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The Journal of American Science

<u>ISSN 1545-1003</u> Volume 7, Issue 2, Cumulated No. 35, February 25, 2011 <u>Cover Page, Introduction, Contents, Call for Papers, am0702</u> Welcome to send your manuscript(s) to: <u>americansciencej@gmail.com</u>.

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2	 Biological Investigation of Persian Gulf blue swimmer crab (portunus pelagicus) In Khuzestan coasts Ashraf Jazayeri^{1*}, Forough papan¹, Ahmad savari², Tayeb Saki Nejad³ Shahid Chamran University of Ahwaz 2. Khoramshahr University of Marine Science and technology	Full Text	2

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	Devising an instrument to assess human resources productivity in an Iranian context	Full Text	
5	Amir Ashkan Nasiripour ¹ , Fardin Mehrabian ² , Pouran Raeissi ³ , Jamaledin Tabibi ⁴ 1. Associate Professor, Department of Health Services Management, - Science and Research Branch, Islamic Azad University, Tehran, Iran(Corresponding author). 2. PhD student of Health Services Management, - Science and Research Branch, Islamic Azad University, Tehran, Iran 3 - Associate Professor, School Of Management and medical information services, , Tehran University of Medical Sciences, Tehran, Iran, 4 - Professor, Department of Health Services Management, Science and Research Branch, Islamic Azad University, Tehran, Iran, Email: nasiripour@srbiau.ac.ir; Tel: 00982144869701 ABSTRACT: Background: There are various factors affecting human resources productivity. Moreover, it is a necessity to identify the most important and efficient ones. Therefore, there should be an appropriate instrument to determine the factors in the most comprehensive way. Accordingly, this study has employed Factor Analysis to devise a locally-based instrument to assess human resources productivity in Guilan University of Medical sciences. Materials and Methods: To achieve the mentioned goal, two stages have been taken during the fall of 2009. One was qualitative and the other was cross-sectional. In the former step 45 expert managers were included as the sample of the research to determine productivity factors and in the latter 321 staff members of scientific society, training and human resources departments of Guilan University were selected to establish the productivity variables. Results : To enclose, One questionnair evith 5 headings and 42 questions has been obtained as follows: Organizational culture with 18 questions / Environmental conditions with 7 questions / Motivation factors with 10 questions / Empowerment with 4 questions / Method of leadership with 3 questions. Discussion: The invented device, regarding to it reliability, validity, relevance and indigenousness in assessing of human resource productivity, could be useful for all th	Full Text	5
б	 Aly,M.S¹, Amber.S. Gad*². El Sayed M. Kamel ¹Agriculture Microbiology Dept,² Chemistry of Natural and Microbial Products Dept., National Research Center,EgyptFaculty. of medicine, Pharmcology. Dept. Zagazig Univ *amber2gad@yahoo.cm Abstract: Spirulina platensis is a microscopic blue-green alga in the shape of a spiral coil, living both in sea and fresh water .It is widely used as health food due to its protein content, vitamins and active substances for immune system. Polyunsaturated fatty acids amount to 46.548 %(w/w) of total lipids.Among the essential fatty acids detected in El Khadra lake water body in Waadi El Natroun micro- alga, cholesterol decreasing -linolenic acid with 0.986%(w/w) .Vitamin A amounts to 120.13 µg/100g, vitamin C amounts to 540.34 µg/100g and vitamin D amounts to 105.6 µg/100g were found . Vivo studies revealed Spirulina effectiveness on Triglycerides(TG) ,Total cholesterol(TC),High density lipoprotein- chloestero(HDL-ch), body weight ,serum calcium., serum iron, and serum ferritin after treatment of the experimental rabbits for 30 days. [Aly, M.S, Amber.S. Gad. El Sayed M. Kamel. Production and application of Spirulina platensis rich in fatty acids, and vitamins. Journal of American Science 2011;7(2):36-45]. (ISSN: 1545-1003). 		6

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	Soheir Mahmoud Ali El-Kosery ^{*1} , Khadyga Said Abd El-Aziz ¹ , Nagwa Mohamed Badr ² , Adel Farouk El- Begawy ³ and Randa Osama Mohamed ¹ ¹ Department of Physical Therapy for Gynecology and Obstetrics, Faculty of Physical Therapy, Cairo University. Cairo, Egypt ² Department of Physical Therapy for Cardiovascular/ Respiratory Disorders and Geriatrics, Faculty of Physical Therapy, Cairo University, Cairo, Egypt ³ Department of Gynecology and Obstetrics, Faculty of Medicine, Cairo University, Cairo, Egypt <u>soheir 1212@hotmail.com</u> [*]		
7	Abstract: This study was conducted to determine the effect of inspiratory muscles training in improving ventilatory functions in postmenopausal asthmatic women. Fourty postmenopausal women, complaining from bronchial asthma, one year ago, were participated in this study. They were divided randomly into two equal groups (A and B).Both groups received traditional medical treatment which was consisted of theophyline, salbutamol sulphate(bronchodilator), dexamethasone, carbocisteine (muco-regulator) & antibiotic for six weeks. In addition to traditional medical treatment, Group "A" received the inspiratory muscles training by using inspiratory muscle trainer. Assessment was performed by measuring weight and height then the body mass index was calculated for each woman in both groups (A&B) before treatment. Also, Electronic Spirometer was used to measure ventilatory functions (Forced Vital Capacity, Forced Expiratory Volume, Forced Vital Capacity /Forced Expiratory Volume and Maximum Voluntary Ventilation) before and after 6 weeks of treatment. Results showed a statistically highly significant improvement (P<0.001) in all ventilatory functions in group (A) than group (B) after end of treatment. So, it could be concluded that inspiratory muscle training with traditional medical treatment were more effective than traditional medical treatment only for these cases as it was effective, safe, easy to perform and led to reducing symptoms of asthma.		7
	[Soheir Mahmoud Ali El-Kosery, Khadyga Said Abd El-Aziz, Nagwa Mohamed Badr, Adel Farouk El- Begawy and Randa Osama Mohamed. Efficacy of Inspiratory Muscle Training on Ventilatory Functions in Postmenopausal Asthmatic Women . Journal of American Science 2011;7(2):46-53]. (ISSN: 1545-1003). <u>http://www.americanscience.org</u> . Key words : Inspiratory Muscle Trainer - Ventilatory Functions - Postmenopausal Asthmatic women		
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	Ahmad Reza Ommani Assistant Professor Islamic Azad University Shoushtar Branch, Iran <u>ommani75451@yahoo.com</u>		
8	Abstract: The purpose of research is analyzing efficiency of agricultural extension programs by Participatory Rural Appraisal (PRA). The method of research was qualitative. The research carried out by 4 analytical loops in rural area of Shoushtar township of Khouzestan province, Iran. Each analytical loop consist 6 to 9 rural people and one outsider as facilitator. According to results extension programs based on efficiency was ranked. This ranking respectively include: Farmer Filed School (FFS), Meeting in Farm (MF), Results Farm Demonstration (RFD), Method Farm Demonstration (MFD), Extension classes, Bulletin and Posters, Radio and TV program. Also ranking of educational needs respectively include: productivity indicators, sustainability, farm management, water management, pest and disease, west management. [Ahmad Reza Ommani. Analyzing Efficiency of Agricultural Extension Programs by Participatory Purple Approinal (PPA). (Illustrate: Wheat Farmers of Khouzestan Province, Iran).		8

	American Science 2011;7(2):54-58]. (ISSN: 1545-1003). http://www.americanscience.org.		
	Keywords: PRA, Extension program, Shoushtar		
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	Shadia R. Tewfik*, Mohamed H. Sorour, Abdelghani M.G. Abulnour, Hala A. Talaat, Nihal M. El Defrawy, Joseph Y. Farah and Ismail K. Abdou		
	Chemical Engineering and Pilot Plant Department, Engineering Research Division, National Research Center, Dokki, Cairo, Egypt *Corresponding author email: shadiatewfik@vahoo.com		
9	Abstract: The use of biomass as a source of energy is gaining increasing interest in both developed and developing countries as a renewable source of energy and to avoid the environmental hazards associated with open burning of ligno-cellulosic materials. This work is concerned with the fast pyrolysis of rice straw which is generated seasonally in enormous quantities to produce biooil which could replace fuel oil #2. Experimental investigations on a pilot-scale in an entrained flow reactor demonstrated that the biooil produced is of acceptable characteristics and has a calorific value of about 29kJ/kg. Process design has been developed for a 200 ton/day commercial facility. Material and energy balance and basic engineering have been accomplished using ASPEN PLUS. Techno-economic investigations have been conducted and financial analysis has been performed using ASPEN ICARUS. Results indicated that for a Base Case, the Fixed Capital Cost is about US \$ Million 7.6 and for a pessimistic selling price of US \$ 0.13/kg, the Internal Return of Return (IRR), exceeds 43%. Sensitivity analysis indicates that even for increase of the Fixed Capital to about US \$ Million 10.9 the IRR still exceeds 30%. Further work is needed for technological development and for ensuring processing over the year round using other ligno-cellulosic materials. [Shadia Tewfik, Mohamed Sorour, Abdelghani Abulnour, Hala Talaat, Nihal El Defrawy, Joseph Farah and Ismail Abdou. Bio-Oil From Rice Straw By Pyrolysis: Experimental And Techno-Economic Investigations . Journal of American Science 2011;7(2):59-67]. (ISSN: 1545-1003). http://www.americanscience.org.		9
	Keywords : biomass, fast pyrolysis, bio-oil, entrained reactor, process design, techno-economics		
	Effect of Stitch Geometry on Particle Bypass in Air Filter Bags M. A. Saad and R. F. El-newashy [*] Department of clothing and knitting Industry Research, National Research Center, Giza, Egypt rnewashy@yahoo.com [*]	<u>Full Text</u>	
10	 Abstract: Fabrics used as a filter media in dust control may have satisfactory efficiency. However, the sewn areas of the filter fabric sleeves may cause a bypass of fine particle size dust through needles holes or bent areas of fabric layers. The present study focuses on the filtration and cleaning efficiency of the sewn areas of filter fabrics using different particle size of solid material. Four types of stitch formation type EFa-1, SSa-1, LSc-1 and BSa-1 according to British Standard BS 3870 were used. [M. A. Saad and R. F. El-newashy. Effect of Stitch Geometry on Particle Bypass in Air Filter Bags. Journal of American Science 2011;7(2):68-74]. (ISSN: 1545-1003). http://www.americanscience.org. Keyword: Filter Bag – sewing stitch – sewing machines- sewing threads 		10

	Diagnostic Value of Serum Cystatin C as an Early Indicator of Renal Impairment in Chronic HCV Egyptian Patients with Liver Cirrhosis	Full Text	
11	Mohamed El –Shazly ¹ , Ayman El Shayeb ^{*1} , Pacint Moez ² , Mohamed Samy ³ , Mariam Zaghloul ¹ Tropical Medicine ¹ , Clinical Pathology ² and Radiodiagnosis ³ Departments, Faculty of Medicine, Alexandria University, Alexandria, Egypt, <u>drayman65@yahoo.com</u> * Abstract: Background and aim: Diagnosis of moderately impaired glomerular filtration rate can be diagnosed easily by elevated serum creatinine concentrations but, moderately reduced renal function may be missed. Cystatin C (CysC) has been suggested as a sensitive marker of renal function, independent of sex or muscle mass. Therefore, the aim of this study was to investigate the value of serum cystatin C concentrations for the detection of moderately impaired renal function in chronic HCV Egyptian patients with liver cirrhosis as well as its correlation with Child-Pugh score and renal resistive index (RRI). Patients and Methods: This study was conducted on seventy subjects; group I fifty non azotemic chronic HCV patients with liver cirrhosis (furtherly subdivided according to the Child-Pugh score into group 1 ⁸ , 1 ⁶ , 1 ^c) and group II twenty healthy subjects with matching age and sex as control group. Liver function tests, renal function tests, CysC levels and RRI were measured on the same day for all patients. CysC levels were measured using the automated latex-enhanced immunonephelometric method. Results: Mean serum levels of serum Cystatin C were 0.66±0.05, 1.02±0.28, 1.17±0.32 and 0.65±0.10 mg/dl in groups 1 ^a , 1 ^b , 1 ^c and II respectively. Serum cystatin C was significantly higher in cirrhotic patients than in controls. Moreover, It was significantly higher in Child C cirrhotic patients than in those with Child B and A (F=19.14 and P=0.001). Significant positive correlations were found between serum cystatin C and each of blood urea (BU), serum creatinine, RRI and Child-Pugh score in patients with HCV induced liver cirrhosis.(r=0.454,0.781,0.508 and 0.412respectively) (p≤0.01). On the other hand, significant negative correlation was found bet		11
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12	Qalubia Governorate Fouad El-said El-Debaky ¹ , Naglaa Ibrahim Azab ^{*1} , Naglaa Fathy Alhusseini ¹ , Sanya khairy Eliwa ¹ and Hamed Rashsad Musalam ² ¹ Medical Biochemistry, Faculty of Medicine, Benha University, Benha, Egypt. ² General Surgery, Faculty of Medicine, Benha University, Benha, Egypt. ² General Surgery, Faculty of Medicine, Benha University, Benha, Egypt. [*] naglaa1270@ yahoo.com Abstract: Breast cancer is the most common cancer in women and its impact on morbidity and mortality is significant and well documented. BRCA genes mutation account for most of the cases of familial breast cancer is the most common cancer in women and its impact on morbidity and mortality is significant and well documented. BRCA genes mutation account for most of the cases of familial breast cancer. Female BRCA1 mutation carriers have an 80% to 85% risk of developing breast cancer over their life-time. This study aims to detect 5382insC ,185delAG and C61G mutations in BRCA1 gene in healthy females and breast cancer female patients in Qalubia Governorate and correlate them with the presence or aberast cancer patients k/or ovarian cancer to allow identification of individuals at high risk. Materials and methods: 50 females divided into 20 healthy females and 30 breast cancer patients with or avarian can		12

