversed after removing process.

The normally closed contact of limit switch LS2 acts as the stop for forward controller, and the normally open contact of limit switch LS2 acts as the start contact for the reverse direction .we can use Tressel (metal plate) to fixation and carry of limit switches.

Inner base tool (inner framework): It is the interior base tool that is a small cylindrical form which the surgeon directs towards the desired tissue.

Suction orifice: It is a small hole in the base tool beside the internal cavity; it is used to clear the bloody field during the operation by Suction tube which is a metal tube that connects suction orifice with external suction device, and it is made of stainless steel metal to overcome corrosion metal problem. We can clear bloody field from removed swelling through suction tube without insertion external suction tube so that the surgeon can be easy control during the operation because we make contraction of the number surgical tools in bloody field.

Safety divider/traverse: It is safe casement metal cylindrical form that is located on sides of blade to prevent

remove the undesired tissue during removing desired (adenoids) tissue.

Aggregation cavity: Used to aggregation results adenoidectomy operation to clear the bloody field.

Light led: It is dc small lamp used to light the internal operation location, it is located backward of camera that allows perfect vision during the operation. Also we can use Tressel lamp to fixation of lamp above safety divider/traverse on frontal framework. We can obtain maximum light forward camera when angle between tressel lamp and tressel camera equals to 110 degree to get a perfect vision during the operation.
Lamp casing with glass is used to protect it from internal fluid during the operation.

Camera: Small camera is used to transfer internal picture of operation into external monitor screen, it enables the surgeon to view operation location during adenoidectomy operation. The type of camera is cmos sensor CCTV video camera with self interface circuit. Also Tressel of camera is used to fix the camera on safety divider/traverse on frontal framework and it must be perpendicular on location of operation to obtain perfect vision. Camera is cased in closed glass tube for patient safety and for isolating the camera from internal fluid during the operation.

Wires tube: It is a metal tube used to carry internal wires into camera and lamp. This tube must be perfect electrical isolation to protect the patient from electrical risks. And there is a small hole (Orifice of input wires) in the frontal framework used to insert wires into camera sensor and lamp.

Interface circuit: It is a connection point between internal camera and LCD screen

Control circuit: A reciprocating motion tool process that uses two limit switches to provide control of the motor. Each limit switch (ls1 and ls2) has two sets of contact, one normally open and the other normally closed. The operation of the circuit can be summarized as following: the start and stop pushbuttons are used to initiate and terminate the motion control of the motor by limit switches.
_using the control relay and its start and stop buttons also provides low-voltage protection.
_reversal of the direction of the rotation of the motor is provided by the action of the limit switches. The pushbuttons provide a means of starting the motor in forward direction. When limit switch (LS2) is moved from its normally position, drops out coil R and the normally open contact closed. The reverse action is performed by normally open contact of limit switch (LS2), the reverse direction is stopped when limit switch (LS1) is moved from its normal position, the normally closed contact opens and the drops out coil L.

5. Basic tool Operation

The tool shape and size are kept as close as possible to the classic tool. The surgeon will use this paper tool similar to the classic one. Only one button is to be pressed to turn on the motor to start the automatic cut of the tissue under the blade, directs the tool towards the desired tissue, then presses on pushbutton and second limit switch

N.C terminal point is connected so that the motor is forward direction(R) and the auxiliary contact of the forward starter is connected in parallel with the pushbutton contact to maintain the circuit during the running of the motor in the forward direction so that the rotating blade then shaves the desired (adenoids) tissue. When rack arm contact connect with terminal second limit switch the N.C and connect with N.O at the same time so that the motor reverse direction (L) so blade motion direction is reversed after removing process. When Rack arm contact connect with terminal first limit switch N.C the motor is stopped.

6. Tool results

From our mathematical model the following values of the velocity versus times is tabulated in table 1.

Table 1: relation between the blade velocity (v) and time of tissue removal (t) at distance of blade movement x=26mm

Time in sec	1	10	30	45	60	70	90
V mm/sec	26	2.6	0.86	0.57	0.43	0.37	0.29

From above data we can obtain the more accuracy of the bad tissue removing at the operation time is 60 sec with blade velocity 0.43 mm/sec. Our results are in accordance with those demonstrating a time advantage of this tool in complete adenoidectomy operation was performed with full control over tissue removal, safer and faster than that with curettes, because at this speed there is a perfect matching between blade velocity and adenoid tissue during operation, also the cutting torque of blade is high so that due to greater control over tissue removal.

Results of this study show that research tool has a number of advantages. Visualization sensor allows easy size assessment of the adenoid and improves the accuracy of the adenoidectomy via conventional curette. Also control unit that allows a perfect control during operation. All current problems can be solved by the use of research tool, which allows accurate placement of the adenoid curette at the superior border of the adenoids. This positioning allows the complete removal of the main bulk of the adenoid without the need for nasal punch forceps, Nasal endoscopy and a microdebrider. In all cases, the developed tool is sufficient to remove the main piece of the adenoids in one attempt. In contrast, the adenoid tissue is removed in multiple small pieces in the classic blind curette adenoidectomy method (according to ENT surgeon's experience). In ENT surgeon's experience, there is only a minimal increase in the operating time. Although further studies might be required to confirm the above advantages, we believe this tool is a good alternative method for adenoidectomy.

7. Conclusion

In this paper, developed tool is to overcome the current problems of recent procedures (diathermy, classic tool and endoscopic adenoidectomy) during resection of nasopharyngeal swellings especially during adenoidectomy as follows:

1- A small camera fixed perpendicular on location of operation to obtain perfect vision and control during operation (Otorhinolarngology call blind operation).

2- Safety divider to prevent faulty removal of adjacent tissue structures, like the vertebral muscles , and the precious spinal cord. And also the Eustachian tube which if injured or blocked can lead to repeated middle ear effusion.

3- Control unit contains a Dc motor connected to blade through rack and gearbox to control its speed.

4- The optimum motor and blade velocity were set up according to expert's recommendations and our practical results.

5- In addition, no need for endoscope to view the operation field and also freeing the surgeon's hand used to handle the endoscope.

To summarize tool advantages; Short duration of the operation, Perfect control and more accurate surgery, complete removal of the adenoids, no injury to adjacent structures, less chance of hemorrhage .

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Isolation and Characterization of Chitosanase Enzyme from Different Parts of Some Higher Plants

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Abstract: Isolation and characterization of a chitosanase enzyme with high activity from different parts of some higher plants were studied. Different vegetative plant parts (leaves, fruit peels and dried seeds) representing nine families were screened in order to select the best source for extraction of the chitosanase enzyme. Results of screening experiments indicated that the enzymatic activity levels varied not only according to differences in plant species, but also to their morphological parts. In general, pepper, cabbage and purslane were the best leaves of chitosanases extractions and green bean was the best peels of chitosanase extraction while green bean, cabbage and purslane seeds were the best seeds of chitosanases extractions in this study. Results showed that leaves have high chitosanase activity more than seeds by three times. Seeds have high chitosanase activity more than peels of fruits by two times. Green bean peels, pepper leaves and opuntia peels were chosen for further study because of their high chitosanase activity. The activities of these three sources were measured by two methods. The two sources of chitosanase namely pepper leaves (C_1) and opuntia peels (C_2) were chosen for further studies. The optimal chitosanase activities of C_1 and C_2 enzymes on chitosan were obtained in 0.05 M acetate buffer, pH 5.8 and 5.4 at 40°C and 60°C, respectively. The isolated C_1 and C_2 chitosanase enzymes were stable on storage for more than three months at -20°C. Chitosanase C_1 and C_2 were stable for 60 minutes at 50°C and 60°C, respectively. The yields of the crude chitosanases C_1 and C_2 with optimum conditions were 47.616 and 59.146 U/g dry tissue, respectively.
[EL-Sayed, M. El-Sayed, Sanaa T. El-Sayed, Wafaa, G. Shousha, Abeer, N. Shehata and Nagwa, I. Omar. **Isolation and Characterization of Chitosanase Enzyme from Different Parts of Some Higher Plants.** Journal of American Science 2011;7(3):713-721]. (ISSN: 1545-1003). http://www.americanscience.org.

Key words: chitosanase - chitosan - pepper leaves – opuntia peels – physicochemical properties.

1. Introduction

Chitosanases (EC. 3.2.1.132) represent a class of hydrolytic enzymes acting on chitosan. Chitosan (a polymer of -1, 4-D-glucosamine) is thought to be absent in plants but it occurs in the cell walls of fungi (**Araki and Ito, 1975**).

Chitosanases from various sources show different hydrolytic action patterns, which are dependent on the degree of polymerization and of acetylation of the substrates. Most of the chitosanases from various sources reported so far were apparently endo-acting in nature, liberating predominantly mixture of dimers, trimers and oligomers from chitosan. Endochitosanase hydrolyze oligomers of glucosamine larger than chitopentaose, and chitosan with 62-100% DDA (degree of deacetylation); but exochitosanase act on chitosan and release glucosamine. Hydrolysis of chitosan with 99% DDA by endochitosanase release chitobiose, chitotriose and chitotetraose as the major products (**Sun et al., 2006** and **Chen et al., 2005**).

Most of chitosanases occur in a variety of microorganisms, including bacteria and fungi (**Chen et al., 2005; Jung et al., 2006** and **Wang et al., 2008b**). Chitosanases are also found in vegetative parts and seeds of some higher plants (**Ouakfaoui**

and Asselin, 1992a). They reported that *Triticum aestivum* and *Hordeum vulgare* seeds are the best monocotyledons sources of chitosanases while *Pisum sativum* seeds and *Cucumis sativus* fruits are the best dicotyledons sources. **Osswald et al. (1994)** isolated chitosanase enzyme from sweet orange callus tissue.

Chitosanase produced chitooligosaccharides from chitosan, ranging mainly from chitotriose to chitohexose (**Sikorski et al., 2006**). By controlling the reaction time and by monitoring the reaction products with gel filtration high - performance liquid chromatography (HPLC), chitooligosaccharides with a desired oligosaccharide content and composition were obtained after endohydrolysis steps (**Choi et al., 2004**). Chain length and the degree of deacetylation are considered the most important factors influencing the biological activities of chitooligosaccharides (**Matheson et al., 1984**). The complete enzymatic hydrolysis of chitosan to free glucosamine is catalyzed by exo- -D-glucosaminidase (EC. 3.2.1.165) (**Côté et al., 2006**). This enzyme was also designated as an exochitosanase. D-glucosamine which is the monomer of chitosan, have many biological, nutritional and pharmaceutical effects such as membrane stabilizing activity, liver protective effect, antioxidant activity and

immunostimulating properties (**Liu Wanshun** *et al.*, **2006 and 2007).**

Chitosanases are generally believed to play an important defense role against invading pathogens because of their potential to hydrolyse fungal wall polysaccharides (**Ouakfaoui and Asselin, 1992b**). Chitosanases have been extensively studied because of their hydrolytic effect on chitosan (**Sikorski** *et al.*, **2006**) and its derivatives (glycol chitosan, carboxymethyl chitosan and colloidal chitosan) (**Dumas-Gaudot** *et al.*, **1992**). Low and high molecular weight chitooligosaccharides perform potent antimicrobial, antitumor and immunopotentiating activities (**Matheson** *et al.*, **1984**). Also chitosanase show applications in various fields, such as biomedical, pharmaceutical, agricultural, biotechnological and in food industry (**Tokoro** *et al.*, **1988; Tsai** *et al.*, **2000** and **Qin** *et al.*, **2005).**

The aim of this study is to isolate, determine and characterize of chitosanase enzymes in some plants from different families

2. Materials and Methods
Experimental plants:

The plant materials used in this study consisted of samples of different vegetative plant parts (leaves and fruits peels) and dried seeds. They are leaves of clover, purslane, artichoke, cabbage, pepper and basil, peels of melon, papaya, water melon, green beans and opuntia fruits and dried seeds of green beans, pepper, cabbage and purslane. They represent nine families namely *Fabaceae, Cucurbitaceae, Portulaceae, Asteraceae, Caricaceae, Cactaceae, Brassicaceae, Solanaceae and Lamiaceae.*

Buffers:

Buffers were prepared according to **Gomori (1955)** and the final pH was checked by pH meter Hanna Instruments, Italy.

Extraction of the chitosanase enzyme from:
a- Dry seeds.

Dry seeds were crushed in Braun multimix MX 32 to be in a powder form. The dry powder was mixed with distilled water for 24 hours at 4°C. The extracts were centrifuged (13,000 xg at 5°C) for 15 minutes and the supernatants were dialyzed against distilled water for 48 hours at 4°C.The dialyzates were centrifuged again (13,000 xg at 5°C) for 15 minutes, and the supernatants were used as the crude enzymes.

b- Leaves and fruits peels.

The healthy fresh leaves and fruits peels of the selected plants were collected and cleaned thoroughly with water. These parts were sliced into small parts and homogenized in Braun multimix Mx 32 with certain extracting solution as distilled water at 5°C. The resulting homogenate was filtrated through cheese cloth and dialyzed against distilled water for 48 hours at 5°C.The resulting dialyzates were centrifuged (13,000 xg at 5°C) for 15 minutes and the supernatant was used as the crude enzymes.

Preparation of chitosan substrate:

The chitosan substrate for the production of chitosan oligosaccharides and low molecular weight chitosan was prepared as follow: ten grams of chitosan with 95% DDA powder was suspended in 400 ml distilled water and dissolved while being stirred with 5ml concentrated acetic acid. This solution was made with up to 1 L of water, and the pH was adjusted by using 1N NaOH (**Choi** *et al.*, **2004**).

Enzyme assay:

Chitosan was used as the substrate in the chitosanase assay. Chitosanase activity was determined by quantitative estimation of the reducing sugars produced from chitosan. The reaction mixture contained 0.9 ml of 1% soluble chitosan (in 0.05 M sodium acetate buffer, pH 5.8), adequate amount of enzyme solution and 1 ml of 0.05 M sodium acetate buffer, pH 5.8. The reaction mixture was incubated at 50°C for 60 minutes. The reducing sugars formed in the supernatant were estimated spectrophotometrically by using the modified dinitrosalicyclic acid (DNS) method (**Miller, 1959**), using glucosamine hydrochloride as standard. The amount of D-glucosamine released was also determined by using the specific method of Rondle-Morgan (acetyl acetone method) for D-glucosamine (**Rondle, 1955**). One unit of chitosanase was defined as the amount of enzyme that could liberate l nmol of reducing sugar as glucosamine per minute under the conditions described above. The activity of chitosanase values of samples were average values of three repeated measurements.

Physicochemical Properties of the purified chitosanase
Effect of the pH on the enzymes activity:

Small aliquots of the prepared enzymes were assayed with two buffering agents, namely 0.05 M acetate (pH 4.5-5.8) and 0.05 M phosphate (pH 6.0-7.5), for recording pH profile under the standard assay conditions.

Effect of the temperature on enzyme activity and stability
a- Activity of the enzymes with different temperatures

The maximum activities of the prepared enzymes were determined at different incubation temperatures ranged from 30-70°C.

b- Thermostability

Small aliquots of the prepared enzymes were preheated at different temperatures; 30, 40, 50, 60 and 70°C for varying time intervals; 30 and 60 minutes. The remaining enzyme activity was then assayed using the standard assay conditions.

Activity of the enzymes at different reaction times.

The prepared enzymes were incubated with the substrate for different time intervals up to 180 minutes, then the enzyme activity were estimated.

Effect of the different enzyme concentrations:

The enzyme activity was estimated at different concentrations of the chitosanase enzyme per reaction mixture. Then, the products were estimated and its amount was calculated per reaction mixture (μg / reaction mixture). Relation between enzyme concentrations and reaction products was plotted.

Effect of the different substrate concentrations on the enzyme activity:

The effect of different substrate concentrations were estimated by incubating different substrate concentrations (1 to 15 mg/reaction mixture) with the same amount of the enzyme concentrations. Then the enzymatic activity was plotted against substrate concentration.

3. Results and Discussion:

Screening for production of chitosanase enzyme from plant tissues.

Chitosanases have been isolated, identified and estimated by the amount of reducing sugar. The aqueous extracts of four seeds, five peels of fruits and six leaves of different plants were incubated separately with colloidal chitosan in order to assess the chitosanase activity.

Screening study focused firstly on sources that have high enzymatic activity for producing glucosamine. Table (1) represents the results of screening experiments for the existence of chitosanase activity in different vegetative parts (leaves and peels of fruits) and dried seeds.

The results indicated that the highest chitosanase activities were estimated in peels of fruits of green bean and opuntia and leaves of pepper, purslane and cabbage extracts (Table 1). The activity increased as chitosanase activity found in extracts of leaves of pepper, purslane and cabbage and peels of fruits of green bean (8.42, 3.622, 2.173 and 6.29 U/g

tissue, respectively) than that found in the corresponding seeds extracts (0.2497, 1.954, 2.687 and 3.43 U/g dry tissue). On the other hand, the differences in the chitosanase activity found in cabbage leaves and seeds extracts were not significantly higher or lower. Generally, it was observed that leaves and fruits peels have high chitosanase activity more than seeds of the same plant in comparison with the activity by U/g dry tissue (Table 1).

Table (2) represents the results of screening experiments for three sources with high enzymatic activities of chitosanase as determined by the acetyl acetone and the dinitrosalicylic acid methods.

Chitosanases from pepper leaves and peels of opuntia and green bean fruits have high activity for producing of chitooligosaccharides from chitosan that determined by dinitrosalicyclic acid method (Table 2).

Results showed that chitosanase stability of green bean peels decreased by saving it at 4°C for two months, in contrary chitosanases from opuntia fruit peels and pepper leaves when saved at 4°C for more than three months conserve with its activity. Thus, the enzymatic activities were varied not only with the species of the tested plants, but also according to the morphology of the examined parts.

Opuntia fruit peels and pepper leaves were chosen for further study of the optimum assay conditions of their crude enzymes C_1 and C_2, respectively.

The results are to great extent in accordance to those found by **Ouakfaoui and Asselin (1992b)** and **Osswald *et al*. (1994)**. They found that chitosanase occur constitutively in certain organs and tissues of plants and it was isolated from seeds, leaves and stems of bean, pea, tomato, cucumber, barley and maize leaves. Also they found that chitosanase activity of leaves of plants have high chitosanase activity more than chitosanase activity of seeds.

Assessment of the optimum assay conditions for the crude chitosanases isolated from pepper leaves (C_1) and opuntia fruits peel (C_2).

The optimum assay conditions for the crude chitosanases C1 and C2 such as temperature, pH, thermal stability, time of reaction and effect of different concentrations of substrate and enzyme were studied. Chitosanases activities were estimated by the amount of reducing sugar released by (**Miller, 1959)** and the amount of glucosamine released by (**Rondle, 1955)** when incubated with chitosan.

Table (1): Chitosanase activities of water extracts from seeds and vegetative parts of some plants by using acetyl acetone method

Name of plants	Family	Chitosanase activity				
		Leaves		Peels		Seeds
		(U/g) Wet tissue	(U/g) Dry tissue	(U/g) Wet tissue	(U/g) Dry tissue	(U/g) Dry tissue
Pepper (*Capsicum annuum*)	*Solanaceae*	1.519	8.42	ND	ND	0.2497
Cabbage (*Brassica oleraceae*)	*Brassicaceae*	0.4198	2.173	ND	ND	2.687
Purslane (*Portulaca oleracea*)	*Portulaceae*	0.41	3.622	ND	ND	1.954
Artichoke (*Cynara scolymus*)	*Asteraceae*	0.011	ND	ND	ND	ND
Clover (*Trifolium repens*)	*Fabaceae*	0.0058	ND	ND	ND	ND
Basil (*Ocimum basilicum*)	*Lamiaceae*	0.00032	ND	ND	ND	ND
Green bean (*Phaseolus vulgaris*)	*Fabaceae*	ND	ND	0.8496	6.29	3.43
Opuntia (*Opuntia ficus*)	*Cactaceae*	ND	ND	0.0558	0.6805	ND
Melon (*Benincasa hispida*)	*Cucurbitaceae*	ND	ND	0.0055	ND	ND
Water melon (*Citrullus lanatus*)	*Cucurbitaceae*	ND	ND	0.0033	ND	ND
Papaya (*Carica papaya*)	*Caricaceae*	ND	ND	0.00232	ND	ND

Table (2): Chitosanase activities of water extract by using two methods (dinitrosalicylic acid and acetyl acetone).

Plants	Part used	Chitosanase activity by	
		DNS (U/g dry tissue)	Acetyl acetone (U/g dry tissue)
Green bean	Peels of fruits	67.78	6.29
Pepper	Leaves	47.616	8.42
opuntia	Peels of fruits	59.146	0.6805

Effect of the different pHs on enzymatic activity:

The effect of 0.05 M acetate buffer at different pH values ranging from 4.2 to 7.0 pH are shown in figures (1-2). The results indicated that the chitosanase C_1 has optimum activity at pH 5.8 by the dinitrosalicyclic acid method and 5.4 by the acetyl acetone method (Figure 1). But the chitosanase C_2 has optimum activity at pH 5.4 by the two methods (dinitrosalicyclic acid and acetyl acetone methods)

(Figure 2). Similarly, **Wang *et al*. (2008a)** reported that the optimum pH for chitosanase activity isolated from *Gongronella* Sp. JG was pH 5.6. But **Osswald *et al.,* 1994** reported that chitosanase isoforms isolated from *Citrus sinensis* have optimum pH at 5.0.

Effect of the temperature on enzymatic activity:

The reaction rate of the crude chitosanase C_1 was measured at various temperatures (30-70°C).

Figure (3) showed that chitosanase C_1 has optimum assay temperature at $40^\circ C$ by the dinitrosalicyclic acid and acetyl acetone methods. Chitosanase C_1 activity is quite stable up to $50^\circ C$ and at higher temperatures the reaction rate declined slightly till reaches 30% at $70^\circ C$. Figure (4) indicated that chitosanase C_2 is very stable and has high activity at high temperature. It was showed that chitosanase C_2 has optimum assay temperature at $60^\circ C$ by the dinitrosalicyclic acid and acetyl acetone methods then the activity slightly decrease with increasing the temperature to $70^\circ C$ (only 5 % decrease of its activity). Similarly, the optimum temperature for the chitosanase isolated from *Citrus sinensis* was 60°C (**Osswald *et al.*, 1994**).

Effect of the incubation times:

The effect of the incubation time on the crude extract of chitosanase C_1 activity was studied up to 180 minutes (Figure 5). It was indicated that, chitosanase C_1 activity increased as the incubation time increased up to 180 minutes. There is linear relationship between C_1 activity and incubation time up to 180 minutes. Chitosanase C_2 activity increased with increasing the incubation time of reaction up to 120 minutes (Figure 6).

Effect of the different substrate concentrations on enzyme activity:

Linear relationship was observed between colloidal chitosan concentration and the activity of chitosanases C_1 and C_2, as shown in figures (7-8). The activity of chitosanase enzymes from C1 and C2 increase with increasing the concentration of chitosan up to 13 mg/reaction mixture for both enzymes.
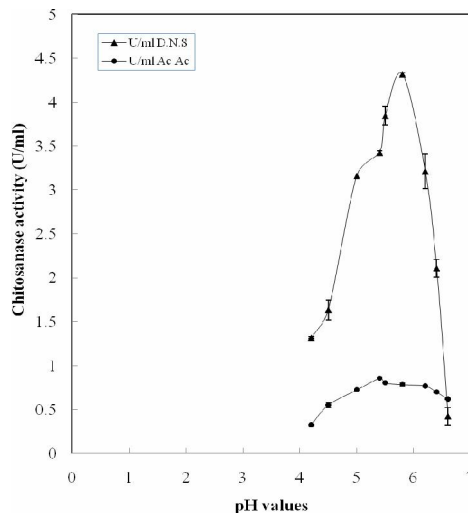
Figure (1): The effect of pH on the crude chitosanase activity (C_1).

Effect of the different enzyme concentrations on enzymatic activity:

The results presented in figure (9) indicated that the enzymatic activity of chitosanase C_1 increased with increasing enzyme concentration up to 240 μg protein per reaction mixture. Figure (10) indicated that chitosanase C_2 increased with increasing the enzyme concentration up to 513 μg protein per reaction mixture.
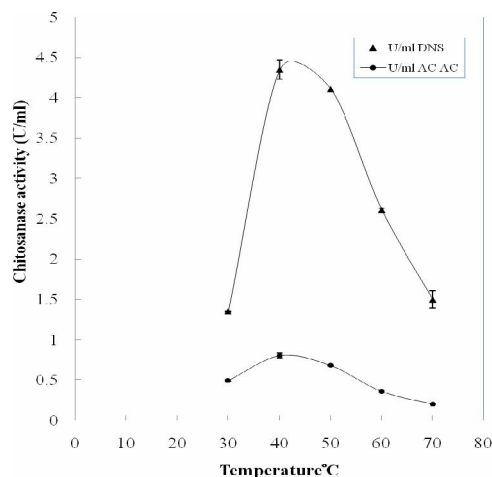
Figure (3): The effect of different temperatures on crude chitosanase activity (C1).

$y = 0.069x + 2.947$

$y = 0.008x + 0.520$
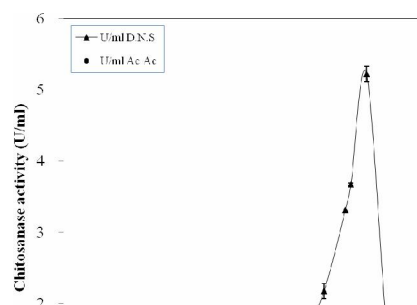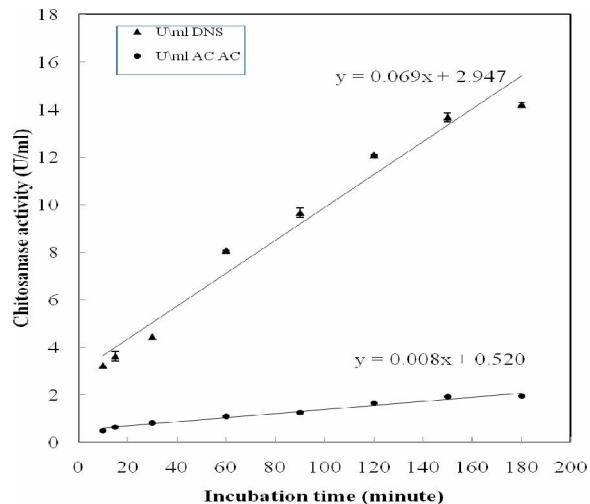
Figure (5): The effect of reaction time on the crude chitosanase activity (C_1).
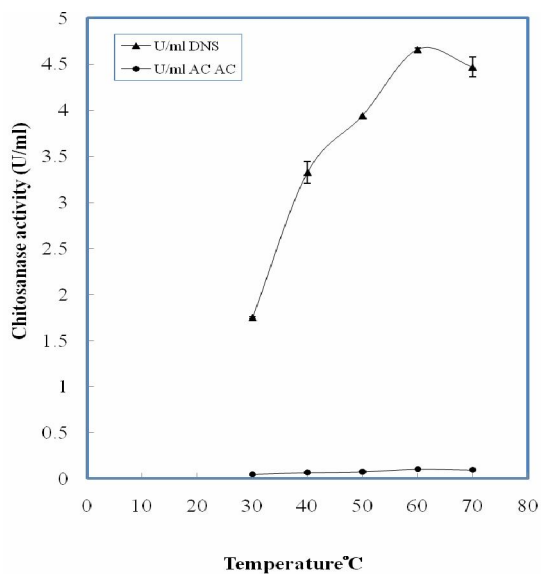
Figure (4): The effect of different temperatures on the crude chitosanase activity (C2).
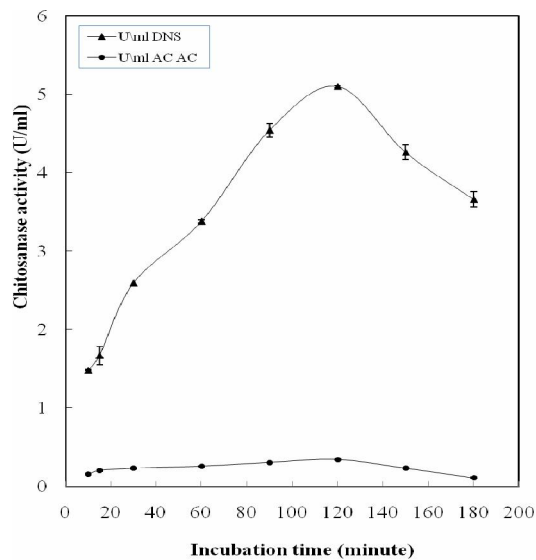
Figure (6): The effect of reaction time on the crude chitosanase activity (C_2).
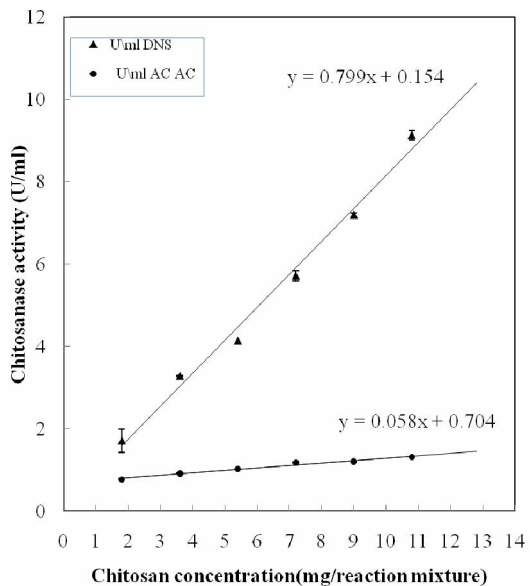
Figure (7): The effect of different substrate concentration on the crude chitosanase activity (C_1).
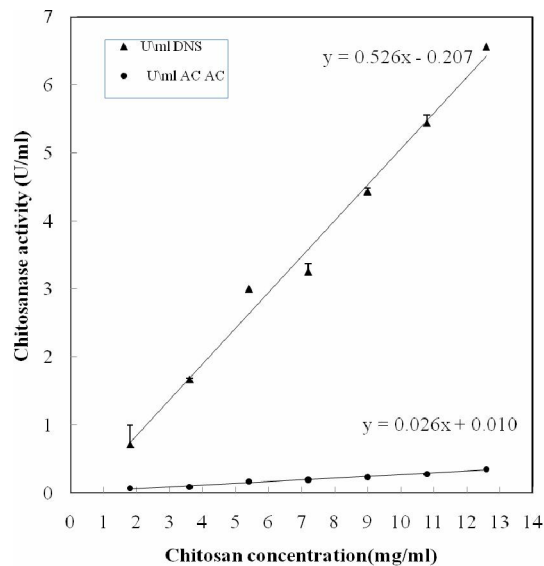
Figure (8): The effect of different substrate concentration on the crude chitosanase activity (C_2).
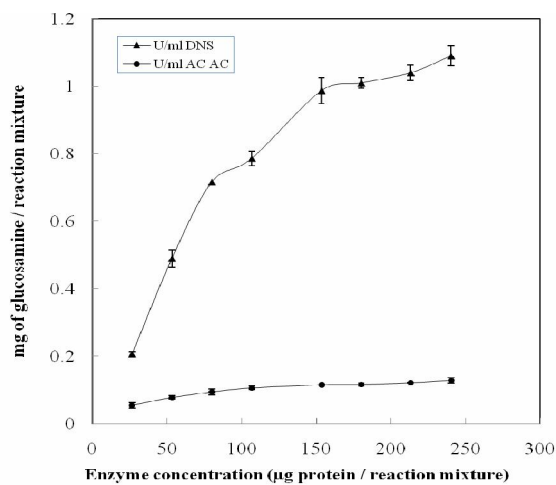
Figure (9): The effect of different concetrations of the crude chitosanase (C_1).
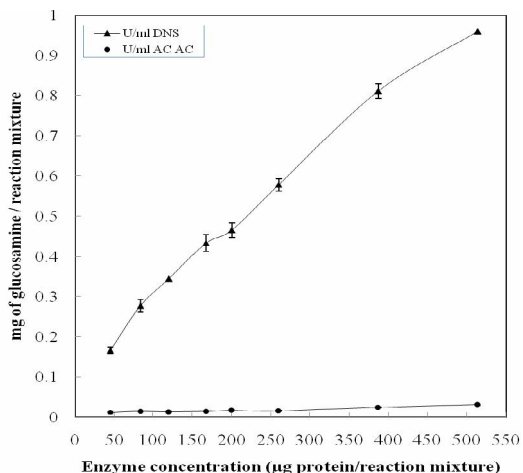
Figure (10): The effect of different concetrations of the crude chitosanase (C_2).
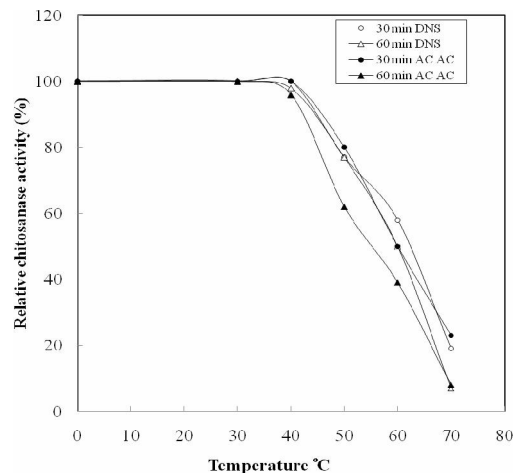
Figure (11): The effect of different temperatures on the crude chitosanase stability (C_1).

Temperature stability of the chitosanase enzymes:

The effect of preincubation of the crude chitosanase enzymes C_1 and C_2 in 0.05 M sodium acetate buffer, pH 5.8 at different temperatures (30-70°C) for 30 and 60 minutes in absence of substrate was carried out. The results illustrated that the activity of the crude enzyme C_1 is stable up to 40°C and the enzyme activity decrease by increasing the temperature (Figure 11). But the activity of the crude enzyme C_2 is stable up to 50°C and then the activity of enzyme highly declined at 60°C by the acetyl acetone method and decrease only by 25% by the dinitrosalicyclic acid method at 70°C (Figure 12). This is in general agreement with the prior results obtained from similar studies with *Streptomyces griseus* chitosanases (Osswald *et al*., 1992).

From these results, it was observed that chitosanase C_1 and C_2 has a high enzyme activity from (50-60°C) by using the dinitrosalicyclic acid method. Chitosanase C_1 has high enzymatic activity more than that of C_2 by using the acetyl acetone method (by eleven times).

The optimum assay conditions that gave the highest chitosanase activity of C_1 was obtained by using 0.05 M sodium acetate buffer, pH 5.8 by the dinitrosalicyclic acid method and pH 5.4 by the acetyl acetone method for 1.5 hours at 40°C.

Also The optimum assay conditions that gave the highest chitosanase activity of C_2 was obtained by using 0.05 M sodium acetate buffer, pH 5.4 by the dinitrosalicyclic acid method and pH 5.4 by the acetyl acetone method for 1.5 hours at 60°C.
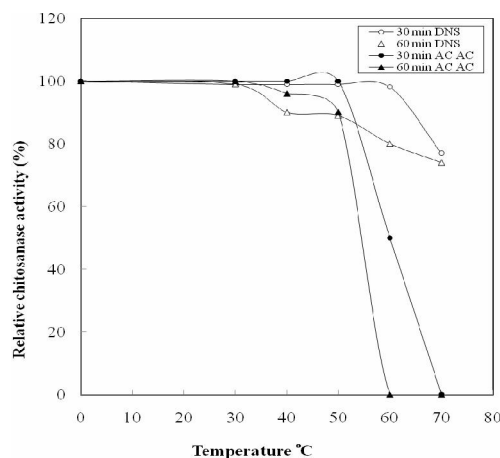
Figure (12): The effect of different temperatures on the crude chitosanase stability (C_2).

Conclusions and Recommendation

From this study, pepper leaves may be the best suitable source for chitosanase extraction with high activity and high heat stability. Moreover, pepper leaves are considered as a very economic source, because of its availability as an agriculture waste.

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Field studies on Prevailing Internal Parasitic Diseases in Male and hybrid tilapia relation to Monosex Tilapia at Kafr El-Sheikh Governorate Fish Farms

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Abstract: The present study was carried out on 1800 specimens of *Oreochromis niloticus* (*phenotypic , hybrids and monosex* of different lengths and body weights. They were randomly collected at different seasons from Kafr El-Sheikh Governorate cultured fish farms. The clinical signs of infested fish revealed no pathognomonic abnormalities on the external body surface .Such fish were shown emaciation. The postmortem showed that the internal organs were appeared anemic with enlargement and congestion. As well as, haemorrhage and ulceration of intestine and stomach mucous membrane. Monogenetic trematode (*Enterogyrus* cichlidarum*),* Adult flukes including *(Orientocreadium batrochoides, Afromacroderoides sp*, *Astiotrema reniferum* and *Eumasenia egypticus*), Nematodes including*(Procamallanas laeviconchus* and *Paracamallanas cyathopharynx),* Cestodes including *Polyonchobothrium sp and* Acanthocephalan including *Acanthocentis tilapiae* were investigated and identified. The highest prevalence possessed in hybrids of *O. niloticus* while monosex *O.niloticus* occupied the last position.. Also some physico-chemical parameters of pond waters represented as alkalinity pH, salinity, ammonia and sulphates were examined in relation to the infestation rate with internal parasites.
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Keywords: Internal parasites,   *O.niloticus, Hybrids, monosex* and   physico-chemical parameters.

1. Introduction

Internal parasitic diseases have the upper hand in fish parasitic diseases regarding the low body gain, high mortality. In addition, such diseases lead to gastrointestinal abrasions which facilitate the invasion of the opportunistic microorganisms. Where unfavourable environmental conditions contribute to stress which was weakens immunity and opens the pathway to pathogens (Kabata, 1985) and (Eissa, 2002). The clinical picture of infested fish revealed no pathognomonic abnormalities on the external body surface .Such fish were shown emaciation. The postmortem showed that the internal organs were appeared anemic with enlargement and congestion Bassiony (2000) and Ibtsam (2004). In addation, Abd El Hady (1998) recorded presence of *Eumasenia aegyptiacus*, *Orientocadium batrchoides, Astiotrema reiferum* and *Afromacroids sp* from *Tilapia sp*. The highest prevalence of *Enterogyrus* was in summer by autumn then followed by spring and the lowest rate of infestation was in winter Osman (2005). Nadia Mahfouz (1991) recorded *Paracamallanus cyathopharynx* with a rate of 1.4 % in *Tilapia sp* while *Procamallanus laeviconchus* 2.8% in *Tilapia sp* at Edfina and Barseek fish farms .The prevalence of nematodes were 9.25, 27.27, 2.65 and 0.0% in winter, spring, summer and autumn respectively.

Omoregie *et al*. (1995) recorded that d *Polyonchobthrium sp* in *Oreochromus niloticus* from Panyam fish farm, Nigeria. Tawfik (2005) recorded that *Acanthosentis tilapiae* isolated from *Oreochromis niloticus* at Abbassa fish farms. The present investigation was planned to studying the clinical signs of the examined affected fish isolation and identification of the internal parasites infesting cultured male phenotypic, hybrids and monosex *O.niloticus* in kafr El-Sheikh fish farms, analysis of some water hydrochemistery of affected aquacultures, Studying the relationship between the seasonal prevalence of parasitic diseases and the water quality and investigation of histopathological alterations of infested male phenotypic, hybrids and monosex *O. niloticus*.

2. Material and Methods
Fish specimens:

A total number of 1800 cultured *Oreochromis niloticus (O.niloticus)* of various life stages; fry, fingerling and adult Tilapia of different Male fish types (phenotypic, hybrids and hormonally treated monosex were collected from special fish farms in Kafr El Sheikh Governorate. The length of fry, fingerlings and adult specimens were ranged from 1- 1.5, 2 - 8 and 20 - 30 cm .While as their body

weights were ranged from 0.9 – 15.0, 17 – 28.7 and 105 – 220 g respectively.

Water samples:
A clean 48 water sample flasks, one litre volume were equipped with a cork stopper. The flasks were rinsed several times with distilled water and sterilized in a hot air oven at 180 C for one hour.

Clinical examination:
The collected fish types were examined clinically for clinical signs and P.M. lesions according to the methods described by Noga (2010).

Parasitological examination:
The alimentary canal of each fish was separated, dissected and divided into three main parts: fore, mid and hindgut. Each part was washed with physiological saline for several times to get rid of mucus and coarse particles that may be adherent to the parasites, then each part was opened and examined in a Petri dish under binocular dissecting microscope. The parasites were collected by Pasteur pipette and dissecting needle and transferred into Petri dishes containing warm saline solution was obtaining a fully relaxed and extended parasites.

Preparation of permanent samples
The collected helminthes (trematodes, cestodes and acanthocephalan) were left overnight in refrigerator to allow the worm to die; then compressed gently between two glass slides and fixed in 4 % formalin. The worms were washed in running water then soaked in alum carmine for 3 hrs. After staining; worms were washed in distilled water and passed through ascending grades of ethyl alcohol 50, 70, 90 and 100%, then transferred into xylol and clove oil respectively. Finally mounted with Canda balsam and covered with cover slide. Then slides were incubated at 60ºC for 24 hrs to driving of the air bubbles (Kabata, 1985). The collected nematodes were washed in saline then relaxed in refrigerator at 4C˚ and then immersed in hot alcohol-glycerin mixture until all alcohol was evaporated and the specimens remained in absolute glycerin. Worms was cleared in lactophenol and mounted in glycerin-gelatin according to Schmidely (1993).

Identification of parasites:
The helminthic parasites were morphologically and parasitologically identified according to Yamaguti (1985).

Physico – chemical analysis of water:

pH value of water was measured at the different locations in the fish pond by means of a digital pH meter (Ph CP. Hanna instruments. Italy). Total salinity was estimated by DR 2010 (at wave length 530, programs 88),also ammonia (mg /l) was measured at wave length 655, programs 342). Alkalinity was tested using (Chest own Maryland) alkalinity test kits 21620.Sulphate was measured , using HACH reagent, code 4630 in HACH apparatus, model CC. PS, (Adams 1990).

3. Results
Clinical examination:
The infested fishes showed signs of emaciation with sunken eyes and petechial haemorrhage on the surface of abdomen and intestinal wall was congested with the presence of ulcer and protruded from anus accompanied with large amount of catarrhal mucoid secretion. Also, they revealed internal organs of naturally infested fish were pale, aneamic with enlargement and congestion of spleen, liver with distended gallbladder, enteritis, haemorrhage and ulceration of stomach as well as intestinal mucous membrane. Some fishes showed slight bulging of the stomach, congestion and haemorrhage on the mucous membrane with watery food especially in heavily infested cases. White nodules in posterior kidney (plate, 1).
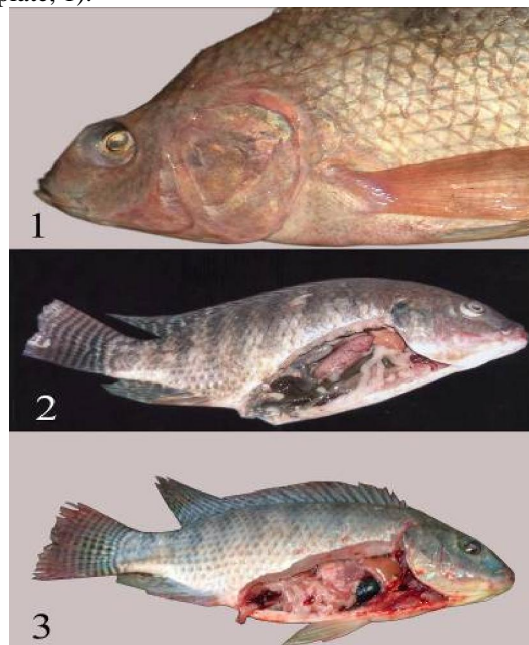
Plate (1):1- Hybrid *Oreochromis niloticus* with sinking eye. **2-** Hybrid *Oreochromis niloticus* with slightly turgid stomach and inflammation of the intestines.**3-** Monosex *Oreochromis niloticus* showing distended of gall bladder

Parasitological examination:

Adult worms were isolated form the stomach of infested fish. Such adult worms identified as *Enterogyrus cichlidarum* and adult worms were isolated from midgut of infested fish. Identified as *Orientocreadium batrochoides and Afromacroderoides sp.* Also, adult worms were collected from foregut of infested fish identified as *Astiotrema reniferum, Eumasenia egypticus, Procamallanus laeviconchus and Paracamallanus cyathopharynx*. While, thorny-head worms were isolated from hindgut of intestine of infested Tilapia sp identified as *Acanthosentis tilapiae* (plate2).
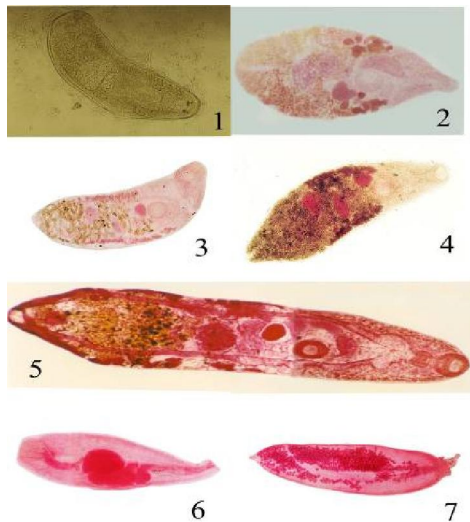
Plate (2): 1- *Enterogyrus cichlidarum* from stomach of hybrid *Oreochromis niloticus*. Wet mount. X 200.**2-** Adult fluke, *Eumasenia aegypticus*. Stain: Acetic acid alum Carmine X 4.**3-** Adult fluke, *Afromacroderoides sp*. Stain: Acetic acid alum carmine X 4.**4-** Adult fluke, *Astiotrema reniferum*. Stain: Acetic acid alum Carmine X 4.**5-** Adult fluke, *Orientocreadium batrochoides*. Soalted from midgut of *O. niloticus*. Stain: Acetic acid alum Carmine X 4.**6-** Adult fluke, *Acanthocentis male*. Stain: Acetic acid alum Carmine X 4. **7-** Adult fluke,

Acanthocentis female. Stain: Acetic acid alum CarmineX4.
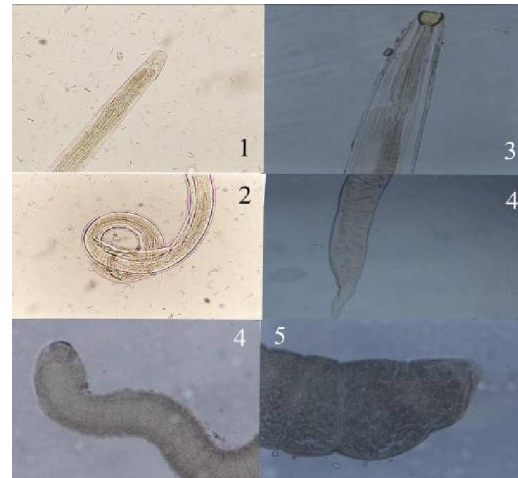
Plate (3): 1- *Procamallanus laeviconchus*. (Anterior end).Wet mount X10. **2-** *Procamallanus eviconchus*. (Posterior end). Wet mount X 10.**3-** *Paracamallanus cyathopharynx*. (Anterior end).Wet mount X10. **4-** *Paracamallanus cyathopharynx*. (Posterior end). Wet mount X 10. **4-** *Polyonchobothrium sp*. (Anterior end).Wet mount X 10. **5-** *Polyonchobothrium sp*. (Posterior end). Wet mount X 10.

Prevalence and distribution of helminthes:

Internal parasitic infestations of phenotypic, hybrids and monosex *O. niloticus* with *Enterogyrus cichlidarum Orientocreadium batrochoides Afromacroderoides sp Astiotrema reniferum , Eumasenia egypticus , Procamallanus laeviconchus and Paracamallanus cyathopharynx and Acanthosentis tilapiae* were recorded in Table (1 and 2).

Water analysis and seasonal prevalance:

The prevalence of parasitic infestation with different water parameters were recorded in Table (3)

Table (1): Prevalence of parasitic infestations among males of phenotypic, hybrid and monosex *O.niloticus*.

Tilapia		No, of exam. fish	Internal parasites							
			Adult trematodes		Nematodes		Cestode		Acanthocephala	
			N.	%	NO	%	NO.	%	NO	%
Male phenotypic	*O. niloticus*.	600	37	6.2	11	1.8	2	0.3	21	3.5
Male hybrids	*O. niloticus*.	600	66	11	17	2.8	4	0.6	31	5.2
Male monosex	*O. niloticus*.	600	27	4.5	5	0.9	0	0	9	1.5
Total		1800	130	21.7	33	5.5	6	1	61	10.2

Table (2):Distribution of helminthes in different types of Tilapias.

Site of infection	Class of parasites	Genus of parasites	Phenotypic *O. niloticus*		Hybrids of *O. niloticus.*		Monosex *O. niloticus*	
			No.	%	No.	%	No.	%
Stomach	Trematodes	*Enterogyrus cichlidarum*	2	0.3	3	0.5	1	0.2
	Nematodes.	*Procamallanus laeviconchus*	6	1	8	1.3	3	0.5
		Paracamallanus cyathopharynx	5	0.8	9	1.5	2	0.4
Intestine	Trematodes	**Adult trematodes**	7	1.2	13	2.2	0	0
	Cestodes	*Polyonchobothrium sp*	2	0.3	4	0.6	0	0
	Acanthocephala	*Acanthocentis tilapiae*	21	3.5	31	5.2	9	1.5

Table (3): The seasonal correlations between the average of some water parameters in different fish cultures with Endo parasitic infestations.

Types of Tilapia culture	No of Exam. fishes	No of Infested. fishes	Types of parasites	Spring	Summer	Autumn	Winter	Seasons	Salinity ppt	pH	ammonia mg/L	D o	Alkalinity ppt	Sulphate ppt
Male phenotypic *O.niloticu s.*	600	202	Trematodes	1.3	1.3	1.2	2.3	sp	2	7.2	0.3	8	210	90
			Cestode	0	0.3	0	0	su	1.3	6.8	0.4	6	180	50
			Nematodes	0.3	0.7	0.8	0	Aut.	1..2	6.2	0..3	8	190	53
			Acanthocephala	1	1.2	0.5	0.8	Win.	2	7.5	0.2	10	215	110
Male hybrids of *O. niloticus*	600	302	Trematodes	1.5	1.8	3.2	4.5	sp	2	7.2	0.3	8	210	90
			Cestode	0	0.3	0.3	0	su	1.3	6.8	0.4	6	180	55
			Nematodes	0.8	1	0.5	0.5	Aut.	1.7	6.3	0..2	8	193	52
			Acanthocephala	0.8	1	1.7	1.7	Win.	2	7.6	0.1	10	209	115
Male Monosex *O. niloticus.*	600	96	Trematodes	0.7	3	0.8	0	sp	1.0	7.5	0.1	10	160	50
			Nematodes	0.3	0.5	0	0	su	1.2	6.4	0.3	12	170	50
			Cestode	0	0	0	0	Aut.	1..2	6.2	0.2	8	173	41
			Acanthocephala	0.3	0.7	0.5	0	Win.	1.0	7.1	0.1	10	210	93

4. Discussions

The present study deals with most of different internal parasitic diseases among naturally infested the cultured *O. niloticus* (phenotypic , hybrid and monosex) in relation to the seasonal prevalence and water parameters in Kafr El- Sheikh fish farms. The internal organs of naturally infested fish appeared pale, anemic with enlargement and congestion of spleen, liver with distended gallbladder. Signs of emaciation with petechial haemorrhage on the surface of abdomen and slight bulging of stomach was obseved. While, intestinal wall was congested with the presence of ulcer and protruded from anus accompanied with large amount of catarrhal mucoid secretion. This clinical picture nearly was similar to that recorded by Osman (2005). This picture may be explained due to the presence of Enterogyrus, nematodes, cestodes and thorny headed worms which cause harmful effect as they embedded themselves between the villi of intestine causing local damage to the intestinal mucosa and possibly peritonitis. Proteolytic enzymes may be discharged from some adult worms degrading the intestinal tissues (Woo, 1995). Regarding the internal monogenea

(*Enterogyrus cichlidarum)* was morphologically and parasitologically described and was nearly similar to the descriptions given by Khidr (1996). Concerning to the cestodes (*Polyonchobothrium sp*) was isolated and identified from infested *Tilapia sp*. Such identification is nearly similar that recorded by Yamaguti (1985). Regarding to the isolated nematodes from naturally infested Tilapia fishes, isolation and identification of *Procamallanus laeviconchus* and *Paracamallanus cyathopharynx* were undertaken that nearly similar to those of original descriptions by Woo (1995). Finally, morphological and parasitological examinations of Tilapia fishes revealed isolation and identification of *Acanthosentis tilapiae* whose descriptions are nearly similar to those of original description by Yamaguti, (1985).

In the present study a total prevalence of Enterogyrosis in phenotypic, hybrid and monosex was 0.3, 0.5, and 0.2% respectively. Such results are lower than recorded by Eid and Negm, (1987) who reported that the prevalence of *Enterogyrus cichlidarum* from *O.niloticus* collected from (Bahr Mouise) was 13.3%, Ibtsam (2004) who recorded

60% of Entergyrosis. Also, disagree with that recorded by Osman (2005) who found a prevalence of Entergyrosis as 67.2%. These variations may be attributed to the water quality criteria and age of fish as such worms are stomach flukes need aged fish have well developed stomach and its wall was thicker for adaptation and fixation.

Regarding the total prevalence of adult flukes in phenotypic, hybrid and monosex were 1.2, 2.2, 0 % respectively. These results disagreed with the finding of Hassan (1992) who recorded the peak of adult trematodes in *O. niloticus* were 6%. Such result was lower than recorded by Abd El- Hady (1998) who recorded   that a prevalence of adult flukes was 15.58 %.

Concerning the total prevalence of *Procamallanus laeviconchus* was 1.8, 2.8 and 0.9% from phenotypic, hybrid and monosex respectively. These findings nearly agree with that met by Nadia Mahfouz (1991) who recorded a prevalence of *P. laeviconchus* as 2.8% in *O.niloticus* and lower than that recorded by El- Naffer *et al.* (1983) who recorded a prevalence of *P.laeviconchus* was in *Tilapia sp* 39%. While in *Paracamallanus cyathopharynx* were 0.8, 1.5, and 0.4%. These finding nearly agree with that recorded Abd El- Wahed (1992) who recorded a prevalence of P. cyathopharynx was 1.4% in *O.niloticus*. These results may be attributed to different types of fish, the presence of intermediate host (Snails), the suitable temperature which consider the main survival factors for these intermediate host and aquatic birds.