	 gene mutation among the females of Qalubia governorate followed by C61G mutation and 185 delAG mutation. Conclusion: In conclusion, BRCA1 gene mutation and multiple BRCA1 exons mutations play an important role in the pathogenesis of familial breast cancer in Qalubia Governorate, Egypt. [Fouad El-said El-Debaky, Naglaa Ibrahim Azab, Naglaa Fathy Alhusseini, Sanya khairy Eliwa and Hamed Rashsad Musalam. Breast Cancer Gene 1 (Brca 1) Mutation in Female Patients with or without Family History in Qalubia Governorate. Journal of American Science 2011;7(2):82-93]. (ISSN: 1545-1003). http://www.americanscience.org. Key words: familial breast cancer, BRCA1 gene, 5382insC mutation, 185delAG mutation, C61G mutation 		
		Full Text	
	Serum Visfatin in patients with chronic hepatitis C		
13	Abd El Fattah Hano ¹ , Akram Deghady ² , Sahar Shaaban ^{*1} and Marwa Abd El Rahman ¹ ¹ Tropical Medicine Department, Faculty of Medicine, Alexandria University, Alexandria, Egypt ² Clinical Pathology Department, Faculty of Medicine. Alexandria University, Alexandria, Egypt sahoram@hotmail.com [*] Abstract: Background: The role of visfatin in non alcoholic fatty liver diseases (NAFLD) is now well known accordingly, the aim of this work was to study the serum level of Visfatin in patients with chronic hepatitis C (HCV) and their relations to the nutritional state of patients as well as the biochemical markers of liver disease. Subjects: This study was carried out on 75 male subjects classified into five groups all of them were subjected to measurement of body mass index (BMI), lipid profile, liver function tests, PCR for HCV, serum visfatin level & liver biopsy when ever possible was done. Results: Mean serum Visfatin level was significantly elevated in group II (HCV & cirrhosis) and group IV (HCV & steatosis) than in group V. (P < 0.05). Significant positive correlation was found between serum visfatin & BMI, degree of inflammation & fibrosis. (P<0.05) On the other hand, significant negative correlation was noted between serum visfatin & apolipoprotein A1. (P<0.05). Conclusion: High levels of visfatin in patients with HCV and steatosis than other patients' groups suggest its involvement in the process of steatosis and schistosomiasis suggest its role in liver fibrogenesis. [Abd El Fattah Hano, Akram Deghady, Sahar Shaaban and Marwa Abd El Rahman. Serum Visfatin in patients with chronic hepatitis C. Journal of American Science 2011;7(2):94-101]. (ISSN: 1545-1003). http://www.americanscience.org. Keywords: Serum; Visfatin; patient; chronic hepatitis C		13

	Characterization of Poly-isoprene Rubber Layer Backed with Porous Material as Sound Absorber and Vibration Damper	Full Text	
	Abd-elfattah A. Mahmoud [*] and Mohamed Abd-elbasseer		
	Acoustics Department, National Institute for Standards. Geiza, Egypt. yy_abd_elfattah@yahoo.com*		
14	Abstract: There are several methods to decrease acoustic noise. Sound absorption is one of the noise control methods, commonly, multi-layer sound absorbers are applied to absorb broad band noise. , the sound absorption coefficient of different materials are measured in impedance tube using two-microphone transfer-function method according to ISO 10534-2 and ASTM E1050-98 international standards. Multi-layers sound absorbers effectiveness depends on their construction. The effects of different porous materials (R, S &F), and layers backing on the sound absorption and of poly isoprene rubber will be studied. The treatment for Multi-layered with sponge and rubber increasing the sound absorption coefficient value at about 0.9 and shifted towards a lower frequency range 315-800 Hz. [Abd-elfattah A. Mahmoud and Mohamed Abd-elbasseer. Characterization of Poly-isoprene Rubber Layer Backed with Porous Material as Sound Absorber and Vibration Damper . Journal of American Science 2011;7(2):102-109]. (ISSN: 1545-1003). http://www.americanscience.org. Keywords: Characterization; Poly-isoprene; Rubber Porous; Material; Vibration Damper		14
	The Effect of Boiling on Milk Microbial Contents and Quality	Full Text	
	Ahmed M.M. Metwally ¹ , Nadia M.A.Dabiza ^{*2} , Wagih I.El-Kholy ² and Zeinab I.Sadek ² ¹ Dairy Technol.Dept. Faculty of Agric. Cairo University, Giza, Egypt ² Dairy Science Department, National Research Center Dokki, Giza, Egypt <u>nadiadabiza@yahoo.com</u> [*]		
15	Abstract: Though milk boiling is a widespread heat treatment in number of countries, the process was not thoroughly studied. In this study, the effect of boiling buffaloes' and cow's milk samples for different periods on their microbiological contents, keeping quality and bacterial ecology contents and chemical changes were determined. Lethality rate of 6.53, 6.77, 7.301 and 7.44 in buffaloes' and 6.76, 7.059, 7.012, 7.15 and 7.159 log ₁₀ cfu/ml in cows' milk were obtained on boiling the samples for 0.5, 1,2 and 5min., respectively. Boiling milk for 0.5 and 1min decreased the bacterial count from 3.6×10^9 in cow's milk into 6.3×10^2 and 3.2×10^2 and from 7.8×10^9 in buffaloes 'milk into 2.26×10^3 and 1.3×10^3 cfu/ml, respectively. On cold storage, the microbial content of boiled milk, not only did not increase but also declined on the first week. Boiling destroyed bacterial vegetative cell leaving behind spores of the sporeformer which were dominated with <i>B.cereus</i> and <i>Micrococcus leteus</i> . Boiling affected milk quality far less than the effect occurred in UHT milk as determined by O.D- value measurements. [Ahmed M.M. Metwally, Nadia M.A.Dabiza, Wagih I.El-Kholy and Zeinab I. Sadek. The Effect of Boiling on Milk Microbial Contents and Quality . Journal of American Science 2011;7(2):110-114]. (ISSN: 1545-1003). <u>http://www.americanscience.org</u> .		15

	Evaluation of the Effects of Bagasse on Tensile and Compressive Strength of Lightweight Concrete	Full Text	
16	Mojtaba Labibzadeh ¹ , Mehdi Nasirifar ¹ , Amin Khajehdezfuly ¹ ¹ Department of Civil Engineering, Faculty of Engineering, Shahid Chamran University, Ahvaz, Iran <u>Labibzadeh m@scu.ac.ir</u> Abstract: Mechanical characteristics of lightweight concrete contained bagasse, including splitting tensile and compressive strength have been examined. Bagasse as an agricultural waste was obtaining in the form of small wood chips after extracting cane sugar in the Khouzestan state of Iran. In this research, at first a fixed mix design was considered according to ACI-21. Then some samples were prepared corresponding to the above mentioned design code and by inserting 20,30,40 and 50% bagasse as a replacement for aggregates in concrete mixture and consequently these samples were tested. The results showed that by increasing of the content percentage of bagasse, compressive strength decreased and this decrease for concrete containing 20% bagasse is about to 36%. The concrete containing 20% bagasse has more splitting tensile strength in compared to normal concrete approximately up to 13%. Finally, based on the obtained findings, it can be concluded that concrete with 20% bagasse could be introduced as an alternative lightweight concrete regarding to its lower unit weight and higher splitting tensile strength. [Mojtaba Labibzadeh, Mehdi Nasirifar, Amin Khajehdezfuly. Department of Civil Engineering,Shahid Chamran University, Ahvaz, Iran. Journal of American Science 2011;7(2):115- 120]. (ISSN: 1545-1003). http://www.americanscience.org. Keywords: Bagasse; Lightweight concrete; Compressive strength; splitting tensile strength		16
17	 Sulphur efficiency in rising of pollution soil by heavy metals qualification under conditions of lettuce plant cultivation Abd El Fattah, M.S. Khaled, S.M. and Safaa. A.M. National Research Centre, Plant Nutrition Department, Cairo, Egypt. Abstract: Pot experiment was established in green house at National Research Centre, Dokki, Egypt, in polluted soil from industrial wastes by heavy metals. Sulphur elemental was used at different rates (100, 200 and 300 ppm). Lettuce plant of class (lactuca sativa var. Capitata). Loamy sand soil type from Helwan region at south of Egypt. Some parameters in fresh plant were performed. From plant analysis showed existence positive relationships between sulphur concentrations were added and chlorophyll concentrations, (N,P and K) and (Fe, Mn, Zn and Co) while a negative contact between sulphur rates were 	<u>Fuil Text</u>	17
	added and (Cd, Ni, Pb and (Cu) contents by comparison with control. Water filtering from water irrigation was analyzed and shown that heavy metals were leached from soil. Soil was analyzed at experiment end shown that nonexistence heavy metals in soil except slight traces. All the differences between treatments were significantly. [Abd El Fattah, M.S. Khaled, S.M. and Safaa. A.M. Sulphur efficiency in rising of pollution soil by heavy metals qualification under conditions of lettuce plant cultivation. Journal of American Science 2011;7(2):121-128]. (ISSN: 1545-1003). <u>http://www.americanscience.org</u> . Key words: Sulphur. Loamy sand soil. Lettuce plant. Heavy metals. Macro-micro nutrients-chlorophyll		

18	 Incorporation Jatropha Curcas Meal on Lambs Ration and It's Effect on Lambs Performance Abo El-Fadel .M.H., Hussein, A.M. and Mohamed, A.H. Animal Production Research Institue, Agricultural Research Center, Giza, Egypt. <u>alaaapri@link.net</u> Absract: This study was conducted to determine the effect of heat (HJM), or biologically with lactobacillu bacteria (BJM), treatments of Jatropha <i>curcas</i> meal with on concentrate ion of anti-nutritive compounds. I order to replacement of costly imported soybean meal and find out their effects on rumen fermentatio characteristics degradability and consequently lambs performance. Seven concentrates feed mixtures (CFM contained soybean meal was replaced with untreated Jatropha meal (UJM) by 0%, JMU (CFM⁰), 25% JMI (CFM¹), 50% JMU (CFM²), or heated Jatropha meal (JMH) by 25% (CFM⁴) and 50% JMH (CFM⁵) of biological Jatropha meal (JMB) by 25% (CFM¹⁰) and 50% JMI (CFM¹¹), were formulated to study the degradation kinetics in the rumen, concentration of anti-nutritive compounds and performance of lambs fee tested rations. Biological treated (BJM) was more effective in decreasing anti-nutritive compounds than heir treatment. These were reflecting on the degradation kinetics, where DM and OM and their effectiv degradability (ED) were higher in (BJM) than (HJM). No significant differences were detected for daily gai of lambs fed rations contained Basel or that contained 50% BJM. Economic cash return was more profit fc BJM ration than the Basel ration. Under the conditions of the present experiment, could be concluded the the bacterial treated JCMB could be replaced up to 50% JMB with Soybean meal at CFM. [Abo El-Fadel .M.H., Hussein, A.M. and Mohamed, A.H. Incorporation Jatropha Curcas Meal on Lambs Ration and It's Effect on Lambs Performance. Journal of American Science 2011;7(2):129-132]. (ISSN: 1545-1003). <u>http://www.americanscience.org</u>. 	Full Text	18
19	 The Effect of Boiling on Milk Microbial Contents and Quality Ahmed M.M. Metwally^a, Nadia M.A.Dabiza^b, Wagih I.El-Kkoly^b and Zeinab I.Sadek^b	<u>Full Text</u>	19