The highest infestation of phenotypic, hybrid and monosex were recorded in summer 3.5%, winter 4.4% and spring 2%. These results disagreed with that recorded by Nadia Mahfouz (1991) who recorded a prevalence of nematodes infestation in winter, spring, summer and autumn were 9.25, 27.27, 2.65 and 0% respectively. This result may be attributed to different types of fish, the presence of intermediate host (*Piscivorous*), and the suitable temperature which consider the main survival factors for these intermediate host and aquatic birds.

In this study, *Polyonchobothrium sp* could be detected with a prevalence 0.3, 0.6 and 0% from phenotypic and hybrid respectively. These findings are lower than that recorded by Hassan (1992) who found up to 7.5%.

The highest infestation of phenotypic, hybrid and monosex were recorded in summer 3.5% and winter 4.4% and spring 2%. These results disagreed with that recorded by Nadia Mahfouz (1991) who recorded a prevalence of nematodes infestation in winter, spring, summer and autumn were 9.25, 27.27, 2.65 and 0% respectively. These differences may be due to variation in climatic and ecological factors

which affect on intermediate host copepods (Cyclops) and aquatic birds.

Finally, the prevalence of *Acanthosentis tilapiae* in cultured (phenotypic, hybrid and monosex) was 3.5, 5.2 and 1.5% respectively. These findings nearly agree with that recorded by Eissa *et al.* (1996) and Rawia Adawy (2000) who recorded a prevalence of *A. tilapiae* in cultured *Tilapia sp* as 2.4 and 3.7% respectively and lower than that recorded by Eid (1997) who recorded a prevalence of *A. tilapiae* in *Tilapia sp* as 37.8%.

Concerning the highest infestation in phenotypic, hybrid were recorded in summer, spring, winter and autumn as 5.8, 3, 1.4 and 0% respectively. These results nearly agreed with that recorded Rawia Adway (2000) a prevalence of *Acanthocentis tilapiae* in cultured *Tilapia sp* in summer as 4.4% and disagree with Bassiony (2002) who mentioned that the highest infestation rate a prevalence of *A. tilapiae* in cultured *Tilapia sp* was in summer, autumn, spring and winter seasons were 39, 24, 21, 16.2 and 17.4% respectively. Also, Ibtsam (2004) who mentioned a prevalence of *A. tilapiae* in cultured *Tilapia sp* as in summer, autumn, spring and winter seasons were 12, 10, 17.4 and 0% respectively and in Tawfik (2005) who mentioned that the highest infestation rate a prevalence of *A. tilapiae* in cultured *Tilapia sp* in summer and winter as 14 and 7% respectively. This result may be attributed to different type of fish the presence of intermediate host (amphipod and isopod), the suitable temperature which consider the main survival factors for these intermediate host.

In the present study, all of the water parameters namely pH, salinity, ammonia, alkalinity, DO and sulphates in cultured fish farms, were within the permissible limits throughout the period of study according to APHA (1985). The epizootiological point of view to be important, are the predisposing factors for the wide spread of parasites among different Tilapia fishes in this study.

There was a positive correlation between the pH and alkalinity in water and the prevalence of each of (*Protozoa, Monogenea* and *Acanthocephala*). Negative correlation with prevalence of each of (nematodes and cestodes). These may be attributed to the fact that increasing the pH and calcium carbonate in water of fish ponds represent a stress factor on respiration process of fish, especially the gills and may be facilitate such parasites to infect fish.

The negative correlation between the DO in water and the prevalence of each of *Acanthocephala* may be attributed to the fact that low DO represent a stress factor on respiration process fish, leading to asphyxia and loss of escape reflex leading to entry of fish parasites .The present study, revealed that the

levels of total ammonia and salinity in ponds containing monosex tilapias were within the normal levels. On the other side, there was increase of both parameters in ponds of hybrids and phenotypic. This may be due to receiving agricultural drainage, domestic sewage and misuse of poultry dropping (Sabla) that revealed the poor water quality in this location (ponds) which was accompanied by increase the prevalence of *Protozoa, Ergasilus sp* and trematodes infection. These results agree with that recorded by Naguib and Abu Essa (1999) who found that, the incidence of the encysted metacercariae in muscles of infected fish during the breeding season was 56.2% in fish farms received the agricultural drainage. There is a positively correlation between water sulphate levels and the prevalence of *Acanthosentis tilapiae.* It may be due to limiting factor affecting algal growth (Elewa and Mahdy., 1988).

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Hepatopulmonary Syndrome Evaluation in Egyptian Patients with Portal Hypertension and Hepatitis C Virus Cirrhosis

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Abstract: Background: Hepatopulmonary syndrome HPS was defined as a triad of portal hypertension with or without hepatic dysfunction, intrapulmonary vascular dilatation or shunting, and hypoxemia. HPS was known as an independent predictor of survival in end-stage liver disease patients after hepatic transplantation. Egypt ranked among the highest countries in prevalence and incidence of portal hypertension caused by bilharizial peri-portal fibrosis and/or post- hepatitis HCV induced liver cirrhosis, or both. The frequency of occurrence of HPS clinical and laboratory criteria showed wide variability in the different studies. Therefore detection of clinical and laboratory criteria of HPS in a sample of Egyptian patients was of utmost importance. Design and participants: In a cross-sectional comparative observational hospital based study sixty Egyptian patients with portal hypertension in comparison with age matched control group were subjected to: (1) History taking and physical examination to detect manifestations of portal hypertension, hepatopulmonary syndrome and liver cirrhosis. (2) Laboratory investigations including estimation of 1- liver functions including alanine aminotransferase [ALT], aspartate aminotrasferase [AST], alkaline phosphatase, serum albumin, total and direct bilirubin, and prothrombin time and concentration, and international normalized ratio [INR].2- chronic hepatitis viral markers: anti-hepatitis C virus antibodies, hepatitis B surface antigen, hepatitis B surface antibodies, and hepatitis B core antibodies. 3- HCV RNA- PCR qualitative assessment. (3) Arterial blood gases ABG determination in the recumbent and standing position. (4) Chest x-ray. (5) Abdominal ultrasound to detect splenomegaly, ascites, liver cirrhosis, or portal vein dilatation or abnormal flow. (6) Upper endoscopy UE to detect evidence of portal hypertension. (7) Upright trans-thoracic contrast enhanced echocardiography UTCEE to detect intrapulmonary right to left shunting. Objective of the current study was to determine the percentage of occurrence of HPS in a sample of Egyptian patients [with a mean age of 50 ± 4 years] with portal hypertension and HCV induced cirrhosis based on clinical, ultrasound, and laboratory findings, ABG abnormalities, UE and UTCEE. Results: In a total of 60 Egyptian patients with portal hypertension and HCV induced cirrhosis clinical and laboratory evidence of HPS was positive in 6 patients [10%]. All of them belonged to Child-Pugh C class with serum albumin level below 3 mg/dl and prothrombin concentration of less than 50%. Among clinical features dyspnea showed the maximum sensitivity (100%), followed by cyanosis (83.33 %), spider naevi (83.33 %) and palmer erythema (83.33 %), while platypnea (100 %) and clubbing (94.4 %) were the most specific. Partial pressure of oxygen PO_2 was less than 70 mmHg in (100%) of HPS cases and was less than 60 mmHg in (50%) of them. Orthodeoxia was present in (66.66 %) of HPS versus (0 %) of non HPS patients with 66.66 % sensitivity and 100% specificity. All Child C patients, who were all HPS positive, showed oesophageal varices and congestive gastropathy, denoting severe portal hypertension. UTCEE was a useful non-invasive diagnostic tool for detection of trans-pulmonary abnormal blood shunting characteristic for HPS. Conclusion: The severity of HPS was clearly correlated with the degree of portal hypertension and liver dysfunction. Dyspnea had the maximum sensitivity followed by cyanosis, spider naevi and palmer erythema. Platypnea and clubbing were the most specific clinical features. Orthodeoxia strongly suggested the diagnosis of HPS with 100% specificity. Trans-thoracic contrast enhanced echocardiography in the upright position was a safe, useful semi-quantitative bed-side tool for assessment of shunting evidence to select cases for further quantitative lung scintigraphy based evaluation. [Abir Zakaria, Ahmed El-Mazny, and Tarek Heshmat **Hepatopulmonary Syndrome Evaluation in Egyptian Patients with Portal Hypertension and Hepatitis C Virus Cirrhosis,** Journal of American Science 2011;7(3):729-737]. (ISSN: 1545-1003). http://www.americanscience.org.

Key Words: portal hypertension, liver cirrhosis, hepatopulmonary syndrome, upright trans-thoracic contrast-enhanced echocardiography, hypoxia, dyspnea, platypnea, clubbing, orthodeoxia.

1. Introduction:

Cirrhotic or non-cirrhotic portal hypertension may be associated with arterial hypoxemia caused by pulmonary vascular changes referred to as hepatopulmonary syndrome HPS [1, 2]. This arterial hypoxemia may be due to one or more of the following pathologic mechanisms pre-capillary dilatation, capillary dilatation, or arterio-venous communication [3, 4]. Either due to ventilation perfusion mismatch or due to anatomic shunts bypassing the gas exchange units, these patients have a wide range of presentation ranging from

asymptomatic increase in alveolo-arterial oxygen pressure difference(P[A-a]O2) to severe breathlessness due to hypoxia detected by arterial blood gases [ABG]. Worsening of dyspnea in standing position compared to supine position [platypnea] with associated deterioration of hypoxia [orthodeoxia] may be explained by reduced cardiac output in standing position as well as increased perfusion of middle and lower lung zones by gravitational effects due to associated predilection of arteriovenous shunts to these zones[5,6].

Egypt has the highest countrywide prevalence of hepatitis C virus infection in the world [7]. The majority of cases develop chronic hepatitis that is usually asymptomatic for years. Twenty percent of those with HCV caused chronic hepatitis progress to cirrhosis and a proportion of these die as a result of liver cirrhosis complications [8]. Liver transplantation is a treatment option for end stage liver cirrhosis. HPS with obvious hypoxia was found to be an independent predictor of survival after liver transplantation [9, 10]. Our objectives in the current study were to assess a sample of Egyptian patients with portal hypertension and HCV induced cirrhosis for the percentage of occurrence of HPS clinical and laboratory features in correlation with their Child Pugh classification to determine their prognosis after transplantation. Using a bed side diagnostic UTCEE to screen those patients was suggested to be better than exposing all patients to radio-isotopic lung scanning [11].

2. Participants and Methods:

After informed consents 60 portal hypertension and HCV induced cirrhosis Egyptian patients with a mean age of 50 ± 4 years and 20 age matched control subjects participated in this observational comparative cross-sectional hospital based study. Patients and control group were subjected to:

o *Medical history taking and physical examination* with special attention to evidence of portal hypertension, Child Pugh classification, and clinical features suggested being HPS related. Excluded from the current study were those who suffer from cardiopulmonary diseases that may overlap in their symptoms or laboratory data with HPS.

o *Liver function tests and liver enzymes were determined:* Alanine aminotransferase(ALT), aspartate aminotransferase(AST), alkaline phosphatase, serum albumin ,total bilirubin, direct bilirubin , prothrombin time, concentration and INR.

o *Chronic hepatitis viral markers were assessed:* anti-hepatitis C virus antibodies HCV Ab, hepatitis B surface antigen HBsAg, hepatitis B surface antibodies HBsAb, and hepatitis B core antibodies HBcore Ab.

o *Qualitative HCV RNA-PCR was done for all cases.*

o *Child Pugh classification for liver disease severity assessment was used:* One to three points were given for each of the following items from least to worst affection: 1-history of encephalopathy, 2-detection of ascites clinically or by ultrasound, 3-serum bilirubin, 4-serum albumin, 5-prothrombin time, concentration, and international normalized ratio INR.

    o Child A: till 7
    o Child B: 8- 10
    o Child C: > 10

o *Arterial blood gases [ABG]*: Samples were obtained by radial arterial puncture in recumbent position to determine PO2, PCO2, and pH, and after standing for 20 minutes to detect orthodeoxia.

o *Chest x-ray* was performed complementary to clinical data to exclude those with any cardiac or pulmonary abnormalities.

o *Abdominal ultrasound was done.*

o *Upper endoscopy was done:* The presence of oesophageal varices and congestive gastropathy confirmed portal hypertension.

o *Contrast-enhanced Trans-thoracic Echocardiography CEE in the upright position UTCEE:* 10 ml of hand agitated normal saline were injected in an upper limb peripheral vein with the patient in the upright position. Positivity was defined as late opacification of the left cardiac chambers after more than five cardiac cycles following appearance of microbubbles in the right side of the heart. This finding suggested passage of these microbubbles through dilated pre-capillary or capillary pulmonary vessels or pulmonary arteriovenous shunts. Those with CEE evidence of inter-atrial shunts [early opacification of the left side before five cardiac cycles], valvular heart disease, dilated right side of the heart, or pulmonary hypertension were excluded.

o *HPS was suggested in patients with evidence of portal hypertension, with hypoxia [with a recumbent PO2 cutoff level of 80 mmHg in an arterial blood sample], and positive UTCEE.*

Statistical Analysis:

Clinical data were presented in a descriptive manner. Numerical laboratory data were presented as mean $\pm$ standard deviation. Sensitivity, specificity, and frequency of occurrence of clinical, imaging, and laboratory features were evaluated. One way analysis

of variance [ANOVA] was used to compare patients' groups and control group. P-value < 0.05 was considered statistically significant.

3. Results

Sixty portal hypertension cirrhotic patients were included in this study. Their age ranged from 35 to 60 years, 24 male and 36 female patients. Portal hypertension was proved by physical examination, upper endoscopy, and abdominal ultrasound. According to Child-Pugh classification patients were subdivided into 3 groups:

- Child Pugh A: 10 patients with mean age (39±3), 4 males and 6 females.
- Child Pugh B: 15 patients with mean age(45±7), 6 males and 9 females.
- Child Pugh C: 35 patients with mean age (51±6), 14 males and 21 females.

20 sex and age matched healthy subjects were taken as a control group.

Table (1) showed that in Child-Pugh A group dyspnea was present in 1 patient (10%), spider naevi in 1 patient (10%), palmer erythema in 1 patient (10%), collaterals in 1 patient (10%); while platypnea, clubbing and cyanosis were not present in any patient. In Child-Pugh B group dyspnea, clubbing, cyanosis, spider naevi, palmer erythema and subcutaneous collaterals were present in 3 (20%), 1 (6.66%), 2 (13.3%), 3 (20%), 3 (20%), 1 (6.66%) of patients respectively, while platypnea was not present in any patient (0%). In Child-Pugh C group dyspnea, platypnea, clubbing, cyanosis, spider naevi, palmer erythema and subcutaneous collaterals were present in 14 (40%), 2 (5.71%), 6 (17.1%), 9 (25.7%), 15 (42.9%), 16 (45.7%), 11 (31.4%) of patients; respectively.

Palmer erythema was the most common finding among all cirrhotic patients. It was detected in 20 cases, who represented 33.3 % of cirrhotic patients. It was found in five cases out of the six HPS cases, so represented 83.3 % of HPS patients.

Spider naevi were the next common clinical finding among all cirrhotic patients (31.66 %) with incidence of 83.3 % in HPS cases. Dyspnea was found in 30 % of all cirrhotic patients. It was evident in (100%) of HPS cases, with a sensitivity of 100%.

In our study 6 patients met the suggested diagnostic criteria for HPS, while 54 patients had no evidence of HPS.

Table (2) showed that among patients who met the suggested criteria of HPS, dyspnea was present in 6 patients (100%), platypnea in 2 patients (33.33%), clubbing in 4 patients (66.66%), cyanosis in 5 patients (83.33%), spider naevi in 5 patients (83.33%), palmer erythema in 5 patients (83.33%),

and subcutaneous collaterals were present in 1 patient (16.66%). However, in patients who had no evidence of HPS dyspnea was present in 12 patients(22.22%), platypnea was not present in any patient(0%) clubbing was present in 3 patients(5.55%), cyanosis in 6 patients(11.11%), spider naevi in 14 patients (25.9%), palmer erythema in 15 patients(27.77%), and subcutaneous collaterals were present in 12 patients(22.22%).

Table (3) showed that dyspnea had a sensitivity and a specificity of 100%, and 77.78% respectively with p-value < 0.0001 (i.e. highly significant statistical difference). Platypnea had a sensitivity and specificity of 33.33%, and 100%, respectively with p-value < 0.0001. Clubbing had sensitivity and a specificity of 66.66%, and 94.4% respectively with p-value < 0.001. Cyanosis had sensitivity and a specificity of 83.33%, and 88.88% respectively with p-value < 0.0001. Spider naevi had sensitivity and a specificity of 83.33%, and 74.07%, respectively [p-value < 0.05]. Palmer erythema had sensitivity and a specificity of 83.33%, and 72.23% respectively [p-value < 0.05]. Subcutaneous collaterals had sensitivity and a specificity of 16.66%, and 77.78%, respectively [p-value > 0.05 (i.e. no significant statistical difference)].

Dyspnea had the highest sensitivity (100%) in HPS cases followed by cyanosis (83.33 %), spider naevi (83.33 %) and palmer erythema(83.33%).

Dyspnea (100 %), platypnea (100 %), and clubbing (94.4 %) were the most specific clinical features, respectively.

Table (4) showed that PO_2 in the supine position was 59.83 mmHg ±3.488 in HPS patients compared to 81.35 mmHg ± 9.853 in non HPS patients with highly significant statistical difference [p-value < 0.001]. Also PO_2 in the standing position was 55.166 mmHg ± 5.382 in HPS patients compared to 82.56 mm Hg ± 9.484 in non HPS patients [p-value < 0.001].

Table (5) showed that patients belonging to Child Pugh A and B groups had recumbent PO2 values exceeding 70 mmHg without significant decline in the standing position i.e. no orthodeoxia. On the other hand out of 35 patients belonging to Child Pugh C group five patients (14%) had recumbent P02 readings between 60 and 70 mmHg without significant decline after standing, and four patients (11%) showed readings below 60 mmHg in the recumbent position with significant decline after standing i.e. orthodeoxia.

Table (6) showed that 3 out of 6 HPS patients (50%) had recumbent PO2 readings between 60 and 70 mmHg and another 3 (50%) had recumbent PO2 readings below 60 mmHg. The later 3 patients [PO2 <60 mmHg] plus one from the former group [PO2

between 60 and 70 mmHg] representing together 66.66% out of HPS patients showed orthodeoxia after standing.

Table 7 showed that all cases who belonged to Child Pugh A class had cords of grade I to II oesophageal varices, but nor had fundal varices or congestive gastropathy. In Child Pugh B class upper endoscopy revealed grade II oesophageal varices in 7 patients, and grade III in 17 patients. Fundal varices were found in 2 patients and congestive gastropathy in 7 patients.   All cases positive for HPS manifestations belonged to Child Pugh group C, who

showed grade II oesophageal varices in 7 patients, grade III in 17, and grade IV in 9 patients by upper endoscopy. From these fundal varices were detected in 6 and congestive gastropathy in all 26 cases; respectively.

As shown by table 8 UTCEE study was positive in 6 out of 60 cirrhotic patients (10%). All CEE positive patients belonged to Child Pugh C group only. All patients with positive CEE had recumbent PO_2 readings below 70 mmHg.

Table (1): **The number and the percentage of HPS related symptoms and signs in the three groups of patients:**

	Dyspnea	Platypnea	Clubbing	Cyanosis	Spiders	Palmer erythema	Collaterals
Child Pugh A	1(10%)	0(0%)	0(0%)	0(0%)	1(10%)	1(10%)	1(10%)
Child Pugh B	3(20%)	0(0%)	1(6.66%)	2(13.3%)	3(20%)	3(20%)	1(6.66%)
Child Pugh C	14(40%)	2(5.71%)	6(17.1%)	9(25.7%)	15(42.9%)	16(45.7%)	11(31.4%)
Control	_	_	_	_	_	_	_

Table (2): **Characteristics and diagnostic values of signs and symptoms suggestive of hepatopulmonary syndrome**

	Dyspnea	Platypnea	Clubbing	Cyanosis	Spider naevi	Palmer erythema	Collaterals
Cases HPS	6	2	4	5	5	5	1
Non HPS Cases	12	0	3	6	14	15	12
	100%	33.33%	66.66%	83.33%	83.33%	83.33%	16.66%
% HPS	22.22%	0%	5.55%	11.11%	25.9%	27.77%	22.22%

Table (3): Comparison between symptoms and signs related to HPS as regards their frequency, sensitivity, and specificity.

Symptoms and signs	Frequency in HPS (%)		Sensitivity (%)	Specificity (%)	P-value
	Pos	Neg			
Dyspnea	100	22.22	100	77.78	< 0.0001
Platypnea	33.33	0	33.33	100	<0.0001
Clubbing	66.66	5.55	66.66	94.4	< 0.001
Cyanosis	83.33	11.11	83.33	88.88	< 0.0001
Spider naevi	83.33	25.92	83.33	74.07	< 0.05
Palmer erythema	83.33	27.77	83.33	72.23	< 0.05
Subcutaneous Collaterals	16.66	22.22	16.66	77.78	>0.05

Table (4): **PO_2 in the recumbent and standing positions in HPS and non HPS patients:**

	HPS [n = 6]	Non HPS [n = 54]	p-value
PO_2 [recumbent] in mmHg	59.83 ± 3.488	81.35 ± 9.853	< 0.001
PO_2 [standing] in mmHg	55.166 ± 5.382	82.56 ± 9.484	<0.001

Table (5): **Distribution of PO_2 values in the patients' groups and control subjects:**

	PO_2>70 mmHg	60<PO_2<70 mmHg	PO_2<60 mmHg	Orthodeoxia
Child Pugh A	10	0	0	0
Child Pugh B	15	0	0	0
Child Pugh C	26	5	4	4
Control	20	0	0	0
Total	71	5	4	4

Table (6): Distribution of PO₂ values within HPS and non HPS cases:

	PO₂>70	60<PO₂<70	PO₂<60	orthodeoxia
HPS	0(0%)	3(50%)	3(50%)	4(66.66%)
Non HPS	51(94%)	2(4%)	1(2%)	0(0%)
Total	51	5	4	4

Table (7): Upper Endoscopy data of studied cases: *OV: oesophageal varices;* FV: fundal varices

	Upper endoscopy		
Child Pugh A	*OV* *10 grade I and II*	*FV* *1*	*Congestive Gastropathy* *0*
Child Pugh B	*OV* *6 grade I and II* *7 grade III*	*FV* *2*	*Congestive Gastropathy* *7*
Child Pugh C	*OV* *7 grade II* *17 grade III* *9 grade IV*	*FV* *6*	*Congestive Gastropathy* *26*
Control group	*0*	*0*	*0*

Table (8): Upright Trans-thoracic Contrast-enhanced Echocardiography [UTCEE] findings in HPS and non HPS cases:

	UTCEE positive	UTCEE negative
HPS	6	0
Non HPS	0	54
Total	6	54

4. Discussion:

Hepatopulmonary syndrome HPS was defined as a triad of portal hypertension with or without hepatic dysfunction [12], intrapulmonary vascular dilatation or shunting, and hypoxemia [13].

Bilharzial periportal fibrosis of the liver was one of the oldest parasitic infections in Egypt [14]. Serological and epidemiological studies showed a high prevalence and incidence of HCV among families from Egyptian areas known previously to be endemic for schistosomiasis. Co-infection with schistosomiasis and HCV was found to cause more severe disease due to resultant imbalance in HCV-specific T-cell responses leading to increased viral load, chronicity, and faster progression of complication and end stage liver disease [15]. Therefore Egypt was considered to have the highest countrywide prevalence of hepatitis C virus in the world nowadays [7]. Twenty percent of those with HCV caused chronic hepatitis progressed to cirrhosis [8]. HPS was an independent predictor of mortality and morbidity after hepatic transplantation [10]. Therefore studying the frequency of clinical features of HPS among Egyptian patients with portal hypertension and/or liver cirrhosis was suggested to be of utmost importance, especially when we found a wide variability of prevalence of HPS among end-stage liver disease patients in relation to etiological, racial and geographical factors [16].

In our study we suggested HPS in patients with evidence of portal hypertension, hypoxia and positive UTCEE showing delayed right to left shunting. Portal hypertension was detected by physical examination or ultrasound evidence of splenomegaly, ascites, shrunken cirrhotic liver and portal vein dilatation ± abnormal portal hepatofugal flow. Upper endoscopy was used for detection of oesophageal and/or fundal varices and congestive gastropathy. Hypoxemia was defined by a recumbent PO2 cutoff level of 80 mmHg in an arterial blood sample to pick up these patients for further evaluation by UTCEE.

This arterial PO2 cutoff level was suggested by previous researchers [17], who found that patients with PO2 of more than 80mmHg were unlikely to have HPS. Others defined hypoxia by arterial recumbent PO2 level of less than 70 mmHg [13]. A PO2 value of > 80 mmHg was considered mild, <80 and 60 mmHg moderate, <60 and 50 mmHg severe, and < 50 mmHg very severe HPS by other researchers [21]. Hypoxemia in HPS was explained by intrapulmonary dilatation or shunting through direct arterio-venous communications [24]. As vascular abnormalities were suggested to predominate in the middle and lower lung fields, gravitational effect was expected to increase the blood flow to worsen the ventilation-perfusion mismatch and finally resulted in deterioration of arterial oxygenation when the upright position was attained by the patient (orthodeoxia), hence

worsening of dyspnea in the upright position (platypnea) [25]. The intrapulmonary vascular dilatation or shunting was confirmed by different tools in the various studies interested in this syndrome as Technetium 99m macro-aggregated albumin lung scintigraphy [26], pulmonary angiography [27], and contrast enhanced trans-thoracic echocardiography CEE [28].

In the current study, among the 60 Egyptian patients with portal hypertension and HCV induced cirrhosis, 6 patients (10 %) met the clinical, laboratory and radiological criteria of HPS. This agreed in prevalence with previous studies [28, 33]. However HPS showed a wide variability in prevalence in the different studies, ranging from 4 to 47% among cirrhotic patients [34], depending on the diagnostic criteria and the cutoff levels used for hypoxia. Moreover etiological, racial and geographical factors were suggested to explain this variability by some investigators [16].

All our HPS patients belonged to Child-Pugh C group classification, which suggested a correlation between the severity of liver affection and possibility of development of HPS. This agreed with other studies [35]. It was explained by increased release of endothelin-1 and tumor necrosis factor alpha [TNF-] together with increased vascular shear stress with high levels of nitric oxide [18, 36], and carbon monoxide secondary to liver injury and/or portal hypertension. These mediators resulted in intrapulmonary pre-capillary or capillary vasodilatation considered the main pathogenic mechanism of HPS [34, 36]. As all this cascade was dependent on liver injury, it was suggested to be positively correlated with the degree of liver function deterioration as assessed by Child Pugh classification. On the contrary other studies [13, 37, 38] showed that Child Pugh grade didn't influence the prevalence of intrapulmonary vascular dilatation or HPS among cirrhosis patients.

In our study dyspnea was the most prevalent clinical feature in HPS patients. It was present in 100% of HPS patients. This agreed with other investigators [13, 35]. However ascites by elevating the diaphragm and impairing the ventilation/perfusion match might lead to mild hypoxemia and dyspnea in cirrhotic patients without HPS [35].

In our study platypnea was the most specific clinical feature. These results were closely similar to other investigators [35]. Platypnea and orthodeoxia were explained in HPS by the preferential circulation to the basal areas of the lungs in the upright position, as these areas were assumed to be more affected by intrapulmonary dilatation and/or shunting [39]. However in our study just two out of four HPS

patients with orthodeoxia had platypnea, suggesting that these two conditions did not necessarily go together. Gomez and co-investigators [25] found that HPS patients with orthodeoxia had lower cardiac output in both supine and standing positions in comparison to HPS patients without orthodeoxia [25]. Attaining the upright position was associated with increased minute ventilation with deterioration of ventilation perfusion [V/Q] in orthodeoxia group versus improvement of V/Q in non-orthodeoxia group in their study due to altered pulmonary vascular tone [25]. Platypnea on the other hand is a symptom i.e. more subjective, as dyspnea may be perceived by the HPS patient without special attention to worsening of the condition while standing.

In our study dyspnea (100 %) , platypnea (100 %), clubbing (94.4 %), and spider naevi (74%) were the most specific clinical features; respectively. This is closely similar to Alizadeh et al [35]. It was also in agreement with Anand et al [41]   and Varghese et al [36] who detected that patients with HPS had significantly higher incidence of dyspnea, platypnea, clubbing and spider naevi.

Moreover cyanosis showed in the current study high sensitivity and specificity. This was consistent with previous researchers [13, 16, 35]. Lee and co-workers [16] concluded that only cyanosis could reliably distinguish between shunt positive and shunt negative patients.

Spider naevi showed high sensitivity and specificity. This agreed with Hira and co-workers [13], who concluded that the presence of spider naevi was significantly correlated with intra-pulmonary vascular dilatation. In agreement with Alizadeh et al [35] our study showed a significant specificity of subcutaneous collaterals in relation to HPS.

Dyspnea had the highest sensitivity (100%) in HPS cases followed by cyanosis (83.33%), spider naevi (83.33 %) and palmar erythema (83.33 %). On the other hand platypnea had the highest specificity (100%) in HPS cases followed by clubbing (94.4 %), and cyanosis (88.88 %). These results were similar to Alizadeh and co-workers [35].

In our study all HPS patients had albumin level below 3 and PC less than 50% suggesting that HPS development was related to liver synthetic dysfunction. This agreed with Alizadeh et al [35], but disagreed with Kim et al [42].

In our study recumbent PO_2 was less than 70 mmHg in 100% of HPS patients versus 3.7% of non HPS patients. It was less than 60 mmHg in 50% of HPS patients versus 1.9% of non HPS patients. These results were closely similar to Alizadeh et al [35] and Krowka et al [12]. Moreover in their research on HPS patients Pastor and Schiffer [17] recommended that

patients with PO2 between 50-60 mmHg should have the priority for orthotropic liver transplantation. On the other hand 3 months follow up was their recommendation for those with PO2 levels between 60-80 mmHg for early detection of deterioration. PO2 of less than 50 mmHg might preclude liver transplantation [17].

In the current study there was a highly significant statistical difference in PO_2 both in recumbent and standing positions between HPS and non-HPS patients. These results were similar to Hira et al [13]. Orthodeoxia was defined by some authors as PO2 reduction by 5% in the standing position as compared to recumbence [25]. Nevertheless the pathophysiology of orthodeoxia in HPS patients was not fully understood [21, 40]. High progesterone level was suggested as an explanation by some authors [30]. We found orthodeoxia in HPS patients with 66.66% sensitivity and 100% specificity. This agreed with Alizadeh et al [35], Hira et al [13], as well as Wang and Lin [30] who considered orthodeoxia a suggestive indicator of HPS.

In our study all patients with positive UTCEE had arterial $PO_2 < 70$ mmHg and were qualified for the diagnosis of HPS. Among investigators who used CEE for diagnosis of pulmonary shunting some used it in the recumbent and other used it in the upright position. The later method, which was used in our study, was proved by previous investigators to be more yielding as regards diagnosis of HPS than that performed to patients in the recumbent position [32]. They explained this finding similar to platypnea and orthodeoxia, by gravitational effect of the upright position in shifting blood to areas of pulmonary vascular dilatation and/or shunting in HPS. CEE was proved by previous investigators to be useful sensitive and specific screening test for HPS even in early stages of liver dysfunction even in whom the lung scintigraphy was still negative [30,31]. On the other hand no patient had positive lung scintigraphy and negative CEE results [29]. The only privilege of lung scintigraphy over CEE was quantitation of the degree of shunting in relation to cardiac output [31, 32]. Some authors suggested transoesophageal CEE as a goldstandard [44, 45]. However others claimed that transthoracic CEE was as accurate as transoesophageal CEE in determining the presence of right to left shunt. Proper timing of left atrial opacification by microbubbles during the cardiac cycle was considered a distinguishing step in the transthoracic CEE between intracardiac and intrapulmonary shunting by Viles-Gonzalez and Rodriguez-Roisin [47]. Only in rare cases when timing of microbubbles was not clear cut, visualization of the microbubbles traveling through the pulmonary veins using transoesophageal CEE was suggested by both authors [47].

Study Limitations:

In the current study we didn't confirm liver cirrhosis by biopsy. This was not necessary as HPS was described in portal hypertension patients without or with cirrhosis. Portal hypertension was suggested by other investigators [2, 38, 42] as the determining factor for HPS.

Conclusions and recommendations:

The occurrence of HPS among Egyptian patients suffering from portal hypertension and liver cirrhosis was not uncommon. Diagnostic criteria were found in 10% of our studied sample in a small hospital based study. This suggested a mandatory wider scale research of cases with HPS especially after availability of liver transplantation as a treatment option for end stage liver disease Egyptian patients. Severity of HPS correlated with liver synthetic dysfunction evidenced by its positive correlation with Child Pugh class of the patients. Dyspnea had the maximum sensitivity followed by cyanosis, spider naevi and palmer erythema. Platypnea and clubbing were the most specific clinical features. So detection of these clinical manifestations mandated further evaluation of any portal hypertension patient by other investigations to confirm diagnosis of HPS. Arterial blood gases evaluation in the recumbent followed by re-sampling in the upright position should be the next step, as orthodeoxia in the current study strongly suggested the diagnosis of HPS with 100% specificity. Imaging confirmation of transpulmonary right to left shunting using upright trans-thoracic CEE was proved by our study to be an accurate, safe, and useful semi-quantitative bed-side tool for assessment of shunting evidence to select cases for further quantitative lung scintigraphy based evaluation.

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A Study of the Application of Ergonomics in Ready-made Garments Factories in Egypt

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Abstract: Garment industry is one of the most important strategic industries which constitute about 7% of total industrial production in the world and 8.3% of the total trade in industrial materials. Also, occupies more than 14% of the total labor force in the world. It employs about 40 million people in various countries of the world. The garment industry suffers from poor efficiency of workers performance due to stress as a result of the following factors: (1) The inappropriate Design of equipment and tools used by the Group. (2) The inappropriate Design of the workplace. (3) The absence of a suitable work environment. The International Organization for Occupational Safety and Health Administration (OSHA) is concerned about providing protection, safety and occupational health of workers. For that their must be application of human Ergonomic which studies the working environment where there is a mismatch between the materials needed of equipments, tools and the physical capacities of workers, thereby reducing or limiting the Musculoskeletal disorder (stresses on the group during the performance of the work), which had not been applied ideally, till now in the garment industry in Egypt. Garment industry passes through numerous stages represented in the (Design - Action Pattern - cut - sewing of all kinds - Finishing – Ironing. etc.) and this research focuses on the stage of sewing and knowledge of occupational diseases resulting from it. By considering ready- made Garment industry and address the problems faced by the employees of poor organization of the workplace and the provision of (a appropriate chair, sewing Tables with standard specifications, comfortable pedals, and appropriate work place, good handling) we could achieve high added values in which we can develop the structure of the industry in Egypt and so increase the size of Egyptian exports. The research aimed at raising the efficiency of the performance of the garment industry, by examining the application of human ergonomics engineering   which is interested in improving the employment and upgrading the conditions of the appropriate environmental factor (which prevent the stress-causing diseases). A field study was used in this method where a survey form was prepared covered five different garments factories in different places in Egypt in the governorates of Cairo and Alexandria and El-Gharbia in addition to the observations and interviews. Through the data and information collected by the computers in the extraction of statistical indicators which give indications can be used in a comprehensive picture of strengths and weakness points in the garment industry, particularly in the stage of sewing, and through this step we reach the research results.

1. Introduction

Garment industry offers opportunities to engage in competitions due to the participation of appropriate quotas from the global markets. This kind of industry in Egypt faces some obstacles as a result of the stresses experienced by workers at work such as:

- Physical stress of the muscles during the phase of sewing
(http://www.osha.gov/SLTC/ergonomics/outreach.html0.

- Stress due to the continuous work on the computer in various stages of design.

- Stress due to the unsuitable workplace and stress at the stage of ironing, and handling (http://www.osha.gov/SLTC/etools/grocerywarehousing/packaging.htm).

Employment in the garment industry is of the most important factors of production, which cannot be compensated and if it is not preserved, that will affect the future of the industry. By providing comfort for them as a result of poor conditions inside these factories which do not take into account the importance of rest of the human factor. It's obvious that most workers in this industry suffer from many diseases, and thus after a certain period of time, we find that the vast majority of skilled workers abandon the profession and turn to other professions which they might see more comfortable.

Some previous studies that examined the improvement of working conditions and the environment in the garment industry in India noticed that we should expanded research using ergonomics to reach a solution to the diseases affecting workers

in the industry. (P parimalam, N Kamalamma and A K Ganguli (2005).

With a simple look, we find that what the workers needs is simple and inexpensive and at the same time may cause him rest if applied or may cause him health problems, if it is absent, for example a worker who works on a sewing machine needs to:

1. Appropriate chair to sit on during sewing.
2. A table with standard size and specifications for sewing machine.
3. The Pedal used in operating sewing machine should be in appropriate angle for convenient operation and the material made with appropriate specification.
4. The workplace should be appropriate to the process of sewing.
5. Proper Handling.

These factors are affected by the size and nature of the process of sewing. In order to overcome these diseases and troubles, we must find other suitable design alternatives which help to solve all the obstacles that workers face.

Therefore, good manufacturing includes identification of the most dangerous tasks that affect worker health and production quality. By setting controls and afford the resources that help reducing or preventing injury to reach the highest production quality possible.

The Organization of Health and Safety establish rules for reducing industrial diseases for many industries but it did not include some industries such as the garment industry, which raises interest in solving this problem as they are willing to contribute to eliminate remaining occupational diseases.

It encourages employers in the implementation of an effective process of working environment and their role will be as follows: - Provide administrative support – care of workers - identify problems - implement solutions - Develop reports of injuries - provide training for workers – evaluate the place and the work environment. - Use of research centers to participate in resolving this problem.

The scientific strategy to reduce occupational diseases resulting from the sewing process is to identify the group of diseases affecting workers and the reasons that led to these diseases, what are the appropriate design solutions to reduce or eliminate these diseases and to find out the efficiency of what has been applied. The Study of the effect of the new variables (machine - a place of work) on the performance and the efficiency of workers which results on determining the effect of new regulations in increasing the quality of production and on workers as well by not leaving their profession.

2. Practical experiences:

Identify the basic features that define the causes of occupational diseases resulting from the process of sewing in the garment industry in Egypt, as well as the role of ergonomics (human engineering) in achieving the ideal work environment, as well as identify constraints faced by both.

To achieve that, a survey form has been designed and developed previously referred aiming at renovating the garment industry in Egypt, which came in three themes, where the data was analyzed and statistically tabulated in appropriate manner. These three themes are: First: occupational diseases resulting from the process of sewing.
 Second: tools used in sewing (chair - Desk - pedals .... etc). Third: work environment includes (the distribution of the space - Handling - the appropriate distribution of leisure time - Lighting - Ventilation - Noise - health care). The research team conducted field visits to factories during the study of the project to find out the current situations through a survey form, the observations and in-depth interviews for these factories. The survey has been focusing on the following points: (- Martha j. Sanders, ma, Msosh, OTR /L2004.) [1]

	DISEASES	PAIN%		
		bad	average	simple
1	Neck pain			
2	Shoulder pain			
3	Pain upper back (spine)			
4	Lower back pain			
5	Knee pain			
6	Leg pain			
7	Thigh pain			
8	hip pain			
9	Foot pain (ankle)			
10	Forearm pain			
11	Annex pain (elbow)			
12	Wrist pain (wrist)			
13	Problems of low vision			

The field study of the garment factories tested come out with the following results:

3. Result and discussion

As a result of field study found that a large number of the workers in the garment industry are suffering from diseases of the spine, neck, wrist hands, feet, the mother, the knee joints and muscles of the paragraphs and visual impairment, hearing All this is to AFS design table sewing and the use of a wheelchair is appropriate and random order of the workspace and the distribution of lighting.

3-1 - Diseases caused by the use of a chair in the studied factories.

Through statistical analysis of the results showed significant differences between the studied factories as a result of the use of the chair as table (1) shows:

Table (1) illustrates the moral difference as a result of the use of the chair.

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.488097645	4	0.12202441	5.9087113	0.00463	3.055568
Within Groups	0.309774177	15	0.02065161			

The table shows significant difference with a rate of 0.00463 factories as a result of the use of chairs used by the workers and figure (1) below illustrates these relations.
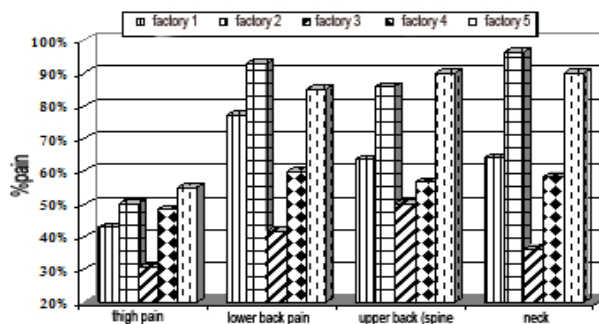
Figure (1): The percentage of pain resulting from the use of chairs

From the previous figure that the most affected factories by the use of chairs are factories number (1, 2, and 5) and the least affected ones are factories number (3, 4) because of the bad settings during the process of sewing due to the quality of the chair. We will review some of these chairs to see their effect on back pain, neck, and thigh of the worker. The Chairs used for sewing machine in factories (1, 2, and 5) are
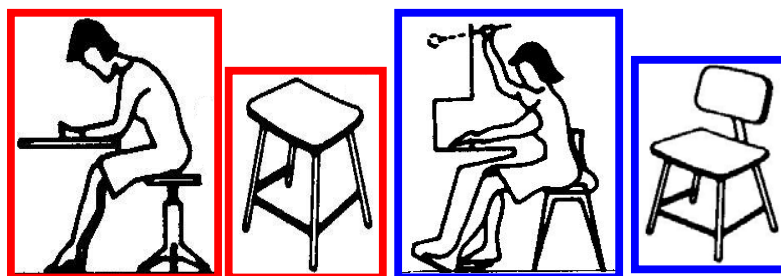
Figure (2) shows a sample of chairs used for factories (1, 2, and 5)

We discover from the figure that the chairs used in these factories are not suitable to sit on during the process of sewing. As it force the worker to stoop forward to complete the process of sewing and thus causing troubles and pains for the back and this is due to the lack of a back support of the chair and others with a wooden back. You cannot adjust the height considering workers tall, and also buttock and thigh pain are high compared to other factories as a result of non-upholstered chair and a small area of seat as shown in the graph. Always each worker uses the appropriate method to sit comfortably on a chair by putting a pillow or fill chair seat with more things in order to be suitable for him only when sitting on the sewing machine, and then come another worker and changes the chair position in a way to suit him, and so on, without founding a fixed system in the

factory that cares of the proper environment for the workplace.

The definition of stress physically is (a force /an area) and in this case uses a weight instead of force thus stress becomes (body weight / area) and the space in question here is the base of the chair the less the base of the chair the more stress and this is what exactly happened in these factories. This is a result of poor specifications of chairs used during tailoring and irregular breaks during the shift which increase stress on the worker and causes lack of desire in the Working and hope to finish his shift in order to get rid of the chair he sits on it and thus affect the production process and quality (the Union of Needle trades, Industrial and Textile Employees, the Institute for Work & Health, and the Occupational Health Clinics for Ontario Workers, Inc. Copyright © 2001 UNITE).[2]

Figure 3: This sample shows chairs used for factories (3, 4)

These factories differ from the previous ones so they use another type of chairs as shown in figure (3).

They seem appropriate for workers to use in sitting on sewing machine and thus reduce the stresses on the back due to the possibility of adjusting its height but its non-upholstered and back shape to the body causes pain in hip and neck area. Also we recognize through figure that the average pain were reduced for knees, legs and forearms lower than the

previous factories and this is due to the improvement of using pedals and regular lengths for all types of machines thus reduces stress on those areas of the body during the process of sewing.

The following figure (4) shows the incorrect posture of sitting on sewing machine, which often causes trouble for spinal cord as a result of bending forward constantly. Figure (5) below shows the allowable limits for the angle of dip backbone.

Figure (4): The wrong posture of sitting down causes curvature and pain on the back
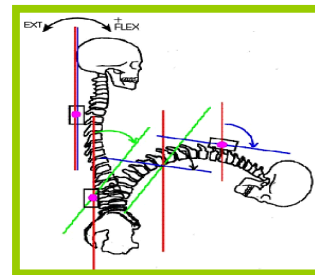
Figure (5): shows the form of curvature of the spine as a result of worker pending during sewing because incorrect sit

The angle of inclination of the spine in figure (5) is more than 60 degrees, which is higher than the permissible limits. The angle of inclination of the spine should not exceed 20 degrees, otherwise it will cause back pain. The inclination angle of previously used chairs in factories was more than 20 degree. By continua ting this situation while sewing; workers in the garment industry will be led to many troubles,

which will affect their health- (P parimalam, N Kamalamma and A K Ganguli (2005).

Therefore, they must change these chairs with other chairs have special specifications fit the conditions of movement of the workers during the process of sewing and has all the ergonomic design requirements appropriate for the formation of the

spine and hip to prevent back, neck and thigh pain of the workers.

3-2 Diseases caused by inappropriate design of sewing table in the studied factories

Through statistical analysis of the results, we have determined significant differences between the studied factories as a result of the lack of inclination angles of sewing table and its sharp edges, as shown in table 2:

Table (2): shows the difference as a result of the lack of inclination angles of sewing table and its sharp edges

Source of Variation	SS	df	MS	F	**P-value**	F crit
Between Groups	0.397335	4	0.099334	13.41624	**0.000499**	3.47805
Within Groups	0.07404	10	0.007404			

The previous table shows there is significant difference between factories with a rate of 0.000499 as a result of the lack of inclination angles sewing table and its sharp edges used by workers. The following drawing illustrates this difference by graphic Relations
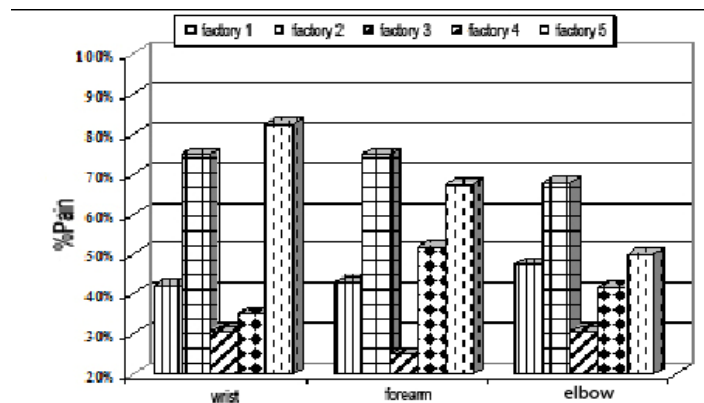
Figure (6): The proportion of pain resulting from the inclination angles of sewing table and its sharp edges

It is clear from the previous figure that the most affected factories are (1, 2, 5) due to the lack of inclination angles of sewing table and its sharp edges, in addition to the lack of an appropriate chair compensate to that lack. The least affected factories are (3 and 4) and this is due to the presence of an appropriate chair. The process of sewing requires that hands hold cloth down and push forward. The process of stretching arms to pull and push the cloth forward needs continuous effort, causing pain in hands**,** arms**,** and bending of the back. The pain increases sharply as a result of inappropriate sewing table design in its inclination angles and sharp edges, which makes the worker exert more effort to flatten the cloth during sewing, so we must search for design alternatives to reduce this pain. Figure (7) illustrates this http://osha.europa.eu

Figure (7): illustrates the effect of the design of inclination angles of sewing table and its sharp edges on hands and arms pain

As for the different heights of sewing table in the studied factories there are differences in height and shape figure (8) below illustrate this:
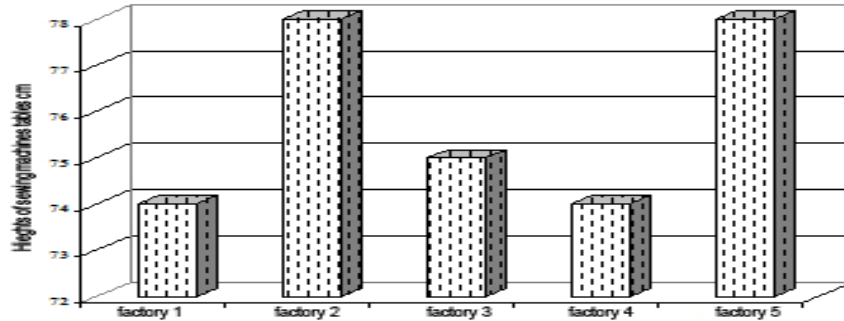
Figure (8): shows the percentage raises of Sewing Table

The previous figure demonstrates a discrepancy varying between heights of sewing machines tables, which in turn affects the rest of people at work. This indicates the lack of commitment of factories with standard heights suitable for sewing machines, considering adjusting heights to match the tall of workers. This causes major problems while working because of discomfort factor. It also increases the hands, arms and back pain. In addition to the above, despite the presence of natural and industrial light, the worker needs to add lighting in the machine to improve the vision of the cloth and maintain the vision of the worker. Beside

the level of noise in sewing machines could exceed the limit 90db, which could lead to hearing impairment or deafness of the worker**?** Furthermore, most sewing workers are of women**,** thus they need to shut the table side to hide their legs which must be taken into account in the design.

3-3 - Diseases caused by the different inclination angle of pedals in the studied factories
Through statistical analysis of the results turned out significant differences between the studied factories as a result of inclination angle of pedal as shown in table (3):

Table (3): shows the difference result of inclination angle of Pedal

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.65316	4	0.1633	15.39386	3.4437E-05	3.055568
Within Groups	0.159112	15	0.0106			

The previous table shows the value of P is almost zero which explains the difference between the studied factories due to the lack of appropriate

inclination angle of the pedal in sewing machines used by the factories and figure (9) below illustrates this difference.
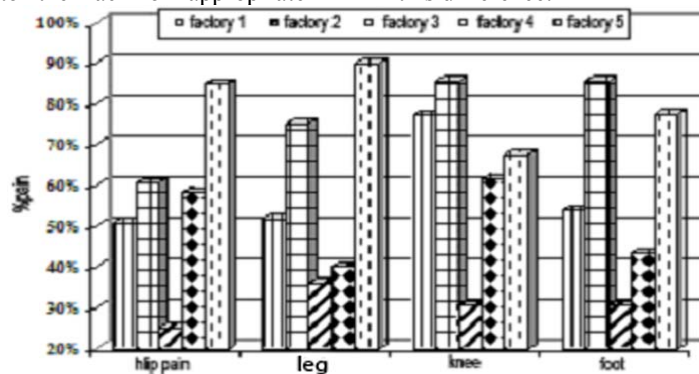
Figure (9): Shows the proportion of pain resulting from the inclination angle of pedal

It is clear from the previous figure how the foot suffers from stress during the process of sewing because of the type and inappropriate inclination angle of pedals in the way of movement carried out by the foot, whether left or right foot or both together.

At the factory number (2, 5), we observed a change in the focal point of pedal. Sometimes the link between motor and pedal is long and other times is short, which leads to a change in the effort made by the worker foot during the process of sewing, and hence lead to stress increase on the foot and the knee together during sewing. And figure (10) below shows a type of pedals used:
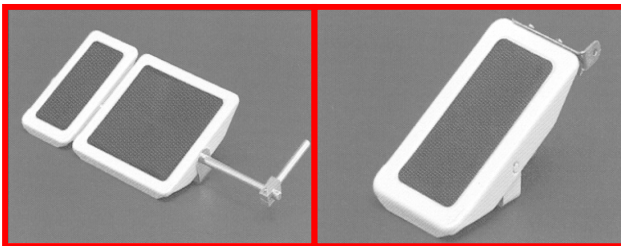
Figure (10): shows the types of pedals used

The previous figure shows the design of the used pedals by the user. As we notice high inclination angle of the pedal, which increases the stress on the worker's foot while running the sewing machine. Figure (11) below shows the ideal inclination angles of pedals that should be followed while using sewing machines.
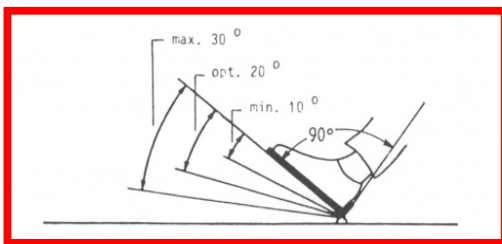
Figure (11): The ideal inclination angles of pedals used in sewing machines.

The previous Figure illustrates some of the different inclination angles of pedals used in sewing machines. The worker's foot must be in a right angle (90 degrees) on the pedal, while running sewing machines. The lowest inclination angle of the pedal should be 10 degrees, while the maximum angle is 30 degrees. In factory no (3) the ideal inclination angle used was 20 degrees, thereby reducing stress on the leg during sewing to 180 Nm, as shown in the following figure (Chris j. Snijders, 1998):
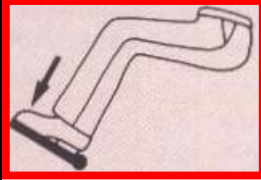
Description of Stress	The stress value in Newton	Type of stress
When the focal point is at the back and you press the pedal with the front part of foot.	180	

Figure (12): Shows similar movement of the foot in the factory

Regarding the legs and feet pain, they were more than 85% in factories (2, 5) followed by factory (1). It was observed that the irregular inclination angle of pedal is sometimes large (greater than 30 degrees), so the worker who sits on the sewing machine is forced to raises his feet up. Therefore, the inclination angle of foot on the leg becomes less than 90 degrees, which causes him tension stress on the heel, pressure on the instep, and thus causing trouble and pain of the foot.
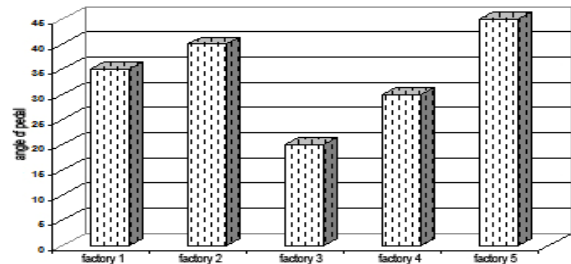
Figure (13): Shows the amount of pain because of difference in angle of pedal in factories

We can observe from the previous figure that the inclination angles used in the factory number (3), followed by factory number (4) is appropriate, as it registered the lowest inclination angle ranged between 20: 25 degrees. Although the proper angle should not exceed 20 degrees, it is the ideal angle during usage.