	Hormesis Influence of Glyphosate in Between Increasing Growth, Yield and Controlling Weeds in Faba Bean	Full Text	
	El-Shahawy, T.A. and Faida A.A. Sharara Botany Dept., National Research Centre, Dokki, Cairo, Egypt		
20	Abstract: This study investigates the response of faba bean and associated weeds to the low rates of glyphosate (1.4, 2.8, 5.6, 11.2, 22.4, 44.8, and 89.6 g a.i./feddan) corresponding to 0.3, 0.6, 1.2, 2.4, 4.8, 9.6, and 19.2% of the usage rate (0.467 kg/feddan), respectively. Two field experiments were conducted in this regard during two successive seasons (2008/2009-2009/2010). The plants were sprayed once and twice, 5 and 15 weeks after sowing. Generally, the one foliar application treatments were more effective in increasing faba bean growth than the two foliar application treatments. The best results obtained were for the concentration 11.2 g/feddan, especially sprayed as once. Plant height, fresh and dry weight as well as yield and its components were significantly increased in response. Total protein and carbohydrate contents were unaffected by the treatments. Weeds in converse were negatively affected recording the highest inhibition rate at the concentration 11.2 g sprayed also as once. It has been suggested that the low doses of glyphosate can induce faba bean growth, and this could indirectly affect weeds growth. [El-Shahawy, T.A. and Faida A.A. Sharara. Hormesis Influence of Glyphosate in Between Increasing Growth, Yield and Controlling Weeds in Faba Bean. Journal of American Science 2011;7(2):139-144]. (ISSN: 1545-1003). <u>http://www.americanscience.org</u> . Keywords: faba bean, glyphosate, herbicides, hormesis, weeds		20
	Neurological Disorders In Shoe-Makers And The Role Of Some Trace Elements	Full Text	
21	Salwa F. Hafez1, Yasser H. Ibrahim2, Amal S. Hussein1 and Mahmoud A. Hassanien2 Departments of 1Environmental & Occupational Medicine and 2Air Pollution Research, National Research Centre, Egypt Salwa999 2000@yahoo.com Abstract: Workers in shoemaking industry are exposed to various risk factors which can result in many health hazards. They include CNS affection, other system abnormalities and biochemical derangements. The aim of this study is to investigate the possible neurological disorders among shoe makers, determinate the levels of Cu, Zn and Se in the workers, and correlate the environmental concentrations of respirable dust, heavy metals, and organic solvents contents in the workplaces with the detected neurological disorders. The study included 62 shoe makers and 72 control subjects who were matched for age, sex and socioeconomic status. Questionnaire and clinical neurological examination were done for all subjects. Serum trace elements (Cu, Se and Zn) were determined. Air samples were collected for environmental monitoring of volatile organic compounds (VOCs), respirable dust and its heavy metal content such as (Cu, Pb, Cr and Ni). Results showed that VOCs level in the ambient air of the workplaces were found to be lower than the OSHA standard. Respirable dust and heavy metal contentrations were alded tha 61% of the shoe makers had neurological disorders, while all the control subjects were normal. Nearly half the shoe makers with neurological abnormality had combined cranial and spinal neurological disorders (47.4%). Olfactory and auditory nerves recorded the highest frequency of affection. Among motor sand horse with cranial problems while there was no significantly longer than that in normal shoe makers and those with cranial problems while there was no significant effect of the age on the incidence of neurological abnormalities, on comparing levels of the trace elements between the shoe makers and controls, Se was significantly lower in the shoe makers. In conclusion, Occupational exposure to signi		21

	development of neurological abnormalities and needs further study. [Salwa F. Hafez, Yasser H. Ibrahim, Amal S. Hussein and Mahmoud A. Hassanien. NEUROLOGICAL DISORDERS in SHOE-MAKERS and the ROLE of SOME TRACE ELEMENTS . Journal of American Science 2011;7(2):145-153]. (ISSN: 1545-1003). <u>http://www.americanscience.org</u> . Key Words : Cranial Neurological Disorders, Spinal Neurological Disorders, shoemakers, Organic Solvents, Zinc, Selenium		
	Ultrastructural Studies On The Effect Of Electromagnetic Field On The Liver Of Albino Rats (<i>Rattus Norvegicus</i>)	<u>Full Text</u>	
	Mohamed El-Hady El-desoky and Marwa Mohamady Department of Zoology - Faculty of Science - Benha University <u>dr.elhady@yahoo.com</u>		
22	Abstract: The aim of the present study was to study the effect of long-term exposure of rats (1 hour per day, 3 days weekly for 4 weeks) to either static or time varying magnetic fields of induced flux densities 2 mT (millitesla) =20G (Gauss), on the ultrastructure of liver tissue. One hundred and ten male rats were divided into three main groups. Animals of the first group (10 rats) were not exposed to the magnetic field and represented the control group. The second group (50 rats) was exposed to static magnetic field (direct current) at strength of 2 mT. The third group (50 rats) was exposed to alternating magnetic field (alternating current) at strength 2 mT. The results revealed structural irregularity in hepatocyte nuclei as the most prominent ultrastructural change in the liver of treated groups. This was manifested as irregularity of nuclear membranes, widening of the nuclear pores and heterogeneous distribution of the chromatin material. Furthermore, swelling and clumping and deformation of mitochondria were observed in the groups exposed to the magnetic field. In addition, the rough endoplasmic reticulum appeared with marked dilation and the lysosomes appeared distorted. [Mohamed El-Hady El-desoky and Marwa Mohamady. ULTRASTRUCTURAL STUDIES ON THE EFFECT OF ELECTROMAGNETIC FIELD ON THE LIVER OF ALBINO RATS (RATTUS NORVEGICUS). Journal of American Science 2011;7(2):154-165]. (ISSN: 1545-1003). http://www.americanscience.org.		22
	Seed Morphology and Seed Coat Sculpturing of 32 Taxa of Family Brassicaceae	Full Text	
23	Wael Taha kasem ¹ , Ghareeb, A ² and Marwa, E. ² ^{1.} Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt ^{2.} Botany Department, Faculty of Science, Zagazig University, Cairo, Egypt <u>ataawael@yahoo.com</u> Abstract: The seed exomorphic characters of 32 taxa were investigated by the aid of LM and SEM. The seed exomorphic characters that is diagnostic at the generic and specific level are, seed shape, dimensions, colour, epidermal cells, and seed coat surface, aspect of anticlinal and periclinal walls. The seed shape among the taxa showed wide range of variations. LM revealed most of the seeds vary from globose to oblong-ellipsoid or elongate. The seed shape as observed in the present study seems to be diagnostic at the generic level. Also, most of the investigated seeds have no wings except <i>Farsetia aegyptia</i> . The SEM investigation at higher magnifications revealed main six types of seed surface sculpture; reticulate, ocellate, foveate, papillate, stellate and domate. The seed exomorphic criteria extracted from LM and SEM were analyzed by the STATISCA program package using the UPGMA clustering method. Produced data facilitate the construction of an dendrogam between the studied taxa. [Wael Taha kasem, Ghareeb, A and Marwa, E. Seed Morphology and Seed Coat Sculpturing of 32 Taxa of Family Brassicaceae. Journal of American Science 2011;7(2):166-178]. (ISSN: 1545-1003). http://www.americanscience.org.		23
24	Keywords: Brassicaceae (Cruciferae); Seed coat sculpture; LM; SEM Evaluation of the Reaction of Major Weeds and Some Rice Cultivars to Colletotrichum graminicola	Full Text	24

	Mohammad Reza Safari Motlagh ¹ and Armin Javadzadeh ² ^{1.} Department of Plant Pathology, Faculty of Agriculture, Islamic Azad University, Rasht Branch, Rasht, Guilan Province 41335-3516, Iran. Tel:00989111384168 ^{2.} Department of Plant Breeding and Agronomy, Faculty of Agriculture, Islamic Azad University, Tabriz Branch, Tabriz, Azerbaijan Province Iran *Corresponding author's email: <u>ssafarimotlagh@yahoo.com</u>		
	Abstract: Alisma plantago-aquatica, Sagitaria trifolia and Echinochloa spp. are among the most important damaging weeds of rice paddies. In this research, Colletotrichum graminicola was isolated from these weeds and studied as a biological agent for controlling weeds. To do so, at first, reactions of five rice cultivars including three indigenous cultivars such as Hashemi, Ali Kazemi and Binam and two bred ones, i.e. Sepidroud and Khazar to Colletotrichum graminicola were evaluated. Thus, a complete random design with three replications and five treatments was used at a greenhouse. Then, Colletotrichum graminicola was inoculated on these weeds. The experimental design was a randomized completed with three replications. Inoculation was done at the 3-4-leaf stage using a spore suspension consisting of		
	10 ⁶ conidia/mL distilled water to which Tween-20 1% was added. Results showed that <i>Colletotrichum</i> graminicola caused high disease ratings in Alisma plantago-aquatica, Sagitaria trifolia, E. crus-galli and E. oryzicola, respectively. In addition, the studied rice cultivars showed a significant reaction in terms of the disease rating among which bred cultivars were less tolerant. Moreover, the fungus reduced fresh weight, dry weight and height in the studied weeds and rice cultivars. Therefore, C. graminicola can be used as a mycoherbicide for the biological control of these weeds only when other cultivars except the above-mentioned rice cultivars are planted. This issue particularly requires producing new tolerant cultivars with a combination of the desired traits. [Mohammad Reza Safari Motlagh and Armin Javadzadeh. Evaluation of the Reaction of Major Weeds and Some Rice Cultivars to <i>Colletotrichum graminicola</i> Journal of American Science 2011;7(2):179-185]. (ISSN: 1545-1003). http://www.americanscience.org.		
	The Theoretical and Conceptual Framework and Application of Community Empowerment and	Full Text	
	Participation in Processes of Community Development in Malaysia		
	¹ Asnarulkhadi A Samah & ² Fariborz Aref		
	¹ Dept. of Social and Development Science, Faculty of Human Ecology, Putra University, Malaysia ² School of Management and Economics, Science and Research Branch Islamic Azad University, Tehran, Iran, <u>fariborz.aref@gmail.com</u>		
25	Abstract: This study defines and discusses the concept of participation in the context of social development in general, and in terms of community development and community work specifically. Community development could not be achieved without community participation. The principles and techniques underlying the empowering process are also elaborated. The study describes the application of the concepts defined in some of the community work and community development activities in Malaysia		25
	[Asnarulkhadi A Samah & Fariborz Aref, The Theoretical and Conceptual Framework and Application of Community Empowerment and Participation in Processes of Community Development in Malaysia, Journal of American Science 2011; 7(2):186-195]. (ISSN: 1545-1003)" <u>http://www.americanscience.org</u> Keywords: community, community participation, empowerment		
	Level set segmentation method in cancer's cells images	Full Text	
26	*Department of Electrical Engineering, Islamic Azad University Central Tehran Branch, poonak Sq.		26

	Corresponding Author: <u>Tel:+98</u> 21 66423022, E-mail: <u>alielyasi15@gmail.com</u> Abstract: For early detection in cancer, it is necessary that cells be monitored on time. One of the first steps in the monitoring process is segmenting the cancer's cells. In this paper, we focused on the level set method and compared with snake active contour that use in image segmentation. Level set method is a fast and accurate approach that can be used in segmentation and reduce human interaction as possible. A set of cancer cells images is selected to serve as the representative test set. The selections are different sizes and resolutions. [Ali Elyasi, Yousef Ganjdanesh, Kave Kangarloo, Mehdi Hossini. Level set segmentation method in cancer's cells images. Journal of American Science 2011;7(2):196-204]. (ISSN: 1545-1003). <u>http://www.americanscience.org</u> . Keywords: Level set; GVF snake; cancer's cell; Image segmentation		
	Determinants of urban Land Price in Freetown, Sierra Leone	Full Text	
28	Victor Tamba Simbay KABBA ^{1, 2} and Jiangfeng Li ³ ¹ Department of Land Resources Management, Faculty of Economy and Management, China University of Geosciences, Wuhan, Hubei Province 430074, China <u>Victor kabba@yahoo.co.uk;</u> 0086-15827480592 ² Institute of Geography and Development Studies, School of Environmental Sciences Njala University, Republic of Sierra Leone ³ Department of Land Resources Management, Faculty of Earth Resources, China University of Geosciences, Wuhan, Hubei 430074, China Abstract: Internal migration has over the last two decades increased demand for residential land in Freetown. Unfortunately, access to land via the government functionary has not kept pace with demands. The majority of the population therefore depends on the unofficial land market. This study addressed two objectives. The first attempted to identify the most important variables that determine urban land price. The second compared the east, and west sections of the city, in terms of which factors are significant in determining land price in each area. Taking eight settlements, four on either side of the city centre, 160 residents were interviewed for objective one. A model was developed using 10 variables, and a regression indicated a strong overall positive correlation between the dependent and explanatory variables, and the regression model highly significant. Positive correlation coefficients were shown by income, population, infrastructure, social status, environmental concerns and neighbourhood. These indeed explained the factors that influenced residential land use in Freetown, especially in the western half of the city. For objective 2, a total of 40 land traders and 40 land buyers were interviewed, and the results presented in percentages. These showed that the two sides agreed only on income, and population as significant in determining land price. We concluded that this had to do with class division, as the west houses the three arms of government, diplomats, and wealthy, as against t		28
	Determination of the Appropriate Time of Relaying Cassava into Pepper in Intercropping System in Nigeria	Full Text	
29	Adeola, R. G. ¹ , Tijani-Eniola, H. ² And Kolawole, G. O. ³ ¹ Department of Agricultural Economics and Extension, Ladoke Akintola University of Technology, P.M.B. 4000 Ogbomoso, Nigeria ³ Agronomy Department, University of Ibadan, Ibadan, Nigeria ³ Department of Agronomy, Ladoke Akintola University of Technology, P.M.B. 4000 Ogbomoso, Nigeria; <u>adeola20022000@yahoo.com</u>		29
	ABSTRACT: Relay intercropping of cassava (<i>Manihot esculenta</i> Crantz) into pepper (<i>Capsicum annum</i> L.) is a common practice among farmers in Nigeria. However, there is high variation in the time of the relay thus leading to variability in yields of cassava and pepper. Field experiments were conducted to		

	determine the appropriate time to introduce cassava into pepper in a relay intercrop. The experiment was a randomized complete block design replicated three times. Five different planting dates of relaying cassava into pepper were evaluated . Pepper and two cassava varieties were the test crops. Sole pepper was included for comparison. Delayed relay planting of cassava into pepper beyond 1 MAT (Months after transplanting) adversely affected the yield of both cassava varieties while simultaneous planting of pepper and cassava significantly (P 0.05) delayed flowering and maturity of pepper with a resultant effect of significant (P 0.05) reduction in fruit yield of pepper. The most appropriate time to introduce cassava into pepper in a relay intercrop was 1 MAT. [Adeola, R. G., Tijani-Eniola, H. And Kolawole, G. O. Determination of the Appropriate Time of Relaying Cassava into Pepper in Intercropping System in Nigeria. Journal of American Science 2011; 7(2):224-231]. (ISSN: 1545-1003). http://www.americanscience.org. Key Words: Capsicum annuum, cropping pattern, Manihot esculenta, relay intercrop, sole crop		
	Enhancement the teaching and learning methods of some zoological courses (invertebrate, parasitology and animal physiology) in Taif University, KSA	Full Text	
30	Ismail Shalaby ^{1,2} , Nahla S. El-Shenawy ^{1,2} , Mahi Ghobashy ^{1,2} and Amaal Mohammadein ^{1,3} ¹ Biology Department, Faculty of science, Taif University, Taif, Saudi Arabia. ² Zoology Department, Faculty of Science, Suez Canal University, Ismailia, Egypt. ³ Zoology department, Faculty of Science, Zagazig University, Zagazig, Egypt <u>clshenawy nahla@hotmail.com</u> Abstract: The main objective of this study is to outline specific manners by which learning and teaching processes can be enhanced in the university campuses for the sake of superior learning capabilities and experiences. This paper describes the advantages of various approaches of improving the teaching of some biological courses (invertebrate, parasitology and animal physiology), including the disciplinary, the problem-oriented and combined approaches. In the disciplinary approach, the previous courses are taught in the classical manner as a coherent subject, covering classification of invertebrate, biology of invertebrate and parasites, molecular biology, pathology and immunology, as well as clinical manifestations, diagnosis, therapy, control and prevention of parasitic diseases. Animal physiological course included the description of different systems in the human body and compared with the other animals. Problem-oriented teaching approaches the subject, starting from diseases in animal species or from organ systems; it also tackles training of skills for problem solving and self-learning. Combined approaches include elements of the disciplinary approach and those of other methods. A list of the developed courses and the way of presentation has been discussed. The course syllabus put in student guide to be given to the student in the beginning of the semester. Improving the practical courses has been done by recording them in video. The strategy in the lectures has been changed with the students to minimize the distance with them. Moreover, three workshops have been hold during improving the courses to enhance the efficacy of t		30
	Water use efficiency in rice hybrid under different water intervals and nitrogen levels	Full Text	
31	Ashouri, M and E, Amiri Islamic azad university Roudsar&Amlash branch, Iran. E-mail: <u>mashouri48@yahoo.com</u> and mashouri@iau-roudsar_amlash.ac.ir Tel: +981426212910; Fax: +981426212911		31

	Corresponding Author: Ashouri, M.	
	Abstract: Shortage of water in rice cultivation area of Iran is going to be a major problem in near future therefore water use in rice production systems has to be reduced and water use efficiency increased. Experiment was conducted in a split plot based on completely randomized block design with 3 replications during 2007-2008. Four levels of nitrogen (N1=0, N2= 90, N3= 120 and N4= 150 kg/ha) were splited on 4 different irrigation managements (I1=continuous submergence, I2= 5, I3= 8 and I4= 11 days interval). Grain yield was 7342, 7079, 7159 and 5168 kg/ha in I1 to I4 and 5303, 6628, 7398 and 7418 kg/ha in N1 to N4 respectively. Water use efficiency was 1.41, 1.53, 1.68 and 1.31kg m3 in I1 to I4 and 1.16, 1.48, 1.67 and 1.63 kg/m3in N1 to N4 respectively. Water used was 5190, 4630, 4270 and 3950 m3 in I1 to I4 and 4590, 4470, 4440 and 4540 m3 in N1 to N4 respectively. The results clearly showed that continuous submergence irrigation is not essential for rice production and we can use irrigation interval 8 days. The application of 120 and 150 kg/ha nitrogen produce same grain yield, therefore consumption of 120 kg/ha nitrogen for rice hybrid advised. [Ashouri, M and E, Amiri. Water use efficiency in rice hybrid under different water intervals and nitrogen levels. Journal of American Science 2011; 7(2):239-243]. (ISSN: 1545-1003). http://www.americanscience.org.	
	Keywords: irrigation, nitrogen, water use efficiency, rice, Iran	
32	 Nilotica (<i>Oreochronis, Bachta's Jabitis</i>, against Fravobacterium commare in Frapha Nilotica (<i>Oreochronis Niloticus</i>) Fish in Sharkia Governorate, Egypt Mohamed H Mohamed and Nahla AG Ahmed Refat Dept of Vet. Pathology, Fac Vet Medicine, Zagazig University, 44519 Zagazig City, Egypt. Nahla_kashmery@hotmail mohamedelariny@yahoo.com Abstract: Fifteen out-of eighty-five of collected <i>Tilapia nilotica</i> fish (17.64%) showing skin lesions, were positive for <i>Flavobacterium columnare</i> with cultural, morphological and biochemical characteristics. These skin lesions were large erosions with loss of scales and red-grayish patches, particularly at the frontal head region and abdomen. All of the positive isolates (<i>Flavobacterium columnare</i>) were molecularly tested by means of PCR. With consistent with <i>F. columnare</i> standard ATCC 49512 strain, these isolates produced a 675 bp band. One hundred apparently healthy <i>Tilapia nilotica</i> fingerlings (30±5 gm) were used to evaluate the effectiveness of probiotic, <i>Bacillus subtilis</i>, in water or diet against the intramuscular challenge with <i>Flavobacterium columnare</i> infection. They were equally divided into 10 groups (10 fish for each group). Five groups were experimental control {placebo (gp 1), intramuscularly infected with 0.2 x10⁸ <i>F. columnare</i> CFU (gp 2), received 0.1 gm/L probiotic in mater (gp 3), 0.2 gm /L probiotic in fish diet (gp 4), or 1 gm/L oxytetracycline (gp 5)}; two were prophylactic experiment {received 0.1 gp 6) or 0.2 (gp 7) gm of probiotic in water (gp 8), diet (gp 9) or 1 gm/L oxytetracycline for a week (gp 10)]. Specimens from the skin, gills, liver, kidney and intestine were collected, fixed in 10% buffered neutral formalin solution and were routinely processed for pathological examination. Exposure of the fish to <i>F. columnare</i> infection produced focal coagulative necrosis, ulcerations besides severe hydropic and spongiosis in the epidermis, particularly at the necrotic areas of the fins and heavily	32

	 effective in amelioration the lesions of <i>F. columnare</i> infections that have wide spread among Egyptian freshwater fish. Oxytetracycline is the drug of choice to treat such disease and minimize the lesions of <i>F. columnare</i>. [Mohamed H Mohamed and Nahla AG Ahmed Refat. Pathological Evaluation of Probiotic, <i>Bacillus Subtilis</i>, against Flavobacterium columnare in Tilapia Nilotica (<i>Oreochromis Niloticus</i>) Fish in Sharkia Governorate, Egypt. Journal of American Science 2011; 7(2):244-256]. (ISSN: 1545-1003). http://www.americanscience.org. Keywords: <i>Flavobacterium columnare</i>, Probiotic <i>B. subtilis</i>, <i>Tilapia nilotica</i>, PCR and Oxytetracycline 		
33	Journal of American Science 2011; 7(2):257-269]. (ISSN: 1545-1003). <u>http://www.americanscience.org</u> . 13	Full Text	33
	Evaluation of Lumbo-Pelvic Stabilizing Exercises in the Treatment of Backache after Normal Labour.	Full Text	
	Hala M. Hanfy ^{1*} , Fayiz F. Elshamy ¹ , Mohamed A. Awad ¹ , Hossam Gad. ² ¹ Department of physical therapy for Obstetric and Gynaecology, Faculty of physical therapy, Cairo University, Egypt. ² Department of Orthopedic surgery, Faculty of medicine, Tanta University, Egypt. <u>*Awad18111972@yahoo.com</u>		
34	Abstract: This study was designed to evaluate the effect of Lumbo-pelvic stabilizing exercises in the treatment of post partum backache. Twenty volunteers women diagnosed with post partum backache, their ages ranged from 25-35 years, they were delivered normally and received stabilizing exercises for lumbo-pelvic muscles (24 sessions), 3 sessions per week for 8 week. All women were evaluated before and after the end of the treatment programe using visual analogue scale for pain assessment and Oswestry disability questionnaire for the assessment of functional disability The obtained results showed highly statistically significant decrease in pain intensity.(p<0.001), and improve the functional disability (p<0.01) at the end of the study Accordingly, it could be concluded that lumbo-pelvic stabilizing exercises appears to be effective in the management of post partum backache. [Hala M. Hanfy, Fayiz F. Elshamy, Mohamed A. Awad, Hossam Gad Evaluation of Lumbo-Pelvic Stabilizing Exercises in the Treatment of Backache after Normal Labour. Journal of American Science 2011; 7(2):270-276]. (ISSN: 1545-1003). http://www.americanscience.org. Key words: post partum – backache – lumbo-pelvic stabilizing exercise -visual analogue scale – Oswestery disability questionnaire		34
	The Association between Lichen Planus and Hepatitis C Virus	Full Text	
35	 Hasnaa A. Abo-Elwafa^{*1}, Esam-Eldeen Abdel-Aziz Nada², Reham Ezz- Eldawla El-Sharkawy², Nesreen Ali Ahmed Abdel-Rahman² Clinical Pathology Department¹, Dermatology and Venereology Department², Faculty of Medicine, Sohag University, Egypt. *aboelwafahasnaa@yahoo.com Abstract: Background: Hepatitis C virus, has been associated with a wide variety of extrah- epatic manifestations (EHM) in the natural history of the disease. Lichen planus LP has also been reported in association with the hepatitis C virus. Objective is to determine the frequency of HCV in patients with LP in Sohag University Hospital. Patients and methods: The study was carried out at Sohag University Hospital in Clinical Pathology Department and Dermatology Venereology Department from October 2009 to April 2010, on 70 patients with LP (34 males, 36 females) their age ranged from 25 to 80 years old, and 20 patients (11 males, 9 females) their age ranged from 22 to 70 years old as a control group with other dermatological manifestations. Patients were recruited from the out patient clinic of dermatology department, full history, clinical examination, biopsy in some cases to confirm the diagnosis of LP and laboratory investigations for anti-HCV detection, liver function tests were done. Results: There is no significant difference between LP group and control group regarding the age or the sex (p = 0.176). There is highly significant increase in HCV infection between LP group (p = 0.000), there is a significant increase in HCV infection among the old age patients (p = 0.008), HCV infection and duration of LP 		35