The difference in inclination angles resulted from the different lengths of the connection between the pedal and the operation motor. If this connection is broken it will be connected again, leading to reduction in the length of the connector and therefore high inclination, and so on. In the beginning, the inclination angle does not exceed 15 degrees and because of the frequent break of connector and reconnecting them up again this angle reaches 45 degrees. This lead to foot, knee, and crippling (hip) pain.

3-4 diseases caused as a result of different means of handling in the study factories

　　　Through statistical analysis of the results we find out significant differences between the studied factories as a result of different means of handling, as shown in table (4):

Table (4): Shows the difference result of different means of handling

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.480377525	4	0.1200944	10.895199	0.00115	3.4780498
Within Groups	0.110226878	10	0.0110227			

The previous table shows, the value of P 0.00115 which explains the difference between the different studied factories as a result of the different means of handling in factories and figure (14) below illustrates this difference in a graph.
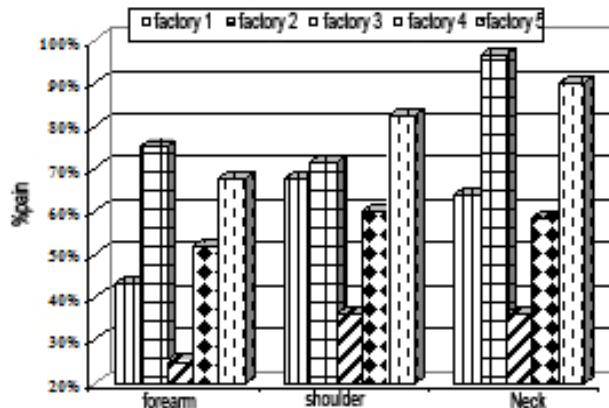
Figure (14): Shows the amount of pain as a result of different means of handling in factories

　　This figure shows the most affected factories because of bad design of handling process in factories number (2,5), followed by factories (4, 1, 3). The reason is that the handling unit is stable and far to the reach to by the worker, the chair is also fixed in place, leading the worker to rotate the upper part only to the left and right which makes him stress in twisting and causes pain in the neck, forearm and the back. But if the handling unit is adjustable to the needs of the sewing process and the seat has steering wheels, and movable, thus the worker will be able to rotate his body a complete cycle easily and avoid the pain of the shoulders and arms. We must look for other designs taking into consideration the upright position of the worker during handling. The following figure shows the position of a worker at the sewing machine during handling of the cloth (http://www.osha.gov/SLTC/etools/sewing_sp/index.html)

Figure (15): Shows the position of a worker at the sewing machine during handling of the cloth
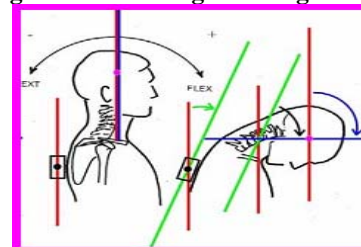
Figure (15): Shows the position of a worker at the sewing machine during handling of the cloth

The previous figure shows the wrong position during the process of handling while sitting on the sewing machine which leads to increase the inclination angle of the neck than the permissible limits (25: 65) degrees. This will lead to vertebra stress of the neck and back. This will be taken into account when designing a new handling method suitable for workers in garment industry to avoid neck pain.

3 - 5 diseases caused by the unsuitable workplace design in the studied factories

In addition to the above mentioned points and as a result of the observation of the research group, it has been noticed that the poor workplace design led to other diseases and reduce the speed of the production process and lack of quality. The design of the workplace contains (arranging the work components in accordance to the dimensions of the human body - Lighting - Ventilation - Noise), in addition to the social dimensions.

Regarding the order of components of the work:

To order the components of the sewing process efficiently around the worker so as to achieve speed, quality of this process, and to avoid any more stress.

While planning the worker position and the components in the workplace, we must take into account the following:
- To accommodate the work surface height and shape with the worker**'s** body and type of work.
- Consider space limits within the body movement.
- All handling and modify process with foot should be in reliable positions within the natural movement of the worker body parts.

The concept in factories in arranging the components of the work is not as the former concept, but regarding the space between the sewing tables and concerning the given area the length of the space is large to an end, what is disproportionate to the speed of the process of sewing, quality, posterior borders, and mobility of the worker body.

For lighting:

It must be appropriate and compatible with the necessary requirements to perform the desired function of the worker, especially during the performance of assembly and finishing. Also the worker should be sitting in the right position during the operation without obstructing the visual perception and accuracy in discrimination. The best way for the light to be parallel with the natural lighting and close to the worker in addition to lighting joined to the machine itself to see the minute pieces. But for lighting in factories, it takes the orthogonal position with natural lighting and high for

the worker and one factory only takes the parallel position with natural light, but the elevation is not suitable for the worker In addition to the lack of a lighting unit connected to the machine (Abdel-Nabi Abu al-Majd, first edition, 1420 E – 2000) figure (17) below illustrates this:
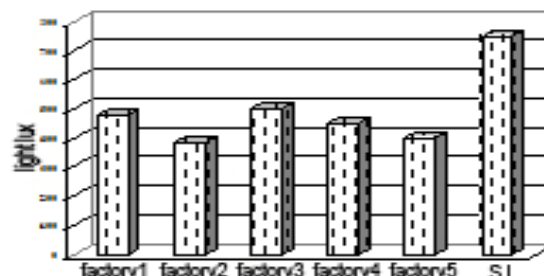
Figure (17): the amount of the impact of lighting on worker

The previous figure illustrates the most closer factories to using the best light distribution is factory number (3) then (1, 4, 5, 2).

For the noise: Noise is generated because of the lack of ongoing maintenance of machines and with the continuous use the noise increases, which lead to reduction or losing hearing. Also, there is absence of audio units next to the worker which send or receive instructions. This requires taking into account the periodic maintenance and running a system to receive instructions.

For ventilation: It relies on the traditional windows .we can also notice the absence of filters for dust suction and they do not give workers covers for their faces to protect their noses, which in turn leads to chest sensitivity.

We will review in the next part of this research designs that concentrate on all these obstacles faced by those workers on sewing machines in the garment industry to maintain their health and comfort and not forsaking the profession.

4-Conclusion

The garment industry in Egypt suffers from poor efficiency of workers performance at work, as a result of using inappropriate design of sewing table, seat, and workspace. The research target is to improve the performance of this industry by applying ergonomic human engineering which is interested in raising the efficiency of labor and improve the environmental conditions by preventing stress-causing diseases. I chose to use a field study method in this research which showed clearly how the workers face many health problems due to inappropriate equipments in performing the sewing process.

5. Recommendations:

1 - From the previous results it was observed that most of the factories of the garment industry do not stick to the standard specifications in the workplace which forces the worker to sit by till the end of the shift and he is not even allowed to complain of the inadequacy of the place or he will be subjected to harassment especially in private sector. As a result of these circumstances, ready-made garment industry has become expelling employment as a result of bad work conditions. Thus, these conditions and nature of work should be improved and this is what will be developed in Part II of the research work by creating design solutions to meet all these obstructions.

2 - We recommend that employers apply a systematic process to identify and resolve the problems of work environment, and integrate this process into the overall work programs to prevent injuries and occupational diseases.

3 - Engage workers in determining the workplaces threats, classification, and participation in resolving these problems. This leads to great success of design solutions and give**s** the worker motivation and satisfaction of the workplace. The worker will be required to do the following:

- Provide suggestions for what they suffer from.
- To discuss methods of work and the workplace.
- Participation in workplace design, equipment, procedures, and training.
- Evaluation of equipment and tools used.
- Respond to surveys employee.
- Participating in preparing tasks with the responsible groups for the work environment.
- Participating in developing the work environment by establishing a place of health care.

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2/1/2011

IT IS NOT ENDOCANNABINOIDS BUT THE TYPE AND AMOUNT OF FOOD ARE THE MAIN CAUSE OF METABOLIC DISTURBANCES IN RATS

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Abstract: Obesity has been identified as a major global health problem. A major cause of the obesity is the changes in feeding behaviour. Many controversy data concerning the role of endocannabinoid system in regulation /or disturbing of the metabolic parameters. The aim of this research is to identify the effect of methanandamide (as a one of CB1 selective agonist) on some metabolic parameters in rats fed by different types of food to clarify which is the cause of metabolic abnormality in obese?. Design: A total number of 56 healthy adult male albino rats were used to study the effect of different types of diet and daily i.p injection methanandamide (CB1 agonist) in a dose of 0.5 mg/kg BW for 6 weeks on some metabolic parameters using pair feeding paradigm. Results: a significant increase in final body weights and a significant dyslipidemia and hyperglycemia with insulin resistance was in both HFD and HFrD fed groups when compared with that of standard chow diet fed group. Moreover, a significant dyslipidemia and hyperglycemia with insulin resistance was observed in methanandamide treated ad libitum group. In addition, our study revealed an insignificant change in all parameters measured between HFD and HFrD fed groups except for TG and VLDL parameters which are significantly higher in HFrD-fed group in comparison with that of HFD fed group. Interestingly, an insignificant change in serum levels of all previously mention parameters in the three different methanandamide treated pair fed groups in comparison with that of the three different fed control groups respectively. Conclusion: we can conclude that endocannabinoid system is not the main responsible for metabolic disturbance in obese rats.
[Kariman E. Slim, Mostafa H. AbdSalam, Abeer A. A. Khalefa and Eman R. H. Abozid. IT IS NOT ENDOCANNABINOIDS BUT THE TYPE AND AMOUNT OF FOOD ARE THE MAIN CAUSE OF METABOLIC DISTURBANCES IN RATS. Journal of American Science 2011;7(3):748-757]. (ISSN: 1545-1003). http://www.americanscience.org.

Key Words: high fat, high fructose, endocannabinoid, insulin resistance, dyslipidemia.

1. Introduction:

The history of marijuana and its medicinal use go back thousands of years, but the endogenous counterparts of cannabis, the endocannabinoid (ECs) have only been known about 18 years ago, their discovery have been triggered by the identification of specific cannabinoid receptors in the brain[1].

Two subtypes of cannabinoid (CB) receptors have been identified to date. Both CB_1 and CB_2 receptors couple to the Gi/o subtypes of G proteins, but can also activate additional, G protein-independent pathways[2].

It is common knowledge that marijuana use improves appetite, presaging the role of ECs as endogenous orexigenic factors. However, findings as early as the 1970's suggested that ^{9}-tetrahydrocannabinol (^{9}THC), the psychoactive ingredient of marijuana, has additional metabolic effects unrelated to appetite[3].

CB_1 receptors are present in skeletal myocytes and are upregulated in obesity[4] and may be one of the targets for cannabinoid-induced insulin resistance. ECs may also influence insulin secretion in the endocrine pancreas, although there are conflicting reports on CB_1 receptors mediating a decrease[5] or an increase in insulin release[6], so ECs may share in the regulation of glucose homeostasis and insulin sensitivity. Metabolic studies have confirmed that calories obtained from fat have a greater role in obesity and have been considered as dietary risk factors for metabolic syndrome (MS)[7,8], moreover, fructose as a common supplement of the packed food has been implicated as a possible cause of the MS[9].

CB_1 receptor inhibition by AM251 is capable of countering insulin resistance in adipose tissue, muscle and liver[10,11]. However, similar beneficial effects on insulin sensitivity were also observed in pair fed animals[12]. The lack of significant improvement in metabolic status in AM251 treated, in comparison to pair fed animals does not mirror this finding. Thus, further studies are necessary to confirm these initial observations. Interestingly, AM251 also increased locomotor activity in agouti mice[13].

Recently, **Sink et al.** [14] reported that all clinically available cannabinoid receptor antagonists (e.g. AM 251 and rimonabant " SR141716A") are inverse agonists that can target CB_1 receptors located in both central circuits regulating appetite and motivation and in peripheral organs regulating

metabolism and energy expenditure. This profile (inverse agonism) complicates understanding of cannabinoid CB_1 receptor blockade as a therapeutic strategy in obesity and metabolic disorders.

Unlike rimonabant, chronic adminstration of LH-21 (selective CB_1 antagonist only) reduces feeding but does not improve hypertriglyceridaemia or hypercholesterolaemia; nor does it reduce liver fat deposits in Zucker rats. These data explain why AM251 increased locomotor activity in agouti mice at low doses[13].

It is still uncertain whether an overactive ECS is an early cause[15] or just one of the several consequences, of HFD and the subsequent development of overweight and obesity.

The present study was done to identify the effect of methanandamide (as a one of CB1 selective agonist) on some energy metabolic parameters concerning glucose homeostasis and lipid in rats fed by different types of food (commercial, high fat and high fructose diet) using pair feeding paradigm in a trial to clarify which is the cause of metabolic abnormality in obese, it is ECS alone, type of food, amount of food intake or crosstalk between them?.

Materials And Methods
Animals
This study was carried out on a total number of 56 adult (4 months; body weight, 180-200 gm) healthy male albino rats. Under hygienic conditions, in the animal house of the faculty of medicine Zagazig University, all rats had free access to water and chow, supplied in separate clean containers. Rats were kept at comfortable temperature (20 to 24 °C) and were maintained on a 12 hr light/dark cycle[16].

Diet
Normal (standard) diet: consists of commercial rat standard chow [it was consisted of 25.8 % protein, 62.8 % carbohydrate and 11.4 % fat[17].

High fat diet (HFD): was consisted of 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[17,18].

High fructose diet (HFrD): commercial rat laboratory chow containing 60% fructose[19].

Methods
The rats were accommodated to the new laboratory conditions for three weeks before the beginning of the experimental regimen[20].
Grouping of the animals
Group I "normal fed group": to study the effect of 6 weeks normal diet. It is consists of 24 rats which further subdivided into 3 equal subgroups (n= 8):

Group IA: vehicle (saline) treated group with access to ad libitum standard chow.
Group IB: Methanandamide (dissolved in sterile saline), treated (0.5 mg/kg BW i.p, daily)[21,22], pair fed group. Rats were given a weighed amount of standard chow each day corresponding to the amount consumed by vehicle treated rats on the previous day[12].
Group IC: Methanandamide treated (0.5 mg/kg BW i.p, daily), ad libitum group, rats had access to ad libitum standard chow.
Group II "HFD fed group: to study the effect of 6 weeks HFD diet. It consists of 16 rats which further subdivided into 2 equal subgroups (n= 8):
Group IIA: vehicle treated group with Access to ad libitum HFD.
Group IIB: Methanandamide treated (0.5 mg/kg BW i.p daily), HFD pair fed group.
Group III " HFrD fed group: to study the effect of 6 weeks HFrD diet. It consists of 16 rats which further subdivided into 2 equal subgroups (n= 8):
Group IIIA: vehicle treated group with Access to ad libitum High fructose diet.
GroupIII B: Methanandamide treated (0.5 mg/kg BW i.p, daily), HFrD pair fed group.

For all groups, body weight was recorded at the beginning and the end of the study period (6 weeks).

Sampling of blood
At the end of the experimental period (at the end of 6th week) after overnight fasting, at 8:00 a.m, blood samples were obtained from sinus orbitus vein of each rat after ether inhalation[23]. The blood samples were allowed to clot at room temperature before centrifuging at approximately 3000 rpm for 15 minutes. The serum was stored at -20° C.

Serum analysis
Determination of serum glucose level: According to **Trinder**[24] using glucose enzymatic **(GOD-PAP)-** liquizyme Kits (Biotechnology, Egypt).

Determination of serum insulin level: By a solid phase enzyme amplified sensitivity immunoassay according to **Starr et al.** [25] using KAP1251-INS-EASIA (Enzyme Amplified Sensitivity Immunoassay) Kits (BioSource Europe S.A., Belgium).

Determination of the Serum total cholesterol (TC): by enzymatic colorimetric method according to **Allain**[26] using Cholesterol RTU 61218 kits: (bioMerieux S.A., Lyon, France).

Determination of the Serum high density lipoprotein cholesterol (HDL): by enzymatic colorimetric method according to **Warnick et al.[27]**, using Stanbio HDL-cholesterol procedure No. 0599 kits (Stanbio laboratory Inc., San Antonio, Texas, USA).

Determination of the Serum Triglyceride levels: It was carried out according to **Naito[28]** using triglycerides ESPAS SL kits (Elttech S.A., Sees, France).

Calculation of very low density lipoprotein cholesterol (VLDL) and Low density lipoprotein cholesterol: According to **Friedewald et al. [29]**.
HOMA-IR was assessed by homeostasis model assessment (where HOMA= fasting serum insulin (µIU/mL) x [fasting serum glucose (mmol/L)/22.5][30]**.** The data obtained in the present study were expressed as mean ± SE for quantitative variables and statistically analyzed by using SPSS program (version 18 for windows) (SPSS Inc. Chicago, IL, USA). P value <0.05 was considered statistically significant.

3. Results:

Table 1&2 and histograms 1, 2, 3, 4, 5, 6, 7, 8 & 9 show the final body weight (gm), serum glucose (mg/dL), insulin (µIU/mL), the HOMA index of insulin resistance (HOMA-IR), total cholesterol (CHO) (mg/dl), HDL-C(mg/dl), triglyceride (TG) (mg/dl), VLDL (mg/dl) and LDL-C(mg/dl) levels in all studied groups. In group IB "Methanandamide treated normal diet pair fed group" the mean values

were found to be non significant (P > 0.05) when compared with that of group 1A"normal diet control group", however group IC "Methanandamide treated normal diet ad libitum" were found to be significantly higher than that of both group 1A and group IB (P < 0.01, P < 0.001, P < 0.001, P < 0.001, P < 0.001, P <0.001, P < 0.001, P < 0.001 & P < 0.001 respectively) except HDL-C levels was significantly lower. In addition, the mean values of group IIB "Methanandamide treated HFD pair fed group" and III B "Methanandamide treated HFrD pair fed group" were found to be non significant (P > 0.05) when compared with that of group IIA "HFD control group" and IIIA "HFrD control group".

It was found that both of group IIA "HFD" and group IIIA "HFrD" showed a significant increase in final body weights, serum glucose, insulin, the HOMA-IR, CHO, TG, VLDL-C LDL-C levels (P <0.001) and HDL-CHO (P < 0.01 & P < 0.05)when compared with that of group IA normal diet group". While, group IIIA "HFrD" showed an insignificant change (P > 0.05) in final body weights, serum glucose, insulin, the HOMA-IR, CHO, and LDL-C levels, however, it had significant increase in TG and VLDL (mg/dl) (P < 0.001) when compared with that of group IIA"HFD".

In addition, there were significant positive correlations between final body weights and serum glucose, serum CHO, serum TG, serum VLDL-C, serum VLDL- C, LDL-C levels and calculated HOMA-IR, accompanied by significant negative correlation between serum HDL-C levels and final body weights in all studied groups.

Table 1: Final body weights, serum glucose, insulin, the HOMA-IR, levels in all studied groups.

parameter	N= 8	Normal Diet			HFD		H Fr. D	
		Group IA	Group IB	Group IC	Group IIA	Group IIB	Group IIIA	Group IIIB
Final body weight (gm)	X̄ ± SE	241.25± 2.13	243.63± 2.93	255.25 ± 3.47	266.86 ± 3.47	267.25 ± 3.06	264.50± 2.49	264.87 ± 2.81
	P	NS		<0.01 *$	NS		NS	
Glucose (gm/dl)	X̄ ± SE	89.63 ± 6.0	93.13 ± 7.75	219.63± 3.64	272.0± 6.67	273.5 ± 5.5	281.75± 4.41	280.75 ± 4.89
	P	NS		< 0.001 *$	NS		NS	
	r P	+.752 < 0.05	+.795 < 0.05	+ .771 < 0.05	+ .890 < 0.01	+ .948 < 0.01	+ .866 < 0.01	+.881 < 0.01
Insulin (IU/mL)	X̄ ± SE	21.04±0.93	21.05± 0.69	36.44± 1.78	40.25± 1.61	39.17± 1.21	38.68± 1.18	39.76± 1.22
	P	NS		< 0.001 *$	NS		NS	
	r P	+ 0.749 < 0.05	+ 0.804 < 0.05	+ 0.920 < 0.01	+ 0.932 < 0.01	+ 0.977 < 0.01	+ 0.775 < 0.05	+ 0.932 < 0.01
HOMA index	X̄ ± SE	4.69 ± 0.44	4.88± 0.49	20.12±1.26	27.57± 1.71	26.93± 1.34	27.35± 1.22	28.00± 1.19
	P	NS		< 0.001 *$	NS		NS	
	r P	+ 0.901 < 0.01	+ 0.921 < 0.01	+ 0.920 < 0.01	+ 0.937 < 0.01	+ 0.981 < 0.01	+ 0.832 < 0.05	+ 0.848 < 0.01

* VS group IA $ VS group IB

Table 2: Serum CHO, TG, VLDL-C LDL-C levels in all studied groups.

Parameter	N= 8	Normal Diet			HFD		H Fr. D	
		Group IA	Group IB	Group IC	Group IIA	Group IIB	Group IIIA	Group IIIB
CHO	X̄ ± SE	106.0± 2.66	101.50± 2.24	133.88± 5.30	186.75± 3.87	190.63± 3.87	189.38± 4.17	191.88± 2.68
	P	NS		< 0.001*$	NS		NS	
	r P	+ 0.839 < 0.01	+ 0.828 < 0.05	+ 902 < 0.01	+ 0.724 < 0.05	+ 0.793 P < 0.05	+ 935 < 0.01	+ 0.925 < 0.01
HDL-C (mg/dl)	X̄ ± SE	51.0± 1.65	47.75± 3.08	38.63± 1.55	38.25±1.75	37.75± 1.33	39.63± 1.05	38.13± 1.74
	P	NS		< 0.001*$	NS		NS	
	r P	- 0.800 < 0.05	- 0.734 < 0.05	- 0.955 < 0.01	- 0.958 P < 0.01	- 0.886 < 0.01	- 0.865 < 0.01	- 0.877 < 0.01
TG (mg/dl)	X̄ ± SE	56.86± 1.8	57.75±2.31	91.37± 4.85	125.63± 2.90	130.13± 2.49	150.75± 4.19	161.5±3.91
	P	NS		< 0.001*$	NS		NS	
	r P	+ 0.716 < 0.05	+ 0.834 < 0.05	+ 0.968 < 0.01	+ 0.737 < 0.05	+ 779 < 0.05	+ 0.831 < 0.05	+ 0.970 < 0.01
VLDL-C (mg/dl)	X̄ ± SE	11.38±0.36	11.55±0.46	18.28±0.97	25.13±0.58	26.0±0.50	30.15±0.84	32.30±0.78
	P	NS		< 0.001*$	NS		NS	
	r P	+ 0.716 < 0.05	+ 0.834 < 0.05	+ 0. 968 < 0.01	+ 0.737 < 0.05	+ 779 < 0.05	+ 0.831 < 0.05	+ 0.970 < 0.01
LDL-CHO (mg/dl)	X̄ ± SE	43.63±4.04	42.20±4.11	76.98±5.75	122.25±5.94	126.85±4.54	119.60±4.41	121.45±3.53
	P	NS		< 0.001*$	NS		NS	
	r P	+ 0.813 < 0.05	+ 0.908 < 0.01	+ 0.925 < 0.01	+ 0.822 < 0.05	+ 0.853 < 0.01	+ 0.933 < 0.01	+ 0.919 < 0.01

* VS group IA $ VS group IB

Histogram 1 : The final body weights (gm) in all studied groups.
* VS group IA $ VS group IB

Histogram 2: illustrates serum glucose levels (mg/dl) in all studied groups.
* VS group IA $ VS group IB

Histogram 3: illustrates serum insulin levels µIU/ml in all studied groups.
* VS group IA $ VS group IB

Histogram 4: illustrates HOMA index of insulin resistence in all studied groups.
* VS group IA $ VS group IB

Histogram 5: illustrates total serum cholesterol levels mg/dl in all studied groups.
* VS group IA $ VS group IB

Histogram 6: illustrates serum HDL-C levels (mg/dl) in all studied groups.
* VS group IA $ VS group IB

Histogram 7: illustrates serum TG levels (mg/dl) in all studied groups.
* VS group IA $ VS group IB

Histogram 8: illustrates serum VLDL levels (mg/dl) in all studied groups.
* VS group IA $ VS group IB

Histogram 9: illustrates serum LDL-C levels (mg/dl) in all studied groups.
* VS group IA $ VS group IB

4. Discussion:

In mammals, body weight and composition are maintained within a narrow range by the integrated control of energy intake, storage and expenditure. The endocannabinoid system (ECs) which consists of cannabinoid receptors, the endogenous lipid ligands (endocannabinoids), and the machinery for their biosynthesis and metabolism share in this control[31].

To explore the exact role of diet and ECs in metabolic disturbance, the present study used the 6 weeks pair-feeding paradigm to ascertain if the metabolic effect of ECs were related to type and/or amount of food intake or not.

As regard HFD ad libitum group, It showed a significant increase in final body weight with preservation of its correlation with all metabolic parameters, and a significant disturbance in glucose metabolism, distinguished by significant increase in fasting serum glucose levels, fasting serum insulin

levels, and HOMA index of insulin resistance. Ad libitum HFD fed group also showed dyslipidemia proved by significant increase in serum total cholesterol, TG, VLDL-cholesterol levels, and LDL-cholesterol levels, accompanied by a significant decrease in HDL-cholesterol levels when compared with that of ad libitum normal diet group.

These data are in consistent with **Alsaif and Duwaihy**[32] who found that in general, HFD significantly increased weight gain, impaired glucose tolerance and increased insulin resistance.

Buettner et al.[33] stated that long term HFD feeding often impairs lipid metabolism by reducing insulin sensitivity of peripheral tissue.

HFD decrease insulin-stimulated glucose disposal in skeletal muscle and increase TG, long-chain acyl-CoA and diacylglycerol contents[34]. In addition, Glut 4 mRNA in WAT is down-regulated by HFD[35].

The HFD induced glucose intolerance and dyslipidemia which proved in the present study can be explained by disturbance of some metabolic hormones such as insulin resistance which has been proved in our results. In addition, **Naitoh et al.[36]** confirmed that mRNA expression level of adiponectin in WAT and plasma adiponectin levels were found to be decreased in HFD-fed mice compared with commercial diet-fed mice. This hypoadiponectinemia was closely linked to insulin resistance and low HDL cholesterol[37], as adiponectin enhance lipid clearance from plasma and increasing fatty acid beta oxidation in muscle[38].

It was found that, diet induced obesity in humans and rodents have very high amounts of circulating leptin, this hyperleptinemia is associated with leptin resistance which neither reduces appetite nor increases energy expenditure[39].

Apelin has been reported as a beneficial adipokine up-regulated in obesity as an attempt to overcome either insulin resistance or obesity-related cardiovascular diseases[40], elevated plasma apelin has been estemated in moderately[41] and in severe obese[42]. It has also been shown that plasma apelin levels were increased in diabetic subjects and positively correlated with BMI, HOMA-IR and fasting plasma insulin[43], suggesting a role of apelin in the pathogenesis of type II diabetes induces by overweight[44].

Watanabe et al.[45] stated that disturbed ECs levels that accompanied HFD are related to onset, duration and its fatty acid composition.

Cannabinoids promote lipogenesis and the storage of adipose tissue via CB_1, and the expression of CB_1 in adipose tissue is up-regulated in rodent models of obesity[15,46].

On the other hand, antagonists of CB_1 promote lipolysis and fatty acid oxidation and increase in insulin sensitivity[47,48].

As regard the comparison between the HFrD and the normal diet fed groups, HFrD fed group showed a significant increase in final body weight with preservation of its correlation with all metabolic parameters and a significant increase in fasting serum glucose levels, fasting serum insulin levels, and HOMA-IR, HFrD fed group also showed dyslipidemia proved by significant increase serum total cholesterol, serum triglyceride, VLDL-cholesterol levels, and LDL-cholesterol, accompanied by a significant decrease in HDL-cholesterol.

These data are in line with studies on rodents that stated that HFrD increases intra hepatocellular lipid and stimulates hepatic de novo lipogenesis within a few days and induce hyperlactatemia and hypertriglyceridemia[49].

Studies of pure fructose fed to laboratory animals show increased plasma free fatty acids, and abdominal adipose tissue, as well as impaired insulin sensitivity[50].

Fructose consumption by adult rats has been shown to produce diminished glucose tolerance and insulin sensitivity as well as elevated TG, cholesterol, and body fat[51]. The insulin resistance in this animal model probably results from the impairment of insulin-stimulated glucose uptake in insulin-responsive tissues as well as changes in hepatic glucose metabolism[52] Insulin resistance may also contributed to this hypertriglyceridemia by reducing the inhibitory effect of insulin on TG secretion rate in the liver of fructose fed rats[53].

The increase in TG level may be due to stimulation of hepatic VLDL-triacylglycerol synthesis and secretion and decreased VLDL-triacylglycerol clearance[54]. **Stanhope et al.[55]** indicate an increase in weight gain on diets rich in fructose, and a correlation between body fat and circulating TG has been established. In addition, HFrD induce leptin and insulin resistance. Taken together, leptin or insulin resistance and elevated TG serum levels may cause food over-consumption and contribute to the corresponding obesity, moreover, ingesting fructose-sweetened food raises calorie intake resulting in an over-consumption of energy which is not balanced by energy output, leading to weight gain[55].

As regard the comparison between HFD and HFrD fed groups, both groups showed dyslipidemia and glucose intolerance, however there were no significant differences between both groups in final body weight, fasting serum levels of glucose, insulin, total cholesterol, HDL-cholesterol, and LDL-cholesterol and HOMA-IR. While a significant increase in serum triglyceride, VLDL-cholesterol in HFrD fed group when compared with HFD fed group. These data are in consistent with that of **Liu and Manson[57]** who proved that diet high in carbohydrates is associated with glucose intolerance and obesity.

One of the main differences between glucose and fructose metabolism is that glucose must advance through a negatively regulated step using phosphofructokinase which regulates glycolysis in the liver. Fructose can bypasses this regulatory step and continue to be metabolized in the liver into glycerol-3-phosphate and acetyl coenzyme, these latter metabolites serve as substrates for glyceride synthesis leading to increased formation of VLDL and TG in the liver[58]. In contrast to glucose, when large amounts of fructose are ingested, the glycolytic pathway becomes saturated, and TG production is facilitated[59].

The present results showed an insignificant disturbance in all metabolic parameters measured in methanandamide treated normal diet pair fed group and methanandamide treated HFD pair fed group and methanandamide treated HFrD pair fed group when compared with that of normal diet fed, HFD fed, and HFrD fed control groups respectively. However, methanandamide treated normal diet; ad libitum showed a significant disregulation of glucose metabolism "significant hyperglycemia, hyperinsulinemia, and increase HOMA-IR and significant dyslipdemia when compared with that of both ad libitum normal diet and methanandamide treated normal diet pair fed groups.

Taken together, this results proved that the ECS affects the metabolism by increasing the substrates that are needed for the lipogenesis, through increasing food intake and this concept is in line with **Williams and Kirkham**[21], as they proved that AEA injections in rats activated CB1 and promoted overeating, similar results were reported in rats injected with 2-AG[60]. ECS is considered to be primarily involved in the regulation of food intake via effects in the hypothalamus and nucleus accumbens[4]. CB_1 is selectively expressed in ventro-medial hypothalamus neurons. Absence of these neurons leads to weight gain, and their excitability is decreased in the presence of CB_1 agonists and increased by leptin[61].

ECs appear to interact with several other anorexigenic and orexigenic factors, clearly implicating the ECS in appetite regulation in a central control mechanism[2]. Cannabinoid and leptin signals are integrated in lateral hypothalamic neurons[62]. **Di Marzo et al.**[63] demonstrated that injecting mice with leptin, an anorexigenic adipokine that acts on the hypothalamus, resulted in a significant decrease in both AEA and 2-AG in the hypothalamus. They also showed that defective leptin signaling in the hypothalamus of obese db/db, ob/ob mice and Zucker rats was associated with an increase in endocannabinoid content.

Some studies stated that CB_1 antagonism has shown an improvement in insulin resistance and plasma glucose parameters, and a decrease in insulin and free fatty acid levels[12,64].

The absence of significant disturbance in methanandamide treated pair fed groups in comparison to other ad libitum control groups is in line with **Irwin et al.**[12] who suggested that the effect of ECS on disturbing the metabolism is due to increase food intake, depending in their study on the anorectic effect of the CB_1 blocker (AM 251) they observed that subchronic AM251 treatment in ob/ob mice caused weight reduction, improved the impaired metabolism and decrease insulin resistance, but these observations were found also in pair-fed control animals suggesting that the beneficial actions of CB_1 receptor antagonism is due to reduced food intake.

Specific central CB_1 blockade decreased body weight and food intake in diet induced obese rats, but had no beneficial effects on glucose metabolism; on the other hand, peripheral CB_1 blockade also reduced food intake and body weight but, in addition, enhanced insulin sensitivity. However, this improvement in insulin sensitivity was also detected in vehicle pair-fed rats, which suggest that decreased energy intake was the major factor responsible for these effects[65].

Finally, collecting data from previous researches concerned with ECS and ECs benefits revealed that ECs have been used therapeutically for alleviating pain[66], And induces neuroprotection in ischemic brain areas[67]. Moreover, endocannabinoid signaling was proposed to protect against the consequences of stress in a certain dose range as low doses of methanandamide was proved to induce anxiolytic effects which are CB_1 receptor-mediated. In addition, CB_1 receptor activation at amygdala promotes fear extinction[68]. Cannabinoids are also effective in treatment of models of nausea and vomiting[69], gastric ulcers, irritable bowel syndrome, ulcerative colitis, Crohn's disease, secretory diarrhea, paralytic ileus and gastro-esophageal reflux disease[70].

In fact, many side effects related to the use of CB_1 receptor antagonists were proved as impairs fear extinction, increase anxiety-related behaviours in the elevated plus maze. In addition, both pharmacological antagonism and genetic inactivation of CB_1 receptors impair extinction of conditioned fear memories[71]. Moreover, acute injections of rimonabant or AM251 (CB1 antagonists) also increase both basal and stress-induced serum corticosterone levels[72]. Furthermore, mice lacking CB_1 receptors are impaired in actively coping with stress in a model predictive for antidepressant-like activity[73]. Thus, CB_1 receptor antagonism may induce psychiatric side-effects, in humans, mainly anxiety- and depression-like states, being in accordance with the notion that the endocannabinoid system acts to keep a set point counteracting aversive emotions[71]. Obese patients already suffer from anxiety or depression more frequently than non-obese subjects[74].

Taken together with results of the present study, it can be concluded that ECS should not be palmed for metabolic disturbances but the type and amount of diet is the main cause and its receptors antagonists must not be the main target for treatment obesity.

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Evaluation of Correction Factors Applied in Photon Calibration of NIS TE Neutron Ionization Chambers

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Abstract: Calibrations of two tissue equivalent (TE) ionization chamber were made in five photon beams (100 kV, 180 kV, 250 kV, ^{137}Cs and ^{60}Co) with two different pure gases namely acetylene $C_2 H_2$ and carbon dioxide CO_2. The different calibration factors were compared both for in-air and in-water phantom, the measurements were performed according to the international atomic energy agency (IAEA) recommendations. For ionization chamber the total absorbed dose can be derived from the charge produced within its cavity employing a number of physical parameters. To discuss the charge produced in the cavity several correction factors which are related to the operational characteristics of the chambers have to be introduced. Information on the operational characteristics of two TE neutron ionization chambers were studied as a function of the effects of the warm-up to 3 hours, polarity, stem scattering, ion recombination, leakage current. Six different caps 1, 2, 3, 4, 6 and 8 mm were used to investigate wall thickness effect. Also, gas flow rate up to 31 ml/min and the radial & axial uniformity were investigated.

1. Introduction:

The increase in the number of centers throughout the world which are using fast neutrons for radiotherapy has led to a need for accurate neutron dosimetry methods which will give comparable results in each of these centers. [Williams and Greening, 1980, Ruedi Risler and Alina Popescu 2010].

The use of calibrated tissue-equivalent (TE) ionization chambers is commonly considered to be the most practical method for total absorbed dose determinations in mixed neutron-photon fields for biomedical applications. [Zoetelief and Broerse, 1983]. Most ionization chambers are usually not employed as absolute instruments due to uncertainties in determining effective cavity volume, and hence the mass of gas therein, and due to uncertainties in the absolute value of *W*, the average energy required to produce an ion pair in the gas or gas mixture [Pszona, S. 2010].

The use of calibrated A-150 plastic TE ionization chamber with TE gas filling is recommended as the practical method of obtaining the tissue kerma in air and the absorbed dose in a TE phantom. This recommendation is based on the fact that TE chambers have been used as the principal dose measuring instrument by the neutron therapy groups in Europe, USA and Japan which are regularly treating patients. IAEA, (1984) and generally accepted as probably the most accurate method for measuring absorbed dose and kerma in most practical situations [Lindborg and Nikjoo 2011].

Although the hydrogen and nitrogen components in the chamber materials can be made to simulate that in tissue, for certain determinations, such as that of kerma in free air, it may be necessary to take into account the disturbance of the neutron fluence produced by the chamber itself. [Podgorsak, 2005].

According to Bragg-Gray principle, chamber homogeneity is achieved for neutron dosimetry using wall, gas, and insulator materials that have the same energy transfer coefficient for the primary radiation and the same stopping power for the secondary particles. Since the principal concern of this work is measurement of neutron absorbed dose in tissue, the ideal material for a homogeneous chamber is one which has an atomic composition similar to that of tissue. Such materials may be called tissue substitutes. [ICRU, 1989, Ferreira et al., 2010].

The absorbed dose, *Dg*, in the gas cavity of an ionization chamber is given by:

$$D_g = Q \frac{W}{e} \frac{1}{m} \qquad (1)$$

Where Q is the total charge produced within the cavity, W is the average energy required producing an ion pair in the gas, e is the charge of the electron and m is the mass of gas within the cavity.

In the SI-system D_g is expressed in Gy, Q and e in C, W in J and m in kg. Absorbed dose in the wall material adjacent to the cavity of the chamber, D_{m*}, can be calculated from the energy absorbed by the gas using the gas-to-wall absorbed-dose conversion factor, $r_{m,g}$, similar to r introduced by Bichsel and Rubach (1978).

$$D_{m^*} = r_{m,g} D_g \qquad (2)$$

For a cavity whose size is not negligible in relation to the range of the secondary charged particles generated in the wall, it is necessary to make more detailed calculations for the values of r as a function of cavity-size and neutron energy.

If the chamber wall is replaced by reference tissue, the absorbed dose in the tissue adjacent to the cavity of the chamber, D_{t*}, is calculated from D_{m*} using the ratio of mass energy absorption coefficients, $(\mu_{en}/\rho)_t/ (\mu_{en}/\rho)_m$, in the tissue and wall material, assuming that there is charged particle equilibrium [Juan G. Miranda et al., 2004]:

$$D_{t^*} = \frac{(\mu_{en}/\rho)_t}{(\mu_{en}/\rho)_m} D_{m^*} \qquad (3)$$

i.e. $\qquad D_{t^*} = \frac{Q}{m}\frac{W}{e} r_{m,g} \frac{(\mu_{en}/\rho)_t}{(\mu_{en}/\rho)_m} \qquad (4)$

For measurements with an ionization chamber the reading obtained from the chamber, R, has to be related to the charge produced within the cavity at a reference temperature and pressure by the product of several correction factors, Πk_R

$$Q = R \Pi k_R \qquad (5)$$

The factors contained in Πk_R include the electrometer calibration factor and correction factors for ion recombination, temperature and pressure, gas flow rate and leakage current. To apply equation (4) the mass of gas in the cavity has to be known. This can be obtained from the calibration factor of the tissue-equivalent chamber with photons ac which is defined as:

$$a_c = \frac{(D_{t^*})_c}{Q_c} \qquad (6)$$

where subscript c refers to the photon calibration beam. If this is substituted into equation (4) we obtain:

$$m = \frac{1}{a_c}\frac{W_c}{e}(s_{m,g})_c \left[\frac{(\mu_{en}/\rho)_t}{(\mu_{en}/\rho)_m}\right]_c \qquad (7)$$

In this equation the gas-to-wall absorbed dose conversion factor, $r_{m,g}$ has been replaced by $(s_{m,g})_c$ since the chambers used for clinical neutron dosimetry are usually small enough to satisfy the conditions for the Bragg-Gray theory at the photon calibration energies normally used.

Absorbed dose in TE plastic adjacent to the cavity of the TE chamber can be calculated [Mijnheer and Williams, 1981] from the measured exposure, X_C:

$$(D_{TE^*})_C = X_C \frac{(W_{air})_C}{e}\left[\frac{(\mu_{en}/\rho)_{TE}}{(\mu_{en}/\rho)_{air}}\right]_c (\Pi k_A)_C \qquad (8)$$

The correction factor $(\Pi k_A)_C$ accounts for the attenuation and scattering by the wall, central electrode and build-up cap of the chamber and also for the radiation scattered by the stem of the chamber into the sensitive part of the chamber. In addition, corrections should be made if there is any radial or axial non-uniformity in the field that is in the plane perpendicular to the central axis of the beam or along the central axis. The calibration factor with photons can now be written as:

$$a_C = \frac{X_C(f_t)_C(\Pi k_A)_C}{R_C(\Pi k_R)_C} = N_C(f_t)_C(\Pi k_A)_C \qquad (9)$$

It should be noted that the product $X_C (f_t)_C (\Pi k_A)_C$ does not represent the absorbed dose in tissue in the absence of the chamber but it represents the absorbed dose in tissue adjacent to the cavity of the chamber. N_C and N_k, are the exposure and air kerma calibration factors, defined as [Wojciech Bulski et al., 2008]:

$$N_C = \frac{X_C}{R_C(\Pi k_R)_C} \qquad (10)$$

$$N_K = N_C\left(\frac{W_{air}}{e}\right)(1 - g) \qquad (11)$$

Where, g is the fraction of energy of secondary charged particles that is converted to Bremsstrahlung in air. The calculation of this fraction for electrons produced by ^{60}Co gamma rays in the graphite wall of an ionization chamber amounts to 0.003 [Boutillon and Perroche, 1985 and Kessler et al., 2010].

The aim of the work is to study the optimizing parameters affecting the sensitivity and stability of two tissue equivalent neutron ionization chambers types 33051 and 33053 in different types of γ- beams, for use as neutron secondary standard dosimeters in National Institute of Standards (NIS)- Egypt.

Also, to determine the correction factors required to eliminate perfectly the γ-component in neutron-gamma mixed fields. Finally, Comparing the obtained calibration factors with most ionization chambers commonly used internationally.

2. Experimental Work

Determination of the calibration factor with photons is usually made with an exposure standard chamber whose calibration is directly traceable to a national standards laboratory. The calibration should be made in air with the geometrical centre of the detectors being taken as the point of measurement [Oliver Ja¨kel 2009]. A build-up cap of the same material as the wall should be added if the wall thickness of the TE chamber wall is not sufficient to achieve charged-particle equilibrium. The chamber should be orientated so that its stem is perpendicular to the beam. [Broerse et al, 1981]

The measurements of air kerma and absorbed dose to water calibration factors for TE neutron ionization chambers are performed against the two NIS secondary standard dosimetry systems.

The calibration of TE neutron ionization chambers performed in two different reference gamma beams, ^{137}Cs, and ^{60}Co Gammatron therapy unit at NIS. The ^{137}Cs source used in this work type Gamma Beam-150B, manufactured by the Atomic Energy of Canada Limited. The present activity is 500 Ci, and dose rate is 1.235 Gy/h at 1 meter from the source center. The ^{60}Co therapeutic unit used in this work is Gammatron manufactured by Siemens, Germany. The present activity is 750 Ci, and dose rate about 5.94 Gy/h at one meter from the center of the source.

The X-ray machine used in this work is MCN-323 metal-ceramic Philips double pole x-ray tube. The MG325 Philips x-ray system is highly stabilized constant potential X-ray system. The H. V. and tube current adjustment range are from 15-320 kV and from 0 to 22.5 mA respectively. [Philips 1998].

The first NIS dosimetry system is Farmer electrometer type (NE-2570/1B) manufactured by Nuclear Enterprises Ltd. made in U.K. Farmer ionization chamber NE 2571 is a 0.6cc cylindrical manufactured by Nuclear Enterprise. This system used in this work in X-ray calibration.

The second dosimetry system used in calibration in both ^{60}Co and ^{137}Cs beams is the secondary standard NPL therapy level dosimetry system. The system is composed of an electrometer of type NE-2560. A 0.3 cm^3 ionization chamber type NE-2561. The system is made in U.K. manufactured by Nuclear Enterprises Ltd, Beenham. A laboratory timer of type NE-2546 with resolution 0.001 second is used for time measurements.

Two types of TE neutron ionization chambers TM33051 and TM33053, which are the thimble shape, are manufactured in Germany by PTW-FREIBURG. Figure (1) show a schematic diagram of two NIS TE neutron ionization chambers illustrating the internal construction and its dimension. For the evaluation of the attenuation in the chamber wall a set of different caps is applied. For chamber type 33051 the additional wall thickness are 1, 2, 3, 4, 6 and 8 mm A-150. Unidos electrometer type 10001 is manufactured by PTW-FREIBURG, Germany.

All chambers employed in neutron dosimetry are provided with gas inlet and outlet tubes. The gas system installation to provide the chamber with a steady, continuance and low gas flow rate consists of the items shown in Figure (2)

Two types of gases tissue substitutes were used, namely acetylene (C_2H_2) and carbon dioxide (CO_2). The two gases were supplied by El- Naser Company for Intermediate Chemicals Egypt. The C_2H_2 is a Technical Grade with purity 99.95 % supplied in specially designed steel cylinders with pressure 13 bar. The CO_2 is a Normal Grade with purity 99.995 % supplied in cylinder with high strength aluminum alloy with pressure 50 bar. The carbon percentage by mass in C_2H_2 and CO_2 are 92.3 and 27.3 respectively. The hydrogen percentage is 7.7 by mass in acetylene while the oxygen in CO_2 is 72.7 by mass. [ICRU, 1989]

The electrical air pump used to push air through neutron ionization chamber to refresh the cavity medium and study the difference between the static and air flow inside the cavity during measurements. The pump flow rate of air is in range from 30 to 1000 ml/min. It has two vents for air inlet & outlet and DC power supply 12 V, manufactured by Genitron Instruments GmbH, Heerstraße, Frankfort, Germany.

The IAEA standard dosimetric calibration phantom is Perspex cubic ($30\times30\times30$ cm^3) with Wall thickness 1.5 cm, water-filled container with open top and two entrance windows for horizontal beams. [ICRU, 1992].

Two types of calibrated thermometer were used during irradiation both in air and water. The first is high quality mercury-in-glass thermometers in the range from 19 to 35 ^{o}C a precision of 0.1 ^{o}C. The second is a digital thermometer measuring in the range from -10 to 80 °C with a precision of 0.1 ^{o}C designed and developed by TFA Germany.

A calibrated digital manometer was used for air pressure measurement; it covers range up to 60 in Hg or 2031.8 mbar. It is a Meriam Instrument, manufactured by a Scott Fetzer Company.

The correction factors, Πk_R for Temperature and Pressure($k_{t,p}$), Electrometer (k_e), Ion

Recombination (k_s), Current leakage (k_l), Polarity Effect (k_p), Gas Flow Rate Effect (k_f) and Humidity (k_h), which convert the reading, R, taken from the chamber, to the charge, Q, produced within an ideal cavity at reference conditions.

Πk_A contains all the correction factors that are valid during measurements in air as well as in the water phantom. Πk_A is a composite factor and equal to the product of the factors Wall and build-up cap (kw), stem effect (k_{st}), Radial non Uniformity (k_{rn}) and Axial non Uniformity (k_{an}). [Niatel et al, 1975]

3. Calibration Methods:
Calibration of TE Neutron Chamber in ^{137}Cs and ^{60}Co Beams :

In-air dosimetry was performed at the conditions where source to chamber distance (SCD) =100 cm with field size equal to 10×10 cm^2 at the position of the chamber. The center of the sensitive volume of the ion chamber was aligned by means of laser beam to the center of the radiation field [Yanxiao Huang et al., 2010]. The ion chamber was oriented with the chamber type and serial number inscribed on the stem facing the source and the build-up cap was used for in-air dosimetry in order to avoid measuring in the buildup region. The air kerma calibration factor to be recorded is calculated by:

$$N_K = K_{air} / M_{Cs} \qquad (12)$$

where K_{air} is the air kerma determined by the standard and M_{Cs} is the charge or reading from the chamber to be calibrated, corrected for the reference values of 20°C and 101.325 kPa of ambient temperature and air pressure [Arbabi et al., 2010].

In-water dosimetry was performed as the conditions recommended by IAEA [IAEA, 2000].The ion chamber axis was perpendicular to the central axis of the beam; the chamber was oriented so that the chamber type and serial number inscribed on the stem facing the source. In-water measurements were performed without the build-up cap because the ion chamber was inserted inside a waterproof sleeve.

The absorbed dose to water calibration factor $N_{D,w}$ for the chamber to be calibrated is calculated from the equation:

$$N_{Dw} = D_w / M_{Cs,f} \qquad (13)$$

Where D_w is the absorbed dose to water derived from measurements by the standard and $M_{Cs,f}$ is the charge or reading of the chamber to be calibrated, corrected for the reference conditions of temperature and air pressure [Zhe Chen et al., 2007].

X-Ray Calibration of TE Neutron Chamber & Farmer Dosimetry System

The quality assurance measurements for the X-ray generator system was carried out using x-ray test device Model 4000M+ manufactured by Victoreen. These quality assurance measurements were routinely performed for testing the applied kV on the tube. It was found that, the applied kV on the tube was constant during all measurements within the uncertainty limit according to manufacture specification of the X-ray generator.
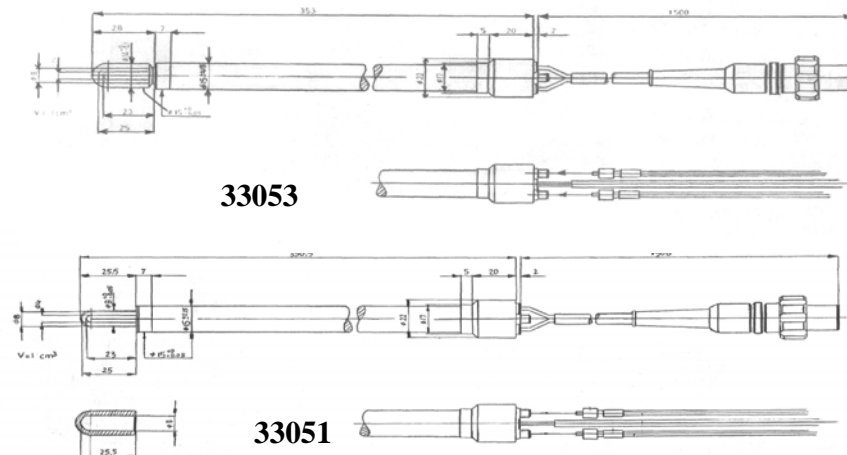
Figure (1): Schematic diagram of two NIS TE neutron ionization chambers
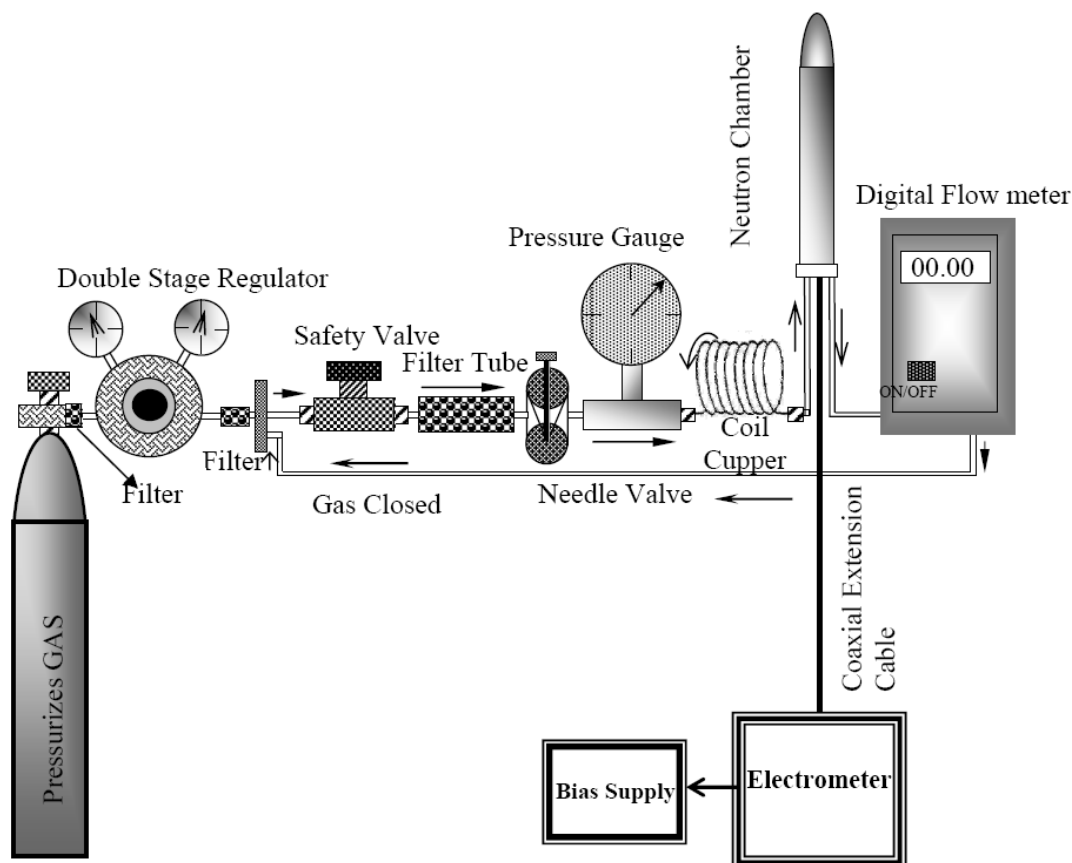
Figure (2): Shows a schematic diagram for developed gas system connected to tissue equivalent neutron ionization chamber.