	showed a significant correlation (p = 0.022). There was an increase in aspartate aminotransferase (AST) in actenic LP patients and mucous LP patients compared with those of classic type (p = 0.028). Conclusion: A possible relationship between LP and HCV, thus, it seems that the HCV antibody test is necessarily required for LP patients. [Hasnaa A. Abo-Elwafa, Esam-Eldeen Abdel-Aziz Nada, Reham Ezz- Eldawla El-Sharkawy, Nesreen Ali Ahmed Abdel-Rahman. The Association between Lichen Planus and Hepatitis C Virus. Journal of American Science 2011; 7(2):277-282]. (ISSN: 1545-1003). <u>http://www.americanscience.org</u> . Key words: Lichen Planus- HCV Infection; dermatological manifestation		
	Protective Effect of some Antioxidants against CCl ₄ -Induced Toxicity in Liver Cells from BRL3A	Full Text	
36	 Kamel, H.H.¹, Azza, H. Abd-El-Rahman¹, Walaa, M.S. Ahmed¹ and Amira, H. Mohamed² ¹Clinical Pathology Dept., Fac. of Vet. Med. Beni-Suef University, Beni-Suef , Egypt ²Clinical Pathology Dept., Fac. of Vet. Med. Cairo University, Giza, Egypt Azza_abdelrahman92@yahoo.com Abstract: An in vitro experiment was conducted to investigate the protective effect of ascorbic acid, mannitol and aminoguanidine at different concentrations against carbon tetrachloride induced toxicity and oxidative stress in hepatocytes cell line (BRL3A) from buffalo rats. Results were compared with those of vitamin E as standard hepatoprotective agent. Treatment of BRL3A with CcL₄ lead to generation of free radicals detected after two hours incubation using ESR technique and produced cell injury demonstrated by increased leakage of LDH, ALT and AST to the media. Exposure to CcL₄ caused apoptosis to cells but did not induce lipid peroxidation as tested by the TBARS technique. Treatment with vitamin E has significant hepatoprotective effect by lowering the leakage of intracellular enzymes, reducing the oxidation of proteins and decrease incidence of apoptosis. Ascorbic acid, mannitol and aminoguanidine were ineffective against CcL₄ toxicity. [Kamel, H.H., Azza, H. Abd-El-Rahman, Walaa, M.S. Ahmed and Amira, H. Mohamed. Protective Effect of some Antioxidants against CcL₄-Induced Toxicity in Liver Cells from BRL3A Cell Line. Journal of American Science 2011; 7(2):283-296]. (ISSN: 1545-1003). http://www.americanscience.org. 		36
37	 Recognition of Geographical diffusion Esophagus Cancer in Southwestern of Caspian Sea Bahman Ramezani Gourabi Associate Professor of Physical Geography Islamic Azad University, Rasht branch, Iran bahman@iaurasht.ac.ir, bahmanr2000@yahoo.com Abstract: Esophagus Cancer is an important disease in Iran and has second rank of death after heart disease, in north of Iran this disease has maximum diffusion and has arrangement of Iran in Asian belt of this disease.Environmental and climatically conditions in each area could be helpful toward diffusion and out breaking diseases, like as Esophagus Cancer. Rate of appearing this disease in recent years in Iran and especially in Gilan province (Southwestern of Caspian Sea) has increased that this problem could be very importance by cost ill and its problems.The aim of this paper is recognition of geographically diffusion of Esophagus Cancer in Gilan and presents it by map.The research method of this paper has used from medical documents diseases from hospital, library document studies (Soil, Geology and climate) and field work from 2001-2005 years.Results of this paper has showed that Esophagus Cancer (most rate) rather than on mans ,and it has more diffusions in central area in province of Gilan(Talesh,Lahijan,Someh sara and Rasht) and environmental factors such as, soil factor (Lithosel and Brown forest , climatically factors (season and cold course) and has relationship effectives on Esophagus Cancer diffusions and its presenting by map. This paper has written in framework at Geography of Health (Medical geography) that Geographical scattering an important of this paper, than this essay must be complete by other specialists (Environmental and medicines). Bahman Ramezani Gourabi. Recognition of Geographical diffusion Esophagus Cancer in Southwestern of Caspian Sea. Journal of American Science 2011; 7(2):297-302]. (ISSN: 1545-1003). http://www.americanscience.org. Keywords: Esophagus Cancer, Geography of heal	Full Text	37

	Development of ELISA Method for primary Detection of HCV using core Antigen	Full Text	
	 Shirin Nourollahi¹, Seyed Mehdi Boutorabi², Ali Mirjalili³, Mahmoud Mahmoudian Shooshtari⁴, Maryam Razaghi¹, Masoomeh Hashemi⁵, Mehrdad Jalalian^{6, 7} ^{1.} Department of Microbiology, Faculty of basic Sciences, Islamic Azad University-Zanjan branch.Iran ^{2.} Director of Research Lab., Pishtaz Teb Inc. Tehran, Iran. ^{3.} Department of Biotechnology, Razi Vaccine and Serum Research Institute, Tehran, Iran. ^{4.} Research Center of Iranian Blood Transfusion Organization, Tehran, Iran. ^{5.} Department of Biochemistry, Payamenour University of Tehran, Tehran, Iran. ^{6.} Khorasan Razavi Blood Center, Mashhad, Iran. ^{7.} Department of Community Health, faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor D.E., Malaysia. 		
38	Abstract: Studies show that Hepatitis C Virus (HCV) antigens appear before antibody while the early days of infection. Therefore detecting antigens could lead us to diagnosing the infection on time. The aim of this study was to develop a simple and sensitive enzyme immunoassay for the detection of hepatitis C virus (HCV) core antigen in order to evaluate the role of core antigen as a marker of HCV infection. A total of 280 samples was tested by third generation anti-HCV, and the reverse transcription polymerase chain reaction (RT-PCR) was performed only when the anti-HCV enzyme immunoassay (EIA) was positive. All samples were tested with HCV core antigen using Elisa kits. Among the 280 samples, 95 samples were anti-HCV positive. Among those 95 samples, 75 samples were RT-PCR-positive. The cut-off value was set at 0.15 unit of optical density (equivalent to 2.5 pg/ml of core antigen based on the distribution of healthy subjects (anti-HCV-negative subjects). The difference between the mean optical density values of HCV-ribonucleic acid-positive (HCV-RNA-positive) samples and HCV-RNA-negative samples in the HCV core antigen assay was highly significant (1.4 us 0.08, p < 0.005). The sensitivity and specificity of the core antigen assay were 88% and 96%, respectively. The pretreatment of the anti-HCV-positive samples with a solution that contained 1.5 M glycin buffer (pH = 2) increased the sensitivity of the assay (from 57.3% to 88%). This assay is a simple, sensitive, and useful method for use as a screening strategy for HCV infection in anti-HCV-positive or anti-HCV-negative individuals. [Shirin Nourollahi, Seyed Mehdi Boutorabi, Ali Mirjalili, Mahmoud Mahmoudian Shooshtari, Maryam Razaghi, Masoomeh Hashemi, Mehrdad Jalalian. Development of ELISA Method for primary Detection of HCV using core Antigen. Journal of American Science 2011; 7(2):303-307]. (ISSN: 1545-1003). http://www.americanscience.org.		38
	Diversity of Medicinal Plants in the Biospherical Reservation Areas of Iran	Full Text	
39	(A Case Study of the protected area of Miankaleh) Abed Vahedi ¹ , Esmaeil Yasari ² ¹ Corresponding author: Department of Agronomy and Plant Breeding, Faculty of Agricultural and Natural Resources, Islamic Azad University, Qaemshahr Branch, Qaemshahr, Mazandaran, 48148-35497. Cell: +98-09356211306. Iran. abedvahedy@gmail.com ² Assistant Prof, Payame Noor University, Sari, Mazandaran, 48189-35455. Cell: +98-9113511510, Iran. e_yassari@yahoo.com Abstract: Awareness of people concerning the side effects of chemical drugs has caused an increasing interest in traditional medicine. This study was carried out to gather and identify medicinal plants, their curative effects and the part of them which is used from the reservation area of Miankaleh. The region under study has an area of 68800 hectares situated 12 kilometers north of the city of Behshahr and		39
	northwest of the city of Gorgan. During numerous visits to the area, plants were gathered and, after their identification using specialized references of medicinal plants, the part used and the curative effects of the plants were determined. Results obtained showed that out of a total of 43 families, 125 genera, and 155 species found in the region, 33 families, 52 genera, and 61 species (39% of all the species) belonged to medicinal plants, among which the class Asteraceae with 6 species and the class Chenopodiaceae with 5		

	species had the most medicinal species. The most used parts of the plants were the leaves with 31%, the whole plants with 19%, and the roots with 15%. [Abed Vahedi, Esmaeil Yasari. Diversity of Medicinal Plants in the Biospherical Reservation Areas of Iran (A Case Study of the protected area of Miankaleh). Journal of American Science 2011; 7(2):308-312]. (ISSN: 1545-1003). <u>http://www.americanscience.org</u> . Key words: Miankaleh, Medicinal plants, Boispherical reservation area, traditional medicine		
	Effective Use Of Teaching Methodologies At Secondary Level In Pakistan	Full Text	
40	Tariq Mehmood ¹ , Zahoor-ur-Rehman ² ¹ Author Tariq Mehmood is Principal at Benevolent Public School, Kohat Road Peshawar and Ph.D. Scholar at Preston University, Islamabad, Pakistan. tariq_619219@yahoo.com ² Al-Khawarizmi Institute of Computer Science University of Engineering & Technology, Department of Computer Science & Engineering, Lahore Pakistan. <u>xahoor@uet.edu.pk</u> , <u>zahoor@kics.edu.pk</u> Abstract: The objective of the study was to investigate the effective use of teaching methodologies at Secondary level in Pakistan. All the participants i.e.100% of Fourth Six week training workshop on "Educational Leadership and Institutional Management" for educational managers at Academy of Educational Planning and Management, Ministry of Education, Government of Pakistan were included in the sample. For the purpose of data collection, a questionnaire was prepared. Data collected through the questionnaire was tabulated, analyzed and interpreted by applying percentage. Major findings of the study reveal that (1) teacher's presents a brief overview of the contents; (2) teacher's uses A.V. aids to enhance the student's comprehension of the concepts; (3) teacher speaks at a rate which allows students time to take notes; (4) teacher evaluates the success of his teaching by asking questions about the topic at the end of the session and; (5) teacher assigns homework and checks it regularly. It was concluded that teachers probe questions answer is incomplete, repeats questions when necessary and also responds students queries politely and carefully; teacher establishes and maintains vigilant contact with the student's body movements do not contradict the speech and takes notes to respond students curiosity and the teachers voice can be heard easily, he raises and lowers his voice for variety and emphasis. It has been recommended that A.V. materials should be used more vigilantly by teachers to make their teaching effective, teacher must pay attention to remove sign of puzzlement to make students l		40
	Importance of Credits for Rural Women	<u>Full Text</u>	
41	Mohammad Abedi ¹ , Seyed Mehdi Mirdamadi ² , Seyed Mahmoud Hosseini ³ , Iraj Saleh ⁴ ^{1, 2 3} Department of Agricultural Extension Education, Science and Research Branch, Islamic Azad University, Tehran, Iran ³ Department of Agricultural Extension Education, Faculty of Agricultural Economics and Development, University, College of Agriculture & Natural Resources, University of Tehran, Karaj, Iran ⁴ Department of Agricultural Economics, Faculty of Agricultural Economics and Development, University, College of Agriculture & Natural Resources, University of Tehran, Karaj, Iran ⁴ Department of Agricultural Economics, Faculty of Agricultural Economics and Development, University, College of Agriculture & Natural Resources, University of Tehran, Karaj, Iran Email: <u>abedi114@yahoo.com</u> Abstract: One of important factor for production and occupation is capital. In developing societies that most of their exploiters are novice, lending and credit projects is suitable tool for accessing purposes such as increasing efficiency and obliterating deprivation from rural society. But at one side, we need credits to make technological changes in productive activities and at the other hand we need suitable technical technologies to use credits with optimum efficiency. Supplying credits and analyzing credits approaches cause opportunity to activate poor men's working power , establishing field for sustainable production and		41