4. Results and Discussion:
4.1 Warm-up

The warm-up time for each chamber was studied and about 30 min warm-up was found to be necessary to give stable and reproducible data. Warming up to 1 hour is desired to reduce the uncertainty values.

4.2 Polarity Correction Factor

Polarity correction factor was measured when the chambers were filled with air or flushed with CO_2 and C_2H_2 in cesium beam whereas it was measured when the chambers were filled with air only in cobalt beam. Polarity correction factor values for the two neutron chambers using different sources and gases are given in Table (I). It is clear from the table that the values of the polarity correction factor when the chambers were filled with air less than 1.0025, which are acceptable [IAEA, 1994]. However, when the chambers were flushed with CO_2 or C_2H_2, the polarity correction factor values in the range 1.0038 % to 1.00421 % for the two chambers used.

4.3 Stem Scattering Correction Factor

Stem scattering correction factor for the two neutron chambers are given in Table (II). The stem effect values of the two neutron chamber [33051 (Al stem) and 33053 (Acrylic stem)] in ^{60}Co beam are slightly higher than that in ^{137}Cs beam and with lower percentage standard deviation. This may be due to the different stem material of two chambers.

4.4 Ion Recombination Correction Factor

The calculated values of ion recombination correction factor are represented in Table (III). It is clear from the table that the two chambers show slight dependence of the ion recombination factor on the type of the filling gas (air, C_2H_2 and CO_2) and the energy of the gamma rays.

Table (I): The polarity correction factor for the two neutron ionization chambers in different gamma sources and its percentage standard deviation.

Source type	Chamber type	Polarity correction factor &Gas type			%σ		
		Air	CO_2	C_2H_2	Air	CO_2	C_2H_2
^{60}Co	33051	1.0011			0.007		
	33053	1.00177			0.043		
^{137}Cs	33051	1.0019	1.00384	1.0038	0.014	0.028	0.047
	33053	1.00244	1.00421	1.00409	0.010	0.028	0.037

Table (II): Stem scattering correction factor and its percentage standard deviation.

Source type	Chamber type	Stem scattering correction factor	%σ
^{60}Co	33051	0.9993	0.015
	33053	0.9991	0.008
^{137}Cs	33051	0.9982	0.050
	33053	0.9961	0.020

Table (III): Ion recombination correction factor in different gamma sources and its percentage standard deviation.

Source type	Chamber type	Ion recombination correction factor			%σ		
		Air	C_2H_2	CO_2	Air	C_2H_2	CO_2
^{60}Co	33051	1.004	1.005	1.022	0.01	0.006	0.001
	33053	1.001	1.002	1.004	0.02	0.011	0.004
^{137}Cs	33051	1.032	1.001	1.028	0.01	0.024	0.015
	33053	1.015	1.001	1.004	0.006	0.12	0.007

Table (IV): The leakage current correction factor (k_l) for two neutron ionization chambers filled with different gases in ^{60}Co and ^{137}Cs beams.

Source type	Chamber type	Leakage current correction factor		
		Air	C_2H_2	CO_2
^{60}Co	33051	1.0002		
	33053	1.0001		
^{137}Cs	33051	1.0005	1.0007	1.0004
	33053	1.0003	1.0003	1.0004

4.5 Leakage Current Correction Factor

Leakage current correction factor was measured when the chambers were filled with air or flushed with CO_2 and C_2H_2 in ^{137}Cs beam whereas it was measured when the chambers were filled with air only in ^{60}Co beam.

The leakage current is divided into two categories, pre-irradiation and post-irradiation leakage. The measured pre-irradiation leakage values of the ionization current produced by minimum air kerma are shown in Table (IV). The results indicate that the percentage pre-irradiation leakage current values below the maximum permissible range (up to 0.5%), IAEA, 1994. The post-irradiation current leakage value was nearly zero because the field size was 10×10 cm^2, that permits no full stem irradiation and all chambers used with guarded stems that permits no leakage.

4.6 Wall Thickness and Build up Cap Correction Factors

The neutron chamber 33051 was used to study the effect of wall thickness and build up cap because it is the only chamber which has the possibility of varying the cavity wall thicknesses with the same material of original wall cavity.

Figure (3a) shows the variation of the relative charge as a function of cavity wall thicknesses for ^{60}Co beam when the chamber was filled with air. It is clear from the figure that there is high dependence of the measured relative charge on cavity wall thickness till 3 mm which is equivalents to about 338 mg/cm^2 in unit mass density. At higher cavity wall thickness the dependence slightly decreased with linear negative slope as shown in Figure (63b). The extrapolation of the linear part of the attenuation curve to zero thickness was found to be at 1.111 ± 0.0002, which is considered as the correction factor k_w.

Figure (4) shows the variation of the relative charge as a function of cavity wall thicknesses for ^{137}Cs beam when the chamber was filled with air and CO_2 gas flow. The results indicate strong negative dependence of the relative charge on the cavity thicknesses till 2 mm (225 mg/cm^2) and 3 mm when the chamber was filled with air and CO_2 gas flow respectively. At higher cavity thickness the charge dependence on the thickness was lower. The extrapolation of the linear dependence of the relative charge on the cavity wall thicknesses to zero value, the values of kw were found to be at 1.004 ± 0.0011 and 1.009 ± 0.0020 for the chamber filled with air and CO_2 gas flow respectively, as shown in Figures (5a) and (5b).

The results showed that the increase of wall thickness increased the sensitivity of the chamber to gamma radiation till a wall thickness of 3mm. High thickness did not show any more increase of the ionic current. This result indicates that the ions are emitted from the walls of the chamber resulting from the interaction with the gamma rays. At thickness higher than 3 mm self absorption of the emitted ions from the walls occur which prevents no more ions of reaching the chamber cavity.

4.7 Gas System Calibration

Plotting a curve of gas system, representing the relation between the pressure in m bar inside the cavity of neutron chamber and the flow rate in ml/min. The result indicate linear dependence of gas flow on the pressure with correlation coefficient R = 0.9999 and the correction factor is 3.14 ± 0.26. The slope of the line is 3.12 ± 0.006.

4.8 Gas Flow-Rate Correction Factor

Figure (6a) shows the effect of gas flow rate on the charge of neutron chamber 33051 flushed with C_2H_2 gas flow, relative to the charge when the chamber was filled with atmospheric air for ^{241}Am-Be, ^{60}Co and ^{137}Cs after a preflush with 100 cm^3 C_2H_2.

It is clear from the figure that up to 1 ml/min, the increase in the sensitivity is proportional to the gas flow rate for all used radiation sources. In the region from 10 ml/min up to 12.5 ml/min slight dependence of the relative ionization chamber reading on gas flow rate. By increasing the gas flow rate up to about 13 ml/min the charge of the chamber remains rather constant. It can be seen that in the gas flow rate region from 13 ml/min to 31 ml/min the sensitivity of the ionization chamber can be increased by about 10 %, 8 % and 3 % for ^{241}Am-Be, ^{60}Co and ^{137}Cs respectively.

Figure (6b) shows the effect of gas flow rate on the relative charge (gas/airatoms) of neutron chamber 33051 flushed with CO_2 gas flow, for ^{241}Am-Be, ^{60}Co and ^{137}Cs. The region from 12.5 ml/min to 31 ml/min the relative charge of chamber was more stable with increasing the gas flow rate. From the figure, it is clear that C_2H_2 gas gave higher sensitivity to detect neutrons than CO_2 and air. This result shows the role of recoil protons in the C_2H_2 gas by fast neutrons. Moreover, the advantage of the use of C_2H_2 as filling gas in the neutron chambers is that it decreases the sensitivity of the chamber to detect gamma rays and increase its sensitivity for neutron detection, which is considered as one of the main objective of the present work.

Figure (7) shows the relation between relative charge (gas/airatoms) and the gas flow rate for neutron chamber 33051 in ^{241}Am-Be neutron beam when the chamber was flushed with C_2H_2, CO_2 and
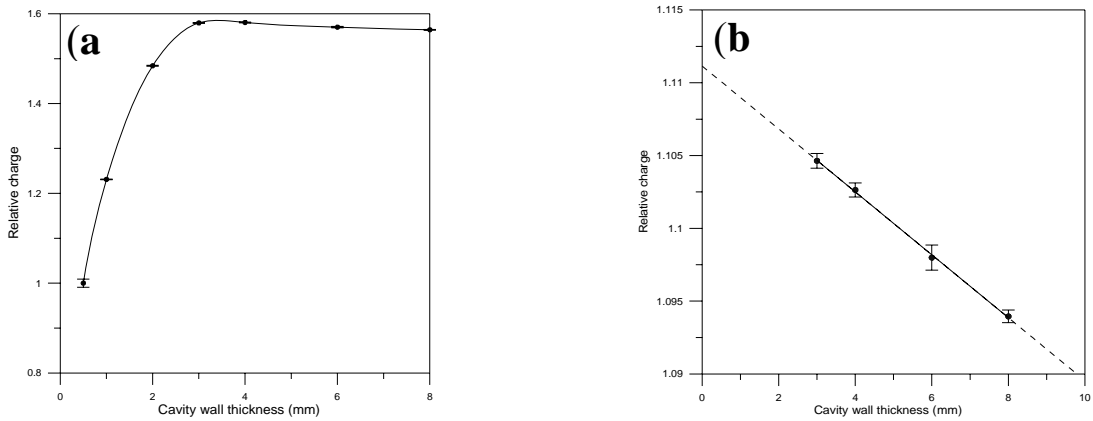
Figure (3): The relation between the relative charge and the cavity wall thickness for chamber 33051 filled with atmospheric air at ^{60}Co source (a) all thickness and (b) the extrapolation of the linear part.
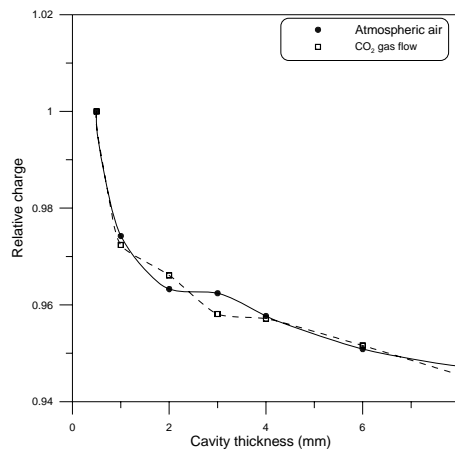

Figure (4): The effect of cavity thickness on the reading of the neutron ionization chamber 33051 in case of CO_2 gas flow and atmospheric air from ^{137}Cs source.
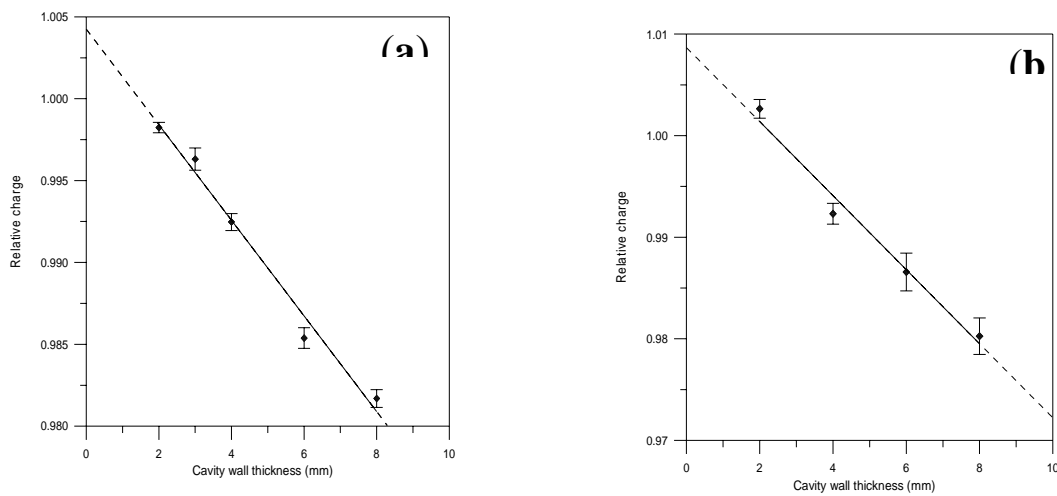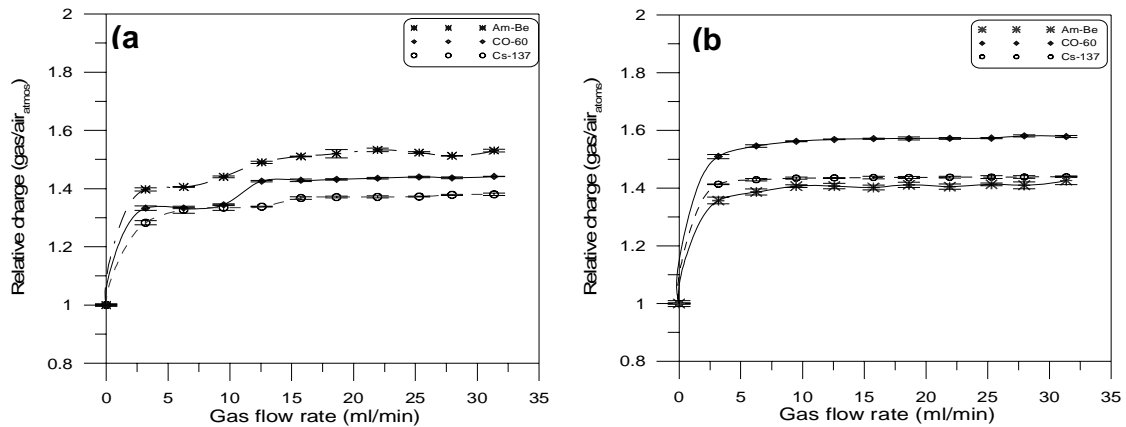

Figure (5): The relation between the relative charge and the cavity wall thickness for chamber 33051 at ^{137}Cs source filled with (a) atmospheric air and (b) CO_2 gas.

Figure (6): Represents the relation between relative charge and gas flow rate (ml/min) for neutron chamber 33051 filled with (a) C_2H_2 and (b) CO_2.
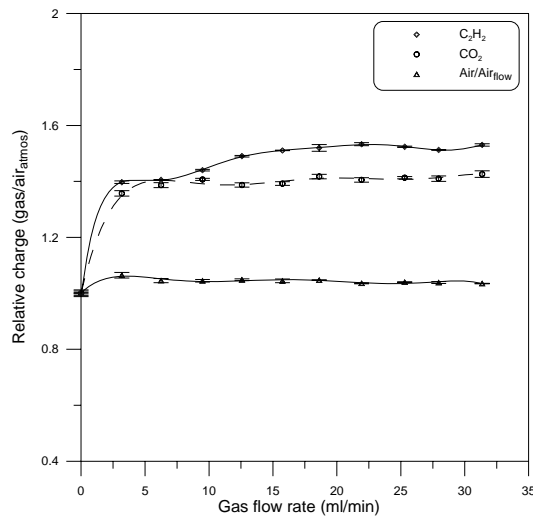
Figure (7): Represents the relation between relative charge and gas flow rate (ml/min) for neutron chamber 33051 using ^{241}Am-Be neutron source.
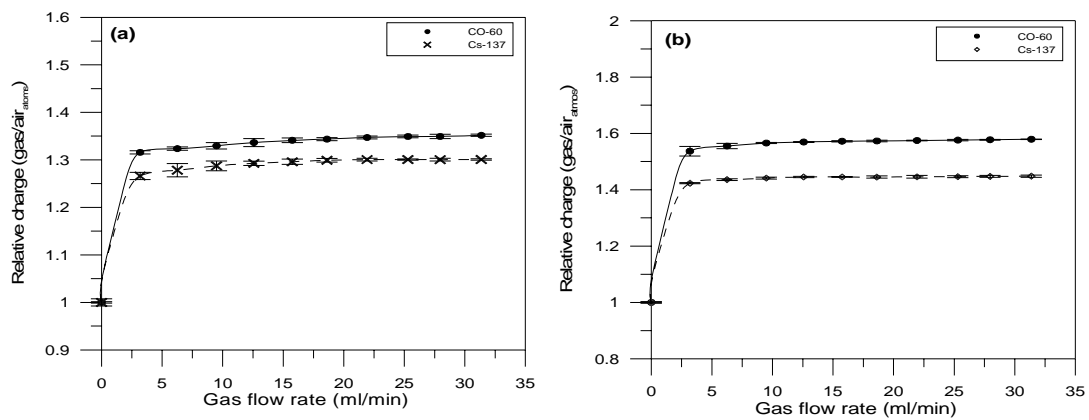
Figure (8): Represents the relation between relative charge and gas flow rate (ml/min) for neutron chamber 33053 filled with (a) C_2H_2 and (b) CO_2.

air. It is clear from the figure that the maximum sensitivity of the neutron chamber 33051 for C_2H_2 gas whereas the minimum sensitivity for air/airflow. Up to 13 ml/min the sensitivity of the neutron chamber 33051 can be increased by about 10 %, 7 % and 4 % for C_2H_2, CO_2 and air/airflow respectively. The ratio of the relative charge for chamber 33051 filled with atmospheric air to air flow rate is about 1.05 which is assumed to be only one. This finding may be due to the recombination reactions between O_2 and N_2 molecules forming atmospheric air which are kept in static form under strong electric field strength of 400 kV/m (applied voltage to the chamber is 400 V and the distance between the internal chamber electrodes is 4 mm). Nitric oxides may be generated within atmospheric air filling the chamber in static form condition under the influence of this strong electric field. These additional growing impurities in the filling air may be a reason for this increase of the chamber reading.

Figures (8 a and b) show the effect of gas flow rate on the relative charge (gas/airatoms) of neutron chamber 33053 using different gases (C_2H_2 and CO_2) and ^{60}Co and ^{137}Cs sources. Comparing these figures with Figures (6 a and b), it can be seen that the two chambers have the same sensitivity when they were flushed with CO_2 for two gamma sources. When the chambers were flushed with C_2H_2 gas, the sensitivity of neutron chamber 33051 is greater than the chamber 33053 with 38 % in the ^{60}Co beam. In the ^{137}Cs beam the sensitivity of the neutron chamber 33051 is less than the chamber 33053 with 2.8 %. Table (V) shows the values of gas flow rate for two neutron ionization chamber with different gamma sources and the percentage of standard deviation.

4.9.1 Radial Uniformity Correction Factor

Figures (9 a and b) represent the radial beam non-uniformity over the cavity thickness in air for 33051 neutron ionization chamber flushed with CO_2 gas and for 33053 neutron chamber flushed with C_2H_2 gas flow using ^{137}Cs gamma beam. From the two figures it is clear that the cesium beam is symmetrical over the studied range. The deviation from the central field point (0,0) to the point ±12 cm is about 1.6 % for 33051 chamber and to the point ± 9 cm is about 1.2 % for 33053 neutron chamber. Beam uniformity is much more homogeneous at the field center.

4.9.2 Axial Uniformity Correction Factor

Figures (10 a and b) represent the axial beam non-uniformity over the cavity thickness in air for 33051 neutron ionization chamber flushed with CO_2 gas and for 33053 neutron chamber flushed with

C_2H_2 gas using ^{137}Cs gamma beam. From the two figures it is clear that the radiation beam is symmetrical over the studied range (±10 cm) in the case of chamber 33051, the deviation from the central field point (0,0) is about 4.24% upward and 4.97 % downward. The studied range in the case of chamber 33053 is (±15 cm), The maximum deviation from the central field point (0,0) is about 4.80 % upward and 5.55 % downward direction. Beam uniformity is noticeable at the field center.

Figures (11) and (12) represent the radiation contour pattern and wire frame mapping for both radial and axial uniformity for the two neutron ionization chambers 33051 and 33053 filled with air in cobalt beam. From the figures it is clear that the radiation beam is not symmetrical over the studied range (±5 cm). For the chamber 33051 filled with air, the deviation from the (0,0) point to the point ±5 cm for the radial direction was 2.7 % and 4.5 %, for axial direction was 3.2 % and 8.5 % for upward and downward direction.

For chamber 33053 the deviation from the (0,0) point to the point ± 5 cm for radial direction was 1.6 % and 2.7 %, for axial direction was 2 % and 6.6 % for upward and downward direction. Beam uniformity is remarkable at the field center. Table (VI) shows the values of Radial & Axial beam uniformity correction factor for two neutron ionization chambers.

4.10 Resultant Calibration of TE Neutron Ionization chambers

Table (VII) shows the results of whole calibration coefficients in air in terms of air kerma (N_k) with unit mGy/nC in different radiation quality. Table (VIII) shows the calibration coefficients in water phantom in terms of absorbed dose to water (N_{Dw}) with unit mGy/nC experimentally and calculated using ^{137}Cs and ^{60}Co, experimental ratio N_{Dw}/N_k in cesium beam for neutron ionization chambers.

The experimental ratio N_{Dw}/N_k for a given neutron chambers give useful information about their uniformity. The usual situation for chambers calibrated only in air having air kerma factor (N_k), the absorbed dose to water factor (N_{Dw}) can be calculated using this ratio which based on a code of practice used. From Table (VII), it is clear that the values of air kerma (N_k) for both neutron chambers types 33051 and 33053 are energy and gas filling dependents.

Figures (13 a and b) illustrate the relation between the relative air kerma N_k normalized to cobalt beam and energies for different radiation quality for two NIS secondary standard ionization chambers (NPL and Farmer) and two neutron

ionization chambers 33053 and 33051 with different gases. It is clear that the results of NPL and Farmer ionization chambers are nearly coincidence and energy independent. The neutron chambers with different gases are not compatible at low X-ray energies while more consistency at higher energies, it is emphasized that energy dependent.

Table (V): The values of gas flow rate correction factor for the two neutron ionization chambers in ^{60}Co and ^{137}Cs beams.

Source type	Chamber type	Flow rate correction factor & Gas type			%σ		
		Air	C$_2$H$_2$	CO$_2$	Air	C$_2$H$_2$	CO$_2$
^{60}Co	33051	0.98154	0.99903	0.99993	0.02	0.070	0.007
	33053	0.99849	0.99928	0.99973	0.01	0.030	0.020
^{137}Cs	33051		0.99991	0.99997		0.030	0.010
	33053		0.99941	1.00000		0.024	0.002

Table (VI): The values of Radial & Axial beam uniformity correction factors for two neutron ionization chambers.

Source type	Chamber type	Radial beam uniformity correction factor & Gas type			Axial beam uniformity correction factor & Gas type		
		Air	C$_2$H$_2$	CO$_2$	Air	C$_2$H$_2$	CO$_2$
^{60}Co	33051	1.004			0.996		
	33053	0.997			1.006		
^{137}Cs	33051			1.000			0.999
	33053		0.9997			1.004	

Table (VII): shows the calibration coefficients in terms of air kerma in X-ray beam, ^{137}Cs and ^{60}Co for neutron ionization chambers.

Radiation quality		N_k, Neutron chamber type 33051			N_k, Neutron chamber type 33053		
		Air static	C$_2$H$_2$	CO$_2$	Air static	C$_2$H$_2$	CO$_2$
		mGy/nC			mGy/nC		
X-Ray	100 kV	40.38	34.03	26.11	35.97	30.30	23.24
	180 kV	35.45	28.72	23.09	32.04	25.82	20.74
	250 kV	33.75	26.34	22.23	30.82	24.01	20.25
^{137}Cs		31.11	22.89	19.65	28.33	22.02	18.85
^{60}Co		30.29	21.24	19.32	28.24	21.00	18.02

Table (VIII): shows the calibration coefficients in terms of absorbed dose to water in ^{137}Cs and ^{60}Co, experimental ratio N_{Dw}/N_k in ^{137}Cs beam for neutron ionization chambers.

Radiation quality	Type of determination	Neutron chamber type 33051			Neutron chamber type 33053		
		N_{Dw}, Air static	N_{Dw}, C$_2$H$_2$	N_{Dw}, CO$_2$	N_{Dw}, Air static	N_{Dw}, C$_2$H$_2$	N_{Dw}, CO$_2$
		mGy/nC			mGy/nC		
^{137}Cs	Experimentally	35.85	27.52	24.74	35.13	27.42	24.42
	N_{Dw}/N_k	1.153	1.202	1.259	1.240	1.245	1.296
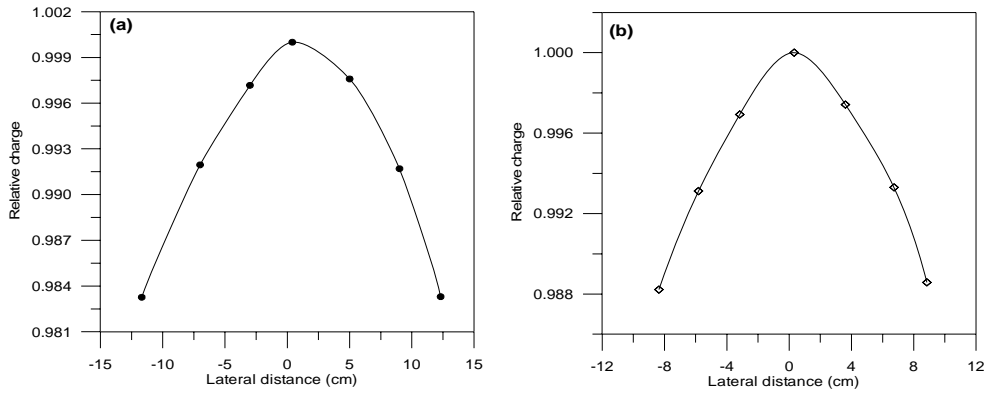
Figure (9): Shows the relation between the relative charge and lateral distance (cm) for neutron chamber at ^{137}Cs source filled with (a) 33051 CO_2 (b) 33053 C_2H_2
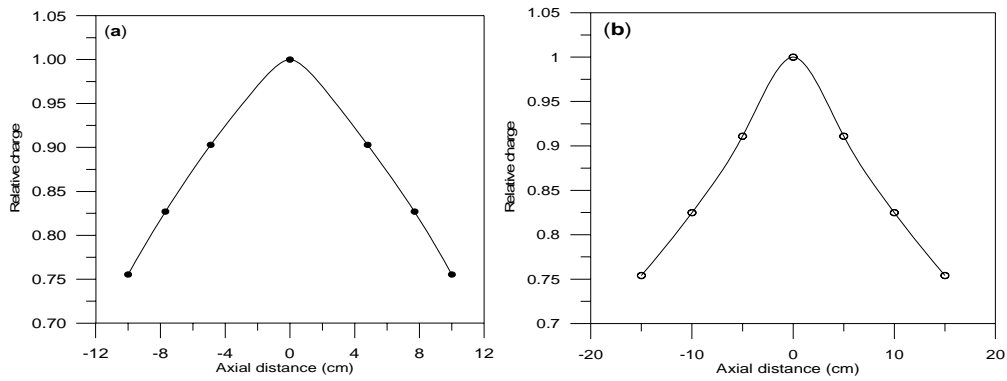
Figure (10): Shows the relation between the relative charge and the axial distance (cm) for neutron chamber from ^{137}Cs source filled with (a) 33051 CO_2 (b) 33053 C_2H_2
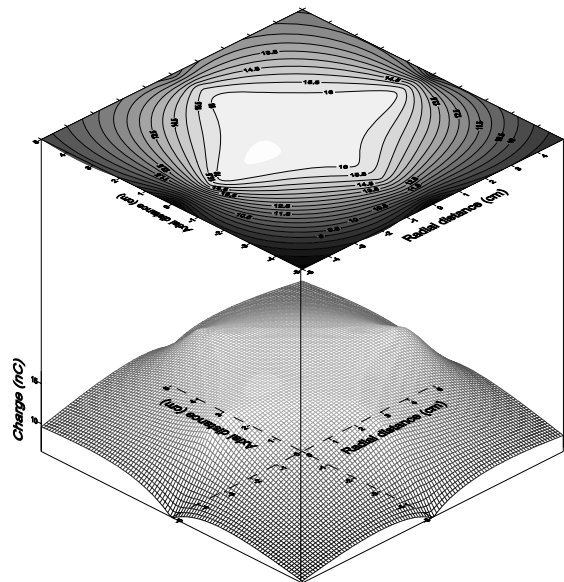
Figure (11): Shows the relation between the charge and both radial and axial distance (cm) from the source center for neutron chamber 33051 with atmospheric air from ^{60}Co source.
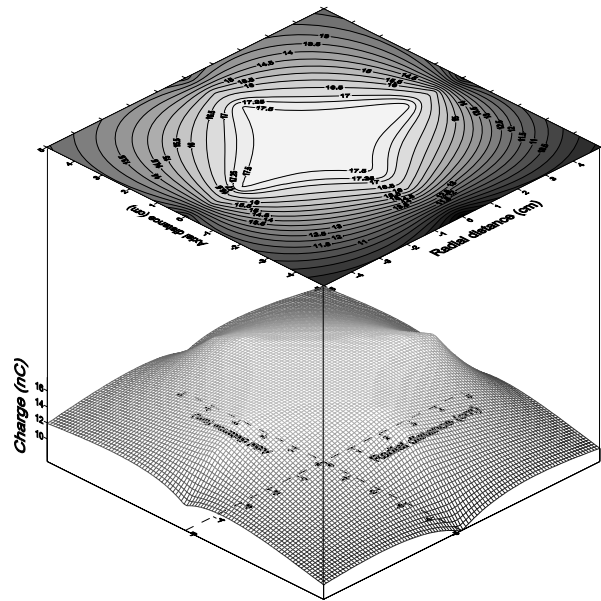
Figure (12): Shows the relation between the charge and both radial and axial distance (cm) from the source center for neutron chamber 33053 with atmospheric air from ^{60}Co source.
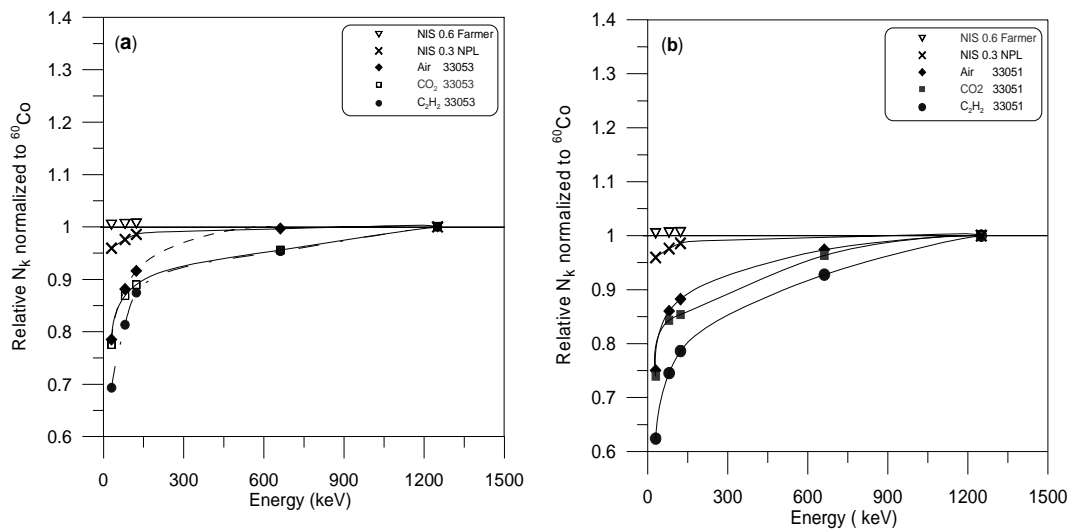
Figure (13): Represents the relation between the relative air kerma (N_k) normalized to ^{60}Co and radiation energies for secondary standard 0.6 cm^3 farmer and 0.3 cm^3 NPL chambers and neutron chamber filled with different gases (a) 33053 and (b) 33051.

4.11 The Sensitivity of Neutron Chamber Compared to Gamma Chamber

Figures (13 a and b) show the relation between collected charge and source -to-detector distance for neutron chamber 33051 compared with the secondary standard gamma ionization chamber (NPL chamber) normalized to its volume in ^{60}Co and ^{137}Cs beams.

The correction factors were 1.085 and 1.098, respectively for ^{60}Co and ^{137}Cs, for neutron chamber filled with atmospheric air. However, when the neutron chamber was flushed with C_2H_2 or CO_2, the response was increased. The correction factors were 0.743 and 0.679, respectively in ^{60}Co beam, in ^{137}Cs beam they were 0.809 and 0.694, respectively.

Figures (14 a and b) show the relation between collected charge and source-to-detector distance for neutron chamber 33053. The correction factors were 0.996 and 1.049 for cobalt and cesium, respectively for neutron chamber filled with atmospheric air. However, when the neutron chamber 33053 was flushed with C_2H_2 or CO_2, the correction factors were 0.735 and 0.633 respectively in ^{60}Co beam, whereas they were 0.774 and 0.666, respectively in ^{137}Cs beam.

5. Conclusion

The sensitivity of the TE neutron ionization chamber to γ-beams must be studied and all the discussed correction factors are implemented to determine the photon calibration factors.

The warm-up time for each chamber was studied and about 30 min warm-up was found to be necessary to give stable and reproducible data. Filling the chambers with C_2H_2 commercial gas gave more stable readings while atmospheric air and CO_2 gas showed fluctuations during the warming-up period demonstrated (160 min).
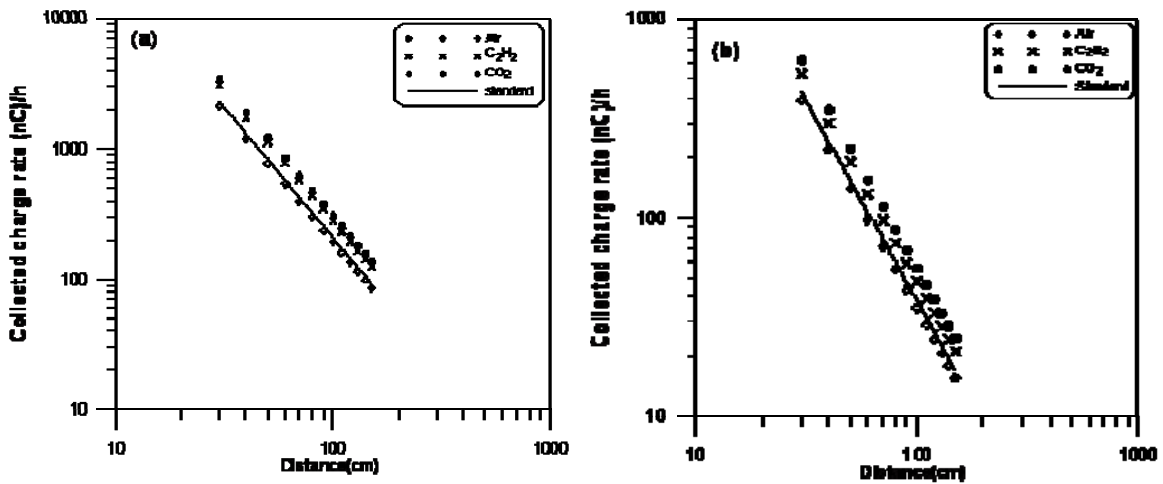
Figure (14): Shows the sensitivity of neutron chamber 33051 with different gas compared with secondary standard gamma ionization chamber in (a) cobalt beam (b) cesium beam.
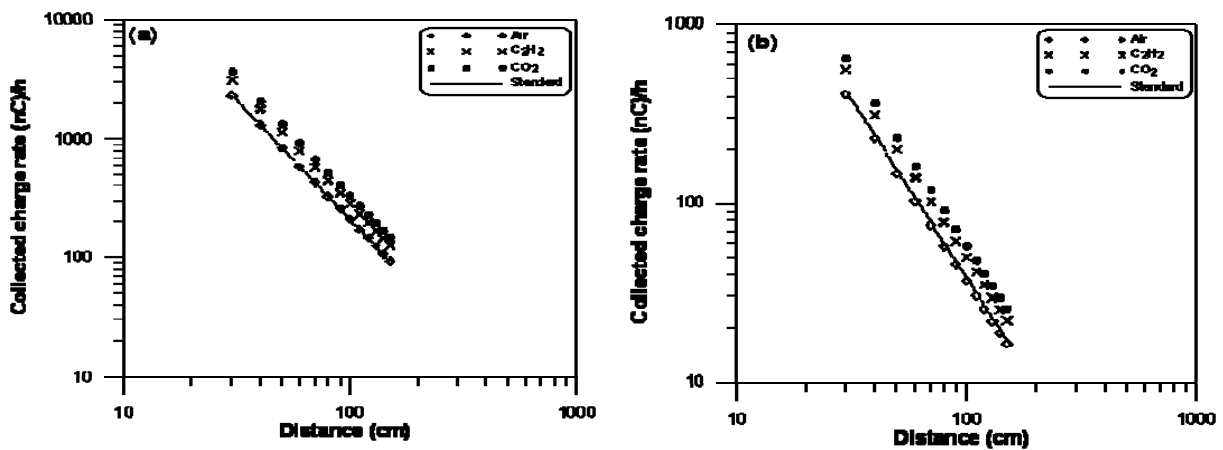
Figure (15): Shows the sensitivity of neutron chamber 33053 with different gases compared with secondary standard gamma ionization chamber in (a) cobalt beam (b) cesium beam.

The results showed that the increase of wall thickness increased the sensitivity of the chamber to gamma radiation till a wall thickness of 3mm. High thickness did not show any more increase of the ionic current. This result indicates that the ions are emitted from the walls of the chamber resulting from the interaction with the gamma rays. At thickness higher than 3 mm self absorption of the emitted ions from the walls occur which prevents no more ions of reaching the chamber cavity. The neutron ionization chambers filled with C_2H_2 gas flow of 18.6 ml/min (as a tissue equivalent gas) can be used as a secondary standard for measuring neutron equivalent dose rates. It has the advantage of increasing the sensitivity of the chamber to detect fast neutrons and decreasing its sensitivity for gamma rays.

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Pushover Analysis for Estimating Resonance Factor of Tall RC Frames with Steel Eccentric Bracing

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Abstract: Since the commonplace designs are directed on linear analyses, a practical estimation on actual displacements and deformations which are taken out by linear analyses could be handled by adding a factor. On the other word, displacement resonance factor (DRF) supplemented to linear analysis responses may be held superior in order to determine actual displacements concerning time and cost concerns. According to provision of Iranian Code for seismic design, displacement resonance factor (DRF) receives same values for all structural systems. To make sure for required modifications on seismic design codes, one hundred concrete moment resisting frames with eccentric braces, designed based on the Iranian National Seismic Standard, has been considered to capture seismic parameters by performing two-dimensional nonlinear pushover analyses. Pushover Analyses have been conducted using SAP-2000 program, which can consider material nonlinearities almost near reality. In this case the applied forces have been considered as the lateral forces of the Seismic Standard. Seismic parameters including overstrength, ductility and behavior factors are excerpted by following Young Theory. Also studies based on Newmark and Hall practice has been pursued to withdraw coefficient of force reduction due to ductility. Concentrating on tall buildings, variation of DRF has been illustrated concerning bracing kind of spans, length of link beam and height of structure. Analytical results show that in the case of reminded frames the value of DRF can be much higher than that recommended by Iranian Code. On the suggestion side, this problem can be devised by multiplying a coefficient of 1.54 to the former resonance factor.

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Keywords: Seismic design, Pushover analysis, Moment resisting frame, Tall buildings

1. Introduction

It's important to understand that seismic provisions in most building codes are intended to protect life and reduce property damage but not completely eliminate losses. That's recommended that structures should be able to resist minor earthquakes without damage, also resist moderate earthquakes without structural damage but with some nonstructural damage. On top of that, structures' attitude toward the major earthquake should be obliged for withstanding structural and non-structural damages. Generally, raising drift initiates structural damages though non-structural ones also leave stability in more critical levels. This put more emphasize to control lateral drift (Yaun et al., 2009). Buildings that are most vulnerable to lateral forces induced by seismic waves are unreinforced masonry, brick and mortar, and adobe constructions. Small wood frame structures are usually the safest as long as they are securely anchored to their foundations.

Steel frame or reinforced-concrete construction methods are least hazardous for multi-story buildings or other tall structures (Hoseinzadeh, 2010). Focusing on tall buildings, this study has been conducted under provisions of both Seismic Resistant Design of Buildings (Iranian Code) and Rehabilitation of Existing Buildings (Code 360). Description of specific earthquake is the point which both criteria are met concerning intermediate important structures. Thus a performance level is established on life security in a way that failure is permitted for a structure providing does not lead to hazard in life safety. On the other side, specific earthquake is referred to a design base earthquake with exceeding probability of less than ten percent in 50 years in accordance with provisions of Iranian Code while Code 360 relates this notion to a risk level-1 earthquake. Presenting probability of ten percent in 50 years, the later comment keeps abreast with a return period of 475 years (Iranian code, 2005; Code

360, 2006). In summing up, considering risk level-1 earthquake can't be greater than design base earthquake, more rigorousness is anticipated on designs under Iranian Code. But lining on more detailed instructions upon checking lateral drifts, Code 360 seems to spend more severity for embracing performance level.

On the other side, under certain conditions the most conventional designs are permitted for linear analyses such as static equivalence method to draw estimation on actual displacements. Based on recommendations of Iranian Code while structures are authorized for static equivalent method, displacement resonance factor of (0.7R) together with displacements fulfilled by assumption of elastic behavior of structure shall be replaced for actual displacements. Provided regarding $P-\Delta$ effects, Equation 1 can be used to confine the displacements:

$$\Delta_m < 0.025h \qquad for : T < 0.7s$$
$$\Delta_m < 0.020h \qquad for : T > 0.7s \qquad (1)$$

Where h and T are height and vibration period of structure respectively. Also Δ_m is referred to actual values of base lateral drift.

This method is placed pivot point of study to make sure on the required modification of the seismic design codes for building systems. In this study one hundred concrete moment resisting frames by eccentric braces designed based on Iranian Code (issued in 1988 and revised in 2005) and Code 360 (2006) has been considered under linear equivalent static and nonlinear static (pushover) analyses.

2. Materials and Methods

Generally speaking actual displacements could be derived based either on non-linear analysis method or simplified methods. Considering time and cost concerns, simplified methods may be held superior in order to determine actual displacements. Static equivalent method supplemented with resonance factor is usually discussed as a conspicuous option. Iranian Code (2005) has broadened this method to the following occasions:

A. Regular buildings with the height less than 50 meters including base story.

B. Irregular buildings with five story limitation or the height less than 18 meters including base story.

C. Buildings with variation in lateral stiffness in which the upper levels hold less stiffness than that of the bottom side and providing first, both parts have regular configurations. Secondly, average stiffness of bottom levels is valued at least ten times greater than that

of above and at the end, Fundamental period of vibration surpasses 1.1 times of the upper level supposing this part is fixed at the end also imagined separately.

These conditions bring large scope of structures into legitimacy of static equivalent method. Thus estimation on actual displacements of these structures could be written as:

$$\Delta_m = 0.7 R \, \Delta_w \qquad (2)$$

Where R is behavior factor of structure, and Δ_w is lateral drift from elastic analysis considering ductility reduction factors. In spite of controversy on the values of coefficients 0.02 and 0.025 (at Eq. 1), more intense is placed here on validation of Eq. 2. Now this equation can be rewritten as:

$$\frac{\Delta_m}{\Delta_w} = 0.7R \qquad (3)$$

Setting right hand of Eq. 3 by displacement resonance factor yields:

$$C_d = \frac{\Delta_m}{\Delta_w} \qquad (4)$$

Now parameter X can be defined as:

$$X = \frac{C_d}{R} \qquad (5)$$

In accordance with Iranian Code the value of X should be taken as 0.7 generally for all buildings designed by static equivalence method without reminding any special structural system. This study is developed to include striking elaborations around the value of X for concrete moment resisting frames with eccentric braces. Accepting general behavior of a conventional structure (Fig .1) also following Young theory set the following equations:

$$C_d = \mu_s \Omega Y \qquad (6)$$
$$R = R_\mu \Omega Y \qquad (7)$$

Where R_μ, μ_S, Ω, Y respectively are force reduction factor due to ductility, ductility of structure, overstrength factor and allowable stress factor. Substituting equations (6), (7) into (5) yields:

$$X = \frac{\mu_s}{R_\mu} \qquad (8)$$
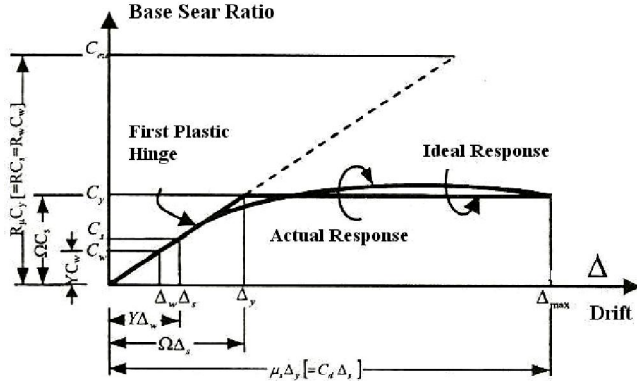
Figure 1. General behavior of a conventional structure (Tasnimi et al., 2007).

By idealizing behavior curve of structure to absolute elastic-plastic phases, ductility factor of structure could be defined as following quotient:

$$\mu_S = \frac{\Delta_{max}}{\Delta_y} \tag{9}$$

Based on Newmark and Hall performance the relation between R_μ and μ_S is expressed as (Tasnimi et al, 2007):

$$T_e \leq 0.03_{Sec} \rightarrow R_\mu = 1.0 \tag{10}$$

$$0.03_{Sec} < T_e < 0.12_{Sec} \rightarrow R_\mu = (T_e - 0.03)\frac{\sqrt{2\mu_s - 1} - 1}{0.09} + 1$$

$$0.12_{Sec} \leq T_e \leq 0.50_{Sec} \rightarrow R = \sqrt{2\mu_s - 1}$$

$$0.50_{Sec} < T_e < 1.00_{Sec} \rightarrow R = 2(T_e - 1)[\mu_s - \sqrt{2\mu_s - 1}] + \mu_s$$

$$1.00_{Sec} \leq T_e \rightarrow R = \mu$$

Where T_e is effective fundamental period of structure. Next step focuses on extracting the coefficients of R_μ and μ_S which are dependant on maximum of roof lateral relative displacement, fundamental period and roof lateral drift at yielding moment. Fundamental period is directly derived by solving the characteristic equation while acquiring aid of Eq. 11 sets value of initial vibration period.

$$\left| [K] - \omega^2 [M] \right| = 0 \tag{11}$$

$$T_i = 2\pi / \omega_i \tag{12}$$

Where $[K], [M], \omega$ respectively are stiffness matrix, mass matrix and modal frequency of structure. T_i is defined as fundamental (initial) period and ω_i is initial frequency of structure. Effective fundamental period needs referring to bilinear diagram belonging to Roof Drift/Shear Base curve (Figs. 2, 3) in case of structure under lateral displacement up to the target point.
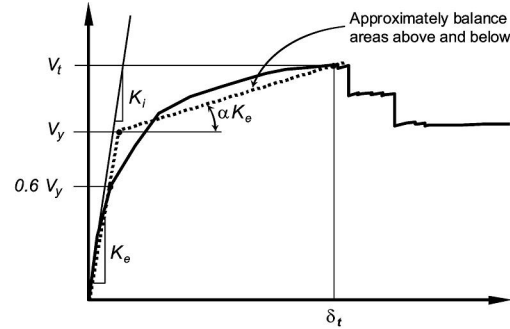
Figure 2. Idealized force displacement curves, Positive post-yield slope (FEMA-356).
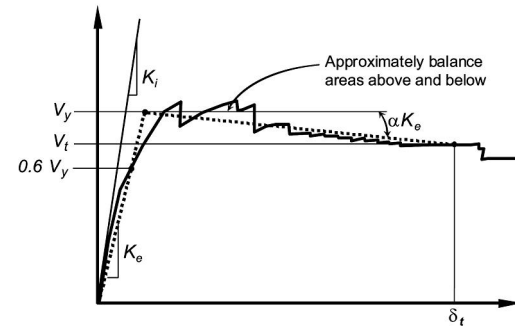
Figure 3. Idealized force displacement curves, Negative post-yield slope (FEMA-356).

Figs. 2, 3 employ K_i, K_e respectively as initial stiffness and effective stiffness. V_y is referred to shear base of total yielding, also δ_y, δ_t are displacement at yield base shear and target displacement. Now effective fundamental period can be calculated from:

$$T_e = T_i \sqrt{\frac{K_i}{K_e}} \tag{13}$$

Roof lateral drift at yielding moment could be derived as:

$$\Delta_y = \frac{V_Y}{K_e} \qquad (14)$$

To satisfy performance level, maximum of lateral drift has been restrained to target displacement of structure and can be written as:

$$\delta_t = C_0 C_1 C_2 C_3 S_a \frac{T_e^{\,2}}{4\pi^2} g \qquad (15)$$

Where g, S_a respectively are acceleration due to gravity and spectral acceleration. c represents a bunch of correction factors which could be extracted according to the Code 360.

3. Results

To draw a concrete conclusion, wide variety of concrete frame structures has been scrutinized under earthquake designs. One hundred braced moment resisting frames giving variety in number of stories (8, 10, 12, 14, 15), bracing kinds of spans, length of link beam (0.5, 1, 1.5, 2 meters) and number of spans (1,3) have been investigated under elastic-inelastic analysis procedures. Analysis methodology covering provision of Iranian Code has been followed by using SAP-2000 (version 12) computer program which consider both gravity and lateral loads. Details of the frames profile in this study are presented at Tables 1, 2.

Table 1. Characteristics of frames.

Zone Type	High Risk Level
Ground Type	Type 2
Ductility of building	Intermediate
Frame Type	Middle
Length of Loading Span	4 m
Length of Spans	4 m
Height of Stories	3.2
Dead Load	550 kg/m^2
Live Load	200 kg/m^2
Equivalent Partition Load	100 kg/m^2

4. Discussion

Because of attending wide variety of buildings and weighty structural elaborations, presenting results has been abbreviated only to averages on ultimate results. Five models applied for bracing the spans are plotted at Fig. 4. Figs. 5, 6, 7 present variation of DRF concerning bracing kind of spans, length of link beam and height of building with this in mind dashed and solid lines represent the results respectively for Iranian Code and analysis.

Table 2. Properties of materials.

Concrete	F$_c$	240 kg/cm^2
	V	0.15
	E	2100000 kg/cm^2
Bar	V	0.3
	E	2100000 kg/cm^2
	F$_y$	3000 kg/cm^2
Brace	V	0.3
	E	2100000 kg/cm^2
	F$_y$	2400 kg/cm^2
	F$_u$	3700 kg/cm^2
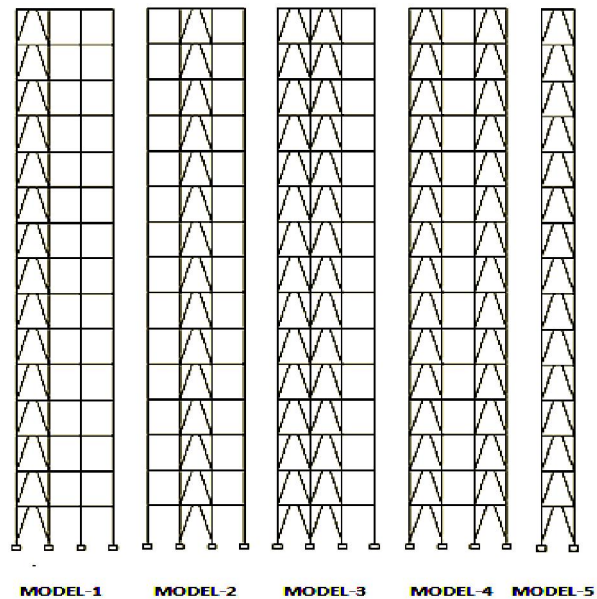
Figure 4. Number of bracing models.

Fig. 5 has represented an approximate equality in displacement resonance factors upon models 1, 2 and separately for the models 3, 4. According to the Fig. 4, these pair models can be marked out because of similarity in number of bracing spans. By this presents, resulting close stiffnesses explain similarity in displacement resonance factors. But locations of bracing spans have been left no tangible effect on DRF. Observed through Fig. 7, increasing height of building is accompanied with demoting DRF. In explaining, obligation to confining roof displacements to the

target displacement acquires more stiffness together with raising height of building. This resulted in a reduction in displacement resonance factor.
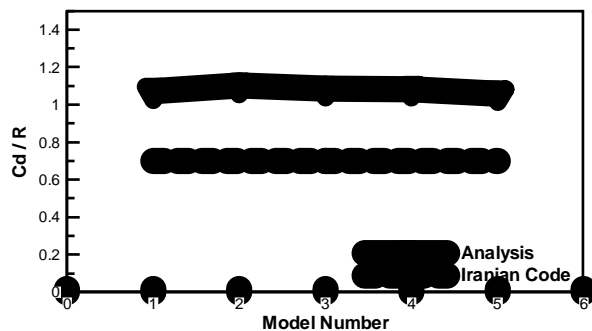
Figure 5. Variation of DRF ratio with Bracing kind of spans.
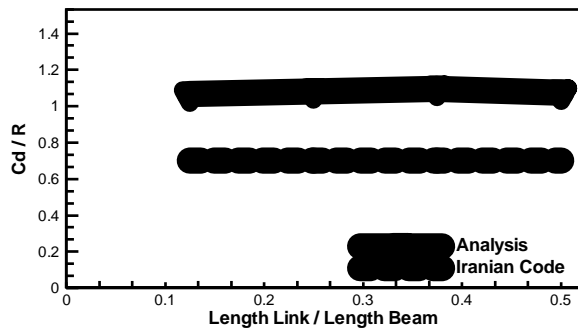
Figure 6. Variation of DRF ratio with Length of link beam.
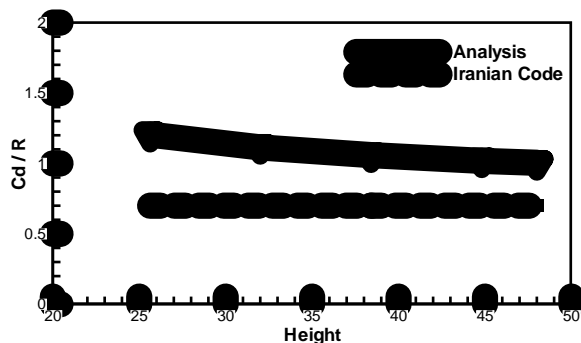
Figure 7. Variation of DRF ratio with height of building.

5. Conclusions

The main conclusions of this project can be drawn as follows:

Contemplating above discussions, seismic design based on Code 361 should be placed in priority than that of Iranian Code.

Giving a grade based on ability to present displacement resonance factor addresses equations $C_d = 1.16R$, $C_d = 1.03R$ respectively for buildings higher and lower than 10 stories.

It seems Iranian Code provisions on postulating the equation $C_d = 0.7R$ are not prepare to provide minimum strength required resistance to earthquake because of undervaluing actual displacements. To mitigate large displacement potential of existing buildings designed based on Iranian Code, retrofitting techniques should be considered.

Increasing either number of braced spans or height of structure leads declining in DRF.

Similarity in displacement resonance factors which are appraised by effect of symmetric and anti-symmetric bracing type is well observed throughout the analytical results.

Affected by increasing length of link beam over the span beam, the values of DRF grow in gradual steps (Fig. 6).

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Assessment of the susceptibility of polyculture reared African Catfish and Nile tilapia to *Edwardsiella tarda*

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Abstract: The study aimed to clarify the relative susceptibility of polyculture rearing African sharptooth catfish (*Clarias gariepnus*) and Nile tilapia (*Oreochromis niloticus*) , the two main reared species in Egypt to Edwardsiella tarda. Experimental infection of catfish and Nile tilapia with *E. tarda* was carried out after determination of the Mean lethal dose in African catfish (LC50). Infectivity pattern and pathology of *E. tarda* in catfish and Nile tilapia were tested via intra-peritoneal inoculation of 0.2ml of 10^4 CFU/ml of the bacteria at 25 °C. The mortality rates were 70% and 60% in catfish and Nile tilapia respectively. Congestion and hemorrhages in fish body were detected in both species. African catfish showed abdominal distention together with ulcers and excessive mucus in the skin. Internally; pinpoint white nodules in the liver was the main lesions observed. Histopathological examination of organs of both species revealed presence of myositis, and degenerative changes in liver and kidneys. Establishment of infection was confirmed with the laboratory diagnosis; culture characters; biochemical reactions; API -20E test kits in addition to molecular studies based on detection of the 1106-bp PCR product in tissue samples from experimentally infected fishes at 24 hr post experimental infection. In conclusion: *E. tarda* can express a potential role in polyculture fish farming. The African catfish exhibited severe pathological lesion and histopathological changes in comparison to Nile tilapia which show moderate to mild lesions. Direct probing for the presence of *E. tarda* in infected fish by PCR is reliable and helpful in diagnosis, anticipating and rapid interference.
[M. D. Ibrahem, Iman, B. Shaheed, H. Abo El-Yazeed, and H. Korani**. Assessment of the Susceptibility of polyculture reared African Catfish and Nile tilapia to *Edwardsiella tarda*].** Journal of American Science 2011;7(3):779-786]. (ISSN: 1545-1003). http://www.americanscience.org.

1. Introduction:

Intensification of fish culture has made the producers think wisely of developing suitable husbandry strategies, based on novel nutritional and management practices that enhance performance without significant increase in the cost of production (Lovell, 1989). The majority of fish farms in Egypt can be classified as semi-intensive water pond farms. Current developments in fish culture production are centered on the changes in the structure of the fish farming community (GAFRD, 2006). The polyculture fish farms are well established systems that proved both productive and economical efficiency. Nile tilapia (*Oreochromis niloticus*) and African Catfish (*Clarias gariepinus)* are two major species that are mixed reared in the aquaculture. Bacterial agents are among the highly encountered causes of diseases in stressed warm water aquaculture (Pavanelli etal., 1998. and Noga, 2000) *Edwardsiella tarda* infection is considered a dangerous septicaemic disease with high economic losses (Meyer and Bullock, 1973), the seriousness of *Edwardsiella tarda* infection is its expanding fish host range (Alcaide et al.,2006 and Mohanty and Sahoo, 2007). It infect catfish causing emphysematous putrefactive disease (EPD) [Darwish et al., 2000] Despite the fact that, *E. tarda* is a

bacterium of fish, it infects humans posing a public health threats; causing gastroenteritis, meningitis, liver and skin abscesses and valvular endocarditis in patient with acquired immune deficiency syndrome (AIDS) (Mikamo et al., 2003 and Mizunoe et al., 2006). With respect to the most recent diagnostic methods for Edwardsiellosis, polymerase chain reaction (PCR) represents a widely-used alternative to traditional identification methods. Although pathogenic and virulent genes have been used as target regions; it is now accepted as a tool for identification purposes (Chen and Lai 1998). The Goal of this study was to determine the relative susceptibility of African catfish *(Clarias gariepinus)* and Nile tilapia *(Oreochromis niloticus)* two fish species that are commonly cultured together in Egyptian aquaculture to E. tarda . Furthermore we assessed pathological and histopathological features of *E.tarda*. Tentative characterization and confirmation of the *Edwardsiella tarda* isolate using molecular identification (PCR), conventional biochemical assays and the API -20 E system.

2. Materials and Methods:
Fish:

The fishes used for the experimental infectivity were obtained from semi-intensive farms, Egypt. Two hundred and twenty African catfish *(Clarias gariepinus)* with average body weight of 10 g $\pm$ 1.0 g and 120 Nile tilapia *(Oreochromis niloticus)* with average body weight of 20 g $\pm$ 1.0 g were used in the pathogenicity assays and the experimental infection. Ten fish were submitted for bacteriological examination to verify the absence of E. tarda. The fishes acclimatized in 16 separate glass aquaria (30x 40x 80cm) contain chlorine-free tap water for 14 days before the onset of the experiment according to Best et al., (2002). Temperature was thermostatically controlled to $24^{0}C \pm 2^{0}C$ simulating the water temperature of the fish pond when the E. tarda strain was isolated. Aquaria were supplied with oxygen through electrical air pumps (Sicc- Alis and Pieters, Italy). Fish were fed to satiation twice daily (09:00 and 15:00) with 35% protein commercial fish pellets.

Bacterial isolate:

Edwardsiella tarda strain was isolated in the summer of 2008 from diseased African catfish farm in Fayoum governorate; Egypt. Confirmation was achieved using the conventional biochemical assays and the API 20 E system. Five typical colonies of the MacConky's agar cultured isolate were picked up; suspended in Mueller Hinton broth (Oxoid). The broth was incubated at 27°C for 24 hours until its turbidity exceeds that of the standard McFarland tube number 0.5. The turbidity was adjusted to match the McFarland tub number 0.5 (1×10^{4} bacteria/ml) by adding sterile saline according to Sahoo et al., (2000).

Pathogenicity assays:

The pathogencity of *Edwardsiella tarda* was determined following the methodology described by **Nieto et al., (1984)**. Groups of 5 catfish (10±1 g body weight) were inoculated intraperitoneally with the bacterial isolate using 0.1 ml of serial bacterial dilutions containing 10^{3}-10^{8} CFU. A control was provided using same number of fish inoculated with 0.1 ml PBS. Inoculated fish were observed daily for 7 days, all mortalities were recorded. Freshly dead fish were submitted for bacterial isolation and re-identification of E. tarda to verify specificity of mortality.

Experimental infection:

The experiment was carried on 3 replicate. Three groups of catfish; 40 fish/group; with a total of 120 catfish. Another 3 groups of Nile tilapia*; 40* fish/group; with a total of 120 were used. The assigned groups were subjected to intraperitoneal injection using a dose of 0.2 ml of 10^{4} bacteria ml^{-1} at 25 ± 1 °C, after Darwish et al., (2000). A group of 40

fish/ group from each species were reared under the same experimental conditions, injected I/P with 0.1 ml PBS and served as controls.

Clinical and post mortem examination:

The experimentally infected catfish and Nile tilapia were grossly examined to determine any abnormalities. The fish were opened under aseptic condition; interior of the body was exposed and examined for changes according to the Noga, (2000) and Kimberley (2004).

Sampling:

Fish samples from the experimentally infected catfish and Nile tilapia*;* were collected from each group daily, starting on day one until day 7 post infection. Liver, spleen, kidney and muscle tissues were tested for bacteria using the method provided by Buller, (2004). Parts of the collected samples were collected in sterile cry- tubes and stored at -80 C for PCR test.

Isolation and Identification:

For bacterial re-isolation, all samples were streaked on MacConky's agar, and the selective medium, EIM (*Edwardsiella ictaluri medium*), according to Shotts and Waltman, (1990). The identification were performed by the biochemical tests and the API -20E rapid identification system test strips (Biomerieux 20 100 Marcy-l' Etiole, France) bacteriological diagnosis (Austin and Austin, 1999).

PCR detection of the hemolysin gene fragment in visceral organs of fish:

PCR was applied for detection of the hemolysin gene fragment in visceral organs (liver, spleen & kidneys) of experimentally infected catfish and Nile tilapia 24 hour post experimental infection. Chromosomal DNA was extracted from 100 μl of tissue samples (tissue sample suspended in 100 μl of sterile saline) using DNeasy tissue extraction kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions. The PCR targeted the hemolysin gene using the method reported by **Chen and Lai,** (1998). The forward primers — hem – F (5'CCTTATAAATTACTCGCT3) and the reverse primers hem — R –5'TTTGTGGAGTAACAGTTT3 were used. Buffers and reagents used prepared according to (Paton and Paton, 1998) .

Histopathological examination:

Fresh tissue specimens from the liver, kidneys, spleen, skin and muscle were collected from morbid experimentally infected fishes; Specimens were fixed in 10 % neutral buffer formalin ,processed by

conventional method , embedded in paraffin, sectioned and stained with Hematoxyline and Eosin stain according to Bancroft and Gamble (2008).

3. Results:
The Pathogenicity assay

The mean lethal dose 50% (LC_{50}) of the *Edwardsiella tarda* in African sharptooth catfish was 10^4 CFU/ml.

Experimental infectivity of catfish and Nile tilapia *with E. tarda*:

Clinical abnormalities were clear in experimentally infected African catfish and Nile tilapia with *E. tarda*, from day one tell the end of 7 days post infection at 25 ± 1 °C. Regarding catfish infected groups; mortality rate was 70%. Grossly, there were petechial hemorrhage and cutaneous ulcers (Figs, 1 and 2); raised edematous areas with liquefaction of underlying tissues in the caudal peduncle and the site of injection. Congestion of the fins and petechial hemorrhages allover the body surface was also observed.

The experimental infection of Nile tilapia resulted in 60% mortality and range of abnormalities as sluggish movement and loss of escape and defense reflexes. Scale detachment and pale coloration (Fig, 3), severe edematous swelling at the site of injection, swollen abdomen with yellowish ascetic fluid, protruded hemorrhagic anus with opaqueness eyes were constant findings. Internally, both catfish and Nile tilapia showed severe hemorrhagic enteritis with adhesion between organs, the body cavity was filled with abundant yellowish mucoid fluid; in catfish; the liver showed a multiple tiny white foci (Fig, 4) while the intestine contained thick white opaque mucus.

Histopathological Examination.

The main lesion in liver of African catfish was congestion of central veins. The hepatocytes showed disorganization and disorientation of hepatic plates. Some hepatic cells showed hydropic degeneration in which the cells swollen with irregular vacuoles in the cytoplasm. Others showed fatty changes in which the cells showed circumscribed vacuoles in the cytoplasm (Fig 5). Some hepatic cells revealed signs of coagulative necrosis. There was increase in the melano-macrophages cells. The kidneys showed congestion of renal blood vessels with multi-focal areas of hemorrhages. The renal tubules showed different necrobiotic changes as cloudy swelling, hydropic degeneration and even necrosis (Fig 6).The spleen was studded with large numbers of erythrocytes. There were increase in numbers of melano-macrophages centers, depletion of lymphocytes and congestion of blood vessels (Fig 7).

The skin showed focal areas of necrosis in epidermal layer forming erosions or even the necrosis extended to the basement membrane leaving an ulcer (Fig 8). The dermis showed signs of dermatitis in which there were congested blood vessels with large areas of hemorrhages and inflammatory cells mainly macrophages and lymphocytes. Some areas of skin showed hyperplastic activity of epidermal cells including the mucus cells (Fig 9). The muscles showed Zenker's necrosis of muscle bundles in which the sarcoplasm stained deeply eosinophilic with losing of striation. There was inflammatory cells infiltration mainly macrophages and lymphocytes in-- between the muscle bundles (Fig 10). The histopathological examination of Nile tilapia revealed hydropic degeneration in most of hepatocytes (Fig11). The kidneys revealed necrobiotic changes in the convoluted tubules. There were increase in melano-macrophages centers and depletions in lymphoid follicles of spleen.

Bacteria recovered from mortalities and euthanized fish of both species had phenotypic characteristic consistent with E. tarda. Genotypic confirmation of E. tarda was performed and amplifying an 1106 bp portion of hemolysin gene was detected (Fig 12).

4. Discussion:

Edwardsiella septicemia is an economically important bacterial disease affects mainly catfish, the disease incidence increases with the rise in water temperature as environmental stress (Palacois and Ghittino, 1987; Noga, 2000 and Roberts, 2001). As a result of the aggressive behavior of the catfish; injuries can be awaited from Catfish toward tilapia. Skin injuries are a potential route of pathogen entry and disease occurrence (Noga, 2000). In order to clarify the possible negative impact of the polyculture rearing of catfish and Nile tilapia, experimental infectivity of the two species *with E. tarda* isolated from African catfish using the intra-peritoneal route was carried out. The route of infection was in accordance to Amandi et al., (1982) and Eissa and Yassien, (1994) who recorded that the pathogenicity of *E. tarda* was demonstrated experimentally in salmon by I/P injection.

The LC50 of Edwardsiella was found to be 10^4 CFU/mL. Mekuchi et al., (1995) recorded following LC50 7.1×10, 1.7×10^2, 3.6×10^6 and 1.3×10^6 CFU/ml by intramuscular injection, interpertioneal injection, immersion and oral administration respectively in Japanese flounder. The difference in calculated dose may by due to differences in types of fish and conditions of experiment.
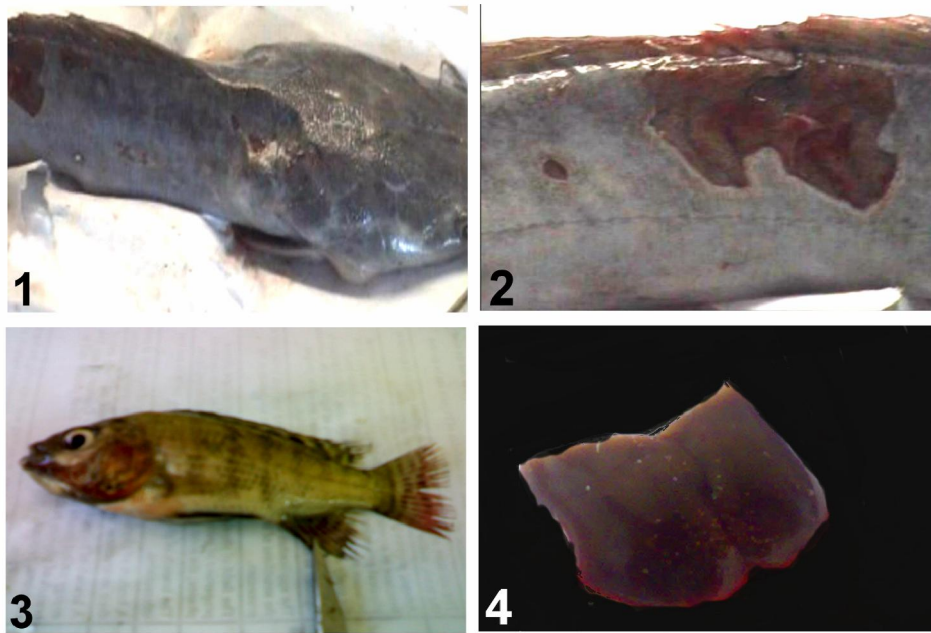
Fig 1: catfish experimentally infected by E tarda showing external skin hemorrhages and ulcers and fin rot
Fig 2: catfish experimentally infected by E tarda showing deep ulceration reached the dorsal muscles
Fig 3: Nile tilapia experimentally infected by E tarda showing signs of septicemia
Fig 4: liver of catfish showing a multiple tiny white foci and congestion
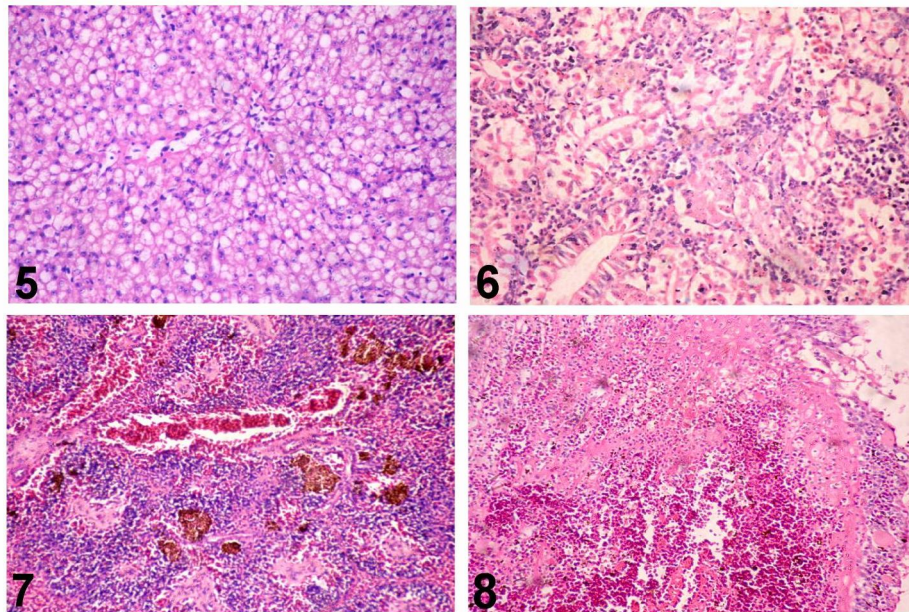

Fig 5: Liver of African catfish experimentally infected I/p by E. tarda showing fatty changes in most of hepatocytes. H&E X200.
Fig 6: Kidney of African catfish experimentally infected I/p by E. tarda showing necrobiotic changes in renal tubules. H&E X400.
Fig 7: Spleen of African catfish experimentally infected I/p by E. tarda showing congestion of blood vessels and increase in melano-macrophage center. H&E X200.
Fig 8: Skin of African catfish experimentally infected I/p by E. tarda showing necrosis of epidermal layer with hemorrhage and congestion of dermal layer. H&E X200.
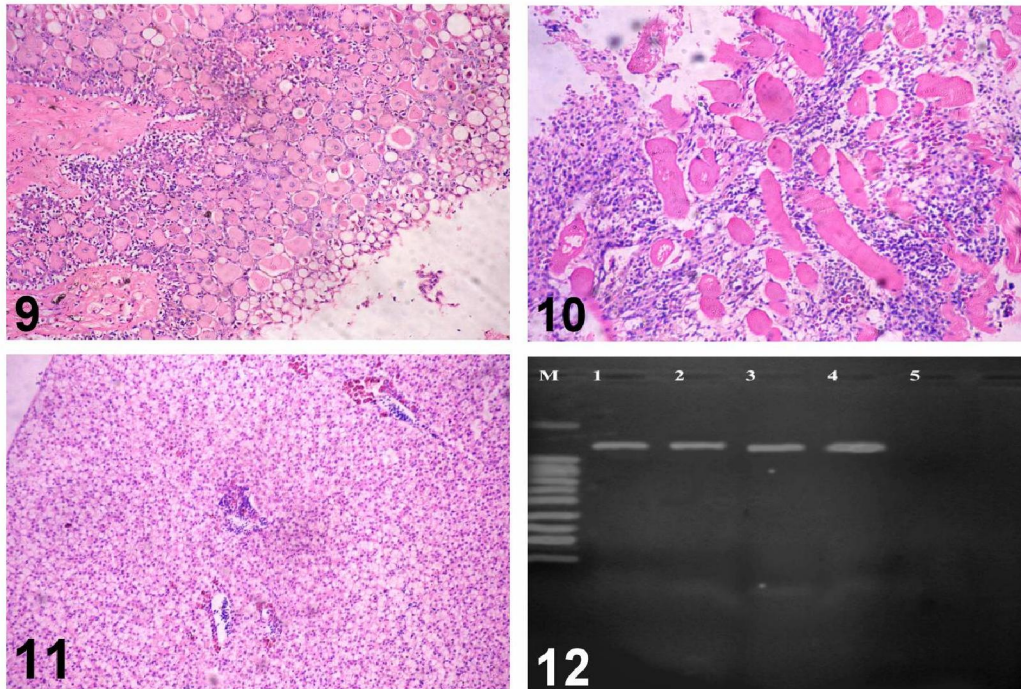
Fig 9: Skin of African catfish experimentally infected I/p by E. tarda showing hyperplasia of epidermal layer especially mucus secreting cells with increase of its activity. H&E X200.

Fig 10: Muscle of African catfish experimentally infected I/p by E. tarda of showing severe infiltration of mononuclear cells in-between the muscle bundles with necrosis of some bundles. H&E X200.

Fig 11: Liver of Nile tilapia experimentally infected I/P by E. tarda showing hydropic degeneration in most of hepatocytes. H&E X100.

Fig 12: Agarose gel electrophoresis: lane 1: positive Control (DNA from ATCC E. tarda); lane 2: liver sample; lane 3: spleen sample; lane 4: kidney sample showing characteristic 1106-bp PCR product; lane 5: control negative. Marker: 100 increment DNA ladder.

There is no available literature dealing with *E. tarda* in African cat fish but the clinical signs and gross lesion observed in the experimentally infected African catfish were in agreement with Meyer and Bullock (1973) who reported that with mild infections of channel catfish,the external signs of disease were small cutaneous lesions. With progression of the disease, abscesses develop in the muscle of the body and tail. These abscesses may enlarge and develop into gas-filled hollow areas. The ulcers seen in our experiments were also similar to those reported in largemouth bass ,*micropterus salmoides*, infected with *E. tarda* (Francis-Flody et al., 1993). Soliman et al., (1991) reported that Catfish injected with *E. tarda* showed hemorrhagic raised edematous areas with liquefaction of underlying tissues, congestion of the fins and petechial hemorrhages allover the body surface. Much evidence from the present study, *E. tarda* is a fish pathogen that causes systemic infections in fish. The

results were in accordance with Stoskopf, (1993) and Ling et al., (2000) who stated that infected fish showed hemorrhages allover the fish body, pale skin areas with detached scales, hemorrhagic protruded vent, and abdominal dropsy.

The experimental infection of Nile tilapia resulted in 60% mortalities, sluggish movement and loss of escape and defense reflexes and scale detachment, severe edematous swelling at the site of injection, the presence of swollen abdomen filled with yellowish ascetic fluid, protruded hemorrhaged anus and opaqueness to the eyes the findings are much likely reported by Kubota et al.,(1981). Internally, both Catfish and Nile tilapia, showed severe hemorrhagic enteritis with adhesion between organs, the body cavity was filled with abundant yellowish or sanguineous mucoid fluid; in catfish; the liver showed multiple tiny white foci; while the intestine contained thick white opaque mucus. The findings were in agreement with Baya et al., (1997)

and Noga, (2000). The pathogenicity mechanisms were investigated by Ullah and Aria (1983a, b) and Suprapto, et al., (1995), they reported that *E. tarda* secrete hemolysins and dermatotoxins, (exoenzymes) which may confer pathogenicity on *E. tarda* as it produce both an exotoxine extracellular products (ECP) and endotoxin intracellular components (ICC) that are lethal to eels and flounders and play an important role in the pathogenicity of this organism. Hemolysin, which is identified as an endotoxin because it is not secreted outside of the cell (Watson and White 1979, Ullah and Arai 1983a, b; Janda et al.,. 1991 and Janda and Abbott 1993 a, b).

Recently, Singh and Singh (1997) revealed that all the pathogenic *E. tarda* had either type 1 fimbriae or colonization factor. On iron deficient medium *E. tarda* produced siderophore, which permit the pathogen to scavenge for iron in the blood of the host (Park et al., 1983; Kokubo et al., 1990 and Ouyang et al., 1998). Certainly the ability of *E. tarda* to acquire iron or produce toxin is an important part of the infection process. The clinical and P.M lesions may be attributed to the action of bacterial toxins. This explanation supported by the statement of Braude (1964) and Nowotny (1979). They reported that the nature of pathogenesis caused by all gram-negative bacteria was almost similar and the disease process was caused by endotoxins and exotoxins. Meanwhile, Ullah and Arai (1983 b) reported that, E. tarda did not produce endotoxins as other Gram-negative bacteria did, but it produced two extoxins which could be responsible for the lesions. It was noticed also that E. tarda infection spreads from lesions of visceral organs into the musculature and then to the skin, where large lesions develop in the musculature and dermis. These observations agreed with those reported by (Eissa and Yassien (1994), Baya et al., (1997) and Miwa and Mana, (2000). Postmortem findings may be due to septicemia induced by two exotoxins that cause diseases and the most important one of them haemolysin secreted by E. tarda microorganism (Hirono et al., 1997 and Mathew et al., 2001). Liver and kidneys of the most examined fish showed congestion and this may be due to the nephric and hepatic virulence factors of E.tarda (Miwa and Mana, 2000 and Mathew et al., 2001).

The re-isolation, phenotypic and biochemical characterization of E. tarda from fishes were similar to those reported by other investigators (Ling et al., 2001 and Roberts, 2001 and Quinn et al., 2002).

The 1106-bp PCR product was detected in tissue samples obtained from the experimentally infected catfish and Nile tilapia (liver, spleen and kidneys) .The PCR gave positive results as early as 24 h post infection, by which the same samples gave negative for the bacteriological detection of the bacteria from experimentally infected catfish and Nile tilapia. This result suggests that live bacteria residing in the visceral organs of infected fish can be rapidly and easily detected by using the oligomers of the hemolysin gene as primers for PCR assay. Direct PCR detection of the hemolysin gene of live bacteria is potentially simple, accurate, rapid and helpful in anticipating and preventing epidemics which otherwise occur so frequently on fish farms.

The investigation of tissues alterations different predilection body organs and tissues could be an important aid for diagnosis of infectious diseases.

regarding the histopathological studies, the lesions in internal organs observed in the present work could be attributed to potential virulence factors of E. tarda such as siderophores, cell adhesion factors and cell invasion activity and two types of hemolysin, dermotoxin, cytotoxin , entertoxin which induced the necrosis and the degenerative changes in most organs especially liver , kidney , skin and muscle (Chen et al., 1996 and Mathew et al., 2001). Also the capability of a pathogen to infect its host is partly due to its ability to detoxify various toxic forms of oxygen (Free radicals) present in the host body by producing enzymes such as catalase, peroxidase, and superoxide dismutase (Yamada and Wakabayashi 1999).Lymphoid depletion observed in our study may be due to extensive apoptosis in lymphocytes induced by E. tarda. Also it may be due to systemic immunosuppression result in this lesion (Pirarat et al., 2007).

The histological alterations in infected Nile tilapia in this study consistent with those reported by Areechan and Plump (1983) and Soliman et al., (1991).

5. Conclusion:

Based on the present study, *E. tarda* may evoke serious ecological and economical problems. Polyculture rearing of catfish and Nile tilapia may lead to disease outbreaks. The experimental infection cleared that E, tarda in African Catfish induce clinical signs and pathology were more or less the same as others species of catfish. Oreochromis niloticus can get infected by *E. tarda* isolated from catfish and expressed clear septicemia and mortalities. The African catfish exhibited severe pathological lesion and histopathological changes in comparison to Nile tilapia which show moderate to mild lesions. Early detection of *E. tarda* using PCR technique is recommended in fish farms in the warm areas and/or under stress; as early diagnosis of the disease before the onset of the clinical picture evokes a better prognosis and allows the rapid interference and treatment.

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The General Equation Of The Pipe To Soil Potential At All Humidity Conditions By The Use Of Both Soil Factor and Stray Potential Of The Pipe-Soil-Earthing Grid System

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Abstract: For pipe-soil-earth system, the buried pipe line segment with soil surrounding medium could be simulated electrically by an electric circuit where the system is subjected to the law: charge = capacitance $\times$ voltage between the pipe surface and earth. This is where each of circuit electric parameter (electrolytic stray capacitor between pipe & earth, the stray potential across the stray capacitor, surface charge and the protection current of the cathodic protection system passed through the pipe segment) could be obtained by an equation which is function of the measured electrochemical properties of the soil (soil factor), 4th degree polynomial at room temperature but the A's constants are different for each electric quantity .The constants of each equation (A's) considered to be as a print of such pipe-soil-earth system . The useful of these prints is to obtain complete electrical data correlated with many cathodic protection levels**.** One of the most critical problems in CP systems is the presence of the earthing network beside the protected pipe line**.** The behavior of the stray potential between the external surface of the pipe and earth could be plotted as stray potential print which will be always valid in all times as the pipe-soil-earth system is maintained and without any external interference. This paper tries to calculate pipe to soil potential along the pipe line without the need of Cu/CuSO$_4$ half cell by the deduction of a general equation of the pipe to soil potential which is function of an electric quantities and system's print. In other words, the aim is to deduce a correlation between pipe to soil potential and both of the measured stray potential of the pipe segment and the measured soil factor around it in the presence of an earthing grid.

1. Introduction:

The behavior of the electrical parameters (stray potential V$_{P-PE}$, stray capacitor C$_{P-PE}$, surface total charge Q and protection current I$_P$) of the pipe-soil-earth system, during the change of the electrochemical properties of the soil, with and without applying cathodic protection system, could be plotted as an electrical parameter PRINT which will be always valid in all times as the pipe-soil-earth system is maintained and without any external interference. Once the system is changed by replacement another pipe with different dimension and/or the replacement of the soil (or by humidity change), there will be another new electrical parameter PRINT for the new pipe-soil-earth system. Also, the buried pipe line segment with soil surrounding medium could be simulated electrically by an electric circuit where the system is subjected to the law Q = C×V between the pipe surface and remote earth. This is where each of circuit electric parameter could be obtained by an equation which is function of the measured electrochemical properties of the soil (soil factor), 4th degree polynomial at room temperature but the A's constants are different for each electric quantity. The constants of each equation (A's) considered to be as a PRINT of such pipe-soil-earth system [10] [12]. The

useful of these PRINTS are to obtain complete electrical data correlated with many cathodic protection levels which help, after complete erection of the pipeline, in defining the cathodic protection level of any pipe line segment through its length by measuring the protection current and / or the stray potential with of course computing of the soil factor at the pipe segment from direct field measurements [14]. The average error of the electrical parameters equations reduced to be less than ± 5%. The most important advantage of such electrical analogue circuit of pipe-soil-earth system is the possibility to simulate a complete pipeline-soil-earth system by an electric circuit and to convert the corrosion problem and cathodic protection of the pipeline to an electric problem [11] [13]. In the near future after completing such electrical studies of the pipe-soil-earth systems, this will help in corrosion monitoring and the maintenance of c.p systems. Not only has that but also to define the most suitable route of the pipe line, before the erection process, which generates the minimum surface charge. The most important result is that: the pipe to soil potential of any buried pipeline could be obtained segmental along its route without the need of both the test point and Cu/CuSO$_4$ half cell. This is by the use of the new electric concept of pipe-

soil-earth system [14]. One of the most critical problems in CP systems is the presence of the earthing network beside the protected pipe line. The question now is: To what extent, the earthing grid would affect the cathodic protection system? This paper tries to calculate the pipe to soil potential along the pipe line without $Cu/CuSO_4$ half cell by measuring both the stray potential of the pipe and the soil factor around the pipe. In other words, this paper tries to find a direct correlation between the pipe to soil potential and the stray potential of the pipe for all boxes under test.

2. Literature Review
2.1 The Soil Factor
As the electrochemical properties of any soil are changed by the change of humidity but returns back to its initial conditions after some time required for soil dryness, we can define a new factor named the soil factor as: "The soil factor (S_f) is the instantaneous value of the electro-chemical properties of the soil based on the electrical properties at Humidity equal to 10% "[1] [2] and is equal to:

$S_f = (1 / K_S)$ pH H log   at room temperature (1)

Dimension of $[S_f] = [1/K_S]$ [pH] [H] [log] = .m %

Where:
S_f = soil factor   .m %
K_S = dielectric constant of the soil at H = 10%
pH = power of Hydrogen of the soil
H = humidity of the soil %
  = soil resistivity in   .m at H = 10%

Figure 1 shows the range values of the soil factor due to humidity change for 10 soil samples under test.
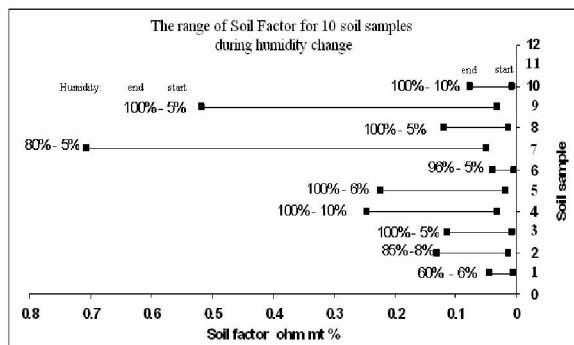
Figure 1: The range of the soil factor & humidity range for the soils under test

The importance of the soil factor is that it is combining all parameters which can affect directly on

the cathodic protection level or in corrosion process. Such factors which can be obtained by a direct measurements from the field. This means that if it is possible to study the relationship between the soil factor and the electrical parameters of the bare pipe segment, then the print curves and the print constants of the electrical parameters of the pipe-soil-earth system could be obtained at natural condition with and without applying cathodic protection system. The soil factor can be considered to be as the key of many studies based on the new proposed electrical concept of corrosion. For an example, the general equation of the natural stray capacitance between external surface area of bare pipe segment and earth is obtained in terms of the soil factor with an average error ± 30% and its print curves and constants are obtained for pipe-soil-earth system for 10 different soils [3]. Also, the general equation of both the natural stray potential and the natural created charge are obtained in terms of the soil factor with an average error ± 30% and their print curves and constants are obtained for pipe-soil system for 10 different soils [4] [5]. Finally, the error of the general equation of the electrical parameters reduced to ± 5% [6] [7] [8] [9].

2.2 Stray Potential General Equation For Pipe – Soil – Earth Under Test Without Applying CP System [10] [12]

For each soil under test and from natural stray potential curves and equations, it can easily observe that the general equation of the natural stray potential from pipe segment to the earth during humidity change is a 4th degree polynomial equation which is function of the soil factor, $V_{n\ stray} = f (X = S_f)$. The stray potential general equation is given by Eq.2:

$$V_{n\ stray} = A_{4vn}X^4 + A_{3vn}X^3 + A_{2vn}X^2 + A_{1vn}X + A_{0vn}$$
(2)

Where:
A's: = $A_{()V}$ are the natural stray potential print constants of the pipe soil under test
X = is the value of the soil factor at certain humidity

As an example, figure 2 shows the natural print of the stray potential of the pipe segments of boxes 1 & 18.

2.3 Natural Stray Potential Print Constants For Pipe-Soil-Earth Under Test
Now, the natural stray potential print constants of the pipe-soil-earth system under test are A_{4VN}, A_{3VN}, A_{2VN}, A_{1VN} and A_{0VN}. This means that these print values are valid for these pipe-soil systems under test at any time at the correspondent electrochemical properties (soil factor). Table 1 shows result example of the natural stray potential print.
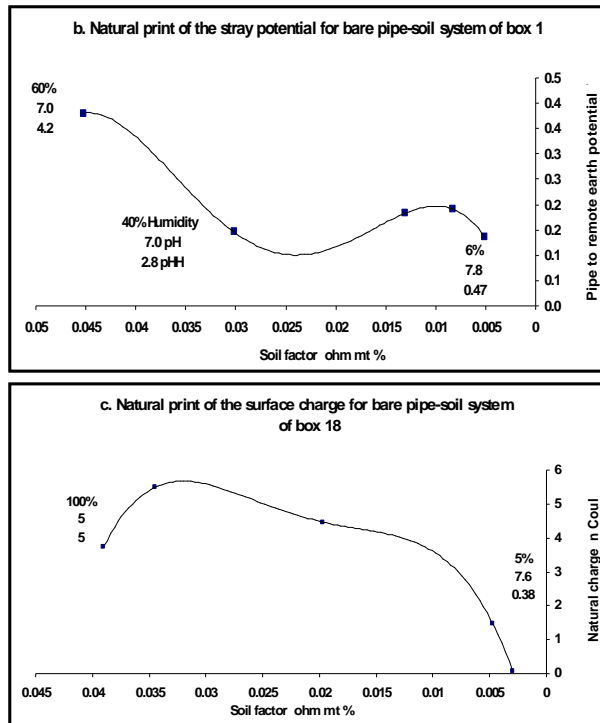
Figure 2: Stray Potential Prints for buried bare pipe-soil-earth of boxes 1&18

2.4 Stray Potential For Pipe-Soil-Earth Under Test With Applying CP System

From the stray potential PRINT curves and trend lines equations, as shown in figures 3b, 4b and 5b, it can easily observe that the general equation of the stray potential of a cathodically protected bare pipe segment during humidity change under multi level of cathodic protection levels is a 4th degree polynomial equation which is function of the soil factor $V_{Str.} = f (X = \text{soil factor})$. The stray potential general equation is equal to Eq. 3:

$$V_{Str.} = A_{4V}X^4 + A_{3V}X^3 + A_{2V}X^2 + A_{1V}X + A_{0V} \quad (3)$$

Where:

A's: = $A_{()V}$ are the stray potential print constants of the pipe - soil under test

X = is the value of the soil factor at certain humidity

As an example, figure 3 shows the natural print of the stray potential of the pipe segments of boxes 1 & 9.

2.5 Stray Potential Print Constants For Pipe-Soil-Earth Under Test

Now, the stray potential PRINT constants of the pipe-soil-earth systems under test are A_{4V}, A_{3V}, A_{2V}, A_{1V} and A_{0V} at a definite cathodic protection level.

This means that these print values are valid for these CP levels for these pipe soil systems under test at any time at the correspondent electrochemical properties (the soil factor). Table 2 shows result example of the stray potential print constants at CP level equal to -0.85 volt.
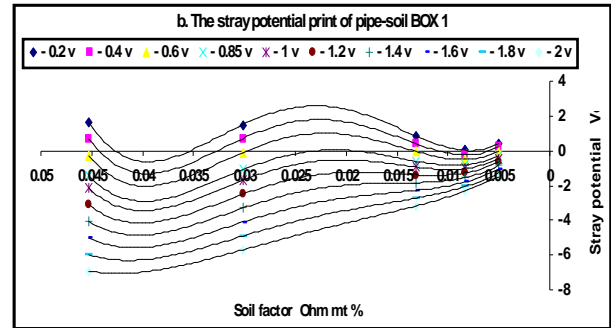
Figure 3a: Stray Potential PRINT curves of pipe-soil-earth of box 1 at multi of cathodic protection levels.
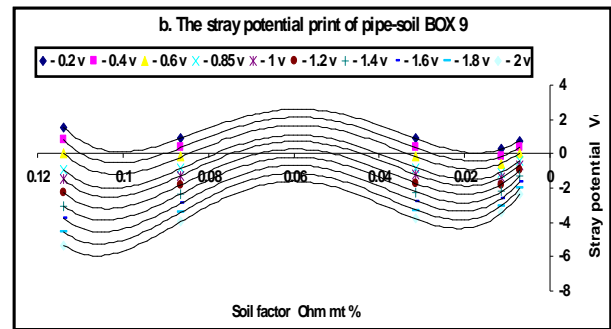
Figure 3b: Stray Potential PRINT curves of pipe-soil-earth of box 9 at multi of cathodic protection levels.

3. Analysis

As we said before, the print A's of the stray potential of the pipe segment to the earthing grid could be obtained from the general equation of the stray potential (3) and easily we can construct the print of the stray potential A's table for all boxes under test at pipe to soil potential, by the use of $Cu/CuSO_4$ half cell, from -0.2V up to -2V as per tables 2 as an example. The question now is: is it possible to rearrange the table results such that to be as stray potential A's for each box against the pipe to soil potential? This is as per tables 3 & 4 as an example for the stray potential A's against pipe to soil potential for boxes 10 & 13 respectively. What would be the results for all boxes under test?

Table 1: Natural PRINT constants of the stray potential for 10 different soil under test

Soil	1	2	3	4	5	6	7	8	9	10
Error	0	±30%	0	0	0	0	0	0	0	0
A_{4VN}	-2.E+06	0	321339	5620	8822.1	0	118.12	140960	0	546856
A_{3VN}	202688	698.68	-78007	-3154.1	-3710.9	-48181	-153.09	-37414	-110.64	-110280
A_{2VN}	-6782.5	-157.71	6025.9	599.26	407.17	2278.7	54.029	3218.8	100.61	7753.7
A_{1VN}	82.539	9.8253	-159.72	-44.958	-3.3452	-19.695	-3.1804	-99.254	-24.97	-215.37
A_{0VN}	-0.133	0.0009	1.377	1.412	0.0954	0.2352	0.1245	0.9629	1.2357	1.8678

Table 2: Stray potential finger print constants at pipe to soil potential equal to -0.85 volt

BOX	1	2	3	4	5	6	7	8	9	10
	1	4	9	10	13	18	19	24	27	28
A_{4V}	3.00E+07	0	925051	17354	1149.5	7.00E+07	861.17	0	2228.7	1.00E+06
A_{3V}	-3.00E+06	-1858.9	-223313	-9862.3	-4002.1	-7.00E+06	-1531.8	61.62	-2755.3	-194014
A_{2V}	91780	507.49	16975	1905.4	1569.6	211211	926.56	-232.3	1155.8	12478
A_{1V}	-1023	-37.7	-428	-143	-176.7	-2389.4	-204.8	35.484	-179.3	-321.02
A_{0V}	2.9327	-0.61	1.9	2.6	2.37	4.66	7.154	-2.19	4.49	1.6
Error	0	±30%	0	0	0	0	0	±30%	0	0

Table 3: Stray potential print constants of box10 at pipe to soil potential equal to -0.2 volt to -2 volt

	Box 10 4									
	-0.2	-0.4	-0.6	-0.85	-1	-1.2	-1.4	-1.6	-1.8	-2
A_4	2.43E+04	22128	20006	17354	15762	13640	11518	9395.4	7273.2	5151
A_3	-1.32E+04	-12146	-11131	-9862.3	-9100.9	-8085.8	-7070.7	-6055.6	-5040.4	-4025.3
A_2	2.37E+03	2224.1	2082.5	1905.4	1799.2	1657.6	1515.9	1374.3	1232.7	1091
A_1	-1.56E+02	-152.17	-148.12	-143.05	-140.02	-135.97	-131.91	-127.86	-123.81	-119.76
A_0	3.64E+00	3.3314	3.0217	2.6345	2.4023	2.0926	1.7828	1.4731	1.1634	0.8537
error	0	0	0	0	0	0	0	0	0	0

Table 4: Stray potential print constants of box 13 at pipe to soil potential equal to -0.2 volt to -2 volt

	Box 13 5									
	-0.2	-0.4	-0.6	-0.85	-1	-1.2	-1.4	-1.6	-1.8	-2
A_4	-2.52E+04	-17108	-8993.3	1149.5	7235.1	15349	23464	31578	39692	47806
A_3	8.50E+03	4654	806.87	-4002.1	-6887.5	-10735	-14582	-18429	-22276	-26123
A_2	-2.73E+02	293.88	860.87	1569.6	1994.9	2561.9	3128.8	3695.8	4262.8	4829.8
A_1	-8.76E+01	-115.06	-142.47	-176.73	-197.29	-224.7	-252.11	-279.52	-306.93	-334.45
A_0	2.14E+00	2.2143	2.2855	2.3745	2.4278	2.499	2.5702	2.6413	2.7125	2.7837
error	0	0	0	0	0	0	0	0	0	0

4. Results

4.1 A_{0V} print constant

The stray potential from the pipe segment to the earthing grid, A_{0V} print constant is linearly proportional to the pipe to soil potential V_{H-C} measured by $Cu/CuSO_4$ half cell. Figure 4 show boxes 10 & 13 as an example and the correlation between them is governed by equation 4 for all boxes under test as follow:

$$A_{0V} = B_{1A0V} \, V_{H-C} + B_{0A0V} \qquad (4)$$

4.2 A_{1V} print constant

The stray potential from the pipe segment to the earthing grid, A_{1V} print constant is linearly proportional to the pipe to soil potential V_{H-C} measured by $Cu/CuSO_4$ half cell. Figure 4 show boxes 10 & 13 as an example and the correlation between them is governed by equation 5 as follow:

$$A_{1V} = B_{1A1V} \, V_{H-C} + B_{0A1V} \qquad (5)$$

4.3 A_{2I} print constant

The stray potential from the pipe segment to the earthing grid, A_{2V} print constant is linearly proportional to the pipe to soil potential V_{H-C} measured by $Cu/CuSO_4$ half cell. Figure 4 show

boxes 10 & 13 as an example and the correlation between them is governed by equation 6 as follow:

$$A_{2V} = B_{1A2V} \, V_{H-C} + B_{0A2V} \qquad (6)$$

4.4 A_{3V} print constant

The stray potential from the pipe segment to the earthing grid, A_{3V} print constant is linearly proportional to the pipe to soil potential V_{H-C} measured by $Cu/CuSO_4$ half cell. Figure 4 show boxes 10 & 13 as an example and the correlation between them is governed by equation 7 as follow:

$$A_{3V} = B_{1A3V} V_{H-C} + B_{0A3V} \qquad (7)$$

4.5 A_{4V} print constant

The stray potential from the pipe segment to the earthing grid, A_{4V} print constant is linearly proportional to the pipe to soil potential V_{H-C} measured by $Cu/CuSO_4$ half cell. Figure 4 show boxes 10 & 13 as an example and the correlation between them is governed by equation 8 as follow:

$$A_{4V} = B_{1A4V} \, V_{H-C} + B_{0A4V} \qquad (8)$$

Table 5 shows the result table of protection current print constants (A's) in terms of pipe to soil potential for all boxes under test

Table 5: Stray potential print constants (A's) in terms of pipe to soil potential for all boxes under test

|		1	2	3	4	5	6	7	8	9	10
		Box 1	Box 4	Box 9	Box 10	Box 13	Box 18	Box 19	Box 24	Box 27	Box 28
A_{0V}	B_{1A0V}	2.19E+00	1.567	0.911	1.548	-0.355	-2.09E+00	-7.671	2.437	-2.635	1.1
	B_{0A0V}	4.80E+00	0.724	2.688	3.95	2.072	2.88E+00	0.632	-0.117	2.302	2.535
	error	0%	0	0	0	0	3%	0	0	1%	0%
A_{1V}	B_{1A1V}	-468.88	-8.6912	141.17	-20.257	137.08	1660.1	237.32	28.372	135.56	82.588
	B_{0A1V}	-1421.8	-45.09	-308.35	160.27	-60.217	-978.29	-3.0607	59.6	-67.017	-250.82
	error	0	0	0	0	0	0	0	0	1%	0
A_{2V}	B_{1A2V}	58664	865.2	-3960	708.1	-2834	-13567	-1225	-857.8	-493.2	-2781
	B_{0A2V}	14164	1020	13609	2507	-840	95891	-115.2	-961.4	747.3	10114
	error	0	± 70%	0	0	0	0	0	0	0	0
A_{3V}	B_{1A3V}	-2.00E+06	-7698	41703	-5075	19236	4.00E+06	2301	5996	428.4	55331
	B_{0A3V}	-5.00E+06	-8402	-18786	-14176	12348	-4.00E+06	424.3	5158	-2400	-14698
	error	±10%	0	0	0	0	±5%	0	0	±1%	0
A_{4V}	B_{1A4V}	2.00E+07	0	-99143	10611	-40571	-3.00E+07	-1424	0	129	-36192
	B_{0A4V}	5.00E+07	0	83364	26373	-33336	4.00E+07	-349.5	0	2335	66349
	error	10%		0	0	0	3%	0		1%	20%
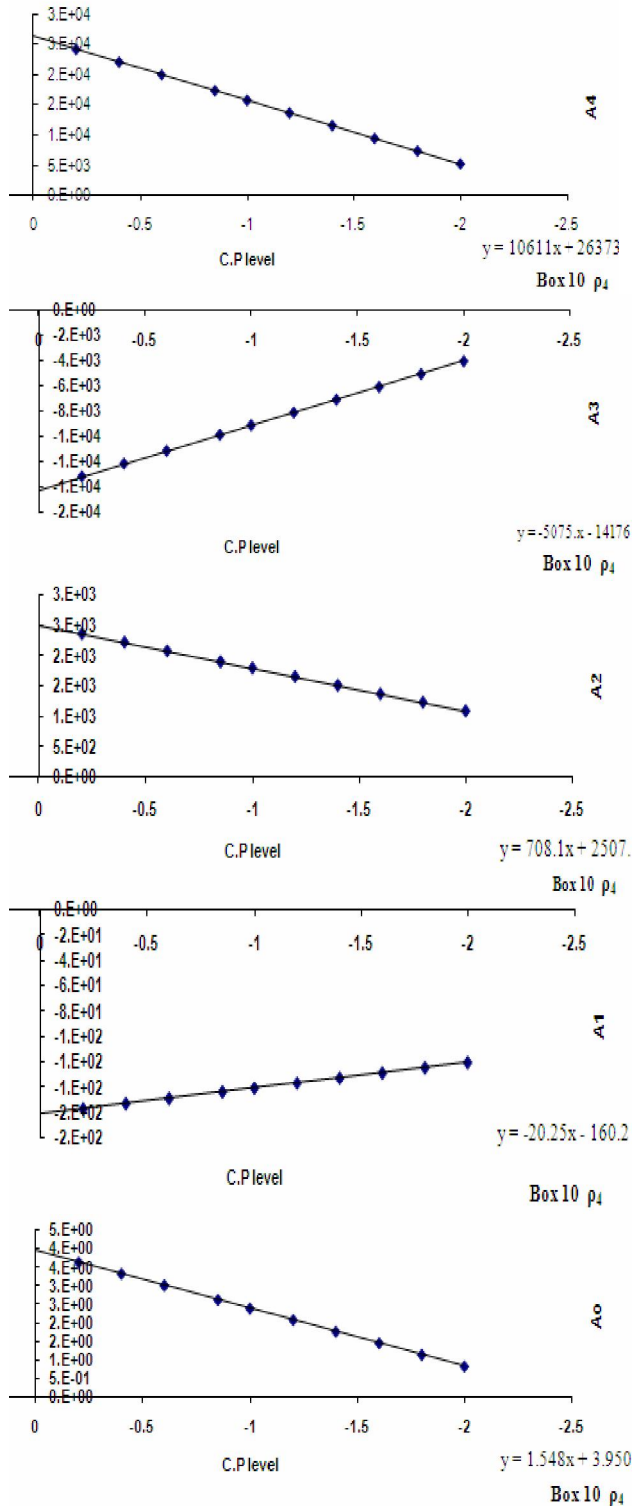
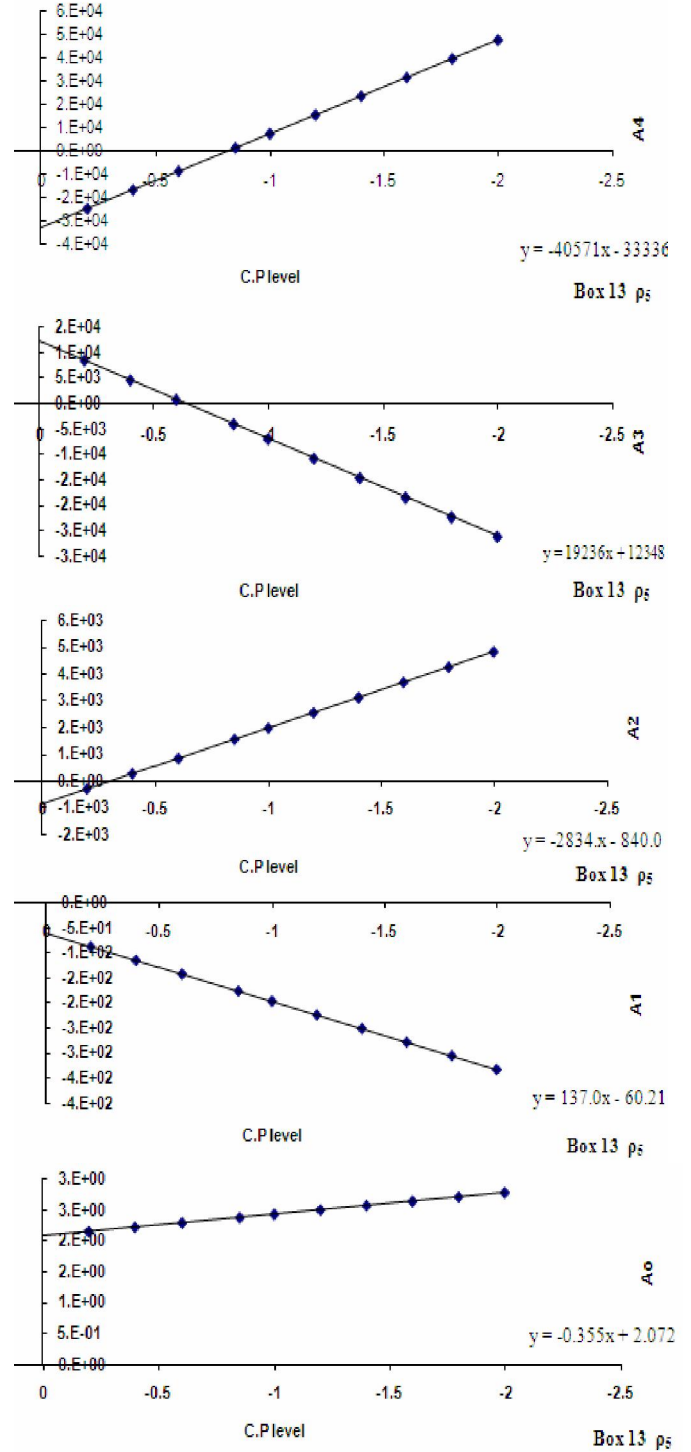Figure 4: The stray potential print constants against pipe to soil potential for box 10

Figure 5: The stray potential print constants against pipe to soil potential for box 13

4.6 The deduction of the general equation of the pipe to soil potential

We have the stray potential general equation from equation 3 as follow:

$$V_{Str.} = A_{4V}X^4 + A_{3V}X^3 + A_{2V}X^2 + A_{1V}X + A_{0V} \quad (3)$$

Where:

A's: = $A_{()V}$ are the stray potential print constants of the pipe - soil under test

X = is the value of the soil factor at certain humidity

By substituting the values of A's from equations 4, 5, 6, 7 and 8 in equation 3 , the general equation of the pipe to soil potential will equal to equation 9 as follow:

$$V_{H.C} = \frac{V_{Stray} - [\, B_{0A4V}X^4 + B_{0A3V}X^3 + B_{0A2V}X^2 + B_{0A1V}X + B_{0A0V}\,]}{[\, B_{1A4V}X^4 + B_{1A3V}X^3 + B_{1A2V}X^2 + B_{1A1V}X + B_{1A0V}\,]} \quad (9)$$

Where:

$V_{H.C}$: The equivalent value of the pipe to soil potential in volt measured by Cu/CuSO$_4$ half cell.