	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 etc. have important role on effectiveness and make effective activities of these credits. [Mohammad Abedi, Seyed Mehdi Mirdamadi, Seyed Mahmoud Hosseini and Iraj Saleh. Importance of Credits for Rural Women. Journal of American Science 2011; 7(2):321-329]. (ISSN: 1545-1003). http://www.americanscience.org. Keywords: women, rural, credit, empowerment		
	Thermo-Statically Safety Control of Dez Dam under Unexpected Lake Level Reduction	Full Text	
	Mojtaba Labibzadeh ¹ , Amin Khajehdezfuly ¹ ^{1.} Department of Civil Engineering, Faculty of Engineering, Shahid Chamran University, Ahvaz, Iran <u>Labibzadeh_m@scu.ac.ir</u> Abstract: Dez dam stability was examined due to unexpected decrease in its reservoir level by performing a 3D finite elements analysis. The dam as well as its foundation and abutments have		
42	been modeled in a relatively exact manner. Moreover, the vertical contraction joints were simulated in the analysis. Hydrostatic, gravity and thermal forces have been taken into account as the main load combinations. 10m reduction of reservoir level from normal water level of the dam reservoir was considered in the modeling and the possibility of initiate and development of cracks in dam body was investigated by means of monitoring of principal stresses. The obtained results revealed that no serious instability would occur in Dez arch concrete dam. [Mojtaba Labibzadeh and Amin Khajehdezfuly. Department of Civil Engineering, Shahid Chamran University, Ahvaz, Iran. Journal of American Science 2011; 7(2):330-337]. (ISSN: 1545-1003). http://www.americanscience.org.		42
	Keywords: Arch dam, Concrete dam, Thermal, Hydrostatic, Gravity, Contraction joints, Dam safety		
	The variational iteration method for exact solutions of fuzzy heat-like equations with	Full Text	
43	 Variable coefficients Hamid Rouhparvar¹, Saeid Abbasbandy², Tofigh Allahviranloo² ^{1.} Department of Mathematics, Islamic Azad University Saveh Branch, Saveh 39187/366, Iran ^{2.} Department of Mathematics, Science and Research Branch, Islamic Azad University, Tehran 14778, Iran: rouhparvar59@gmail.com 		43
J.	Abstract. In this paper, the variational iteration method (VIM) and Buckley-Feuring method (BFM) are applied to find the exact fuzzy solution of the fuzzy heat-like equations in one and two dimensions with variable coefficients. Further a comparison between VIM-BFM and Seikkala solutions is provided. [Hamid Rouhparvar, Saeid Abbasbandy, Tofigh Allahviranloo. Journal of American Science 2011; 7(2):338-345]. (ISSN: 1545-1003). http://www.americanscience.org.		
	keywords: Fuzzy functions; Fuzzy heat-like equations; herative method, variational heration method	I	
	The Zoning of Drought with SIAP model in Sapeedroud valley, Gilan-Iran, South western of Caspian	Full Text	
44	The Zoning of Drought with SIAP model in Sapeedroud valley,Gilan-Iran,South western of Caspian Sea Bahman Ramezani Gourabi Associate professor in Physical Geography,Islamic Azad University,Rasht branch-Iran bahman@iaurasht.ac.ir, bahmanr2000@gmail.com	Full Text	44

	recognition of drought with Standard Index Annual Precipitation (SIAP model) in framework of research project .The results of research paper has shown in Sapeedrud valley drought has occurred in upland and lowland area(12-19% from total frequency) and the other area is 5-12% drought from total frequency and also the focuses of drought years has located in south area in all research period. [Bahman Ramezani Gourabi. The Zoning of Drought with SIAP model in Sapeedroud valley,Gilan-Iran,South western of Caspian Sea. Journal of American Science 2011; 7(2):346-350]. (ISSN: 1545-1003). <u>http://www.americanscience.org</u> . Keywords: Caspian Sea,Drought,Gilan,SIAP model		
	Fundamental challenges of information and communication technology (ICT) in education	Full Text	
	Sharareh Khodamoradi ¹ and Mohammad Abedi ² ¹ Department of Agricultural Extension Education, Science and Research Branch, Islamic Azad University, Tehran, Iran. ² Department of Agricultural Management, Islamic Azad University, Qaemshahr Branch, Iran *Corresponding author: <u>abedi114@yahoo.com</u>		
45	Abstract: There are Fundamental challenges about the role of information and communication technology (ICT) in education. This has led to serious skills shortages in many countries. In turn this has put increasing pressure on policy makers, universities and other training institutions to come up with approaches to inspire young students to choose ICT for their studies. There is also a strong argument for retraining many people who already have pre-service and in-survive education, whether in the workforce or not, to overcome to looming ICT skills crises. This paper reports on the examination of these points. It will also explore appropriate ways to combat this problem through analysis and identification of real prospects for ICT education.		45
	[Sharareh Khodamoradi and Mohammad Abedi. Fundamental challenges of information and communication technology (ICT) in education. Journal of American Science 2011; 7(2):351-355]. (ISSN: 1545-1003). http://www.americanscience.org. Keywords: ICT, education		
	Comparison of Uniaxial Compressive Strength of Light Weight Concrete Prepared with Bagassese between Cubic and Cylindrical Specimens	Full Text	
46	Hassan Tanoursaz ¹ , Mojtaba Labibzadeh ^{2,*} ¹ Civil Engineering Department, Islamic Azad University, Dezful Bearnch, Iran ² Civil Department, Faculty of Engineering, Shahid Chamran University, Ahvaz, Iran *Corresponding author: Tel: +98-0916-311-7745, E-mail: <u>labibzadeh_m@scu.ac.ir</u> Abstract: Sugar cane had been one of the most important agricultural products in the province of Khuzestan in Iran Country. Because of that since a long time ago this region has been called "The sugar Cane Region" or "Khoozestan ". In the process of sugar cane refinery, yellow fibers called Bagasse are generated which are known as the wastes of this product. About one million tons of Bagasse is produced in the province of Khuzestan each year, which could be a source of bioenvironmental problems and environmental pollutants. In this study, this tributary product which had been used before by the author and his co-workers (labibzadeh, et. al., 2011) to generate a kind of Light Weight Concrete (LWC) was more examined in order to investigate its effect on the relationship between the compressive bearing of the proposed LWC with different shape samples (cubic and cylindrical) including Bagasse. To perform this work, at the first a constant mixing plan was considered according to ACI-211.2 standard code, then some samples were prepared according to this strategy, without adding Bagasse and after curing, they were tested. Then, samples with the mentioned mixing plan and 10,20,30,40 & 50 percents of including Bagasse were designed to substitute the aggregates in the mixture and after curing these samples according to standard code manual ACI-211.2, the corresponding tests conducted and the results have been deduced and interpreted. The results showed that the ratio of compressive strength of cylindrical samples to cubic ones of the normal concrete for mass concrete is 0.89 which can be increased to 1 for 20% including Bagasse light weight concrete. Here, the point which should be considered is the noticeable		46

Strength of Light Weight Concrete Prepared with Bagassese between Cubic and Cylindrical Specimens. Journal of American Science 2011; 7(2):356-360]. (ISSN: 1545-1003). http://www.americanscience.org.		
Prevalence of fascioliasis among slaughter sheep in selected abattoirs in Imo State, Nigeria	Full Text	
Njoku-Tony, R.F and Okoli, G.C Department of Environmental Technology, School of Engineering and Engineering Technology, Federal University of Technology, P.M.B. 1526 Owerri, Imo State, Nigeria. Corresponding author: Njoku-Tony, R.F, Email- tonyrosefeechi@yahoo.com Abstract: A 12 months study on fascioliasis in sokoto gudali sheep of Hausa origin (northern part of Nigeria) was carried out in five different abattoirs in Imo State, Nigeria between 2004 and 2005.The		
abattoirs were Obinze, Okigwe, Afor-Enylogugu, Afor-Ogbe, and Orlu .The objective of the study was to determine the prevalence of fascioliasis among slaughter sheep in selected abattoirs in Imo state. Five grams of feces and liver from freshly slaughtered sheep of both sexes, and from different locations and ages were collected, taken to the laboratory and analyzed for fasciola eggs and adult flukes. Direct smear, formol ether concentration and sodium chloride floatation methods were used to harvest the eggs and adult parasites. Out of 367 sheep examined, 64 (17.2%0) were infected. Infection was highest in Obinze abattoir (29.6%) followed by Afor Enyiogugu abattoir with (29.0%) Okigwe (15.6%), Orlu (12.5%) and Afor Ogbe (12.4%). Infection was highest in females than in males and was also sex dependent. Among adult sheep (>2yrs) examined , 59 (14.9%) were infected while out of 38 young goats (<2yrs), 5 (13.2%) were infected. Infection level rose with increased rainfall with the highest level observed at the peak of the rainy season and dropping during the dry season months. The number of parasites were 180, and mean worm load 281. Intensity was higher in males than in females. This result therefore calls for an improved disease control and adequate sanitation programme for sheep rearers in the study area. [Njoku-Tony, R.F and Okoli, G.C. Prevalence of fascioliasis among slaughter sheep in selected abattoirs in Imo State, Nigeria. Journal of American Science 2011; 7(2):361-366]. (ISSN: 1545-1003). http://www.americanscience.org.		47
Geoenvironmental Study Of Groundwater Contamination In A Dual Aquifer Environment Using Earth Resistivity Imaging ¹ Samson Idowu Jegede, ² Isaac Babatunde Osazuwa, ³ Nasir Khalid Abdullahi ¹ Department of Physics, Ambrose Alli University, Ekpoma. Edo State, Nigeria. ² Department of Physics, Ahmadu Bello University, Zaria. Kaduna State, Nigeria. ³ Applied Science Department, College of Science & Technology, Kaduna Polytechnic Kaduna State, Nigeria. <i>samidojegede@yahoo.com</i> , <u>nasirasman@yahoo.com</u> Abstract: The variation of electrical resistivity as a function of soil properties was used as a vital tool to study groundwater contamination in the vicinity of some selected solid waste disposal sites in the municipal town of Zaria. The Abem Lund Imaging system with Terrameter SAS 4000 was used for the resistivity data measurements and the Res2dinv software was used for the processing and interpretations of the data. Due to the high conductivity of the contaminant plumes it was possible to delineate their pathways into the regolith and fractured aquifer environments. Resistivity data from inverted models obtained from profiles near monitoring wells, correlated well with electrical conductivity (EC) and total dissolved solid (TDS) values of water samples taken from theses wells. The inferred water resistivity and the soil resistivity obtained from the resistivity tomosections at depths of water table revealed that the samples, which were collected from hand dug wells whose depths are within the overburden (regolith aquifer), are more polluted than the samples which were collected at deeper levels corresponding to the borehole samples (fractured aquifer). The findings of this study suggest the potentiality of the resistivity imaging technique as a pre-characterization tool for mapping subsurface contamination in the vicinity of waste disposal sites.	Full Text	48
	 Strength of Light Weight Concrete Prepared with Bagassese between Cubic and Cylindrical Specimens. Journal of American Science 2011; 7(2):356-360]. (ISSN: 1545-1003). http://www.amtericanscience.org. Prevalence of fascioliasis among slaughter sheep in selected abattoirs in Imo State, Nigeria. Njoku-Tony, R.F and Okoli, G.C Department of Environmental Technology, School of Engineering and Engineering Technology, Federal University of Technology, P.M.B. 1526 Owerri, Imo State, Nigeria. Corresponding author: Njoku-Tony, R.F. Email- tonyrosefeechi@yahoo.com Abstract: A 12 months study on fascioliasis in sokoto gudali sheep of Hausa origin (northern part of Nigeria) was carried out in five different abattoirs in Imo State, Nigeria between 2004 and 2005. The abattoirs were colloczted, Kigwe, Afor-Enyiogug, Afor-Deybe, and Oftu. The objective of the study was to determine the prevalence of fascioliasis among slaughter sheep in selected abattoirs in Imo state. Five grams of feces and liver from freshly slaughtered sheep of both sexes, and from different locations and ages were collected, taken to the laboratory and analyzed for fasciola eggs and adult flukes. Direct smear, formol ether concentration and sodium chloride floatation methods were used to harvest the eggs and adult parasites. Out of 367 sheep examined, 64 (17.2%0) were infected. Infection was highest in Obinze abattori (29.6%) followed by Afor Enyiogug abattori with (29.0%) (okigwe (15.6%). Oftu (12.5%) and Afor Ogbe (2.4%). Infection was higher in males than in males and was also set dependent. Among adult sheep (2.4%). Infection have lighter in locations are interved in the study area. (21.4%). Infection lovel rose vith increased trainfall with the highest level observed at the peak of the rain solitation programme for sheep reares in the study area. (23.4%). Infection level rose sanitation programme for sheep reares in the study area. (Nigeria. Journal of American	Strength of Light Weight Concrete Prepared with Bagasses between Cubic and Cylindrical Specimens. Journal of American Science 2011; 7(2):356-360]. (ISSN: 1545-1003). http://www.americanscience.org. Prevalence of fascioliasis among slaughter sheep in selected abattors in Imo State, Nigeria Nigeria Technology, PAMB. 1526 Owerri, Imo State, Nigeria. Corresponding author: Nigku-Tony, R.F. and Okoli, G.C Department of Environmental Technology, PAMB. 1526 Owerri, Imo State, Nigeria. Corresponding author: Nigku-Tony, R.F. Email-tonvorsecfeechi@valuoc.com Abstract: A 12 months study on fascioliasis in sokoto gudali sheep of Hausa origin (northern part of Nigeria) was carried out in five different abattoris in Imo State, Nigeria as carried out in five different abattoris in Imo State, Nigeria battoris of the not state. Five grans of feces and liver from freshly slaughtered sheep of both sexes, and from different locations and ages were collected, laten to the laboratory and analyzed for fasciola gegs and adult fayes. Direct smear, formol ether concentration and sodium chloride floatation methods were used to harvest the eggs and adult parsites. Out of 367 sheep examined, 64 (17,260) were infected. Inflection was highest in floate abattoris ri Imos State, Nigeria (12,5%), Infection was highest in famales than in males. and was also sex dependent. Among adult sheep (237s) examined, 59 (14,27%) were infected. Infection was highest on Misze abattoris (257s), 51,25%) were infected. Infection and adequate Sanitation programme for sheep rearres in the study area. [Nigku-Tony, R.F and Okoli, G.C. Prevalence of fascioliasis among slaughter sheep in selected abattoris in Imos State, Nigeria. (Nigku-Tony, R.F and Okoli, G.C. Prevalence of fascioliasis among slaughter sheet) in selected abattoris in Imos State, Nigeria. (Nigku-Tony, R.F and Okoli, G.C. Prevalence of fascioliasis among slaughter sheet) and Agouder Sanots Tokowas (Nigeria. "Applied Science Department of Physics, Anmada Bello University, Zaria, Kaduma State, Nigeria.

	Groundwater Contamination In A Dual Aquifer Environment Using Earth Resistivity Imaging. Journal of American Science 2011; 7(2):367-377]. (ISSN: 1545-1003). <u>http://www.americanscience.org</u> . Key words: Resistivity imaging · Groundwater · Contamination · Dual-Aquifer · Environment		
	Synthesis, Characterization and Biological Studies on some Derivatives of <i>N</i> -(4- Aminobenzenesulphonyl)Morpholine Carrying Amino Acid, Alkoxy and Triazole Moieties	Full Text	
	H.M.Hassan ^{1*} , M. M.Abdelall ¹ , A.M.El-Naggar ¹ , M.E.Tamer ¹ and R.A.Bayoumi ² ¹ Department of Chemistry, Faculty of Science, Al-Azhar University, Cairo, Egypt ² Department of Botony, Faculty of Science, Al-Azhar University, Cairo, Egypt * hassanomar61@gmail.com		
49	Abstract: The reaction of <i>N</i> -[4-(chloroacetyl)aminobenzenesulphonyl]morpholine (IV) in acetone or dimethyl-formamide with amine derivatives, 4-aminotriazoles (I,II) or sulpha drugs yielded the corresponding <i>N</i> -[4-(substituted glycyl)aminobenzenesulphonyl]-morpholine derivatives (V-XV). Moreover, some derivatives of <i>N</i> -[4-(alkoxyacetyl)aminobenzenesulphonyl]morpholine (XVI-XXI) were synthesized. The reaction of XI with the requisite aromatic aldehydes in methanol gave Schiff bases (XXII,XXIII). Coupling reaction between (III) and Pht- or Tos-amino acids using the phosphorus oxychloride method furnished the corresponding <i>N</i> -[4-(pht- or Tos-aminoacyl)aminobenzenesulphonyl]morpholines (XXIV-XXIX). All the synthesized compounds were characterized by IR, ¹ H-NMR, MS spectral data and elemental analyses and investigated their antibacterial and antifungal activities. [H.M.Hassan, M. M.Abdelall, A.M.El-Naggar, M.E.Tamer and R.A.Bayoumi. Synthesis ,		49
	Characterization and Biological Studies on some Derivatives of N-(4- Aminobenzenesulphonyl)Morpholine Carrying Amino Acid, Alkoxy and Triazole Moieties. Journal of American Science 2011; 7(2):378-384]. (ISSN: 1545-1003). <u>http://www.americanscience.org</u> . Keywords: Synthesis; Derivative; N-(4-Aminobenzenesulphonyl)Morpholine; Amino Acid; Alkoxy; Triazole Moieties		
	Identification of Cryptosporidium Species Infecting Camels (Camelus dromedarius) in Egypt.	Full Text	
	[*] Abdel- Wahab, A. and Abdel -Maogood, S. Parasitology Department, Faculty of Veterinary Medicine, Cairo University, 12211 Giza, Egypt <u>dr.azza1973@yahoo.com</u>		
	Abstract: Cryptosporidium species was investigated among 145 camels (5-8 years old) from Egypt. The		
50	prevalence of infection was 19.3%. The detected oocysts were ellipsoidal in shape with a mean length and width 7.5×5.6 um. Ten <i>Cryptosporidium</i> free mice were orally inoculated each with 350.000 oocysts (camel isolate). The prepatent period in mice was 2 days and the patent period could not be determined since they were still shedding oocysts until day 100 post- infection. The camel isolate of <i>Cryptosporidium</i> and the same isolate propagated in mice was non-infective for lambs during an examination period of 3		50
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	daughter concentration in underground uranium mines to safe levels, a computer program has been developed to calculate ventilation parameters e.g. : local pressures, flow rates and radon daughter concentration levels. The computer program (actually two parts, one for mine ventilation and other for radon daughter levels calculations) has been validated in an actual case study to calculate radon concentration levels, pressure and flow rates required to maintain the acceptable levels of radon concentrations in each point of the mine. The required fan static pressure and the approximate energy consumption were also estimated. The results of the calculations have been evaluated and compared with similar investigation. It was found that the calculated values are in good agreement with the corresponding values obtained using "REDES" standard ventilation modelling software. The developed computer model can be used as an available tool to help in the evaluation of ventilation systems proposed by mining authority, to assist the uranium mining industry in maintaining the health and safety of the workers underground while efficiently achieving economic production targets. It could be used also for regulatory inspection and radiation protection assessments of workers in the underground mining. Also with using this model, it could be effectively design, asses and manage underground mine ventilation systems. Values of radon decay products concentration relative to the recommended levels, at different extraction points in the mine and fan static pressure could be estimated which are not available using others software. [M.M.El – Fawal. Mathematical Modelling for Radon Prediction and Ventilation Air Cleaning System Requirements in Underground Mines. Journal of American Science 2011; 7(2):389-402]. (ISSN: 1545-1003). <u>http://www.americanscience.org</u> .		
52	Impact of Structured Nursing Measures Pre and Post Epidural Lumbar Anesthesia on the Occurrence of Post-Epidural Anesthetic Headache ¹ Zeinab Hussain Ali and ^{*2} Nadia Mohamed Taha ¹ Adult Health Nursing, Faculty of Nursing, University of Helwan, Helwan, Egypt ² Adult Health Nursing, Faculty of Nursing, University of Zagazig University, Zagazig Egypt <u>dr nadya mohamed@yahoo.com</u> Abstract: Lumbar puncture is a frequently performed procedure in medical emergencies and anesthesia. Headache after lumbar puncture is a frequently performed procedure in medical emergencies and anesthesia. Headache after lumbar puncture is a common occurrence (32%) and carries a considerable morbidity, with symptoms lasting for several days, at times severe enough to immobilize. The aim of this study is to assess the impact of structured nursing measures on the occurrence of post-epidural anesthetic headache. This quasi-experimental study was conducted in El-naser Health Insurance Hospital; in Helwan city in Egypt on of 60 adult patients admitted for lower abdominal surgery using epidural anesthesia was recruited. The only exclusion criterion was pregnancy in female patients. Participants were alternatively assigned to either the intervention or control groups, ending with 30 patients in each group. The data collection tools consisted of two tools. Tool (1) was concerned with characterization of the pain and patient's personal data. The second tool was a Visual analog scale (VAS). The researchers designed a structured pre-spinal anesthetic nursing intervention to be applied to the study group. The control group received the routine nursing intervention only. The results revealed that the incidence of headache became significantly lower in the study group, reaching its lowest rate (3.3%) by the end of the third day, compared to 76.7% in the control group (p<0.001). the mean duration of headache was shorter in the study (22.1±34.0 hours) than in the control (111.2±55.9 hours) group, p<0.001. as well Patients in the study and control	Full Text	52

	Evaluation of Some Quality Aspects in Pediatric Intensive Care Services at Benha University Hospital	Full Text	
	Hussein Abdelshakour Abdelkader Hamed ^{1,} Ahmed Nabih El-Shazly ¹ ; Mahmoud Abdullah Al-Azzouny ² ; Doaa Refaey Soliman ¹ ¹ Faculty of Medicine - Benha University, pediatric department, ² Faculty of Medicine - Benha University, Clinical and Chemical Pathology Department		
53	Abstract: Objective: This study was conducted to evaluate the quality practice of some aspects of pediatric intensive care services in Benha University Hospital through systematic surveillance approach for situation and gap analysis at PICU. Methods: The surveillance procedures were based on observational scoring meeting with staff and data collection by questionnaires. The surveillance activities were repeated for 9 times (from November, 2009 to March, 2010) for assurance of accuracy of collected data. Results: In the current study the overall assessment of infection control standards in all surveillance cycles (nine cycles) revealed that 6 audits were interpreted as "moderate compliance" (66.7 %) and 3 audits were interpreted as low compliance (33.3 %). As regards total sterilization standards, surveillances revealed, moderate compliance was achieved in 6 audits (66.7%) and high compliance was achieved in 3 audits (33.3%). The repeated nine audits were interpreted for personnel and structure as having moderate compliance (100%). The current study assessment of mechanical ventilation standards revealed variation through different audits that could be summarized as follows; high compliance of 6 audits (66.7%) and moderate compliance of 3 audits (33.3%). Conclusion: There are variable degrees of compliance with the national and international standards of infection control, sterilization and personnel and mechanical ventilation in Benha University Hospital PICU. [Hussein Abdelshakour Abdelkader Hamed Ahmed Nabih El-Shazly; Mahmoud Abdullah Al-Azzouny; Doaa Refaey Soliman. Evaluation of Some Quality Aspects in Pediatric Intensive Care Services at Benha University Hospital. Journal of American Science 2011; 7(2):412-422]. (ISSN: 1545-1003). http://www.americanscience.org.		53
	Detection of Community Acquired Methicillin Resistance Staphylococcus aureus among Staphylococcus aureus isolates.	Full Text	
54	Abeer Ghazal ¹ , Ola Kader ¹ , Samia Ebid ² , Nancy Mostafa ² and Shimaa El Sayed ² ¹ Microbiology Department and ² Applied Medical Chemistry Department, Medical Research Institute, Alexandria University, Alexandria, Egypt. *a_ghazalus@yahoo.com Abstract: The rates of Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA) infections in the hospital, as well as the disease in the community, have continued to rise. Staphylococcal cassette chromosome <i>mec</i> (SCC <i>mec</i>) is a variable genetic element that contains the methicillin resistance determinant, <i>mecA</i> . SCC <i>mec</i> typing is one of the most important molecular tools available for distinction between community- acquired MRSA (CA-MRSA) and hospital-associated MRSA (HA-MRSA) occurring on a worldwide basis. CA-MRSA has been reported to carry the loci for Panton Valentin leukocidin (PVL) in high frequency in association with the type IV <i>SCCmec</i> . The present study aimed to differentiate between HA- MRSA and CA-MRSA by detection of SCC <i>mec</i> and determination the prevalence of PVL gene among MRSA isolates. A total of 34 <i>Staphylococcus aureus</i> isolates were included in this study. Susceptibility of Staphylococci was determined by, Disc diffusion method including methicillin, oxacillin and cefoxitin discs. Penicillin Binding Protein (PBP _{2a}) Latex Agglutination test was done to detect the presence of PBP _{2a} responsible for methicillin resistance. In addition genotypic identification of MRSA was carried out by detecting <i>mec</i> gene by real time PCR. Conventional PCR was carried using different set of primers for the amplification of SCC <i>mec</i> for differentiating the HA-MRSA and CA-MRSA; moreover detection of <i>PVL</i> as virulence factor was also done. The antibiotic sensitivity of CA-MRSA ranged from 11.76% for ceftazidime to 47.06% for Imipenem, Erythromycin and Gentamycin; while the sensitivity of HA-MRSA ranged from 2.94% for Amoxicillin and Ampicillin/sulbactam to 29.41% for Amikin. Out of 34 <i>S. aureus</i> strains; 26(76.47%) isolates were found t		54