$V_{Str.}$: Stray potential of the pipe segment in Volt.

X: Segmental soil factor in .m%.

B's: New print constants of pipe-soil-earth system

Table 6 shows the error for all boxes under test while tables 7 & 8 are showing the detailed comparison between the pipe to soil potential obtained by equation 9 and the pipe to soil potential obtained by direct measurement by the use of Cu/CuSO$_4$ half cell.

Table 6: Comparison between theoretical and experimental values of pipe to soil potential of box 10

Box	Electrical Parameters			pH	H	Pipe to Soil Potential		
	V_{P-PE}	C_{P-PE}	I			Theoretical	Experimental	Error
	Volt	nF	mA		%	V_{HC} - Volt	V_{HC} - Volt	%
10	0.49	0.172	0.0015	7.8	10%	-0.314839657	-0.3000	4.7134014
10	-0.461	0.172	0.0060	7.8	10%	-0.9620273	-0.9600	0.2107321
10	-4.33	0.172	0.0175	7.8	10%	-3.595012572	-3.6000	-0.1387319
10	-5.07	0.172	0.0220	7.8	10%	-4.098607584	-4.1400	-1.0099141
10	-8.06	0.172	0.0335	7.8	10%	-6.133403645	-6.1000	0.5446184
10	0.2723	7.900	0.0046	7.7	18%	-0.182218469	-0.3063	-68.094926
10	-0.1923	7.900	0.1000	7.7	18%	-0.431641808	-0.4550	-5.4114757
10	-4.37	7.900	1.2600	7.7	18%	-2.674465499	-2.4960	6.6729408
10	-6.79	7.900	1.9600	7.7	18%	-3.973657238	-3.8500	3.1119251
10	-9.38	7.900	2.7500	7.7	18%	-5.364114513	-5.3500	0.2631285
10	-10.5	7.900	3.1400	7.7	18%	-5.965393335	-6.1300	-2.7593598
10	0.21	33.400	0.0023	7.0	65%	-0.43028885	-0.5010	-16.433414
10	-0.315	33.400	0.5000	7.0	65%	-0.657031363	-0.6600	-0.4518258
10	-3.38	33.400	5.5400	7.0	65%	-1.980775742	-1.8600	6.0973961
10	-6.1	33.400	11.2100	7.0	65%	-3.1555179	-3.2000	-1.4096608
10	-9.5	33.400	17.0000	7.0	65%	-4.623945597	-4.6500	-0.5634669
10	0.145	49.600	0.0018	6.5	88%	-0.432519024	-0.5380	-24.387592
10	-0.29	49.600	0.1850	6.5	88%	-0.615311165	-0.6400	-4.0124146
10	-3.93	49.600	10.6000	6.5	88%	-2.144882184	-1.9900	7.2210112
10	-6.31	49.600	17.6000	6.5	88%	-3.144986311	-3.0900	1.74838
10	-9.6	49.600	30.0000	6.5	88%	-4.527483193	-4.6500	-2.7060687
10	0.244	89.000	0.0048	6.0	100%	-0.564233649	-0.6350	-12.542029
10	-0.006	89.000	0.1530	6.0	100%	-0.656040154	-0.6700	-2.1278951
10	-3.56	89.000	18.8000	6.0	100%	-1.961161418	-1.9000	3.1186325
10	-5.42	89.000	34.2000	6.0	100%	-2.64420181	-2.5300	4.3189521
10	-6.72	89.000	44.0000	6.0	100%	-3.121595632	-3.1800	-1.870978
10	-8.45	89.000	55.0000	6.0	100%	-3.756896641	-3.8400	-2.2120214

Table 6: Error table between theoretical and experimental values of pipe to soil potential for all boxes under test

Resistivity	1	2	3	4	5	6	7	8	9	10
Box No.	1	4	9	10	13	18	19	24	27	28
Av. Error	H	± 5 %	± 40 %	± 5 %	± 5 %	H	± 5 %	± 40 %	± 5 %	± 5 %

Table 7: Comparison between theoretical and experimental values of pipe to soil potential of box 13

	Electrical Parameters					Pipe to Soil Potential		
Box	V_{P-PE}	C_{P-PE}	I	PH	H	Theoretical	Experimental	Error
	Volt	nF	mA		%	V_{HC} - Volt	V_{HC} - Volt	%
13	0.145	0.000	0.0019	7.3	6%	-0.505960215	-0.4730	6.5143887
13	-0.705	0.000	0.0067	7.3	6%	-1.163633299	-1.1390	2.1169297
13	-4.06	0.000	0.0224	7.3	6%	-3.759507649	-3.9000	-3.736988
13	-4.9	0.000	0.0275	7.3	6%	-4.409443403	-4.4100	-0.0126228
13	-7.13	0.000	0.0394	7.3	6%	-6.134868083	-6.0700	1.0573672
13	0.2796	8.700	0.0044	7.6	10%	0.315257985	-0.2900	191.98815
13	-2.575	8.700	0.0800	7.6	10%	-1.358426955	-0.4860	64.223325
13	-3.81	8.700	0.7000	7.6	10%	-2.082521715	-2.1100	-1.3194717
13	-5.66	8.700	1.0100	7.6	10%	-3.167198076	-3.2220	-1.7302967
13	-8.49	8.700	1.5900	7.6	10%	-4.826459752	-4.8500	-0.4877332
13	-10.79	8.700	2.0200	7.6	10%	-6.174976309	-6.3600	-2.9963466
13	0.288	31.900	0.0024	6.9	80%	-0.441743481	-0.4960	-12.282359
13	-0.211	31.900	0.9200	6.9	80%	-0.670961813	-0.6590	1.782786
13	-3.77	31.900	10.8000	6.9	80%	-2.305807595	-2.2900	0.6855557
13	-6.44	31.900	18.8000	6.9	80%	-3.532286449	-3.7100	-5.0311195
13	-9.11	31.900	27.4000	6.9	80%	-4.758765302	-4.8600	-2.1273312
13	0.146	109.200	0.0019	5.2	100%	-0.648122287	-0.6880	-6.1528069
13	-0.244	109.200	1.0800	5.2	100%	-0.793995223	-0.7700	3.0220866
13	-3.4	109.200	30.0000	5.2	100%	-1.974443905	-2.0000	-1.2943439
13	-6	109.200	56.0000	5.2	100%	-2.946930144	-3.0400	-3.1581969
13	-9.5	109.200	85.0000	5.2	100%	-4.256046236	-4.3200	-1.5026567
13	0.133	243.000	0.0014	5.0	100%	-0.660302144	-0.7010	-6.1635202
13	-0.14	243.000	0.1870	5.0	100%	-0.761080019	-0.7840	-3.0115074
13	-2.87	243.000	19.1000	5.0	100%	-1.768858767	-1.7500	1.0661545
13	-4.6	243.000	60.5000	5.0	100%	-2.407487791	-2.4200	-0.5197206
13	-6.3	243.000	87.0000	5.0	100%	-3.035042323	-3.0800	-1.4812867
13	-7.55	243.000	108.0000	5.0	100%	-3.496479478	-3.6000	-2.9607073

5. Conclusion

One of the most critical problems in CP systems is the presence of the earthing network beside the protected pipe line. Electrochemistry helps to determine the integrity of buried pipe from corrosion by measuring pipe to soil potential by the use of Cu/CuSO$_4$ half cell. In electrical study of pipe-soil-earth system we are now able to calculate that value from the stray potential of the pipe segment as an electrical parameter , the electrochemical properties of the soil around this pipe segment as the soil factor and finally by the use of B's print constants of the pipe –soil - earth system. This will help to calculate the correspondent value of the pipe to soil potential for each segment of the pipe however long it is in the presence of earthing grids. This will help in pipeline both mentoring and maintenance, not only that but also to define both of the most proper rectifier output voltage and the proper distance between the protected pipe line and the earthing grid.

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In Defense Of Thermoluminescence Dosimeter Zero Dose Readouts

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Abstract: Zero dose readout of thermoluminescence dosimeters is a very important parameter which is considered in all accurate dosimetry procedures in order to correct for the additive doses arising from other sources than irradiation processes, however, in many cases this parameter is neglected. In this paper, effects of zero-dose readings for three different thermoluminescence dosimeters glow curves were investigated. Dosimeters included in this study are: sensitized TLD-700, sensitized TLD-600, and CaF_2:Tm (TLD-300). Deconvolution of glow curves was performed in order to investigate individual behavior of each glow peak using a GCAFIT glow curve analysis software. It was found that readout of zero doses usually accompanied by changes in glow curves quantitatively (i.e. area under the curve increases), and qualitatively (relative changes in glow curve peaks intensities and their maxima positions). It is recommended that, even if the zero dose value is to be neglected as an added value to be subtracted, zero dose readouts should be performed for enhancements arise in thermoluminescence glow curves and hence better performance. This behavior is verified even LiF detectors were sensitized or not. In contrary, for CaF_2:Tm (TLD-300), a little effect is noticed because there is no thermal quenching effect and competing deep trap in this material.

1. Introduction:

Dosimetry is a fundamental part in quality programs assuring that the irradiation procedure is carried out according to standard regim. To assess the radiation doses precisely, the sources of dose errors should be identified and minimized (Robertson 1981 and Rocha et al., 2003). There are several techniques for radiation dosimetry (Schönbacher H. et al., 2009), among all these techniques, thermoluminescence dosimetry (TLD) is now the most widely used technique in many fields like personal and environmental radiation exposure (Gilvin and Perks, 2010, Sandouqa et al., 2011), geochronology and space dosimetry. In addition, TLDs are extensively used in both of diagnostic and therapeutic medical applications (Abd El-Hafez et. al., a and b, Czopyk et al., 2007, Gual M. et al., 2011, Sharma et al., 2011 and Shousha et. al., 2011), and in other health related aspects (Vandana et al., 2011) as well.

There are many of well-known thermoluminescent (TL) dosimeters used in radiation monitoring due to their high sensitivity, stability and tissue equivalency (Haiyong Jung et al., 2003). Thermoluminescence phenomena results in forming the characteristic glow curves for the phosphor material. The glow curves obtained for each material are different, and each glow peak is ascribed to the recombination centers and is related to traps (Furetta, 2011).

Typically, standard practice procedures for TL dosimetry involve pre-irradiation background readings of TLDs especially at low dose levels, to be subtracted from readings of irradiated dosimeters (ASTM 1998, Izewska, et al., 2007). However, some experimentalists do skip this procedure at different circumstances: (1) if dosimeters are being irradiated to high doses compared to which background values could be neglected, (2) when high level of dosimetry with minimized uncertainties is not within the scope, (3) when dealing with large number of dosimeters, it was thought to be enough to select randomly few dosimeters for pre-irradiation background measurements instead of reading all dosimeters for saving time, and (4) when the glow curves is a subject of an analytical study regardless of doses delivered (Ixquiac-Cabrera, et al., 2011, Harvey, and Kearfott, K. 2011).

Recently, (Abd El-Hafez and Maghraby 2011) noticed that readouts of LiF based TL dosimeters background before irradiation (regardless of values of background) can enhance TL output and may cause changes to the glow curves both qualitatively in terms of peaks positions and peaks relative intensities, and quantitatively in terms of areas under the curve. Hence, it is of some

importance to investigate if this enhancement in glow curves is observable in other TL materials and the effect of sensitization.

One of the major problems that afflict TLD in general is the complexity of the glow curve obtained with many TLD materials. Hence, a glow curve analytical toolkit (GCAFIT) was used in this study for assessing individual behavior of each peak in terms of both intensity and position (Abd El-Hafez et al., 2011).

2. Experimental Work
2.1. Radiation source and dose determination:
The irradiation were performed using the ^{137}Cs gamma source model GB-150, was manufactured by Atomic Energy of Canada Limited on April 1970 with original activity of 1000 Ci.

Air kerma (K_{air}) was determined according to the International Atomic Energy Agency (IAEA) code of practice TRS-(381) (IAEA, 1997). K_{air} determination was performed using the secondary standard dosimetry system of the National Institute of Standards (NIS) - Egypt, which is composed of the NPL-2560 electrometer (UK) and its NE2561 ionization chamber (UK),. The secondary standard calibration system was calibrated at the Bureau International des Poids et Mesure (BIPM). The expanded uncertainty associated to K_{air} determination was about 0.9 % at 95% level of confidence (coverage factor = 2).

2.2. Thermoluminescent dosimeters and reader:
Three types of TL dosimeters were incorporated in this study, those types are sensitized TLD-700, sensitized TLD-600, and CaF_2:Tm (TLD-300) in the form of chips with dimensions of 6.4 x 6.4 x 0.9 mm^3. The Harshaw 4500 TLD Reader is equipped with two photomultiplier tubes that can read independently; the reader operates on WinREMS software, which runs under Windows® on a separate computer. All dosimeters and reader were manufactured by Harshaw Chemical Co. (USA).

2.3. Experimental procedures:
The sensitization process was performed through exposing the TL dosimeters to about 20 KGy gamma rays. TL dosimeters were annealed, and kept in dark. All dosimeters were divided into two sets; each set is composed of eight dosimeters. Experimental procedures are composed of two main steps, in the first step: background readings were performed for the first set (S_1), while the second set (S_2) were left without reading of the background. The two sets were grouped together and exposed to a

well-defined radiation dose from ^{137}Cs gamma source. Dosimeters readings were acquired using linear heating rate equals to 1 °C /s over the range 100 - 400 °C. This low heating rate was used to avoid extreme overlapping in glow peaks (Abdel-Hafez, 1999, Yazici. 2004). After readout of dosimeters, annealing was performed as one hour at 400 °C, and slowed down at room temperature, followed by two hours at 100 °C for LiF and 1 hour for CaF_2.

The second step involves the reverse of the two sets, where pre-irradiation background was evaluated for S_2 while S_1 were left without reading of the background. The two sets were grouped together again and exposed to almost same radiation dose. Dosimeters readings were acquired using the same conditions.

A control set of TLD-100 was used during the two steps to assure that irradiation process in the two steps was similar; annealing and readout conditions were performed in the same way as the investigated dosimeters.

Thus the same dosimeter passed the two cases; the first case (Case A) represents the readout of dosimeters irradiated without experiencing pre-irradiation background measurements, while the second case (Case B) represents the readout after gamma irradiation for dosimeters experienced pre-irradiation background measurements (Abd El-Hafez and Maghraby 2011).

2.4. Deconvolution:
Glow curves were deconvoluted using GCAFIT software which is a computerized glow curve analysis program was written using the MATLAB technical computing language and developed at the National Institute. of Standards (NIS), Egypt (Abd El Hafez et al., 2011). GCAFIT software uses the nonlinear least-square method with the Levenberg–Marquardt algorithm. The results of the fitting process are estimates of the model coefficients, It worth to confirm that deconvolution processes were performed over the experimentally studied range of temperature (100 - 400 °C), hence, whole first peak (P_1) was not involved.

3. Results and Discussion:
3.1. Sensitized TLD-700:
Figure (1) represents the two cases (A and B) for sensitized TLD-700 dosimeters where triangles represent case (A) while circles represent case (B). Obviously there are several changes occurred, first of all the averaged total area under the curve has been increased almost twice (211 % ± 1.1 %) when the pre-irradiation background readings were considered. When comparing the two glow

curves, it is easy to notice that the increase in sensitivity is not uniform over the heating range; hence a glow curves deconvolution was a necessity for estimating individual behavior of each peak separately.
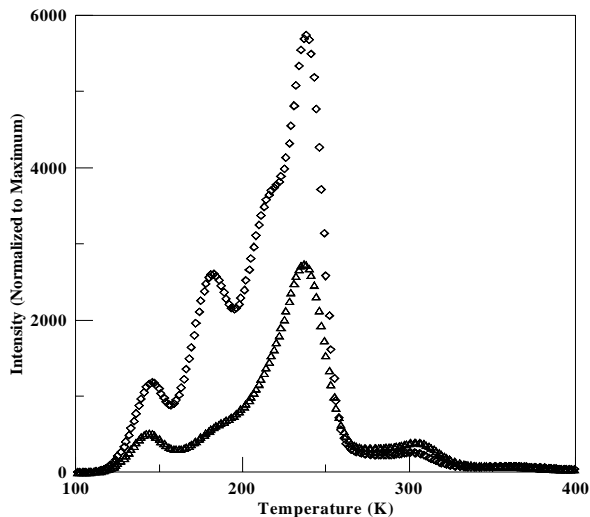

Figure (1): LiF (TLD-700) glow curve with heating rate 1 °C/s, Case A (triangles), and Case B (circles).
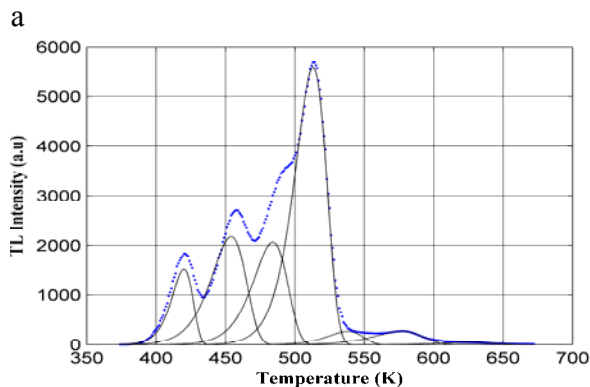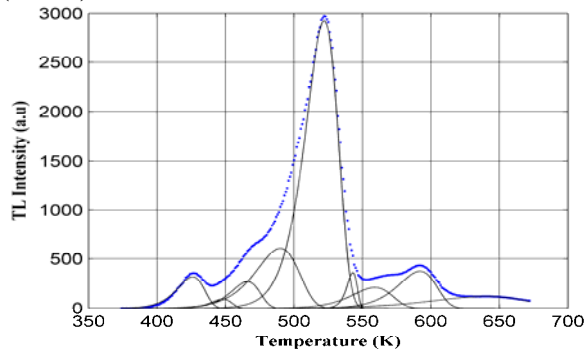

a

b

Figure (2): Computerized Glow Curve Analysis (GCAFIT) of one of TLD-700 samples, a- Case A, b- Case B.

Figure (2) represent the deconvoluted glow curves of gamma irradiated TLD-700 sensitized dosimeters: Figure (2-a) represents the deconvoluted glow curve of dosimeters irradiated without performing pre-irradiation background readouts, it has been deconvoluted into nine peaks (P_2 , P_{2a}, P_3, P_4, P_5, P_6, P_{7a}, P_7, and P_8). The regression coefficients of fitting (R^2) were ranged from 0.898 to 0.998. On the other hand, case (B) dosimeters glow curves were deconvoluted into seven peaks (P_2: P_8) as shown in Figure (2-b), and The regression coefficients of fitting (R^2) were ranged from 0.910 to 0.999.
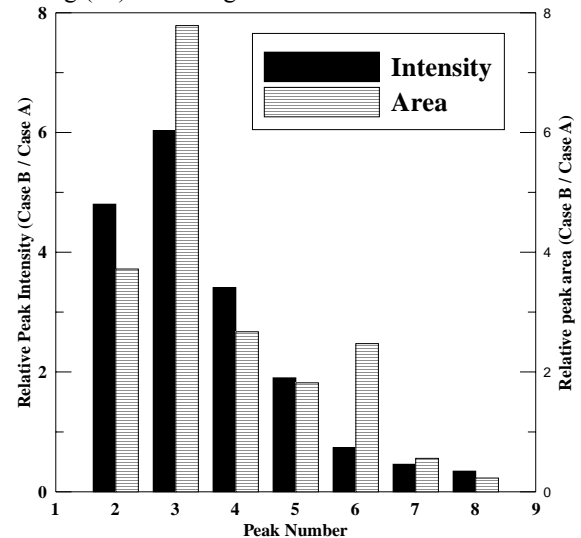
Figure (3): Changes in intensities and areas of resolved peaks in one of TLD-700 dosimeters, Solid denotes to intensity, and striped denotes to area.

Figure (3) represents the relative changes both in peak intensity and peak area (Case B / Case A). From the Figure, it is clear that maximum change either of peak intensity or peak area point of view was for the third peak (P_3), where peak intensity was increased six times and peak area was increased by a factor of 7.79 as a result of zero dose readout. Other peaks show remarkable increments in both of peak intensity and peak area. Those peaks are P_2 with relative change in peak intensity and peak area factors of 4.8 and 3.7 respectively, followed by P_4 whose intensity has been increased by a factor of 3.4 and 2.7 for peak intensity and peak area increment. P_5 was increased in peak intensity by a factor of 1.9 and in peak area by a factor of 1.8. P6 was increased in peak area by a factor of 2.5 while its intensity decreased a little and the ratio is 0.74. Remaining peaks (P_7 and P_8) showed a reverse behavior where both have been decreased in their intensities (ratios are 0.46 and 0.36) and their areas (ratios are 0.56 and

0.23) respectively, noting that P_7 in case (B) has been compared to the sum of P_7 and P_{7a} in case (A).

Other changes in glow curves arose after switching to case (B) were changes in peak maxima positions (T_{Max}), as represented by Figure (4) which clarifies the shift of two peaks (P_3 and P_7) toward higher temperature direction, which has been shifted by 6 ^{o}C and 18 ^{o}C respectively. Remaining peaks showed a shift toward lower temperature direction: 6 ^{o}C for P_2 and P_4, 5 ^{o}C for P_6, and 13 ^{o}C for P_8.

This shift in the temperature was held responsible for the increase in the contribution of non-radiative transitions due to the presence of the competing traps. It was inferred that the glow peaks occurring at higher temperatures must exhibits higher thermal quenching. This is attributed to that the energy dissipated in an indirect transition, however, is much less than the band-gap energy and may thus be dissipated either radiatively (via photons) and non-radiatively (via phonons). Non-radiative capture of free carriers takes place because the lattice vibrations cause the energy level to change its position in the forbidden gap. For large enough vibrations, the energy level crosses into the conduction band and captures a free electron. The lattice relaxation which follows the capture lowers the position of the level back into the energy gap, the excess energy being propagated as lattice phonons (McKeever, 1985). However, the glow curve is controlled by the release of the charge carriers from traps and not by the properties of the luminescence centers (Horowitz, 1984).
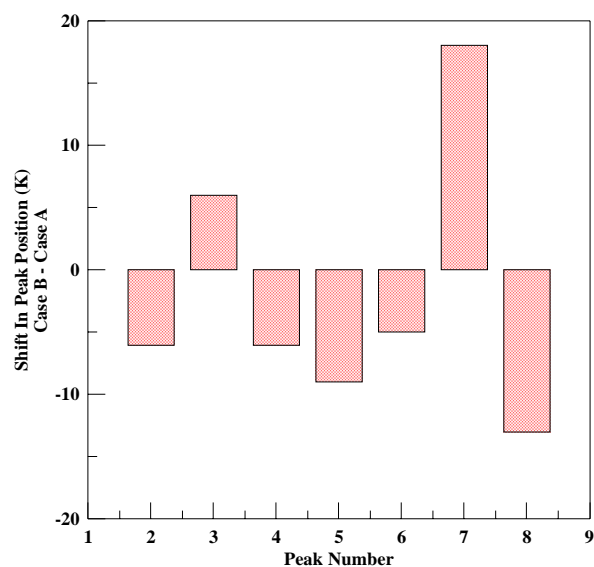
Figure (4): Possible shift in peak position (T_{Max}) of resolved peaks in one of TLD-700 dosimeters.

3.2. Sensitized LiF (TLD-600):

Figure (5) represents glow curves of irradiated TLD-600 sensitized dosimeters, where triangles denote to case (A) and circles denote to case (B). As was the case in sensitized TLD-700, averaged total area under the glow curve has been increased dramatically by a factor of 2.06 ± 0.06 when performing pre-irradiation background readouts (Case B). As shown in Figure (6-a), best fit of case (A) glow curve results in eight peaks (P_2, P_3, P_4, P_5, P_6, P_{7a}, P_7, and P_8). Coefficients of determination (R^2) of fitting were in the range 0.878:0.998, while case (B) glow curve deconvolution results in seven peaks (P_2: P_8) with (R^2) in the range of 0.847: 0.999 as represented in Figure (6-b) in which peak P_{7a} is not seen.
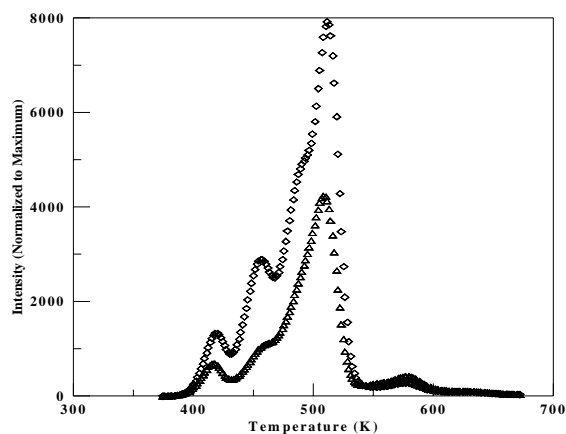
Figure (5): TLD-600 glow curve with heating rate 1 K/s, Case A (triangles), and Case B (circles).

Figure (7) represents the changes in glow curves of LiF (TLD-600) sensitized dosimeters in terms of peak intensities and areas. Maximum increase belongs to P_3, where peak intensity has been increased by a factor of 5.39 and peak area has been increased by a factor of 4.60 after switching to case (B), followed by P_4 which has been increased in intensity by a factor of 3.45 and in area by a factor of 2.87. P_5 has been almost doubled in both of peak intensity (factor of 2.12) and peak area (factor of 2.04). Also, a slight increase was noticed in P_8 intensity and area by factors of (1.17 and 1.39) respectively. P_2 and P_6 shows a reasonable stability where P_2 intensity has been changed only by a factor of 0.82 and its area by a factor of 0.91, while P_6 intensity has been changed by a factor of 0.91 and a factor of 1.26 for its area. On the other hand, P_7 was the only peak which exhibited a decrease in intensity and the factor is 0.58 and in area the factor is 0.50 after switching to case (B).

The change in resolved peaks maxima positions (T_{Max}) as a result of considering pre-

irradiation background reading is represented by Figure (8). All peaks maxima positions have been shifted toward higher temperature direction except for P_6 which shows a shift (3 ^{o}C) toward lower temperature direction. P_8 showed maximum shift (10 ^{o}C), followed by both of P_4 and P_5 (9 ^{o}C), P_2 (7 ^{o}C), P_3 (6 ^{o}C), and a slight shift was in case of P_7 (2 ^{o}C).
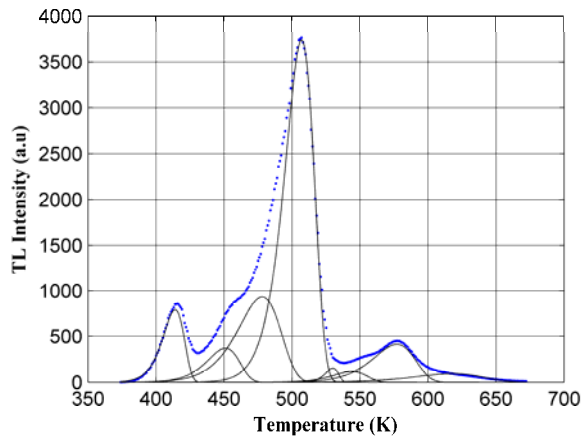
a
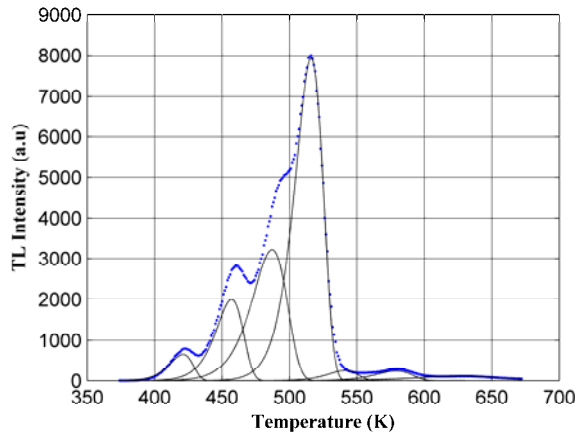
b

Figure (6): Computerized Glow Curve Deconvolution (GCAFIT) of one of TLD-600 samples, a- Case A, b- Case B.

3.3. CaF_2 (TLD-300)

Figure (9) represents the two cases: A, and B, triangles curve represents case A, and the circles one represents case B. The average of the total area under the glow curve after considering pre irradiation readouts has been increased by a factor of about 1.13 with percentage standard deviation σ equals to ± 7.8 %. As TLD models can be classified as models in which the critical processes occur during the absorption of radiation, and models in which the critical processes occur during heating (i.e. during

TL readout). The evidence, at least for LiF-based materials, clearly shows that the critical mechanism is that of competition-during the stage of TL readout. During heating, electrons released from traps, may either recombine with trapped holes to produce TL or be retrapped in deeper traps, which act as competing centers. The competition – during heating process was first suggested by Rodine and Land 1971 and was later analysed mathematically by Kristianpoller et. al. 1974.
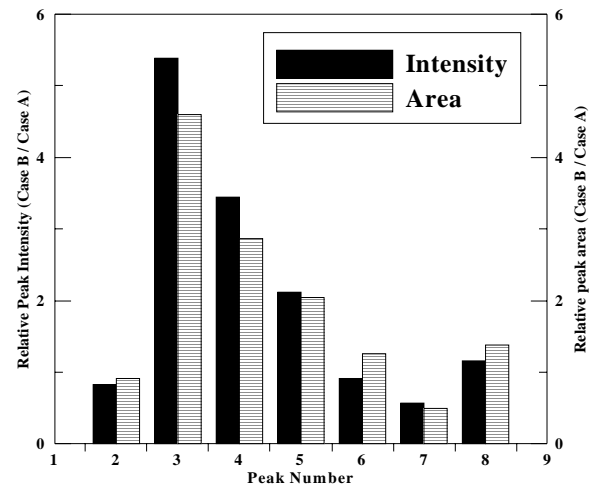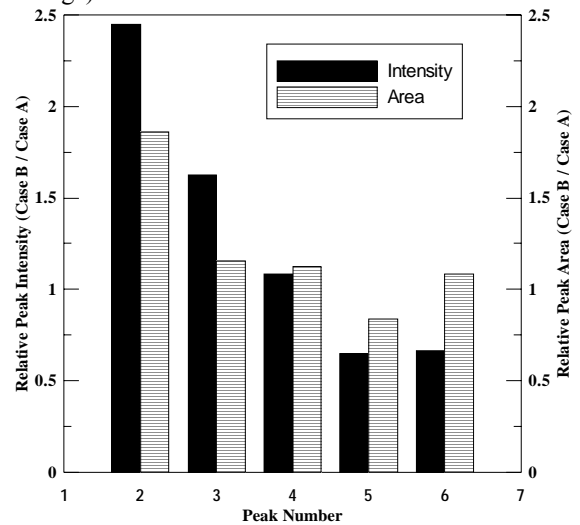

Figure (7): Changes in intensities and areas of resolved peaks in one of TLD-600 dosimeters, Solid denotes to Intensity, and striped denotes to Area.
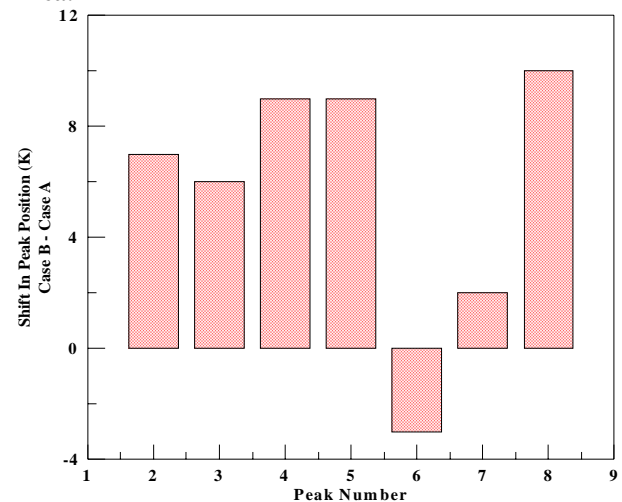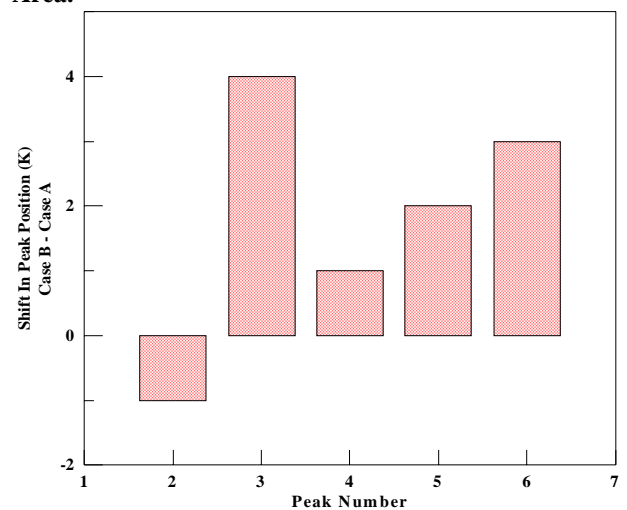

Figure (8): Possible shift in peak position (T_{Max}) of resolved peaks in one of TLD-600 dosimeters.

Deconvolution of TLD-300 dosimeters glow curves results at best fit in five peaks namely (P_2 : P_6) in both cases: Case (A) in Figure (10- a), and case (B) in Figure (10-b).
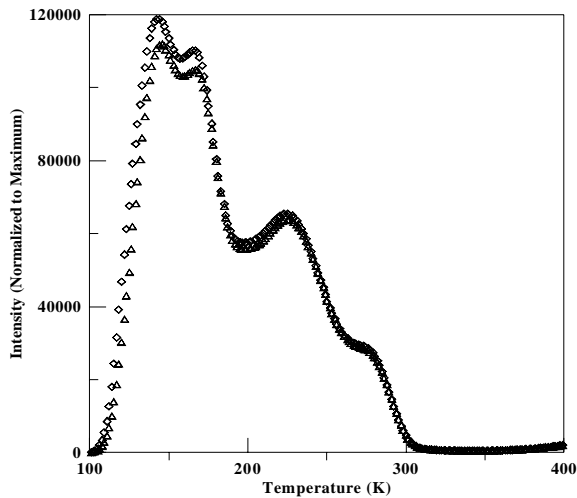
Figure (9): TLD-300 glow curve with heating rate 1 K/s, Case A (triangles), and Case B (circles).
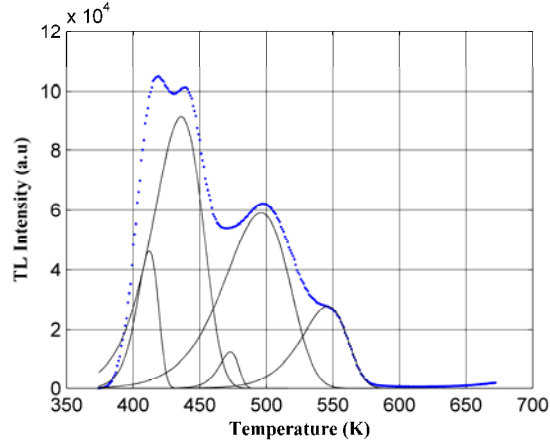
a
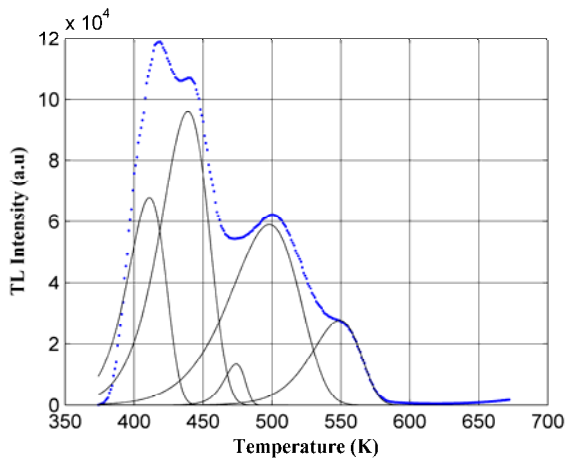
b

Figure (10): Computerized Glow Curve Deconvolution (GCAFIT) of one of TLD-300samples, a- Case A, b- Case B.

As shown in Figure (11), the relative changes in averaged intensities of different peaks were as the following: P_2 intensity has increased by a factor of 2.45 in case B compared to its value in case A, and its area has been increased by a factor of 1.86, Also, P_3 intensity has increased by a factor of 1.63, and its area has been increased by a factor of 1.57, while P_4 shows a slight increase as a result of zero dose readout in intensity and area by factors of 1.08 and 1.13 respectively. On the other hand P_5 exhibited a decrease in intensity and the factor is 0.65 and a slight decrease in Area and the factor is 0.8. P_6 intensity has been decreased and the factor is 0.66 while its area almost was not changed (1.08 factor of change).

Figure (11): Changes in intensities and areas of resolved peaks in one of TLD-300 dosimeters, Solid denotes to Intensity, and striped denotes to Area.

Figure (12): Possible shift in peak position (T_{Max}) of resolved peaks in one of TLD-300 dosimeters.

Figure (12) represent possible shift in peaks maxima positions (T_{Max}) after performing zero-dose readings for TLD-300 dosimeters. Maximum shift toward higher temperature side was in P_3 (4 ^{o}C), followed by P_6 (2 ^{o}C), then P_5 (2 ^{o}C), and P_4 (T_{Max}) has been shifted by 1 ^{o}C only. Toward lower temperatures, a slight decrease in (T_{Max}) of P_2 has been occurred (1 ^{o}C). Kafader et. al., 2009 showed that the linearity of glow peaks of TLD-300 crystal is not affected with the change of heating rate. i. e. there is no thermal quenching. Thermal quenching was understood to be due to the increased probability of nonradiative transitions competing with the radiative transitions.

4. Conclusion

It may be concluded that pre-irradiation background measurements for the studied TLDs is not only important for evaluation of residual doses when considering high level of accuracy, but its most importance (as a form of heat treatment) in the enhancement of their sensitivities and adjustment of glow curves peaks distribution and relative ratios. Impacts on glow curves enhancement were dramatically in cases of sensitized TLD-700 and sensitized TLD-600, and were minor in case of TLD-300 dosimeters.

As TLD models can be classified as models in which the critical processes occur during the absorption of radiation, and models in which the critical processes occur during heating (i.e. during TL readout). The evidence, at least for LiF-based materials, clearly shows that the critical mechanism is that of competition-during the stage of TL readout. CaF_2:Tm (TLD-300) clearly shows that the critical mechanism is that of competition-during the stage of absorption of radiation. Hence, it is much recommended to perform background measurements before irradiation regardless how high the radiation dose is, and even the background value will be neglected either if the thermoluminescent dosimeters were sensitized or unsensitized. The condition of better enhancement is that the TL material should have the critical mechanism is that is that of competition during the stage of readout. Further studies should be performed soon regarding behavior of each resolved peak as a function of radiation quality and dose level effects.

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Early Post-Percutaneous Coronary Stent Intervention Period: Is The Tooth Extraction Safe?

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Abstract:

 Objectives: Several risk factors can affect the cardiovascular outcome resulted from minor dental surgical procedures in patients received Percutaneous Coronary Stent Intervention especially in the early postoperative phase. So, the aim of this study was directed to evaluate the cardiovascular changes and post-operative bleeding occurred during the early post- PCI period (early six months) for patients subjected to tooth extraction and received L.A drug with or without vasoconstrictors in the presence or absence of preoperative sedation.

 Patients & Methods: Fourty four patients included within this study were subjected to tooth extraction during the early 6 months following percutaneous coronary stent intervention (PCI).   Patients were divided equally into four groups. In 1st group, patients received L.A with vasoconstrictor under preoperative sedation. While, 2nd group was similar but without the presence of preoperative sedation. In 3rd group, patients received L.A without vasoconstrictor under the presence of preoperative sedation. The 4th group was similar to 3rd group but without the presence of preoperative sedation. Systolic, diastolic blood pressure, heart rate and ST segment deviation were recorded for patient assessment.

 Results: No significant difference between the 1st and 4th group regarding to Mean Bp (P=0.130), Mean HR (P=0.080)and Mean ST segment deviation(P=0.205)and Sys Bp.(P=0.417). A significant difference between the 2nd group and 3rd group regarding to Sys. BP. (P=0.000) Diastolic Bp (P=0.004), Mean HR (P=0.000) and Mean ST segment deviation (P=0.000)

Conclusion :Combined role of presence or absence of presedation and vasoconstrictor in PCI patients subjected to tooth extraction can play a dramatic effect on cardiovascular parameters rather than each of them separately for the same type of patients.

1. Introduction:

The reported incidence of cardiovascular disease (CVD) among dental patients is 15%. [1] In general, elective dental treatment has traditionally been contraindicated, except for conservative emergency procedures, in patients with unstable angina pectoris (UAP) and within 6 months after onset in patients who have experienced acute myocardial infarction (AMI).[2] Such relative contraindication was attributed to the presence of vasocontrictors which is considered as one of the most essential prerequisite to success in dentistry through achieving good-quality local anesthesia (LA). [3] However, vasoconstrictors have several physiological responses including changes in heart rate and blood pressure. [4] These changes are regulated by the net balance between sympathetic and parasympathetic activity, and both stress and pain will further modify autonomic response.[5]

On the other hand, all physiological responses toward vasoconstrictors beside patients stress can share as a risk factors in aggravating the complications that might be resulted especially for patients received percutaneous coronary stent intervention. Stent thrombosis in percutaneous coronary stent intervention (PCI) are most commonly occurs in the first month after stent implantation, and in this interval, it is referred to as "subacute stent thrombosis." However, numerous cases of "late" stent thrombosis, particularly in patients who have been treated with DES, have been described as occurring months or even years after stent implantation. [6]

In the majority of cases, stent thrombosis is a catastrophic event, resulting in life-threatening complications. Spertus et al., declared that mortality rates due to presumed or documented stent thrombosis range from 20% to 45%.[6] In the current

era of dual antiplatelet therapy, the average reported occurrence of subacute stent thrombosis is 1%.[7]

However, recommendations for use of epinephrine in clinical dental practice are not in full agreement, because of the unwanted side effects.[8] Opinion about the use of epinephrine in patients with cardiovascular diseases and hypertension is also divided. To avoid adverse cardiovascular effects, especially in high-risk patients, some authors have recommended the use of epinephrine free anesthetics.[9] On the other hand, inadequate anesthesia results in stressful pain for the patient, whose body then releases greater amounts of endogenous catecholamine than that used in dental anesthesia.[10]

So, the aim of this study was directed to evaluate the cardiovascular changes and postoperative bleeding occurred during the early post-PCI period (early six months) for patients subjected to tooth extraction and received local anesthesia drug with or without vasoconstrictors in the presence or absence of preoperative sedation.

2. Patients & Methods:

Fourty four patients were included within this study received Bare Metal Stent (Liberte monorail coronary stent system Boston scientific)who required single tooth extraction under local anesthesia; they were selected from out patient clinic, Cardiology Department, Faculty of Medicine, Mansoura University .

All patients included within this study were subjected to tooth extraction during the early 6 months following percutaneous coronary stent intervention (PCI) under coverage of adenosine diphosphate P2Y12 receptor antagonist clopidogrel 75mg (Plavix, Sanofi Aventis) combined with 100mg aspirin (Bayer, Leverkusen) to minimize the risk of thrombotic PCI formation. Patients were divided equally into four groups. The 1st group consisted of eleven patients received local anesthesia with vasoconstrictor (Mepivacaine HCl 2% with Levonordefrin 1:20.000. Alexandria Co. for Pharmaceuticals and Chemical Ind. Alexandria. Egypt) and subjected to dental extraction under preoperative sedation (one hour before starting procedures) using 3mg bromazepam (Calmepam, Glaxo Smithkline, S.A.E, El Salam city, Cairo, A.R.E). The 2nd group consisted of eleven patients received local anesthesia with vasoconstrictor and subjected to dental extraction without the presence of preoperative sedation. The 3rd group consisted of eleven patients received local anesthesia without vasoconstrictor and subjected to dental extraction with the presence of preoperative sedation.

The 4th group consisted of eleven patients received local anesthesia without vasoconstrictor and subjected to dental extraction without the presence of preoperative sedation.

Most patients were given nitrates, angiotensin-converting enzyme inhibitors, calcium antagonists,and/or beta blockers. The patients were scheduled for single tooth extraction. After dental treatment, each patient was carefully observed for vital signs. For postoperative dental pain control, Diclofenac Potassium(Oflam, Mepha Pharma Egypt S.A.E) 50mg tablets was prescribed.

Inclusion & Exclusion Criteria

Inclusion criteria were the following: (1) controlled hypertension (HTN) with blood pressure not exceeding values of 160/100 mm Hg.

Exclusion criteria were the following: (1) cardiovascular instability including unstable angina pectoris, refractory dysrrhythmias, untreated or uncontrolled hypertension, untreated or uncontrolled CHF, uncontrolled hyperthyroidism; (2) uncontrolled diabetes mellitus; (3) sulfite sensitivity; (4) steroid dependent asthma; (5) pheochromacytoma; (6) tricyclic antidepressant treatment; and (7) history of psychiatric illness, chronic use of central nervous system depressants or antidepressants or mental instability.

Furthermore, the exclusion criteria included patients with anaemia, liver disease or any medical condition which might affect the coagulation process and subjects with a history of bleeding episodes or epistaxis.

Treatment protocol

The study time frame for each patient included a baseline period extended from beginning of monitoring 5minutes before administration of local anaesthesia and extrended for 30 minutes after completion of tooth extraction. Anesthesia was induced carefully with aspiration and slow injection to prevent injection of the anesthetic drug into the vasculature and care was taken to inject as painlessly as possible. A standard dose 1.8 mL (Mepivacaine HCl 2%, Alexandria Co. for Pharmaceuticals and Chemical Ind. Alexandria. Egypt)) was injected in all patients. A minimum of 5 minutes was allowed to attain LA effectiveness. [11]

Cardiac Monitoring

A noninvasive E.C.G recorder (SCHILLER MT 200 Holter ECG version 2.04) was used to record all cardiovascular parameters which were used for patients assessment at standard time points and time intervals. Then the mean of recorded data at these time intervals for every parameter of each patient was calculated. Standard time points and time intervals were defined: (1) Baseline: beginning of

monitoring; (2) Baseline_5 minutes: end of stabilization period; (3) LA: injection timing; (4) LA_5 minutes: 5 minutes after the injection; (5) Treatment: beginning of dental treatment; (6) End: completion of dental treatment; (7) Rest: end of the rest period following the completion of the dental treatment (after 30minutes from completion of tooth extraction) (8) _ (LA_5)-LA: ECG changes occurring in the interval between the time of LA and 4 minutes later (this time period presents changes that may be attributed to the LA); (9) _ (Treatment_4)-LA: ECG changes occurring in the interval between the time of LA and 5 minutes after the beginning of the dental treatment (this time period presents changes that may be attributed to the initiation of the treatment and to a lesser extent late effects of the LA solution). [11]

For all patients included within this study a strict cardiac monitoring at the different nine time intervals extending from the beginning of monitoring till 30 minutes from completion of tooth extraction. During this standardized time intervals systolic and diastolic blood pressure (Sys.BP and Dia.BP, respectively), heart rate (HR) and ST segment deviation (_2 mm) or T-wave inversion were recorded for each patient.

Assessment of postoperative bleeding

The patients were then asked to apply pressure on a piece of sterile gauze for 30 minutes and were then re-evaluated for bleeding. If the subjects did not have any signs of bleeding at that time, they were discharged and contacted by phone 12 h, 24 h, 48 h and 5 days post-operatively. If there was any bleeding, they were re-examined, new gauze was placed and re-evaluated after 30 min. Any active oozing from the socket after 30 min was considered immediate bleeding. If the patients reported during

the phone communication that there was bleeding, they were instructed to return for further evaluation.[12]

Statistical analysis

Statistical analysis was done using computer software SPSS version 15. Data were expressed as mean ± standard deviation. The variables distribution was tested for normality assumption using Kolomogrov Smirnov test. One way analysis of variance was used to test for significant difference between groups examined. Bonferonni Post-Hoc test was used for comparison between groups.

3. Results:

During dental treatment, no patients complained of symptoms such as chest pain and dyspnea or showed marked hemodynamic change that necessitated discontinuation or postponement of the dental treatment. The patients ages ranged between 40y and 64 with a mean age 52.78. Among groups patients ages within 1st group ranged between 48 -59 with mean age 54.75, within the 2nd group ranged between 40-64 with mean age 54.45y, within 3rd group ranged between 42-55y with mean age 48.72y and within the 4th group 46-62y with mean age 53.19year.

The average interval between percutaneous coronary stent Intervention and dental treatment was 38.0 days. In the present study, 5 patients underwent single tooth extraction during the time period (17-30), 27 patients at time period (30-60), 9 patients at time period (61-89) and the remaining 3 patients at time period (90-180 days). All patients were kept on the combination between clopidogrel (Plavix) 75mg and 100 mg aspirin to allow metal stent struts to become adequately endothelialized to reduce the risk of stent thrombosis.

Table (1) Showing Mean, Standard Deviation, Standard Error, Minimum and Maximum recorded data among patients regarding to Systolic & Diastolic Blood Pressure

Dependant Variable	Systolic Blood Pressure						Diastolic Blood Pressure				
Patient Groups	N	Mean	±Std. Deviation	Std. Error	Minimum	Maximum	Mean	±Std. Deviation	Std. Error	Minimum	Maximum
G1	11	140.18	3.736	1.126	135.00	147.00	86.72	3.349	1.009	82.00	93.00
G2	11	158.09	3.910	1.179	152.00	165.00	93.36	5.239	1.579	85.00	100.00
G3	11	127.45	4.227	1.274	120.00	132.00	87.81	3.250	0.980	82.00	93.00
G4	11	138.72	4.692	1.414	129.00	145.00	90.59	4.969	1.498	83.50	98.50
Total	44	141.11	11.799	1.778	120.00	165.00	89.62	4.897	.738	82.00	100.00

Regarding to systolic blood pressure, the highest recorded value was presented in the 2nd group 165mmHg while the lowest recorded value was presented among patients within the 3rd group 120mmHg. The same findings were presented during

assessment of diastolic blood pressure since the highest value 100 mmHg was recorded among patients within the 2nd group. However, the lowest value was recorded among patients within the 1st and 3rd group 82 mmHg.**(Table 1)**

Table (2) Showing Mean, Standard Deviation, Standard Error, Minimum and Maximum recorded data among patients regarding to Mean Blood Pressure & Pulse Pressure

Dependant Variable	Mean Blood Pressure						Pulse Pressure				
Patient Groups	N	Mean	±Std. Deviation	Std. Error	Minimum	Maximum	Mean	±Std. Deviation	Std. Error	Minimum	Maximum
G1	11	104.54	2.956	.891	100.67	109.67	53.45	3.908	1.178	49.00	63.00
G2	11	114.93	3.866	1.165	108.00	119.67	64.72	6.165	1.859	52.00	75.00
G3	11	101.03	2.272	0.685	98.33	105.67	39.63	5.937	1.790	30.00	50.00
G4	11	106.63	3.361	1.013	101.33	111.33	48.13	7.500	2.261	34.50	58.50
Total	44	106.78	6.009	0.905	98.33	119.67	51.48	10.87	1.639	30.00	75.00

Regarding to Mean blood pressure, the highest recorded value was presented in the 2nd group 119.67mmHg while the lowest recorded value was presented among patients within the 3rd group 98.33mmHg. The same findings were presented during assessment of pulse pressure since the highest value was 75 mmHg in the 2nd group. However, the lowest value was recorded among patients within the 3rd group 30 mmHg.(**Table 2**)

Table (3) Showing Mean, Standard Deviation, Standard Error, Minimum and Maximum recorded data among patients regarding to Mean Heart Rate & Mean ST Segment Deviation.

Dependant Variable	Mean Heart Rate						Mean ST Segment Deviation				
Patient Groups	N	Mean	±Std. Deviation	Std. Error	Minimum	Maximum	Mean	±Std. Deviation	Std. Error	Minimum	Maximum
G1	11	85.09	4.346	1.310	78.00	93.00	-0.5308	0.357	0.107	-1.26	-0.08
G2	11	111.18	3.572	1.077	103.00	115.00	-1.310	0.522	0.157	-2.07	-.51
G3	11	73.00	4.219	1.272	68.00	83.00	7.928E-02	0.487	0.147	-0.73	0.57
G4	11	88.27	4.429	1.335	81.00	95.00	-.291	0.349	0.105	-.80	0.52
Total	44	89.38	14.53	2.191	68.00	115.00	-0.552	0.631	9.519E-02	-2.07	0.57

During comparing 1st group versus 2nd group, a high statistical significant difference was recorded between both groups regarding to Sys. BP.(P=0.000), Mean Bp (P=0.000), Mean pulse pressure (P=0.000), Mean HR (P=0.000), Diastolic Bp (P=0.001) and Mean ST segment deviation (P=0.000)(**Table4,5**)

A high statistical significant difference was recorded between 3rd group versus 4th group regarding to Mean Sys. BP.(P=0.000)Mean Bp (P=0.000), Mean pulse pressure (P=0.002), Mean HR (P=0.000).(**Table 4,5**) However, no statistical significant difference between both groups regarding to mean Diastolic Bp (P=0.138) and Mean ST segment depression (P=0.261) (**Table 4,5**) revealing the significant effect of secreted endogenous catecholamine within the 4th group which may be expected to have an remarkable effect on the cardiovascular parameter when compared with 3rd group that was covered by presedation.

Table (4) Showing the level of significance when comparing different groups between each other regarding to Systolic and Diastolic blood pressure

Dependant variable	Systolic Blood Pressure				Diastolic Blood Pressure		
Patient Group	Patient Group	Mean Difference I-J	St. error	Sig	Mean Difference I-J	St. error	Sig
G1	G2	-17.9091(*)	1.7728	0.000	-6.6364(*)	1.8331	0.001
	G3	12.7273(*)	1.7728	0.000	-1.0909	1.8331	0.555
	G4	1.4545	1.7728	0.417	-3.8636(*)	1.8331	0.041
G2	G3	30.6364(*)	1.7728	0.000	5.5455(*)	1.8331	0.004
	G4	19.3636(*)	1.7728	0.000	2.7727	1.8331	0.138
G3	G4	-11.2727(*)	1.7728	0.000	-2.7727	1.8331	0.138

On the other hand, no statistical significant difference was recorded between 1st and 4th group regarding to Mean Bp (P=0.130), Mean HR (P=0.080)and Mean ST segment deviation (P=0.205)and Sys Bp.(P=0.417). However, there was low statistical significant difference between both groups regarding to Diastolic Bp (P=0.041) and Mean pulse pressure (P=0.045).**(Table4,5)**

A high statistical significant difference was recorded between 2nd and 3rd group regarding to Sys. BP.(P=0.000) Diastolic Bp (P=0.004), Mean HR (P=0.000) and Mean ST segment deviation (P=0.000)**(Table4,5)**

Regarding to Mean heart rate(HR), there was a highly statistical significant difference between 2nd and 3rd group (P=0.000) and all other groups (P=0.000). However, there was no statistical significant difference between 1st and 4th group (P=0.080).

Regarding to Mean Blood pressure, there was a highly statistical significant difference between 2nd and 3rd group (P=0.000) and all other groups (P=0.000). However, there was no statistical significant difference between 1st group and 4th group (P=0.130).

Table (5) Showing the level of significance when comparing different groups between each other regarding to Mean Heart rate & Mean ST Segment Deviation

Dependant variable		Mean Heart Rate			Mean ST Segment Deviation		
Patient Group	Patient Group	Mean Difference I-J	St. error	Sig	Mean Difference I-J	St. error	Sig
G1	G2	-26.0909(*)	1.7719	0.000	0.7794(*)	0.1860	0.000
	G3	12.0909(*)	1.7719	0.000	-.4515(*)	0.1860	0.020
	G4	-3.1818	1.7719	0.080	-.2394	0.1860	0.205
G2	G3	38.1818(*)	1.7719	0.000	-1.2309(*)	0.1860	0.000
	G4	22.9091(*)	1.7719	0.000	-1.0188(*)	0.1860	0.000
G3	G4	-15.2727	1.7719	0.000	0.2121	0.1860	0.261

Regarding to Mean ST segment Deviation there was a high statistical significant difference between 2nd group and all other groups(P=0.000). Also, there was a high statistical significant difference between 3rd group versus 1st group (P=0.020) and 2nd group (p=0.000) except 4th group (P=0.261) this may be attributed to positive effect of presedation measures in decreasing the liability of ST deviation especially when combined with absence of vasoconstrictor. **(Table. 5)**

Assessment of Postoperative Bleeding

In the 1st group, only one male patient out of eleven patients (9.09%) oozed blood within 12 hours post-operatively after initial controlling of post extraction bleeding within the early 30minutes. The patient was seen, re-evaluated and asked only to apply pressure over the gauze which controlled the bleeding. No further oozing of blood was recorded at the other time intervals of follow up either at 24 h and 48 h and 5 days post-operatively. Within, the 2nd group one male patient out of eleven patients (9.09%) revealed bleeding at 30 minutes of follow up. However, instructing the patient for extending the time of proper application of pressure pack for another 30min was able to stop bleeding and the patient revealed no further bleeding at the other time intervals of follow up either at 12h, 24 h and 48 h and 5 days post-operatively.

The 3rd group showed two patients one male and one female out of eleven patients(18.18%) suffered from postoperative blood oozing at 30min of follow up. The classical application of pressure pack was enough to control bleeding with the male patient during the next 30minutes and the other time intervals of follow up. However, the female patient who suffered from postoperative blood oozing was subjected to further using of pressure pack with adequate stabilization through application of eight figure suture of the extraction socket for proper assurance of bleeding control. In the 4th group no patient recorded any sign of postoperative bleeding either at the different time intervals of follow up either at 30min, 12h, 24 h and 48 h and 5 days post-operatively.

4. Discussion:

The recent trend in cardiac rehabilitation is toward aggressive early introduction of exercise instead of prolonged bed rest, which had resulted in physical and mental deconditioning. [13] As a result, early hospital discharge and return to normal community life has become main stream, and the safety of this approach has been well established. These facts demonstrate that even patients with AMI or UAP can tolerate considerable cardiovascular load. Consequently, such patients may safely undergo

minimally invasive dental treatment when appropriate precautions are taken.[14]

Furthermore, through the last 30-year interval since PCI has emerged as a safe, economic and less invasive alternative to surgery, and has changed the face and the delivery of cardiac care and offering patients a reasonable option to relieve cardiac symptoms, while minimizing procedural risks and facilitating a swift return to normal activities. [15]

On the other hand, dental treatment poses an additional risk in that treatment- related pain and stress increase the amount of catecholamine released in blood, which results in elevated heart rate and blood pressure; these in turn can reduce the oxygen demand-supply balance in the myocardium and induce myocardial ischemia. In addition, elevated blood catecholamine levels may induce platelet aggregation and coronary spasms, [13] which can lead to myocardial infarction. Blood pressure reduction due to neurogenic shock or syncope, often encountered during dental treatment, reduces coronary blood flow, which may induce thrombotic occlusion in stenotic portions. Dental treatment must therefore be carefully conducted under rigorous systemic management and with these risks in mind. [14]

A recent Science Advisory from the American Heart Association, American College of Cardiology, Society for Cardiovascular Angiography and Interventions, American College of Surgeons, and the American Dental Association recommended continuing aspirin and clopidogrel therapy for minor dental surgical procedures in patients who have coronary artery stents or delaying treatment until the prescribed antiplatelet regimen is completed, and warned of the significant thrombotic risks of discontinuing therapy. [16]

Such recommendation for the prevention of stent thrombosis after coronary stent implantation state that, at a minimum, patients should be treated with clopidogrel 75 mg and aspirin 325 mg for 1 month after bare-metal stent implantation, 3 months after sirolimus drug-eluting stent (DES) implantation, 6 months after paclitaxel DES implantation, and ideally, up to 12 months if they are not at high risk for bleeding. [17]

These recommendations were based on the antiplatelet regimen used in trials that were conducted to obtain US Food and Drug Administration approval (low-risk lesions in low-risk patients) and the anticipated time it takes for the metal stent struts to become adequately endothelialized to reduce the risk of stent thrombosis.[16]

As a result of all documented recommendation, all patients included in this study were monitored starting from the early preoperative phase and

through the postoperative phase for careful assessment of the associated cardiovascular changes that might result from local anesthesia administration or patient stresses secondary to tooth extraction at a standard time points and time intervals for each patient included in the study then the average reading for each parameter for every patient included in this study was recorded as a proper method of assessment since all patients included in the study were subjected to the same surgical procedure carried out by the same operator under same type and dose of local anesthesia (only1.8ml within the patient tolerance level) and the only two variable that evaluated in this study were the role of VC and preoperative sedation on such risky PCI patients. Beside that, tooth extraction procedure is a collective situation that can not be separated during which such risky PCI patients must be subjected to both of risk factors either local anesthesia (with or without V.C) and extraction process (with its expected associated stress level).

On the other hand, monitoring of postoperative bleeding in this study which is considered also as another risk factor in PCI patients receiving both clopidogrel 75 mg and aspirin 100 mg as a prophylaxis from occurrence of thrombotic stent.

Our finding revealed that there was no significant difference between the 1st group in comparison with the 4th group regarding to the measured cardiovascular and heamodynamic parameters except diastolic blood pressure (P=0.041) and pulse pressure (P=0.045). These findings can be attributed to either the injected dose of V.C was within the cardiovascular tolerance level, beside the suppressive role of patient sedation resulted in decreasing of overall level of the endogenous catecholamine.

Our results were in accordance with, Cintron et al., [18] who investigated the effects of local anesthesia and some dental procedures in 40 patients with histories of myocardial infarction within 3 months previously; the investigators demonstrated the absence of significant hemodynamic change, suggesting good tolerance of dental procedures. Findler et al., [19] reported that no complications were caused by dental treatment in 26 patients with severe ischemic heart disease.

Niwa et al., [20] reported that lidocaine-epinephrine is safe in hemodynamic consequences in patients with cardiovascular diseases. They concluded that a low dose of epinephrine in local dental anesthesia was well tolerated by cardiovascular patients. Tolas et al., [21] reported no significant cardiovascular changes after injection of a single cartridge of an anesthetic containing epinephrine, although there was a slight increase in

plasma epinephrine level. Furthermore, authors revealed that local anesthesia have a limited cardiac side effects and within normal physiological variation.[11]

A high statistical significant difference between the 2nd group in comparison with the 3rd group regarding to the measured cardiovascular and heamodynamic parameters. Such finding can be explained by the simple fact revealed that patients within 2nd group can be considered as the highest risk group since they received vasoconstrictor beside endogenous catecholamine resulted from such stressful condition especially, in absence of any presedation in contrast to the 3rd group which might represent the lowest risk group.

This result was in agreement with Muller et al.,[22] who stated that the amount of catecholamine released in blood, which resulted from dental treatment can induce myocardial ischemia and aggravate platelet aggregation and coronary spasms. Niwa et al.,[20] demonstrated significant cardiovascular changes 10 minutes after the injection of lidocaine 2% with a higher adrenalin concentration of 1:80,000. In this study, the concentration of Levonordefrin vasoconstrictor was 1:20000 that may explain our findings especially within these two groups.

Regarding to mean ST segment deviation, non of all patients included in this study showed an remarkable ischemic changes or ST segment deviation that might affect the continuation of tooth extraction. However, there was a high statistical significant difference between 2nd group and all other groups(P=0.000). Also, there was a high statistical significant difference between 3rd group and other groups except 4th group (P=0.261). On the other hand, no statistical significant difference between 1st and 4th group (P=0.205). Such inter-group variation of level of significance may be attributed to positive effect of presedation measures in decreasing the liability of their effect on ST segment deviation. Furthermore, it can help operators in directing them toward the way of minimally neutralizing the effect of these risk factors in those compromised PCI patients.

Our findings were in accordance with a study that was carried out on cardiovascular patients using two different types of local anasethesia and revealed a lack of clinical presentation of the ischemic changes indicateing that the risk for severe ischemic emergency is low. The ECG changes noted in this population do not represent a life-threatening condition. They mentioned that the dentist should be alert to these ischemic changes as the patient is at risk for ischemic deterioration, especially in extensive surgery. [11]

Regarding to assessment of post extraction bleeding, only four patients showed a relative bleeding within the 1st 24 hours and the operator was able to control bleeding by either simple and proper application of pressure pack or adequate stabilization of pack with eight figure suture even without application of any local hemostatic material. Such finding was in accordance with an abstract presented at the American Academy of Oral Medicine meeting in 2006,[23] that reported the results regarding 36 patients randomized to 325 mg aspirin or placebo 2 days before and 2 days after a single tooth extraction. There were no differences in any bleeding outcomes between the 2 treatment groups. This appears to be the first randomized, double-blind, placebo-controlled trial evaluating the impact of aspirin on bleeding complications from invasive dental procedures.

Yokoyama et al., [24] have shown that a low dose of ASA has an effect on bleeding time. However, others have shown that there were no effects on bleeding after extraction of teeth.[25] In accordance with this finding, a minimal aspirin dose was used to minimize the risk of both post-operative bleeding and prevent stent thrombosis.

5. Conclusion:

Combined role of presence or absence of presedation and vasoconstrictor in PCI patients subjected to tooth extraction can play a dramatic effect on cardiovascular parameters rather than each of them for the same type of patients.

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Evaluation of Abrasion Behaviour of Knitted Fabrics under Different Paths of Martindale Tester

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Abstract: The Martindale tester is used for both the abrasion, pilling resistance of fabrics, and straight line test by adjusting three moving parts, each one has three setting levels; making twenty seven paths possibilities. According to the standards there are only three types of motion to perform different tests. Therefore the aim of this study is to evaluate the effect of other setting possibilities on abrasion behaviour. The Lissajous patterns which consist of the Path traced by the fabric over the abradent in Martindale tester have been drawn in continuously changing directions at different setting. The total numbers of working conditions are sixteen patterns, since some adjustments did not work or gave the same path or lines. Then the areas of all patterns have been calculated and analyzed. Three samples of knitted fabrics produced from three counts have been tested at the combinations of different path of the Martindale tester. Therefore forty eight results of abrasion resistance for all fabrics at different settings have been measured and analyzed. Using Martindale standard testing setting is not enough to determine the actual abrasion behaviour of knitted fabrics. Other probabilities of setting, producing other different patterns in area and shape, could be simulated to the actual abrasion behaviour of fabrics during the end use. It could help the textile designer and producer to understand and improve their products according to the actual performance requirements.
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1. Introduction

However abrasion is generally only one of several factors contributing to wear performance or durability as experienced in the actual use of the material [3], but abrasion behavior is an important property of textile materials that governs the quality and efficiency of processing and the performance of products [6].

Abrasion is the mechanical deterioration of fabric components by rubbing them against another surface [8]. Therefore it is affected by many factors in a very complex, and as yet little understood manner [5]. Many researchers have investigated the influence of raw material, yarn production technology, yarn twist and chemical treatment on the abrasion resistance property of woven, knitted and nonwoven fabric [2, 5, 6, 7, 8 12, 14, 15, 16, and 17].

The measurement of resistance to abrasion is also greatly affected by the nature of the abradant, variable action of the abradant over the area of specimen abraded, the tension of the specimen, and the dimensional changes in the specimen [9].

It is quite clear that no test can be made to forecast the service life of a fabric in so many hours, but the test conditions should imitate the required service conditions as far as possible [10].

Various types of devices have been created for testing abrasion resistance which is often defined in terms of the number of cycles of abrasion applied by a specified machine, using a specified technique to produce a specified degree or amount of abrasion. In general, abrasion resistance test findings are unreliable for prediction of actual wear life in specific end uses unless data exists showing the specific relationship between the abrasion resistance test results and actual wear in the intended end use [1].

Martindale Abrasion Tester can be used for a variety of purposes. The way in which it is used depends on the operator who must decide which method appears most applicable and suitable for the problem in hand [10]. The Path traced by the test specimens over the Abradant is known as Lissajous figure [11, 13]. This family of figures was investigated by Nathaniel Bowditch in 1815, and later in more detail by J., A., Lissajous in 1857 [4]. It changes from nearly a circle shape to gradually narrowing ellipses, until it becomes a straight line, from which progressively widening ellipses develop, in a diagonally opposite direction, before the pattern is repeated [13].

The resistance to abrasion in Martindale test method is affected greatly by the conditions of the tests, such as the nature of abradant; variable action of the abradant over the area of specimen abraded, the tension on the specimen, the pressure between the specimen and abradant, and the dimensional changes in the specimen. The Martindale tester contains three

moving parts. Consideration should be given to the nature of these moving parts. However, only three types of motion to perform different tests by Martindale Tester are used for abrasion Test, Pilling Test, and Straight Line Test. The set up for each of the three tests is described in figure 1 [11].
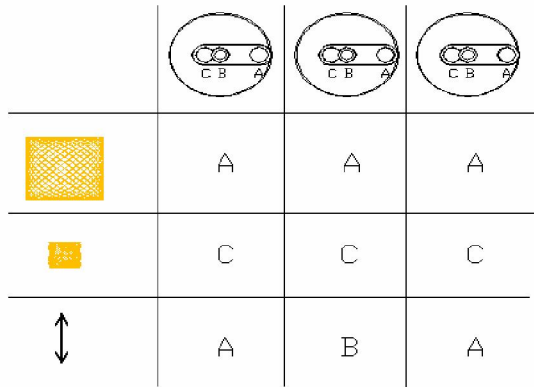

Figure 1: The standard setting for Martindale tester

For an abrasion test: All 3-bearing support blocks must be in position A, and for a pilling test: All 3-bearing support blocks must be in position C, however for a straight line test: The two outer bearing support blocks must be in position A and the inner bearing support block in position B [11].

Therefore this study was undertaken to evaluate the effect of the other setting possibilities on abrasion resistance of knitted fabrics.

2. Experimental work:

As mentioned above The Martindale instrument contains three moving parts to perform different tests by Lifting out the bearing support block and move it into the correct position on the drive crank. Adjusting each one of these three bearing support blocks (P1, P2, and P3) could be at three levels (A, B, and C); making twenty seven possibilities. According to the standards only two are applied, for testing abrasion and pilling.

The complete Lissajous Motion Diagrams for all setting possibilities were drawn by inserting the instrument pen into a specimen holder spindle bearing, so as the ball tip of the pen resting on the surface of the paper was used firstly to draw the twenty seven possibilities of paths.

The total numbers of working paths for testing fabric abrasion have been only sixteen, since some adjustments don't work or give the same path or line. The abrasion areas of the sixteen figures have been calculated using MATLAB program.

For evaluating the effect of different figures on abrasion of the fabrics, three samples of single knitted fabrics produced from three counts(Ne) 24/1, 30/1, 40/1Ne have been tested at the abrasion sixteen figures which results from the combinations of different path of the Martindale tester. The characteristics of the three knitted fabrics were determined as shown in table (1).

Table (1) the characteristics of the produced knitted fabric

Sample No.	Ne*	Weight/ m2 (gm)	Thickness (mm)	course/ cm	Wales /cm
1	24/1	187	0.61	20	15
2	30/1	153	0.55	20	15.5
3	40/1	121	0.50	20.5	15.5

*Ne is cotton yarn count

The M235 Martindale Tester- SDL Atlas was used to determine the abrasion resistance of fabrics according to ASTM D 4966 standard. The sand paper for abradant used in testing was P1000, and the specimens were abraded under low pressure (250gm) at the standard speed (47.5r.p.m.). The abrading was continued until a hole was occurred on the fabric when one thread is broken.

Therefore the different areas of abrasion geometric pattern and abrasion resistance of the knitted fabrics were measured and analyzed.

3. Results and discussion:

The experiment has been consisted of sixteen patterns on three different fabrics depending on the yarn count. Hence the results of abrasion test for all fabrics are 48 results, rubbed in a figure eight-like motion(the Lissajous pattern) to evaluate the effect of setting possibilities of Martindale tester on abrasion resistance of weft knitted fabrics.

After drawing the abrasion paths pattern on paper using the Martindale pen as shown in figure (2), the MATLAB program was used for measuring the area of each pattern. The results are different of each pattern shape and area. It is also clear that the patterns no. 1,13 in figure(2) which are the standard for ASTM test method for testing abrasion and pilling resistance have only the square Lissajous shape, their path shapes are big and small basic Lissajous square pattern (16 Revolutions) in order. The pattern no.1 (AAA) in figure (2) has the greatest area compared with other patterns.
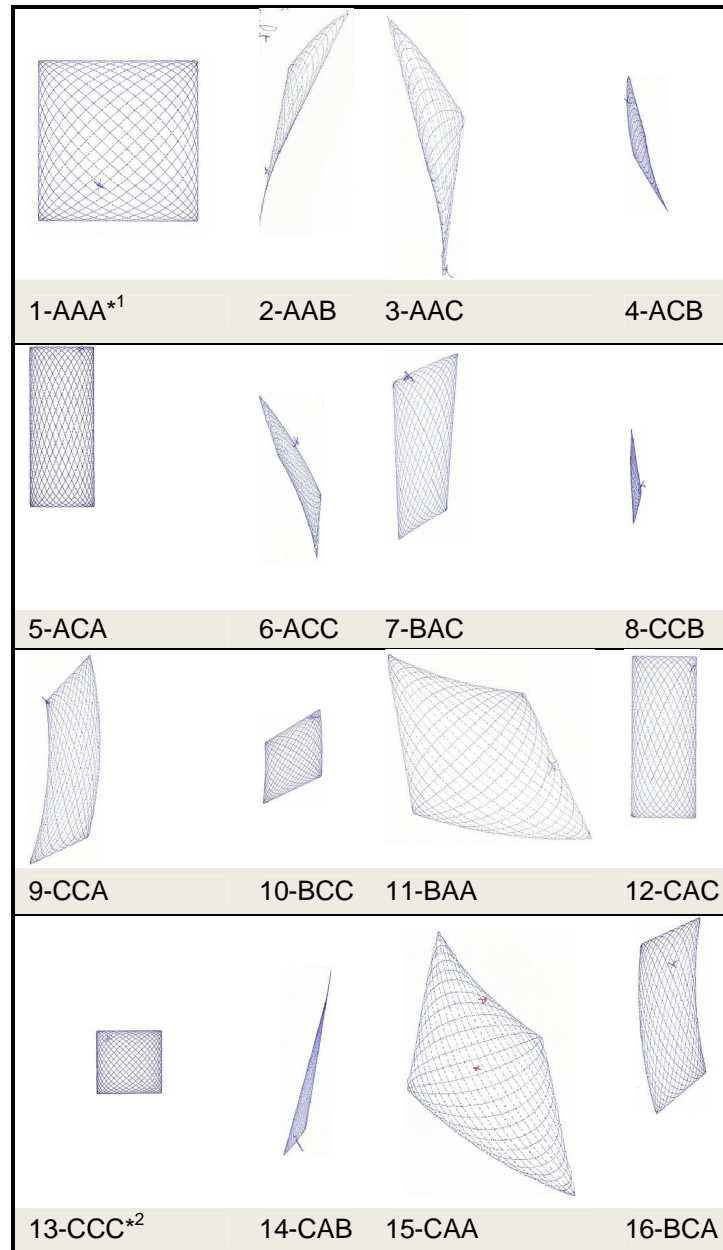
Figure 2: The patterns of the drawn paths by pen using different setting of Martindale tester P1,P2,and P3 the moving parts 1,2, and 3 in order

A,B, and C the setting levels of the moving parts where A is level 1, B is level 2, and C is level 3

*1 the ASTM standard motion setting for testing abrasion

*2 the ASTM standard motion setting for testing pilling

Once the areas of abrasion pattern were recorded for each path pattern, the relation between the area of abrasion patterns and the setting of Martindale tester using the regression analysis has been performed. The result is shown in equation (1).

Path Area= 0.43- 0.07P$_1$ -0.19P$_2$- 0.29P$_3$+ 0.17 P$_1$*P$_3$+0.88P$_3$*P$_2$- 0.06 P$_1$* P$_2$* P$_3$ -0.03P$_2$*P$_3$ Multiple R =0.99 (1)

From the above equation it could concluded that the pattern area is increased by using the setting position (A) for the three moving parts in the tester. Therefore the largest area is occurred in setting the moving parts at (AAA) position which is the standard path in testing the abrasion resistance (path pattern no.1) in figure (2). The third moving part (P3) has also the greatest effect in the equation that has also

interacted and quadratic effect. When the data of paths area is arranged and drawn in figure (3), it has been confirmed that the standard path in testing the abrasion resistance (path pattern no.1) in figure (2) has the largest area of all patterns.
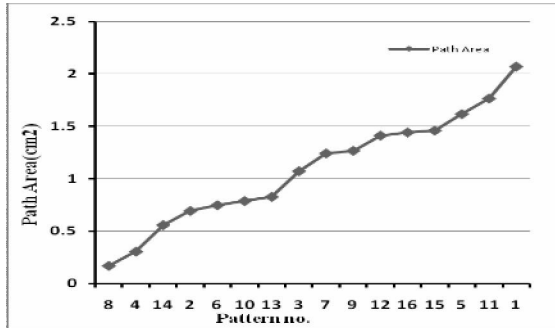
Figure 3: Arranged areas of different abrasion paths
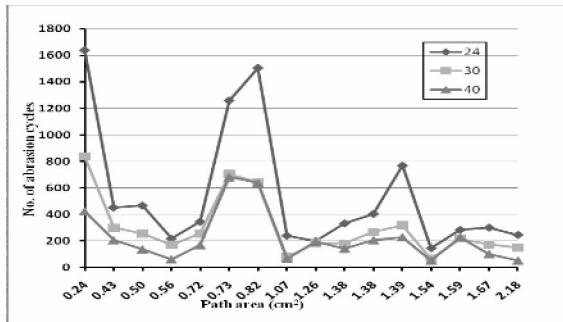
Figure 4: The relation between paths area and fabric abrasion

The results of abrasion test reveals the differences in abrasion between fabrics of the different yarn count in the experiment for each path pattern of the Martindale tester. The relation between the abrasion resistance of fabrics produced from different yarn counts and abrasion pattern areas is shown in figure (4). It could be observed that the abrasion properties of the weft knitted fabrics are varied depending on yarn count of the fabric and abrasion path area. There is no doubt that increasing the path area and yarn fineness tend to decrease the abrasion resistance of fabrics but this relation is not linear. As it was expected the abrasion resistance of weft knitted fabrics is increased by increasing yarn thickness since the weight and thickness of fabric increased as yarn thickness increased as shown in table (1).

The regression analysis has been performed for investigating fabric abrasion resistance using variables include: the setting level of the three moving parts, path area and yarn count of fabric for all samples, and is indicated in equation (2).

Abrasion=
$$431-120Area+118P_1+147P_2+81.7P_3+139P_1*P_3+85.3P_1*P_2+77.9P_2*P_3-163Ne+86.9Ne^2$$
Multiple R =0.87   (2)

Trying to reveal the effect of Martindale moving setting on the abrasion resistance separately, the results of regression analyzing to abrasion resistance of fabrics of each count are summarized in equations (3), (4), and(5).

$$Abrasion=550.4+214.3P_1+255.7P_2+178.2P_3+188.4P_1*P_3+161.9P_1*P_2+130.9P_2*P_3 \quad MultipleR=0.89 \quad (3)$$
$$Abrasion=300.5+90.4P_1+132.3P_2+85.5P_3+86.6P_1*P_3+58.4P_1*P_2+62.3P_2*P_3 \quad MultipleR=0.89 \quad (4)$$
$$Abrasion=224.4+72.3P_1+122.1P_2+87.1P_3+80.2P_1*P_3+36.7P_1*P_2+52.4P_2*P_3 \quad MultipleR=0.93 \quad (5)$$

The following results have been obtained from the initial regression analysis;

Using the setting position (A) of Martindale moving parts decreases the fabric abrasion resistance. Weft knitted fabrics have more resistance to rubbing in setting the moving parts at (C) position than (A) position. These results are in agreement with the paths pattern areas.

However the path pattern no. 1 in figure (2) (the standard setting for Martindale tester) does not have the lowest number of abrasion cycles for all fabrics although it has the largest area. That means this setting is much related to abrasion resistance but not enough to determine the actual abrasion behavior for all fabrics during the end use, where the shape of the path and the yarn count of the fabric beside to the path area have also played an important role in determining the abrasion behavior.

The surface rubbing properties of weft knitted fabrics do not show similar tendencies with the Martindale setting. These effects may be correlated with the path shape during the abrasion test.

Using Martindale standard testing settings which draw the square patterns are not enough to determine the actual abrasion behavior of knitted fabrics. Because the lissajous patterns produced of different settings could be simulated to the actual abrasion behavior of fabrics during the end use, it could help in studying the fabric abrasion behavior in the laboratory and helping the textile designers and producers to improve their products according to the actual performance. The changes in the surface of a fabric during processing, use, and care could be realized also.

Many researches should be conducted to predict the actual abrasion behavior of fabrics of the combination parameters of Martindale abrasion tester and fabric, and examine this phenomenon since

evaluating surface wear has been a challenging and contentious issue.

4. Conclusion:

Martindale abrasion tester is most widely accepted tester although it may be the most complex. The Path traced by the test specimens over the abradant is known as Lissajous pattern. The instrument contains three moving parts each one has three setting levels. The test method standard is using only three settings for abrasion, pilling and straight line test in order. However, according to the setting of the three moving parts in the Martindale tester, other probabilities of setting could be used producing other different patterns in area and shape. This study has been undertaken to determine the area and shape of the produced patterns and evaluate the abrasion behavior of fabrics at different abrasion setting motions.

Only sixteen patterns have been produced, drawn and illustrated. The areas of all patterns have been measured and analyzed using regression analysis. The effects of Martindale abrasion motion settings on three weft knitted fabrics produced of yarn count (24/1, 30/1, 40/1) have been investigated and characterized. Therefore the experiment consisted of 48 abrasion results.

The abrasion properties have been showed remarkable differences with the patterns of abrasion paths and their areas. The increase in abrasion is occurred at the setting of Martindale moving parts at (A) position since the area of the abrasion decreases. Finer yarns have been expected to wear more quickly than larger yarns.

Using Martindale standard testing settings which draw the square patterns are not enough to determine the actual abrasion behavior of knitted fabrics. Other probabilities of setting, producing other different patterns in area and shape, could be simulated to the actual abrasion behavior of fabrics during the end use. It could help the textile designer and producer to understand and improve their products according to the actual performance requirements.

Many researches should be conducted to predict the actual abrasion behavior of fabrics of the combination parameters of Martindale abrasion tester and fabric, and examine this phenomenon since evaluating surface wear has been a challenging and contentious issue.

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3/3/2011

The relation between fabric construction, treatments and sewability

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Abstract: In this study, the effects of fabric construction and treatments on the sew ability of cotton/polyester woven fabric are investigated. Fabric mechanical tests are measured using FAST (Fabric Assurance by Simple Testing) system, for assessing aspects of the performance in garment manufacture and garment appearance after wear. The optimization construction are used to carry out treatments impart to improve pilling and antimicrobial activity. The effect of fabric construction and treatments on sewing needle penetration of untreated and treated fabric is measured for determine any damage which appears in garment. It was found that formability, bending rigidity and shear rigidity decrease with decrease the weft count, but extensibility increase consistently. Also, the construction of plain has count No.40/1 gave low force penetration. **The relation between fabric construction,treatments and sewability.** Journal of American Science 2011;7(3):818-826]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Construction, FAST, Cotton/polyester blend fabric, sewability, ant pilling and antimicrobial.

1. Introduction

The idea of using the objective measurement of properties to predict fabric performance is not new. Measurements have been used to predict some aspects of fabric performance for many years. Recently, techniques have been developed to measure the mechanical properties of fabrics and use these measurements to quantify handle[1] and predict performance in both garment manufacture and its appearance[2].

The first experimental work on the objective measurement of fabric mechanical properties dates back to the 1930s [3]. The prediction of fabric performance in garment manufacturing from mechanical properties was first extensively examined by Lindberg[4] research teams in the 1960s. These teams identified many properties of fabric associated with performance in garment manufacture. These were included extensibility, bending and shear properties as well as fabric weight. Several measurements are required to fully describe tensile, shear or bending behavior of fabric. Those used to describe resistance to deformation[5]. The Fabric Objective Measurement can be used for various purposes; the main ones are:- fabric quality and hand;- evaluation of the formability of fabrics and the appearance of finished garments; creation of specific techniques to enable the making-up industry to select fabrics, as well as for quality and process control[1].

Pilling is defined as entangling of fibers on a fabrics surface during wearing, washing, dry- cleaning or testing to form fibers balls or pills that stand on the fabric surface and are of such density that light will not pass through them and they cast a shadow[6]. Pilling phenomenon has always been a major drown back in

using acrylic fibers for making woven goods, it affects not only the fabric appearance, but also its texture and quality. Pilling in general is a self limiting process which emerges at three consumptive different stages, formation of surface fuzz, entanglement, and transformation into pills[7].

The problem of pilling became even more serious with the emergence if synthetic fiber such as polyester, especially these fibers such are present in the form of a blend with some fiber of lower tensile strength[8]. Sivakelmar etal found that twill weaves tend to pill more compared with plain weaves[9]. Polyester / viscose blend woven fabric was subjected to different softening and sanforising treatment[10].

The sewing needle penetration force is the quantitative measure of the damage which appears in agreement as the result of the sewing process[11,12]. A high penetration force means a high resistance of fabric and thus a high risk of damage[13].

Sewing needle penetration force is one of the most significant technical parameters in the sewing process which affected by various factors such as type and amount of layers of the sewing material, needle size, needle point shape, speed of the sewing machine, and treatment of the sewing material, among others[13].

It is also used as a binder and thickener in the pigment printing of both polyester and polyester/cotton fabrics[14]. Pretreatment of acrylic and polyester fabrics with chitosan in presence of binder enhances ability to acid dyes. A new technique is used for improving the adhesion of chitosan molecules to the surface of these fabrics which is manifested by the higher color strength[15]. Waterborne polyurethane is prepared and reacted with chitosan as chain extender. The prepared polyurethane chitosan was studied as antimicrobial

agent of acrylic fabrics[16]. The chitosan- modified acrylic fibers showed excellent antimicrobial activity against Staphylococcus aureus, compared with the original acrylic fibers. The modified acrylic fibers treated with chitosan showed high durability to laundering probably due to strong ionic interaction between chitosan and modified acrylic[17].

The aim of this study carried out to found the relation between the construction and the sew ability of garment as well as the effect of some treatments on performance properties of garment. In these study fabric mechanical properties tests were measured using FAST.

2. Material and Methods
2.1 Fabric
Twelve cotton/blend woven fabric samples (75% cotton 25% polyester) with two fabric structures (plain & Twill weaves) and different construction properties were used. The constructions properties of woven fabrics are given in Table 1.

Table 1: Constructions properties of woven fabrics

S. CO	F. ST	E	W.C	Pick/cm
1	plain 1/1	24	20/1	18
2	twill 1/7	24	20/1	25
3	twill 1/7	24	20/1	18
4	twill 1/7	24	20/1	23
5	plain 1/1	24	30/1	20
6	plain 1/1	24	30/1	25
7	twill 1/7	24	30/1	19
8	twill 1/7	24	30/1	27
9	plain 1/1	24	40/1	23
10	plain 1/1	24	40/1	34
11	twill 1/7	24	40/1	24
12	twill 1/7	24	40/1	33

S.Co: Sample code   F. ST: Fabric structure   W.C: weft count   Warp cotton yarn 100%
  Warp Count: 4o/1   Weft blended yarn ratio cotton /polyester 75/25%   E: Ends/cm   Twist factor: 4

2.2 Chemicals
Chemicals of technical grades vis chitosan of molecular weight (N 150000), Citric acid, β-Cyclodextrin hydrate (CD), monochlortriazinyl β-cyclodextrin Na-salt (CD-T) were products of Wacker –Chemie GmbH, Munich, Germany. Acetic, citric acid and copper sulphate were of used.

2.3 Dyestuffs
Reactive dyestuff Remazol Red violet R (C.I. Reactive violet 4) and disperse dyestuff Samaron B. Pink HFG (C.I. Disperse Red 185) were used.

3. Treatments

Chitosan solution (0.4 % owf) was prepared by stirring the material in water containing acetic acid (2% v/v) overnight at room temperature. The fabrics were immersed at room temperature for 1 h., liquor ratio 1:10, then padded to pick up of 100% using a laboratory padder and cured at 150°C, for 5 min in a laboratory drying chamber (Type 450 MM model HT–V- from RBE – India). Some treated fabric was immersed in copper sulphate solution (2% w/v), at room temperature for 30 min., liquor ratio 1:10, then padded to pick up of 100%, and cured at 120°C, for 10 min.

The treatment carried out by fabric was immersed in 10% citric acid at room temperature for 1 h., liquor ratio 1:10, then padded to pick up of 100% using a laboratory padder and cured at 150°C, for 10 min. Also, the fabric was treated with was immersed in 10% citric acid at room temperature for 1 h, then solution of 15 g/l (o. w .f) of CD, or CD-T adjusted to pH 4 using citric acid. It was immersed in immersed at room temperature for 1h, liquor ratio 1:10 and padded to pickup 100 % using a laboratory padder, cured at 150°C, for 10 min.

4. Dyeing
The blend fabric was dyed with tow dyes in tow steps. The first step carried out to dye the cotton fibres with reactive dye in the blend fabric, the second step carried out to dye the polyester fibres with disperse dye in the blend fabric.

4.1 Dyeing with Reactive dye
The dye bath was prepared by accurately weighing the dyestuff to give the prescribed shade. The paste was then dissolved by adding hot boiling water. The dye solution was adjusted to pH 5 using acetic acid. The dye bath of C.I. Reactive violet 5 was heated to 60°C and added the sample to the dye bath, the temperature was then raised gradually up to 90°C through 30 minutes then added 40g/l sodium sulphate after 30 min., and the dyeing continued for 60 min., at liquor ratio 1:50. The dyed sample was thoroughly washed in warm and cold water and air-dried.

4.2 Dyeing with disperse dye
The dye bath of C.I. Disperse Red 185 was prepared by pasted the dye with 1% acetic acid in hot water, then added 2g/l of carrier. The dye bath was gradually heated to 100°C. The sample fabric was added to the bath and the dyeing continued for 60 min., at liquor ratio 1:50. The dyed sample was thoroughly washed in warm and cold water and air-dried.

5. Methods
In this work, fabric mechanical properties tests were measured using FAST (Fabric Assurance by Simple Testing) system, for assessing aspects of the performance in garment manufacture and garment appearance after wear. The samples were dyed only

before carried out the FAST system. Fabrics mechanical properties have been calculated, three different weft counts cotton blended have been used (20/1, 30/1, 40/1) with two fabric structures (plain1/1&twill1/7).FAST tested properties are given in Table 2.

5.1 FAST - Fabric Assurance by Simple Testing

FAST is a set of instruments and test methods for measuring mechanical, physical, and dimensional properties of fabrics. These measurements allow the prediction of fabric performance in garment manufacture and the appearance of the garment during wear[20]. The instruments were developed by the Australian CSIRO.The FAST system was designed for measuring properties of fabrics important to the intended performance and the appearance of tailored.

FAST consists of three parts and a test method:

FAST-1 is a compression meter that measures fabric thickness[18, 19].

FAST-2 is a bending meter that measures the fabric bending length.[20]

FAST-3 is an extension meter that measures fabric extensibility.[21, 22, and 23]

FAST-4 is a test procedure for measuring dimensional properties of fabric.[24, 25]

Table 2: FAST test (Fabric Assurance by Simple Testing) of cotton/polyester blend fabrics

S. CO	P	T	F-1 (mm²)	F-2 (mm²)	E100-1 %	E100-2 %	B-1 μ-Nm	B-2 μ-Nm	G N-m	T2 mm	ST mm	W (g/m²)
1	18	4	1.27	0.24	7.7	2.7	13.1	6.7	29.4	0.643	0.206	122
2	25	4	0.76	0.32	6.4	2.2	8.2	9.3	32	0.626	0.226	133
3	18	4	1.15	1.3	6.3	11.2	9	5.1	7.1	0.82	0.275	115
4	23	4	1.53	1.43	6.9	10.8	10.9	6.9	24.5	0.93	0.34	138
5	20	4	0.43	0.34	6.2	4.2	6.1	5.2	45	0.624	0.258	91
6	25	4	0.44	0.17	4.3	3.5	8.1	4.3	35.8	0.535	0.211	108
7	19	4	0.74	0.74	5.3	10.4	7.4	3.2	7.7	0.813	0.356	94
8	27	4	0.98	1.84	5.3	9.7	9.4	8.9	11.5	0.848	0.337	116
9	23	4	0.52	0.62	6.7	7.6	7.3	5.5	23.4	0.488	0.181	87
10	34	4	0.35	0.37	2.8	4.2	17.3	5.6	47.6	0.494	0.185	98
11	24	4	0.94	0.93	7.1	11.5	9.3	4.2	9.8	0.868	0.368	112
12	33	4	0.81	0.59	8.4	12	5.1	2.3	7.5	0.769	0.338	88

S.Co: Sample code   Formability Warp = F-1   Formability Weft = F-2   Extensibility Warp = E100-1   Extensibility Weft = E100-2   Bending Rigidity Warp = B-1 Bending Rigidity Weft = B-2   Shear Rigidity = G   Thickness = T2   Surface Thickness = ST   Weight = W

5.2 Roughness

Surface roughness was measured according to JIS 94 Standard by Surface roughness measuring instrument.

5.3. Colour Intensity

Spectral reflection measurements of the dyed fabrics were carried out using a recording filter spectrophotometer. The colour intensity expressed as K/S values of the dyed samples were determined by applying the Kubleka -Munk equation. (28)

$$K/S = \frac{(1-R)^2}{2R} - \frac{(1-R_0)^2}{2R_0}$$

Where R: is the decimal fraction of the reflectance of the dyed substrate.

R_0: is the decimal fraction of the reflectance of the undyed substrate

S: is the scattering coefficient   K: is the absorption coefficient.

5.4 The L&M sew ability tester:

Fig 1 The L+M sewability Tester [27]

Testing fabric sewing properties, US patent 3979951, 1976), a device used in many studies on needle penetration force. This equipment simulates a sewing machine by penetrating the tested fabric with an unthreaded needle, at a rate of 100 penetrations per min., with needle count 100/16 DB Singer. Sewability tested properties are given in Table 3.

Table 3: Sew ability tested properties

S. CO	Sew ability at weft /N	Sew ability at warp/N
1	7	8
2	28	29
3	8	8
4	8	7
5	6	8
6	17	23
7	9	9
8	8	9
9	7	7
10	21	18
11	9	9
12	8	8

S.Co: Sample code

5.5 Pilling test

Pilling tester is used to determine the pilling resistance of all kinds of textile structures. Sample was rubbed against the same material of sample at low pressures and the amount of pilling is compared against standards parameters. Pilling taste was carried out according to standards ASTM standard D4970 (pilling), all samples and standard fabrics should be conditioned in the standard atmosphere for testing (20c - , + 2 and 65% RH - , + 2%). The specimens assed are using the following 5 point scale.

5.6 Antimicrobial activity

The antibacterial activity of the NMA-HTCC was evaluated against the Gram positive - Staphylococcus aureus and the Gram negative bacteria Escherichia. coli (AATCC 6538). (29)

3. Results and Discussions
Fast Results

Table2 illustrated FAST tested properties. Six samples were selected from the total samples. The chosen samples have almost the same picks/cm in the different fabric structure. Selection was on the basis that there are two samples of each weft count include the two fabric structure. (a) To assess the effect of different fabric structure at the same weft counts on fabric properties. (b) To evaluate the effect of the difference of the weft count of the same fabric structure on fabric properties. (c) To evaluate the best fabric structure on fabric properties. The selection samples code are (1,3,5,7,9,11). In figures (2-6) fabrics mechanical properties have been calculated, three different weft counts cotton blended have been used (20/1, 30/1, 40/1) with two fabric structures (plain1/1&twill1/7).

Fig.2 compared formability values in both fabric structures for weft direction. FAST meter tends to provide lower values of plain weave compared with twill weave in the three weft counts. In the same fabric structure, the weft formability decreases as the weft count decrease. A possible explanation for this trend may be the higher values of extensibility of twill weaves.

Fig. 3 compared fabric extensibility values in case of plain weave and twill weave. For plain weave FAST meter tends to provide lower values compared to that obtained from twill weave. Extensibility increases as a decrease of weft count diameter.

Fig. 4 compared fabric shear rigidity values in both fabric structures for weft direction. FAST meter tends to provide higher values of plain weave than twill weave in the three weft counts.

Fig. 6 illustrated fabric bending rigidity values in both fabric structures. FAST results recorded almost the same values in the both fabric structures, exception in the case of weft count 20/1 in the two fabric structures. This result attribute to, the bending rigidity values increases as fabric weight increase regardless of fabric structure.

Fig. 6compared fabric thickness values of two fabric structures. FAST meter tends to provide higher values of twill weave than plain weave. These differences in thickness values may be attributed to the nature of fabric structure.
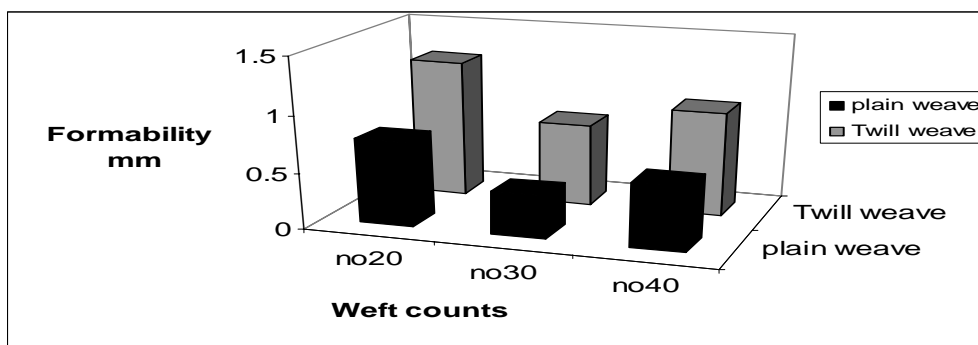
Figure 2: Effect of fabric structure on fabric formability with different weft counts
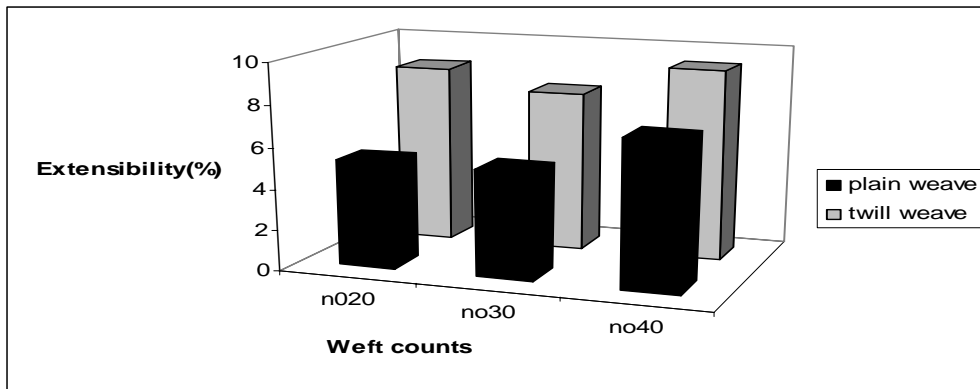
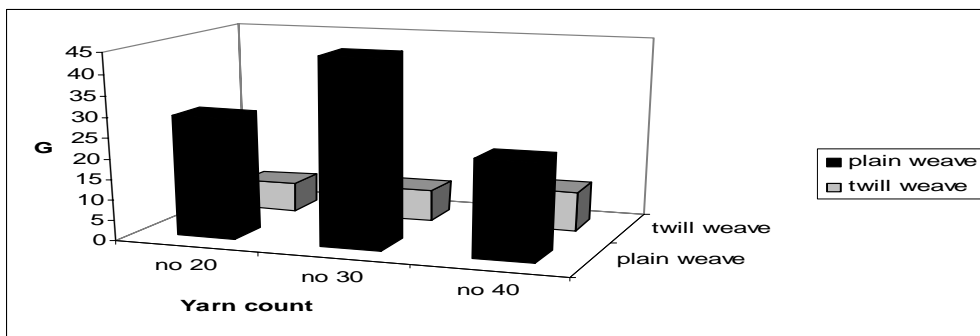**Figure 3: Effect of fabric structure on extensibility with different weft counts**

Figure 4: Fabric shear Rigidity with 2 fabric structure
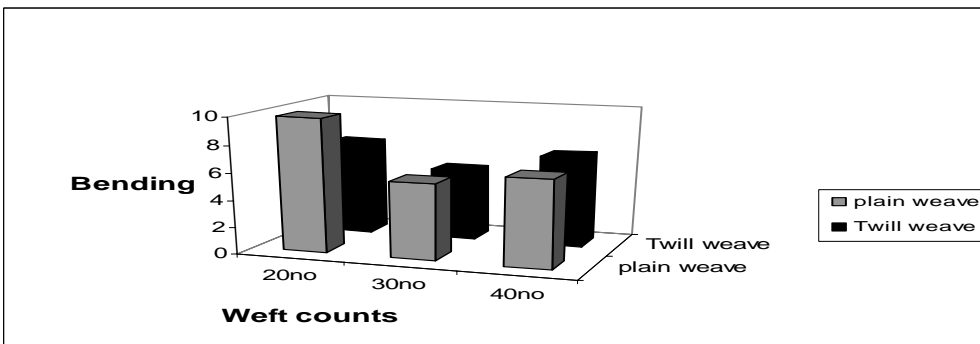

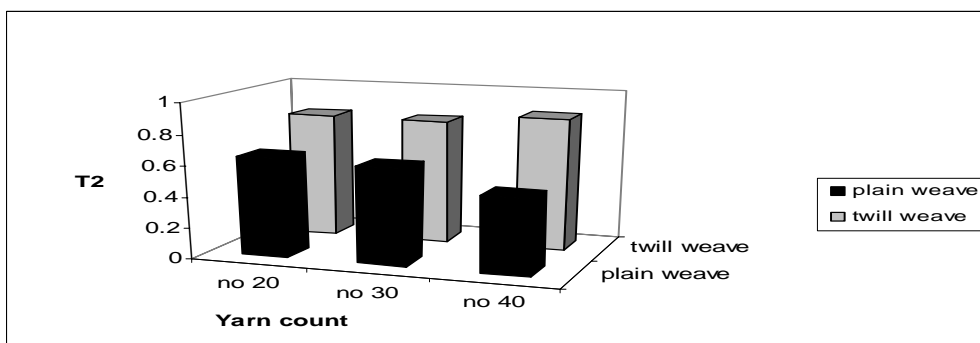**Figure 5: Effect of fabric structure on fabric bending with different weft counts**

Figure 6: fabric thickness with 2 fabric structure

In figures (2-6) it can see that the increasing of weft count caused the fabric mechanical properties such as (formability, bending rigidity, thickness and shear rigidity) decrease, while fabric extensibility increases consistently. This can explained as a result of increasing consolidation of fibers within the fabrics.

Also, in the same figures, it can be seen that the formability, bending rigidity, extensibility and thickness of twill fabrics have increased than plain fabrics, while shear rigidity of plain fabrics have increased than twill weave.

Regard to the specific fabric structure used or the fabric weight per unit areas. An explanation for the observed results may be that the nature of fabric structure permitted easy fibers movement such as twill weave 1/7 than plain weave 1/1. And as a result, the mechanical properties produced using this combination, are different of their results.

Sewability (Sewing needle penetrations force) results
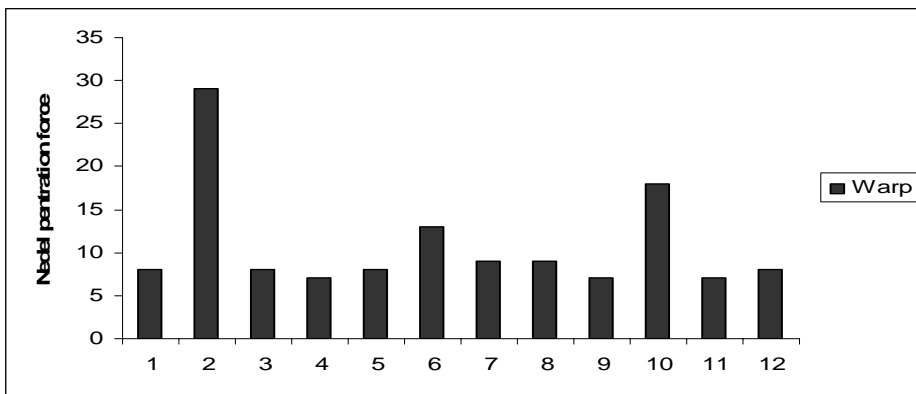
Figure 7: Sewing needle penetrations force/N at warp direction
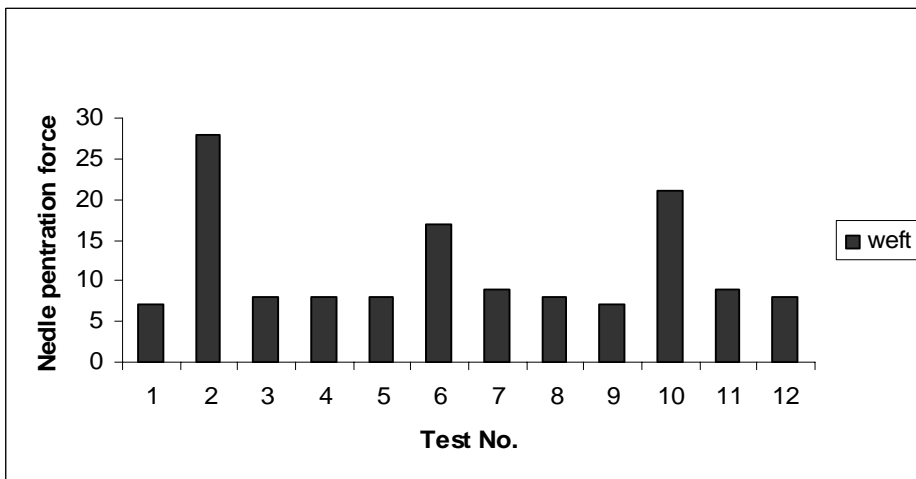
Figure 8: Sewing needle penetrations force/N at weft direction

The figures (7, 8) illustrated the sewing needle penetration force, which have been calculated, three different weft counts cotton blended have been used (20/1, 30/1, 40/1) with two fabric structures (plain1/1&twill1/7). It can see that needle penetrations force increase for sample no 2 (fabric weft count 20/1, 25 pick/cnm) than others, that due to structure plain, density of weft at (weft &warp) direction. And sample no 6 (fabric weft count 30/1 – 25 pick/cnm) that is due to the structure plain, density of weft at (weft &warp) direction - sample no 10 (fabric weft count 40/1 -34 pick/cnm) that is due to the structure plain, density at (weft &warp) direction as shown in figures (7, 8).
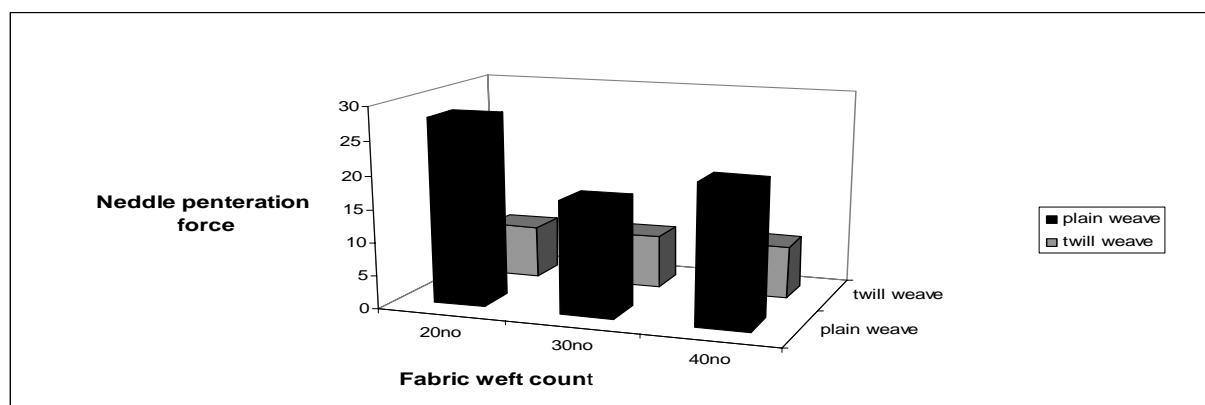
Figure 9: The relation between sewing needle penetrations force/n and fabric structure

In figure (9) it can see that needle penetrations force decrease for twill weave than plain that is due to density, structure.

Pilling, color intensity and roughness of untreated and treated fabrics

Table 4 shows   pilling, color intensity and roughness of untreated and treated fabrics with chitosan on fabrics have different construction. It was found that the treatment enhanced anti pilling and gives little change in roughness. The color intensity don't give different The results show that various constructions don't affect on treatment results, this means that the treatment results are affected only by chemical structures of fibers.

Table 4: Pilling, colour intensity and roughness of untreated and treated fabrics with chitosan

Samples constriction		Pilling		colour intensity K/S		Roughness μm	
W.C	P./cm	Untreated	Treated	Untreated	Treated	Untreated	Treated
20/1	18	1-2	3	1.8	1.9	18.8	19.2
30/1	25	1	2	3.2	3. 2	18.9	19.6
20/1	34	1	3	3.03	3.01	18.6	19.2
20/1	15	1	3	2.48	2. 48	18.7	19.8

W.C   P./cm   Treatment: immersed in 0.4% o.w.f chitosan, at room temperature for 1 h., liquor ratio 1:10, padded to pick up of 100%, curing at 150°C, for 10 min.

Table 5: Roughness, pilling and colour intensity of untreated and treated fabrics

Constriction of treatment	Pilling	Colour intensity K/S	Roughness μm
Untreated	1-2	2.9	18.8
0.4% (o.w.f.) chit+1% o.w.f. cit. acid	4-5	3.1	23.83
0.4% (o.w.f.) chit	4	3	17.12
1% (o.w.f.) cit. acid	4	3	16.59
1% (o.w.f.) sodium iso-phthalic acid	2-3	2.9	20.55
0.4% o.w.f. chit+1% o.w.f. cit. acid, 2% CuSO4	4-5	3	24.25
15 g/l (o. w .f) CD+1% o.w.f. cit. acid	2-3	3.1	17.59
15 g/l (o. w .f) CD	2	3	17.03
15 g/l (o. w .f) CD-T+1% o.w.f. cit. acid	2-3	3	16.12
15 g/l (o. w .f) CD-T	2	3.1	16.91

Dyeing: C.I. Disperse Red 55 (1% o.w.f), 100°C, pH 5, liquor ratio 1: 50
    C.I. Reactive violet 5 (1% o.w.f), 90°C, pH 5, liquor ratio 1: 50

Pilling, color intensity and roughness of untreated and treated fabrics have the same construction (No. 1) with different chemicals materials illustrated in table 5. It can found that the treatments with chitosan, chitosan/copper sulphate, citric acid and 1**%** (o.w.f.) sodium iso-phthalic acid gave higher roughness and higher ant pilling than the untreated one. But the treatment with 15 g/l (o. w .f)

CD+1% o.w.f. cit. acid and 15 g/l (o. w .f) CD-T+1% o.w.f. cit. acid gave little enhancement in roughness ant pilling. The treatments don't give effect in color intensity between untreated and treated polyester/ cotton blend fabric. This change color intensity may be attributed to different construction structure, and different thickness of fabrics only.

Table 6: Antimicrobials activity for the untreated and treated fabric.

Samples	Inhibition zone diameter (mm/1 cm sample)	
	Escherichia Coli (G$^-$)	Staphylococcus aureus (G$^+$)
Untreated	0	0
0.4% o.w.f. chit+1% o.w.f. cit. acid	6	7
2% CD+1% o.w.f. cit. acid	8	7
2% CD-T+1% o.w.f. cit. acid	8	8
0.4% o.w.f. chit+1% o.w.f. cit. acid, 2% CuSO4	9	9

Table 7 shows inhibition zone diameter (mm/1 cm sample) for Esherichia coli (G$^-$) and staphylococcus aureus (G$^+$) for the untreated and the treated fabrics. The treatments give antimicrobials activity than untreated one. The highest antimicrobials activity was observed with treatment with (0.4 % w/v) chitosan in presence of citric acid and (2 % w/v) copper sulphate. This may attributed to copper sulphate**.**

5. Conclusion
- FAST results recorded almost the same values in the both fabric structures, exception in the case of weft count 20/1 in the two fabric structures. FAST meter tends to provide higher values of twill weave than plain weave.
- It can see that needle penetrations force increase for sample no 2 (fabric weft count 20/1, 25 pick/cm) than others, that due to structure plain, density of weft at (weft &warp) direction.
- It can found that the treatment with citric acid, CD and citric acid CD-T was reduced roughness and give enhance than the untreated one. But the treatment with chitosan and chitosan/copper sulphate gave higher roughness and higher ant pilling than the untreated one.
- The highest antibacterial activity was observed with treatment with (0.4 % w/v) chitosan in presence of citric acid and (2 % w/v) copper sulphate than the untreated one.

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Performance Optimization of Field Oriented Vector Control Drive using PSO and GA Techniques

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Abstract: In this paper optimization of flux vector control (FOC) drives performance are studied. Genatic algorithm (GA) and Particale swarm optimization (PSO) are used for this purpose. Optimuim flux referance identifection by using GA and PSO are used to minimize the motor input power to have the optimuim motor efficiency. Selecting of the optimal gains using both methods are done to improve the motor response and behavior. A comparison between the simulation results were illustrated to evaluate the Performance for the developed controller adopting (GA) and (PSO) algorithms.The results show that, the proposed PSO controller algorithm has better optimization performance more than the proposed GA in both for gain tunning and also for the selection flux set point.

I. Introduction:

Induction motor is an electromechanical device that converts electrical energy to mechanical energy, Field oriented vector control drives are capable of both speed and torque regulation because they control both current components and the angle (vector sum) between them. They provide excellent torque characteristics plus tighter speed regulation. These types of drives give independent torque and flux control and allowing a continuous regulation of the motor speed and torque. These types of drives give the best performance in controlling the AC motors. In recent years, optimization algorithms have received increasing attention by the research community as well as the industry to solve various complex control problems as an alternative or complement to the conventional methods [1]. Optimization techniques using analogy of swarming principle have been adopted to solve a variety of engineering problems in the past decade. Swarm Intelligence (SI) is an innovative distributed intelligent paradigm for solving optimization problems that originally took its inspiration from the biological examples by swarming, flocking and herding phenomena in vertebrates. A population of particles exists in the n-dimensional search space in which the optimization problem lives in. Each particle has a certain amount of knowledge, and will move about the search space based on this knowledge. The particle has some inertia attributed to it and so it will continue to have a component of motion in the direction it is moving [2]. Parsopoulos and Vrahatis attempted to improve the search efficiency in PSO by performing two stage

transformation of the objective function which eliminates and elevates the neighborhood of the local minima [3]. Alternative runs and tumbles in Ecoli bacteria found in the human intestine constitute chemo taxis and this foraging mechanism was imitated by Kevin Passino for solving optimization problem in control system [4]. In the earlier PSO algorithms, each particle of the swarm is accelerated by its best previous position and towards the best particle in the entire swarm. Here, the underlying assumption is that each particle in the swarm remembers the best position already visited and also it is informed about the best particle position. After letting the particles to search adequate number of times in the solution space independently for the best possible positions, they are attracted to the basin containing the best particle by establishing proper communication among them about the search environment [5]. Genetic Algorithm also is considered as the famous evolutionary tuning method which has been implemented in twin rotor modeling and controller parameter tuning through recent literature [6-7]. Although GA can provide good solutions in tuning controllers that has a complex model, it requires huge memory and faster processing units with large word lengths to execute huge number of repeated computations [8-9]. Moreover, for highly multi-modal problems, the solutions may lose diversity and get trapped in local minima at some points unless special method is adopted to avoid premature convergence to suboptimal region of the search space [10]. This paper is organized as follows; Section II gives a description of the used induction

motor model while, section III gives field oriented vector control (FOC) drive overview. The proposed GA algorithm is presented in section IV and the proposed PSO Algorithm is presented in section V. Simulation results adopting GA and PSO are provided in section VI and finally the conclusion of this work is presented in section VII.

II-Induction Motor Model

The overall dynamics of the induction motor under assumption of equal mutual inductances and linear magnetic circuit are given by the following fifth- order model [11-13]:

$$\frac{d}{dt} = \mu \quad {}_d i_q - \frac{T_l}{J}$$
$$\frac{d}{dt} {}_d = - \quad {}_d + M i_d$$
$$\frac{d i_d}{dt} = - \ i_d + \quad {}_d + n_p \ i_q + M \frac{i_q^2}{{}_d} + \frac{1}{L_s} u_d \quad (1)$$
$$\frac{d i_q}{dt} = - \ i_q - \ n_p \quad {}_d - n_p \ i_d - M \frac{i_q i_d}{{}_d} + \frac{1}{L_s} u_q$$
$$\frac{d}{dt} = n_p \quad + M \frac{i_q}{{}_d}$$
$$T = \mu \quad {}_d i_q$$

Where;

${}_d$ and ${}_q$ are the d-q axis components of the motor flux and is the motor speed. $u_d,$ u_q are the d-q axis components of the motor voltgae, $i_d,$ i_q are the stator current components, the n_p is the motor pair poles, M is the mutual inductance and R_s and R_r are the stator and rotor resistance respectivelly. L_s and L_r are stator and rotor self inductance respectivelly and T_l is the load torque.

$$= 1 - (M^2 / L_r L_s); \quad = \left(\frac{R_r}{L_r}\right);$$
$$\beta = \left(\frac{M}{\sigma L_S L_R}\right); \quad \mu = \left(\frac{n_p M}{J L_r}\right); \quad (2)$$
$$p = \arctan \frac{b}{a} \quad \text{and} \quad = \left(\frac{M R_r}{L_S L_r^2}\right) + \left(\frac{R_s}{L_s}\right)$$

Iii- Field Oriented Vector Control Model

The induction motors have various methods of control and the particular method to be adopted depends on the nature of the application. The current in an ac motor can be separated into two distinct components; Id or the flux producing current component and I_q or the torque producing current component. The total current is the vector sum of those two current components, the torque produced in the motor is based on the cross product of these vectors [14]. Different technologies in drive system implement different levels of control over one or more of these components and the vector angle between them [15]. Thus FOC has the flux and torque independent of each other and as a result of the FOC is the advantages of increasing starting torque, increasing low speed torque, increased shock load capability, tighter speed and torque regulation. The FOC de-coupling control is shown in Fig.1[16].
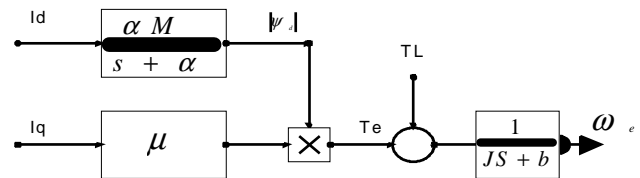

Fig.1: FOC de-coupling control

The voltage is the command action, required to cancel the nonlinearity by using voltage state feedback. So ${}_d$ is regulated directly by V_d, after ${}_d$ becomes constant, the equation of speed becomes linear and voltage V_q regulates the speed directly. The voltage feedback equations [16] are:

$$u_d = \frac{}{L_r}(-p \ i_q - \frac{M}{T_r} \frac{i_q^2}{{}_d} - \frac{M}{T_r} + V_d) \quad (3)$$
$$u_q = \frac{}{L_r}(p \ i_d + \frac{M}{T_r} \frac{I_d i_q}{{}_d} + \frac{M}{T_r} p \quad {}_{rd} + V_q$$

The closed loop system become

$$\frac{d}{dt} = \mu \quad {}_d i_q - \frac{T_l}{J}$$
$$\frac{d \ {}_d}{dt} = - \quad {}_d + M i_d \quad (4)$$
$$\frac{d i_d}{dt} = - \ i_d + V_d$$
$$\frac{d i_q}{dt} = - \ i_q + V_q$$

Where; the time constant is $T_r = \frac{L_r}{R_r}$

The stator current command in current vector control or its desired value in vector voltage control is chosen to satisfy the equation

$$I_S = \frac{T_r}{M} \frac{d\varphi}{dt} + \frac{1}{M}\varphi \quad (5)$$

Whree : is the flux referance value.

The current commands to Voltage commands equations are the following:

$$V_d = (i_{dr} - I_d)(k_6 + \frac{k_7}{s})$$

$$V_q = (I_{qr} - I_q)(k_8 + \frac{k_9}{s}) \qquad 6$$

Where I_d and I_q are the actual d-q stator current components respectively. The current command equations in term of flux and speed set points are the following:

$$I_{dr} = k_1 \int_0^t (f_{ref} - \ _d)dt + k_2(f_{ref} - \ _d) + \frac{\ _d}{M} \qquad (7)$$

$$I_{qr} = (k_3(\ _{ref} - \ ) + k_4 \int_0^t (\ _{ref} - \ )dt + k_5(\ _{ref} - \ ) + \ _{ref})/\mu$$

The main purpose of this paper is to optimize the performance of the FOC technique by improving the motor efficiency by identifing the optimuim refrece flux. Also selecting the optimal flux set point optimal selection of the controller gains (K1, K_2, K_3, K_4 and K5). The optimization is done using genetic algorithm and practical swarm optimization methods.

IV - Proposed Genetic Algorithm

Genetic algorithms (GAs) are global optimization techniques that avoid many shortcomings exhibited in conventional search techniques on a large and complicated search space. The application of GAs to control engineering can broadly be classified into two distinct areas: off-line design and on-line optimization. On-line applications tend to be quite rare because of the difficulties associated with using a GA in real-time and directly influencing the performance of the system. GAs has been applied to controller design problems as well as to system identification techniques. In each case, either the parameters or the structure can be optimized, or potentially both [17]. Other applications include fault diagnosis, stability analysis, sensor-actuator placement, and other combinatorial problems. In any design problem there is a multi dimensional space of possible solutions. Some of these solutions may be acceptable, but not the best (Local Optimum). Optimal mathematical solutions could be obtained from a control system with linear plant dynamics. The GA can be regarded as a research method from multiple directions to solve for problem solutions, since it contains three evolutionary operations: reproduction, crossover, and mutation. In the traditional binary-coded GA, all the

variables of interest must be encode as binary digits (genes) and a collection of binary digits further forms a string (chromosome)[18]. Then three standard genetic operations, i.e., reproduction, crossover, and mutation are performed to produce a new generation. Such procedures are repeated until the pre-specified number of generations is achieved, or the required accuracy is satisfied. Some studies applying traditional GA with binary coding to solve optimization problems such as the PID controller design [19]. After6 a manipulation of binary-coded GA, the final binary digits are then decoded as original real numbers. This is an indirect optimization problem searching .The GA can use single or multiple crossovers algorithm. The most important problem in the design of GA is to choose the fitness function, chromosomes, reproduction, crossover, mutation and the stopping rules of algorithm.

In this paper the optimization process was done using MATLAB/ SIMULINK/GA toolbox parameters as follows;
in case of optimal controller gains , the number of variables are five , the population type is double vector , population size is 20 , the initial range of variables are [200 – 600] for K1 , K2 , [0-20] for K3 and K4 , [500-5000] . For the reproduction , the elite count is 2 and the crossover friction is 0.8 , the mutation function is Gaussian , the crossover function is scattered , the stopping rules is the no of generation is 100 , and the stall time limit is 200 sec , in case of efficiency optimization , the no of variables are one and the initial range is [0.1 – 2] , all other parameters are the same .

A.   FITNESS FUNCTION

The most critical step in applying GA Algorithms is to choose the objective functions that are used to evaluate fitness of each chromosome. In this paper there will be two genetic algorithms, one is to improve the drive performance as in by selecting the optimal gains of K_1, K_2, K_3, K_4, K5 of the equations (7), and the other algorithm is to optimizing the motor efficiency by selecting the optimal flux set point.
The following two fitness functions are used for optimization proccess;

- For the case of improving the motor efficiency, the fitness function will be motor input power.

$$f_2 = \max(p_{in}) = \max(I_{sa} * u_a + I_{sb} * u_b) \quad (8)$$
- For the case of optimum control gains, the fitness function will be

$$f_1 = \int [(f_{ref} - f_r)^2 + (w_{ref} - w_r)^2] dt \quad (9)$$

Such that f_{ref} is the reference flux and f_r is the motor rotor flux, w_{ref} is the reference speed and $_r$ is the motor speed.

B. CHROMOSOMES

Let parent chromosomes are selected to be crossed and parameters (gains) be a random number chosen from [0, 10]. And for the parameter of flux reference will be chosen in the range from [0.1, 2].

C. MUTATION

In this paper, dynamic mutation is applied. The mutation process randomly picks up Pm × N Chromosomes to be mutated. The algorithm stops if the pre-specified number of generations is achieved.Figure 2 shows the outline of design steps and data flow for the real-coded GA.

D. STOPPING RULES

The processes of generating new chromosomes and selecting those with better function values are continued until the given stopping conditions are satisfied. The process can be stopped after a fixed number of generations, or when any significant improvement in the solution ceases to occur. In this paper, GA is run for a fixed number of generations 100 Generations and the size of population or the number of individuals in each generation is
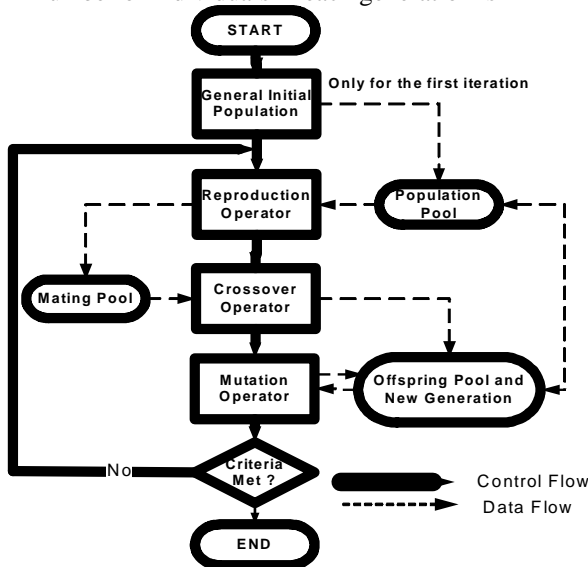
Fig. 2: The data flow of RCGA

V-Proposed Particle Swarm Algorithm

Optimization techniques using analogy of swarming principle have been adopted to solve a variety of engineering problems in the past decade. Swarm Intelligence (SI) is an innovative distributed intelligent paradigm for solving optimization problems that originally took its inspiration from the biological examples by swarming, flocking and herding phenomena in vertebrates. A population of particles exists in the n-dimensional search space in which the optimization problem lives in. each particle has a certain amount of knowledge, and will move about the search space based on this knowledge. The particle has some inertia attributed to it and so it will continue to have a component of motion in the direction it is moving [19-20].

The particle swarm optimization PSO algorithm we employ here is based on that Particles fly through the solution space and are influenced by both the best particle in the particle population and the best solution that a current particle has discovered so far. The best particle in the population is typically denoted by (global best), while the best position that has been visited by the current particle is donated by (local best) [21]. The (global best) individual conceptually connects all members of the population to one another. That is, each particle is influenced by the very best performance of any member in the entire population. The (local best) individual is conceptually seen as the ability for particles to remember past personal success. The particle swarm optimization makes use of a velocity vector to update the current position of each particle in the swarm. The position of each particle is updated based on the social behaviour that a population of individuals adapts to its environment by returning to promising regions that were previously discovered. [22]

Let the ith particle of the swarm is represented by theD–dimensionalvector $x_i = (x_{i1}, x_{i2}, ..., x_{iD})$ and the best particle in the swarm, i.e. the particle with the smallest function value, is denoted by the index g. The best previous position (the position giving the best function value) of the ith particle is recorded and represented as $p_i = (p_{i1}, p_{i2}, ..., p_{iD})$, and the position change (velocity) of the ith particle is $v_i = (v_{i1}, v_{i2}, ..., v_{iD})$. , The particles are manipulated according to the equations;

$$v_{id} = w v_{id} + c_1.r_1.(p_{id} - x_{id}) + c_2.r_2.(p_{gd} - x_{id}) \quad (10)$$

$$\& \quad x_{id} = x_{id} + v_{id} \quad (11)$$

Where; d = 1, 2, . . . , D; i = 1, 2, . . . , N and N is the size of swarm (no of birds); w is the inertia weight; c_1 and c_2 are two positive constants; r_1 and r_2 are the random values . Equation (10) is used to calculate i^{th} particle's new velocity by taking into consideration three terms: the particle's previous velocity, the distance between the particle's best previous and current position, and, finally, the distance between swarm's best experience (the position of the best particle in the swarm) and i^{th} particle's current position. Then, following the second equation, the ith particle flies toward a new position. In general, the performance of each particle is measured according to a predefined fitness function, which is problem dependent. The role of the inertia weight w is considered very important in PSO convergence behaviour. The inertia weight is employed to control the impact of the previous history of velocities on the current velocity. In this way, the parameter w regulates the trade–off between the global (wide–ranging) and local (nearby) exploration abilities of the swarm. A large inertia weight facilitates global exploration (searching new areas); while a small one tends to facilitate local exploration, i.e. fine–tuning the current search area. A suitable value for the inertia weight w usually provides balance between global and local exploration abilities and consequently a reduction on the number of iterations required to locate the optimum solution. A general rule of thumb suggests that it is better to initially set the inertia to a large value, in order to make better global exploration of the search space, and gradually decrease it to get more refined solutions, thus a time decreasing inertia weight value is used.

In this paper, the pso will be used to evaluate the optimal gains of the field oriented vector controller and for selecting the optimal flux set point for efficiency improvement of the motor, the fitness function to be minimized in the two cases are the same as in equations (8) and (9) .

For the optimal gains to be chosen, there will be five variables (r_1 to r_5) which are the controller gains, there will be five constants (c_1 to c_5) to be chosen as $c_1= 0.8$, $c_1= 0.6$, $c_3= 0.5$, $c_4= 0.15$, $c_5= 0.12$, w=0.9 , n= 50 , max no of bird step is 30 and the variable boundary are [0 to 50]

For the optimal flux set point , there will be one variable (r_1) which is the flux reference , there will be one   constants (c_1) to be chosen as $c_1= 0.12$, w=0.9 , n= 50 , max no of bird step is 30 and the variable boundary are [0.1 to 2]

VI- Simulation Results
The simulation targets are:
- From the behavior of the motor without any control during the speed and flux manual change.

For FOC the speed and the flux are independent variables. The opitmuim flux referance value can be identified which has the minimuim input power.The motor behavoir at this case will be shown in part A.

- For the optimization proceess is to identify the optimuim flux referance automatically by GA and PSO techniques. These results are illustarted in part B.

- The optimum gains selction for the controller of field oriented vector control shown in equations (6) and (7) using GA and PSO. These results are introduced in part C.

A.   Performance during Flux Referance Change without Control
Starting the simulation with speed reference 100 r.p.m from zero to 100 sec, then it increased to 200 r.p.m from time of 100 sec to 200 sec as in Fig. 3. While the reference flux was increased from 0.15 weber to 0.45 weber in steps as shown in Fig. 4. As shown in Fig. 5, the motor input power is starting with high value then decreasing with flux reference increasing to reach it's minimum value then increasing. The Id and Iq currents response are illustared in Fig. 6 and Fig. 7 respctively.These results indicate that the optimuim refrenace flux is 0.24 weber which has the minimuim motor input power.
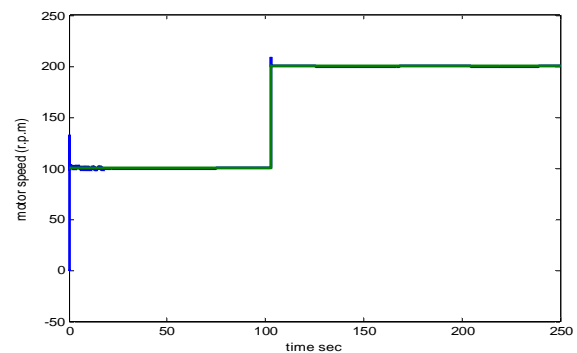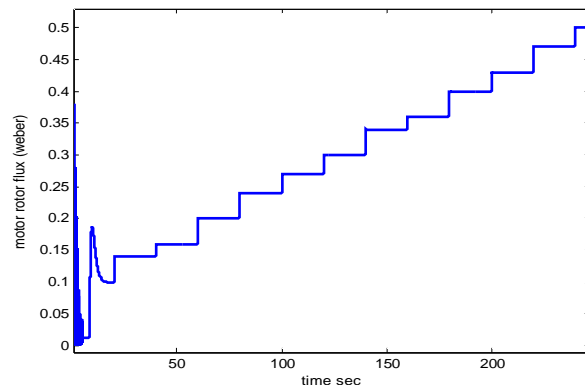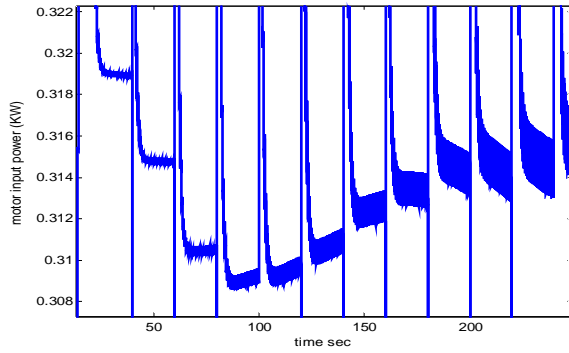

Fig .3: Motor speed

Fig .4: Rotor referance flux
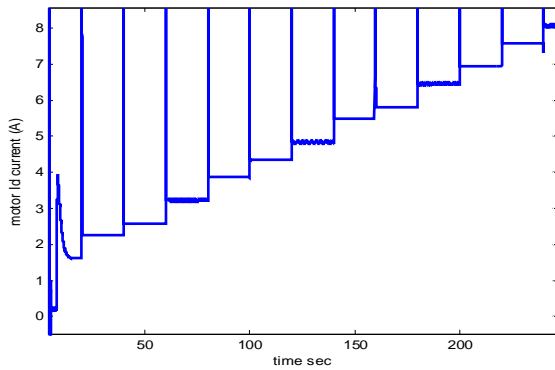
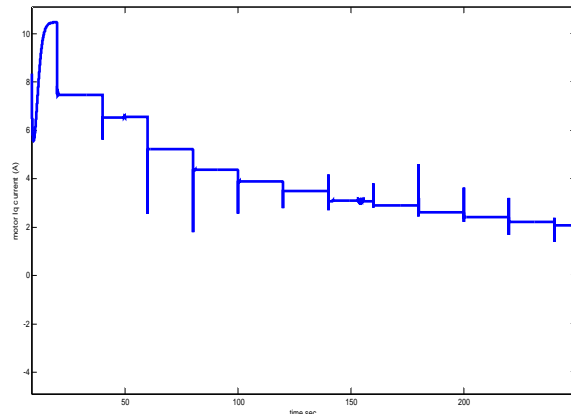**Fig.5: Motor input power**

Fig. 6: Motor I_d current

Fig. 7: Motor I_q current

B. Performance during Optimuim referance Flux Selection by GA and PSO

Using GA and PSO, there will be only one variable in the proposed flux fitness function shown in equation (8), it is the value of the optimal flux referance. The results are illustared in Fig's from (8 – 11) , it show that by using GA, the flux referance , f_{ref}=0.17944, and for PSO, the reference will be 0.244 weber.So The PSO referance value is so closelly to the targt optimuim referance flux that was determined before without control. The input power of PSO gives better motor efficiency than GA with the same

previous parameters and speed reference as shown in Fig. 8.
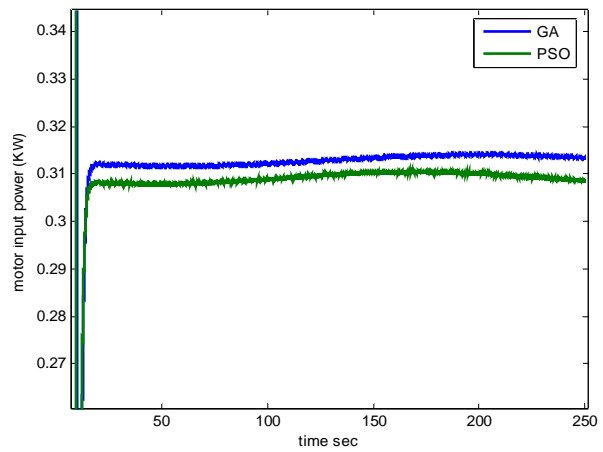
Fig.8: Motor input power with GA & PSO
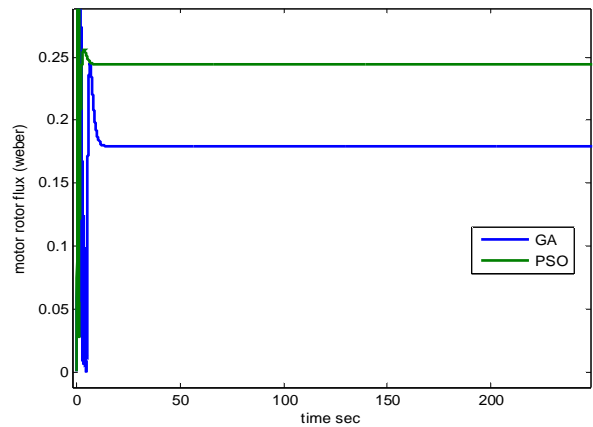
Fig.9: Motor rotor flux using GA and PSO
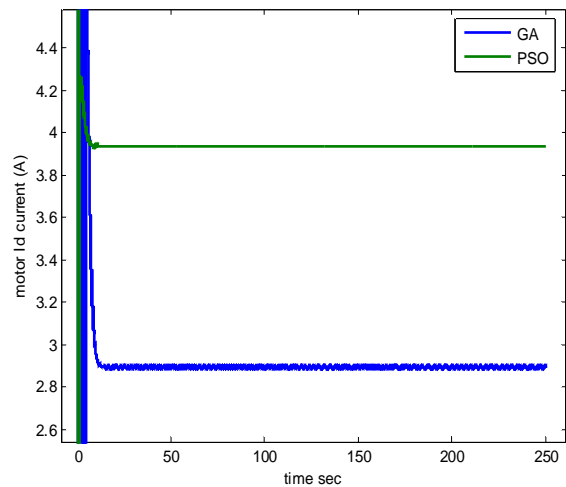
Fig. 10 motor I_d using GA and PSO
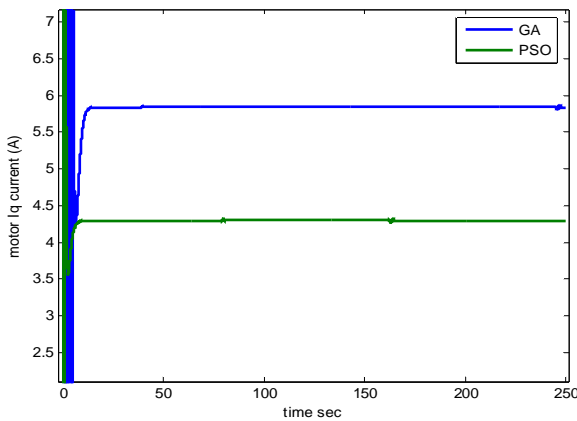
Fig 11: Motor I_q current using GA and PSO

C. Performance during Optimuim Gains Selection by GA and PSO

In this part, selecting of the optimal value of the controller gains (k_1, k_2, k_3, k_4 and k_5) ,is done by using GA with the same motor parameters and with the same speed reference using the gains fitness function as in equation (9). The optimuim gains using GA are ($k1= 450.5$, $k2= 510$, $k3= 9.8$, $k4= 11$, $k5= 960$), while their vaule by using PSO are ($k1= 231.9$, $k2= 355.5$, $k3= 20.6$, $k4= 534.5$, $k5= 737.5$).

The response of the motor speed in the two control methods shown in Fig .12. The motor speed by using PSO is better than the GA performance as it is closely to the referance value. The rotor flux at both optimization methods are shown in Fig.13, the PSO flux has minimum overshoot than in the case of GA.
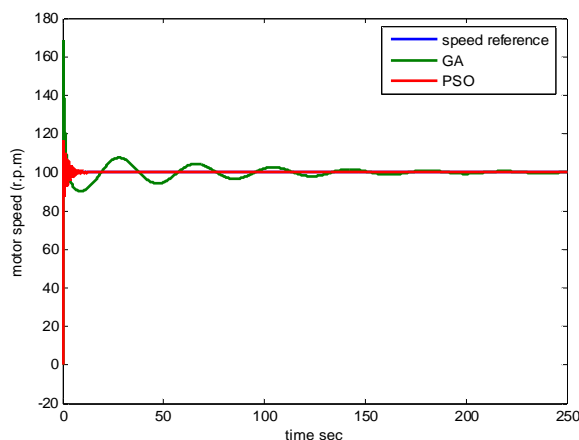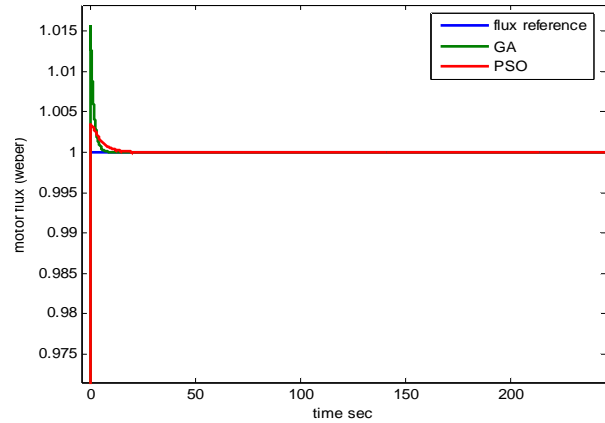

Fig. 12: Motor speed GA and PSO with optimum gains.

Fig. 13: Rotor flux GA and PSO with optimum gain

VII Conclusion:

In this paper, two different techniques of artificial intelligent which are genetic algorithm and partical swarm optimization have been used to improve the performance of field oriented vector control drive. Selecting of the optimuim refernace flux was done by the two methods compared to the uncontrolled methods.Also the optimuim gains selection were done to enhance the motor response. The results show that the proposed are reliable and capable to enhance the motor behavior using the FOC drive by selecting the optimuim gains. The PSO method has better response than the GA method.The proposed techniques minimize the input power which means increasing the motor efficiency by selecting the optimuim referance flux.

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The effects of antioxidants supplementation on haemostatic parameters and lipid profiles in diabetic rats

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Abstract: Diabetes mellitus is a complex, progressive disease, which is accompanied by multiple cardiovascular complications. Oxidative stress is significantly increased in diabetic patients and may lead to great haemostatic disturbances existing in these patients. Antioxidants have been reported to reduce oxidative and haemostatic variables by arresting free radical damage .The aim of this study was to assess the role of antioxidants (vitamin E and C) in modulation of the haemostatic parameters and lipid profiles in experimentally-induced diabetic rats. Blood samples are obtained from control rats (no=24) and diabetic rats (no=24) to estimate haemostatic status by platelets aggregation, fibrinogen levels and prothrombin time. Oxidative status was assessed by estimation of the lipid profiles {Triglycerides (TG), low density lipoprotein (LDL), high density lipoprotein (HDL) cholesterol} and plasma uric acid. Diabetics rats were divided into two sub-groups. The first sub-group (no=12) was orally supplemented with Vitamin E (7mg/rat) daily for 4 weeks and the second sub-group (no=12) was co-administrated Vitamin C (7mg/rat) and Vitamin E daily for 4 weeks. Blood samples are withdrawn from the two sub-groups and the previous parameters were assessed. .Increased levels of TG and LDL cholesterol and reduced levels of HDL cholesterol and plasma uric acid were recorded in the rats after induction of diabetes, compared to prediabetic values. Hypercoagulability state was observed in diabetic rats through percentage increase in platelet aggregation and fibrinogen level. Oral supplementation of Vitamin E to diabetic rats resulted in a significant inhibitory effect on the oxidative stress and partial reduction of the hypercoagulability state, which were more observed by co-administration of vitamin C. It is concluded that hyperglycemia in rats increased oxidative stress which may play a role in induction of hypercoagulable state. Dietary co-administration of vitamin E and C induced protective effects to diabetic rats.
[Zeinab Abdel-Rahman. **The effects of antioxidants supplementation on haemostatic parameters and lipid profiles in diabetic rats.** Journal of American Science 2011;7(3):835-840]. (ISSN: 1545-1003). http://www.americanscience.org.

Key words: Diabetes, oxidative stress ,lipid profile , platelet aggregation, antioxidants.

Introduction:

According to the International diabetes Federation , percent of diabetes in EGYPT was 11.4% in the year 2010 and this likely to increase to 13.7 % by the year 2030 (IDF 2009).Diabetes mellitus is a complex, progressive disease, which is accompanied by multiple complications. It has been recognized as the sole independent risk factor for the development of cardiovascular disease (Jay et al., 2006).

Cardiovascular complications include stroke, myocardial infarction and atherosclerosis which are considered to be important causes of health-related deaths in diabetic patients .Lipid abnormalities in type-2 diabetes are characterized by high triglyceride concentrations, low high density lipoprotein-cholesterol concentrations and normal total and low density lipoprotein-cholesterol concentrations (Valabhji and ElkelesL,2003). Premature atherosclerosis and other vascular disorders are serious complications of diabetes mellitus due to increased

peroxidation of LDL leading to foam cell formation, fatty streaks and plaque formation in the arterial wall, and hyper-reactivity of blood platelets leading to increased platelet adhesion and aggregation. Altered platelet morphology and function have been reported in patients with metabolic syndrome, stroke and diabetes mellitus ((Ferreiro et al.,2010).Mean platelet volume is an independent risk factor for atherothrombosis and cardiovascular disease. Many studies have shown that increased mean platelet volume is one of the risk factors for myocardial infarction, cerebral ischemia and transient ischemic attacks (Chu et al., 2010). Oxidative stress is significantly increased in diabetes mellitus, due to prolonged exposure to hyperglycemia, disturbances in capacities of the antioxidant defense system including uric acid, scavenging enzymes such as superoxide dismutase and glutathione reductase, and deficiencies of antioxidants such as vitamin C and E (Abou-Seif and Youssef, 2004). Uric acid is the most important

circulating antioxidant contributing to about 50% of the total radical trapping antioxidant capacity while other antioxidants such as bilirubin, vitamin E , vitamin C and glutathione contribute to the other 50% (Ceriello et al., 1997). Antioxidants have been reported to reduce the complications in DM by arresting free radical damage. This suggests that treatment with vitamins C and E could ameliorate the oxidative stress caused by hyperglycaemia (Kukner et al., 2009).

Thus, this study was aimed to assess the existence of oxidative stress in diabetic rats by estimating the lipid profile (LDL cholesterol, HDL cholesterol and triglycerides) and plasma uric acid, which is one of the major counteracting antioxidant markers in the circulation. And since vitamins E and C were potent antioxidants nutrients, so this work was performed to evaluate their role in inhibiting diabetic LDL oxidation and to verify whether prevention of free radical might have a role in modifying hemostatic variables in diabetes.

Materials and Methods
Experimental Animals:

Sprague Dawley male rats (n=50) were used in this study, weighing 150-200 gm., obtained from the animal house of National Organization of Drug Control and Research (NODCAR). Animals were kept under standard conditions and allowed free access to food and water. The standard guidelines of NODCAR were used in handling the experimental animals.

Methods:
1) Blood samples were obtained retro-orbitally from the control group (24 rats) to assess the following parameters:
a) Lipid and lipoprotein profile: Triglycerides, LDL cholesterol and HDL cholesterol.
b) Markers of hypercoagulability: Platelets aggregation, fibrinogen level and prothrombin time
c) Marker of antioxidant status: Serum uric acid.
2) Diabetes induction :
Diabetes was induced in rats by using Alloxan® (Alloxan tetrahydrate-Sigma) in a dose of 150 mg/kg for each rat (Chetan et al.,2005).The drug was freshly dissolved in distilled water and was given by intraperitoneal route in two divided doses 75mg/kg each, preceded by 18 hours fasting period. Diabetes was confirmed by the development of hyperglycemia within 36 hours of drug administration using commercially available calorimetric kits.
3) Blood samples were collected retro-orbitally from the diabetic rats and assayed for the lipid profile. Platelets aggregation, fibrinogen, prothrombin time

and uric acid were also assessed and compared to control values.
4)The diabetic rats were then divided into two groups
Group 1 (no.=12): rats received vitamin E, 7mg /rat daily, orally by gavage, for 3 weeks.
Group 2 (no.=12): rats received vitamin C,7mg /rat daily, orally by gavage , in addition to vitamin E for 3 weeks (Maxwell et al., 1993)
5) At the end of 3 weeks, blood samples were collected retro-orbitally from each group and assayed for the same previous parameters.

Preparation of blood samples:
Citrated plasma was prepared by centrifugation of amixture of nine volumes of freshly drawn blood with one volume of trisodium citrate (0.11mol/L) for 30 min. (1600g) then frozen at -80° until assayed.

Chemical Analysis:
Triglycerides concentration in serum were measured using enzyme colorimetric methods (Fossati and Prencipe 1989) .The high-density lipoprotein cholesterol (HDL-C) concentration was measured using the method described by Warnick et al.(1982). Low-density lipoprotein cholesterol (LDL-C) concentration was calculated using the method of Bergmenyer (1985). Uric acid was measured using an enzymatic method (Trivedi et al., 1978).
Spectrophotometer (UV-120-02) was utilized. Commercially available kits (Boehringer Mannheim, Germany) were used.

-Assessment of Platelet Aggregation :
Blood was prepared and used immediately for the assay of platelet aggregation .Collagen produced by chrono-par was used as an agonist for platelet aggregation in a dose of 4 ul/ml blood sample. Platelet aggregation was assayed at 37° C using a platelet aggregometer coultronics (450 dual channel aggregometer) and 540 dual channel recorded. The whole blood impedance method was the technique used during the study. The change in resistance is recorded on a linear strip chart recorder and calibrated as percentage of extent of Platelet aggregation (Cardinal and Flower, 1980).

Assessment of blood coagulation :
Coagulation assays were carried out in ACL 200, a nephlometric centrifugal analyser which measure the intensity of light, scattered by a plasma sample before, during and after clot formation. The increase in light scatter signal at the beginning of clot formation is related to prothrombin time (PT) while the delta scatter reached at equilibrium is proportional to fibrin and therefore to total clottable

fibrinogen (FIB) (Rossi et al., 1988). PT and FIB were determined by IL test TM PT=FIB (97567) kit.

N.B.: Assessment of Platelet Aggregation and blood coagulation were performed in the Clinical and Chemical Pathology Department of Faculty of Medicine, Cairo University.

Statistical Analysis:

Data were processed and statistics were carried out using the student "t" test for paired and unpaired comparison. Correlations were evaluated with the Spearman rank order correlation. P<0.05 was considered significant.

RESULTS
Effects of diabetes on Oxidative and Antioxidative Markers:

Table1, Fig.1 showed that diabetic rats had significantly (p<0.05) higher levels of TG and LDL cholesterol in comparison to control rats. Meanwhile a significant (p<0.05) lower levels of HDL cholesterol and plasma uric acid (Fig.2) had occurred in diabetic rats.

Effects of diabetes on hemostatic parameters:

Table2, Fig.3 showed that diabetes caused significant (p<0.05) higher percentage of platelets aggregation, higher levels of fibrinogen and shorter prothrombin time compared to control values.

Effects of antioxidant vitamins supplementation on diabetic induced changes:

Table (1) showed that supplementation of vitamin E for 3 weeks improved the picture of oxidative stress in diabetic rats as evidenced by a significant (p<0.05) reduction in triglycerides and in LDL cholesterol, and significant increase in HDL cholesterol and in uric acid levels compared to the control diabetic values. Moreover, vitamin E partially reduced the diabetic-induced hypercoagulability state as evidenced by reduction of platelet aggregation and fibrinogen levels accompanied by prolongation in prothrombin time Table (2). By co-administration of vitamin C and vitamin E significant improvement had occurred as evidenced by significant (p<0.05) decrease in platelet aggregation and fibrinogen levels, and prolongation of prothrombin time compared to diabetic levels.

Table (1): Effect of vitamin supplementation on diabetic induced changes in lipid profile {triglycerides (TG), LDL cholesterol, HDL cholesterol} and plasma uric acid. (Mean± S.D)

Parameters	Control rats (n=24)	Diabetic rats (n=24)	Diabetic rats +Vitamin supplementation	
			Vit E (n=12)	Vit E+C (n=12)
TG(mg/dl)	65.3 ±5.2	124± 6.2 *	85± 4*º	81± 8*º
LDL Cholesterol (mg/dl)	118 ±7	134± 2.2 *	123± 7 º	120± 8.6º
HDL Cholesterol (mg/dl)	35.3 ± 3.5	28.2± 3*	31± 3*	32± 2.5*º
Uric acid(mg/dl)	3.7 ± 0.5	3.1± 0.3*	3.5±0.2º	3.6± 0.5º

*Significant difference between control prediabetic and diabetic values (p<0.05).
º Significant difference between vitamin supplemented and non-supplemented diabetic values (p<0.05).

**Table (2):** Effect of vitamin supplementation on diabetic induced changes in hemostatic parameters {platelets aggregation, fibrinogen (FIB) and prothrombin time(PT)}.(Mean± S.D.) .

Parameters	Control rats (n=24)	Diabetic rats (n=24)	Diabetic rats + Vitamin supplementation	
			Vit E (n=12)	Vit E+C (n=12)
Platelet aggregation (%)	70.3±8.2	86 ± 7 *	81 ± 6.4*	78 ± 7 º
FIB (mg/dl)	185.2±14.1	222 ± 13*	203 ± 10* º	200 ± 9* º
PT (sec)	13.5±1.2	11.4 ± 1.1*	11.7 ± 1.5*	11.9 ± 1.6*

*Significant difference between control prediabetic and diabetic values (p<0.05).
º Significant difference between vitamin supplemented and non- supplemented diabetic values (p<0.05).
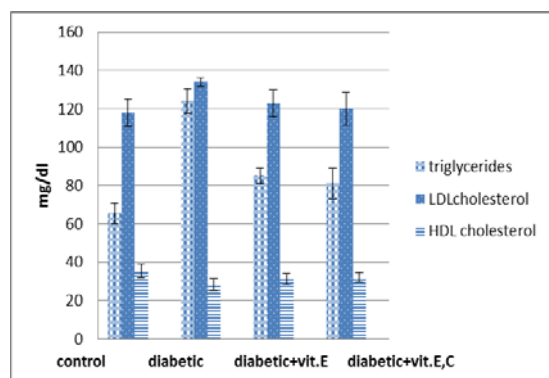
Fig (1): Serum triglycerides, LDL, HDL cholesterol levels in different experimental groups as compared to control.
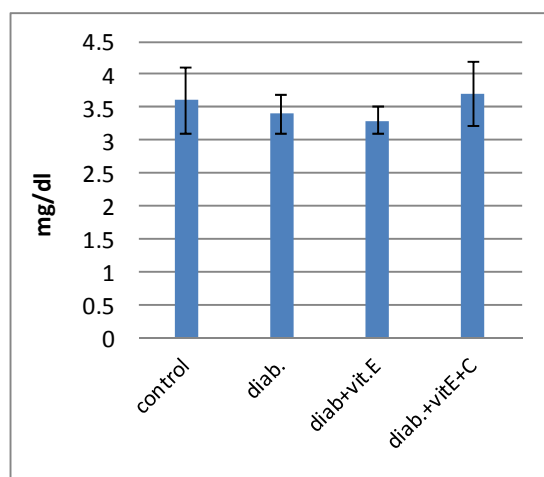

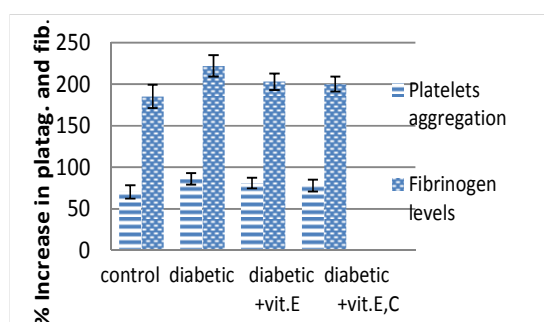**Fig (2):** Serum uric acid level in different experimental groups as compared to control.

Fig (3): Percentage increase in platelets aggregation and fibrinogen levels in the different experimental groups as compared to control.

Discussion

Hyperglycemia has been accepted as an essential factor in the development of diabetic complications (Rosen et al., 2001). Oxidative stress plays an important role in the development of diabetes complications, both microvascular and cardiovascular (Jay et al., 2006). The results of this study supported the relationship of poor glycemic control and higher risk of cardiovascular complications. Administration of alloxan to rats destroyed pancreatic β-cells, leading to inhibited insulin secretion, thus increasing plasma glucose levels. The present study demonstrated that in diabetes, increased production of triglycerides and LDL cholesterol occurred in association with reduced levels of HDL cholesterol and plasma uric acid. This was in accordance with Budin et al., (2009) who studied that both lipid accumulation particularly triglycerides and reduction in antioxidant activity contributed to the development of oxidative stress in diabetes. Hyperglycemia was found to promote lipid peroxidation of low density lipoprotein (LDL) by a superoxide-dependent pathway resulting in the generation of free radicals (Giacco and Brownlee, 2010) .It appears that hyperglycemia could be a driving force for induction of oxidative stress, enhanced leucocyte endothelial interaction and glycosylation of virtually every protein in the body, including lipoproteins and clotting factors (Al-Rawi 2011). Vitamins E and C play an important role in glucose metabolism (Martini et al., 2010). Reduced levels of antioxidants such as ascorbic acid and vitamin E occurred in diabetes (Ceriello et al.,1998). In this study, oral administration of vitamins E and C for 3 weeks improved the picture of oxidative stress in diabetic rats and reduced the diabetic-induced hypercoagulability. Our results were consistent with Rahimia et al., (2005) who indicated that the use of antioxidants reduces oxidative stress in diabetes.

Both vitamin C and vitamin E decreased lipid peroxidation and augmented the activities of antioxidant enzymes in diabetic rats (Kędziora-Kornatowskaa et al., 2003).Vitamin C was found to significantly decrease the elevated levels of blood hydroperoxide, glucose, cholesterol, triglycerides and low-density lipoprotein (LDL) in diabetic rats (Badr et al., 2011). Several studies in diabetic patients, have shown that supplementation with several hundred IU of vitamin E significantly reduced platelet aggregation and lipid peroxidation, so vitamin E may have a therapeutic role in free radical mediated diseases (Gerster;1993). On the other hand, however, a recent study by de Oliveira et al.,(2011), suggested that vitamin E supplementation alone, did not affect the lipid profile of type 2 diabetic patients. In diabetics, platelet vitamin E levels tend to be reduced with concomitant increase in platelet aggregation, this was reversed by correction of the vitamin E status (Garg et al., 2005). In addition, Vitamin E may play a protective role as membrane

stabilizing agent. In platelets it appears to regulate arachidonic acid metabolism (Pazdro and Burgess , 2010). In this study, Uric acid level was significantly reduced in diabetic rats .Uric acid is the end product of purine metabolism; it can act as a pro-oxidant, particularly at increased concentration and may thus be a marker of oxidative stress.Type-1diabetics showed significantly lower serum uric acid levels in comparison to type-2 diabetics. Elevated serum uric acid occurred in type 2 diabetics may lead to significantly higher incidences of coronary artery disease, carotid atherosclerosis, cerebral infarction, diabetic nephropathy and diabetic retinopathy level (Wu et al., 2011). It was observed that uric acid may act as an antioxidant both by binding iron ions and also by directly scavenging reactive oxygen species. In addition , uric acid adds to the enhanced antioxidant profile by preventing ascorbate oxidation and lipid peroxidation (Waugh; 2008).In this study, vitamin E and C supplementation significantly increased the level of uric acid in diabetic rats. Opara; (2002) recommended, that high doses of micronutrient antioxidant vitamins should be administered in combination rather than as single supplement because micronutrient antioxidants interact with each other in a biochemical chain of defence against free radicals. Zhi et al., (2006) proved that there are synergistic antioxidative effects among the antioxidants. On the other hand, however, vitamin C supplementation in healthy dogs doesn't clearly affect blood level of uric acid (Hesta et al., 2009).

In conclusion, the present study provides evidence that hyperglycemia plays a significant role in induction of a hypercoagulable state. Oxidative stress could be the most important factor in the pathogenesis of diabetic complications. Supplementation of vitamins C and E to diabetic rats might assist endogenous antioxidant capacity and ameliorate the oxidative stress caused by hyperglycemia.

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