	methicillin resistant by detection of <i>mecA</i> gene using real time PCR. Out of 34 MRSA strains 32 (94.12%) were PBP _{2a} producer. In the present study, though, the majority (25out of 34) of our strains were not SCC mec typable, yet among the nine typable strains the six hospital strains belonged to type II and III as reported in the literature and the three CA-MRSA belonged to the novel type V reported by other workers to be associated with CA-MRSA and the only PVL positive CA MRSA strain was untypable. [Abeer Ghazal, Ola Kader, Samia Ebid, Nancy Mostafa and Shimaa El Sayed. Detection of Community Acquired Methicillin Resistance Staphylococcus aureus among Staphylococcus aureus isolates. Journal of American Science 2011; 7(2):423-431]. (ISSN: 1545-1003). <u>http://www.americanscience.org</u> . Keywords: Detection; Community; Methicillin Resistance; Staphylococcus; aureus; Staphylococcus; aureus		
	Metabolic Effects of Estrogen and / or Insulin in Ovariectomized Experimentally Diabetic Rats	Full Text	
55	Aziza S. El-Nasr, Faten M.A. Diab, Nihal M. Bahgat, Mona A. Ahmed, Sahar S. Thabet and Soha M.Y. El-Dakkak Department of Physiology, Faculty of Medicine, Ain Shams University, Cairo, Egypt drmona_agha70@yahoo.com Abstract: Postmenopausal adverse metabolic changes increase cardiovascular risk and impair quality of life. This study was planned to evaluate the benefits gained by estradiol treatment alone and insulin treatment alone versus combination of these two hormonal therapies on the metabolic derangements accompanying estrogen deficiency with diabetes. Rats were divided into five groups: control sham- operated group, ovariectomized streptozotocin diabetic group (OVX–STZ), estradiol-treated OVX–STZ diabetic group that received daily subcutaneous injection of insulin (10 or 20 IU/kg) for 2 weeks and combined estradiol-treated, insulin-treated OVX–STZ diabetic group. Rats in all groups were subjected to determination of body weight, body mass index (BMI), blood glucose, plasma levels of total cholesterol, triglycerides, HDL-c, insulin, estradiol, leptin and malondialdehyde (MDA). In addition, <i>in</i> <i>vitro</i> glucose uptake by the diaphragm and glucose output by both kidneys were measured. Insulin treatment alone increased peripheral glucose uptake, reduced renal gluconeogenesis, normalized blood glucose and plasma total cholesterol, decreased triglycerides, LDL-c and MDA and elevated HDL-c. Plasma MDA was reduced however, still higher than controls. Estrogen therapy alone lowered blood glucose although not fully normalized, increased peripheral glucose uptake and decreased renal gluconeogenesis, reduced plasma triglycerides, total cholesterol, LDL-c and MDA and elevated HDL-c. a compared to untreated groups, yet, not completely normalized. Combined estradiol and insulin therapy returned all measured parameters towards control values with complete normalization of peripheral glucose uptake and blood glucose levels as well as plasma triglycerides, HDL-c, atheregoenic index and MDA, while BMI, gluconeogenesis, total		55
56	The Investigation perception of Agricultural Extension Agents about affective factors on effectiveness of Agricultural Advisory Services Companies in Iran Solieman Rasouliazar ¹ , Seyed Mahmood Hosseini ² , Seyed Jamal Farajallah Hosseini ³ and Seyed Mahdi Mirdamadi ³ 1 Department of Agricultural Management, Islamic Azad University, Mahabad Branch, Mahabad Iran	Full Text	56

	2Department of Agricultural Extension and Education, Tehran University, Karaj, Iran 3Department of Agricultural Extension and Education, Islamic Azad University, Science and Research Branch, Tehran, Iran rasouli88s@yahoo.com Abstract: The main purpose of this study was to investigation perception of extension agents about problems that Agricultural Advisory Services Companies (AASC) faced with them and reduced their effectiveness. Statistical population of the study consisted of Agricultural extension agents (N=381). By using the formula Cochrane, sample size was determined at 179. Questionnaire was the data instrument. The appearance and content validity of questionnaire was obtained by comments of extension experts. Reliability coefficient of the questionnaire 0.83 was obtained by Cronbach alpha. The results showed that AASC Increasing farm management skills of farmers. AASC also increasing the specialty of extension services to farmers. By using exploratory factor analysis barriers are classified in four factors, including Policy-making, Socio – cultural, Infrastructural and economical factors. These factors could explain 61% of variance in reduced effectiveness of AASC Services among farmers. [Solieman Rasouliazar, Seyed Mahmood Hosseini,Seyed Jamal Farajallah Hosseini and Seyed Mahdi Mirdamadi. The Investigation perception of Agricultural Extension Agents about affective factors on effectiveness of Agricultural Advisory Services Companies in Iran. Journal of American Science 2011; 7(2):445-451]. (ISSN: 1545-1003). http://www.americanscience.org. Keywords: Perception, Agricultural extension agents, AASC, Effectiveness, Iran		
57	Punjab Land use Classification, Reclassification and Redevelopment Rules: A predicament or new approach to urban management? Rizwan Hameed ¹ , Obaidullah Nadeem ² Department of City and Regional Planning, University of Engineering and Technology, Lahore, Pakistan. ¹ d_rizwan@hotmail.com; ² obaidnadeem@yahoo.com; Abstract: Disorganized and unsustainable patterns of land use change have seriously affected the spatial structure of cities in Punjab and Lahore is no exception to this phenomenon. Realizing the consequent problems of parking, traffic congestion and unfavourable environmental impacts, the Government of Punjab has recently enacted the new set of Rules to regulate commercialization and change of land use. This paper critically reviews the contents of the Punjab Land Use (Classification, Reclassification and Redevelopment) Rules 2009 applicable to Tehsil/Town Municipal Administrations (TMAs) and City District Governments (CDGs) since these Rules embody a system of classification of land uses for the first time in Pakistan. The study involves interviews with the architects of said Rules and a short survey of selected Town Planners working in TMAs and CDGs. The review shows that despite some of the shortcomings, these Rules provide a new approach to mapping and regulating the land use development and conversion activities in urban areas for the benefits of inhabitants. Moreover, the need to prepare Master Plans in future in the light of these Rules appears to be an attempt to introduce bottom-up approach to ground. However, results of brief survey of Town Planners show that effective implementation is likely to be constrained by lack of political will and inadequate technical and financial resources. The circumstances indicate for adoption of facilitative and motivational approach to implementation and enforcement of the said Rules. Rizewan Hameed, Obaidullah Nadeem. Punjab	<u>Full Text</u>	57
58	Quality of Supervision of Ph.D. Program among Public Universities in Malaysia: A Rasch Model Analysis Mikail Ibrahim, Siti Aishah Hassan Faculty of Major Language Studies, Universiti Sains Islam Malaysia, Bandar Baru Nilai, 71800, Nilai, Negeri Sembilan, Malaysia. Faculty of Educational Studies, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.	Full Text	58

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	Abstract: This study examines Ph.D. students' satisfaction with the supervision process at four selected universities in Malaysia. In addition, the study also investigated the psychometric properties of Quality Supervision Scale (QSS); specifically the scale dimensionality, construct validity, endorsibility, and estimation of item and person score reliability of the scales. The participants were 153 Ph.D. students of these universities. The QSS includes many qualities of effective supervision such as supervisor academic competency, research methods competency, attitude towards supervisee, faculty academic and moral supports and supervisees' personal traits was distributed to the respondents. The Rasch model analysis was employed to analyze the data for reliability, fit to the model, estimation of satisfaction levels and possibility of scale to function differentially across gender. Results suggested that generally students were satisfied with the supervision processes at these universities. In addition to that, the scale satisfied psychometrics properties by maintaining unidimensionality, reliability, and internal consistency. Furthermore, Rasch analysis revealed that, for gender, differences in overall satisfaction levels between males and females were marginal. The differential item functioning showed that only 6 of 49 calibrated items function differently. This suggested that students' levels of satisfaction level across different disciplines since previous studies suggested that satisfaction differs across different domains. [Mikail Ibrahim; Siti Aishah Hassan. Faculty of Major Language Studies, Universiti Sains Islam Malaysia; Faculty of Educational Studies, Universiti Putra Malaysia. Journal of American Science 2011; 7(2):562-575]. (ISSN: 1545-1003). http://www.americanscience.org.		
	Cultivation and Detection of Sulfate Reducing Bacteria (SRB) in Sea Water	Full Text	
59	 E. A. Ghazy[*], M.G. Mahmoud, M. S. Asker, M. N. Mahmoud, M. M. Abo elsoud and M. E. Abdel Sami¹ Microbial Biotechnology Department, National Research center, Dokki, Cairo, Egypt <u>*marnoryas@hotmail.com</u> Abstract: Sulfate-reducing bacteria (SRB) represent a class of anaerobic microorganisms that conduct dissimulatory sulfate reduction to obtain energy. The present study aimed to detect and control SRB activities using a very rapid detectable culture medium and reduction of potential economic loss in the petroleum sector. This study is an attempt to isolate SRB from sea water by rapid and sensitive culture media and to control their effect using eight commercial biocides (Aldehydes and quaternaries). The present work studies the effect of composition of four recommended culture media (Postgate medium B, Starkey's, Baar's and API media), besides, the presence of metal coupons in these media to enhance the growth of sessile SRB. Furthermore, the present study evaluates the efficiency of filtration of these culture media on the growth of SRB. The results revealed that modified Postgate medium B was the recommended medium for SRB growth. In addition, the results showed that rapid and abundant growth of SRB when the metal coupons were immersed in the culture media which were deficient in iron. The unfiltered culture media improved the SRB growth. The growth of SRB was depressed by 15 ppm of the commercial quaternaries rather than 20 ppm of the aldehydes. [E. A. Ghazy, M.G. Mahmoud, M. S. Asker, M. N. Mahmoud, M. M. Abo elsoud and M. E. Abdel Sami. Cultivation and Detection of Sulfate Reducing Bacteria (SRB) in Sea Water. Journal of American Science 2011; 7(2):576-583]. (ISSN: 1545-1003). http://www.americanscience.org. Key words: SRB, Culture media, Biocides, Minimal inhibitory concentration 		59
60	Effective Factors on Discontinuance of Sprinkler Irrigation Systems among Farmers in West Azerbaijan Province of Iran Solieman Rasouliazar ¹ , Saeid Fe'li ² 1 Department of Agricultural Management, Islamic Azad University, Mahabad Branch, Mahabad, Iran Ph.D Student of Agriculture Extension and Education, and Member of Youth Research Club-Garmsar Branch, Iran <u>rasouli88s@yahoo.com</u>	Full Text	60

	Abstract: The purpose of this study was to determine effective factors on discontinuance of sprinkler irrigation systems (SIS) among farmers in West Azerbaijan Province of Iran. A causal-comparative design was used and data was collected by means of questionnaire and interview with farmers who had used SIS and at least produced and harvested one agricultural crop in West Azerbaijan Province, as the target population. The sample was obtained through proportional stratified sampling (n=124). Instrument validity was established by a panel of experts and reliability analysis yielded an alpha value of 0.81. Study results showed that approximately 30% (n=36) of farmers discontinued use of SIS. The findings indicated that there was a statistically significant difference between adopters who continued SIS regarding some dependent variables including respondents' personal and farming characteristics and respondents' viewpoints about installing and keeping SIS). The result of discriminate analysis showed that "use of river as water source", "use of Gun system to farm irrigation", and "system design", were identified as the most discriminative factors (99.20% of population), affecting discontinuance of SIS. [Solieman Rasouliazar, Saeid Fe'li. Effective Factors on Discontinuance of Sprinkler Irrigation Systems among Farmers in West Azerbaijan Province of Iran. Journal of American Science 2011; 7(2):584-590]. (ISSN: 1545-1003). http://www.americanscience.org.		
61	 Reduction of Alfatoxin in Clarious lazara Catfish By Ginseng Extract and Nigella sativa Oil Mona S. Zaki¹, Olfat M. Fawzi² and Iman M. Zytuun³ 1. Department of Hydrobiology, National Research Center, 2. Department of Biochemistry, National Research Center, 3. Department of Microbiology,Zagazig University. Abstract: Aflatoxine the major toxic metabolites of fungi which are able to induce chronic liver damages. The antioxidant and hepatoprotective effects of Ginseng extract and Nigella sativa Oil 1% on Alfatoxin was investigated. Alfatoxicosis causes significant increase in liver enzyme SGOT and SGPT, Alkaline phosphatase activity and an increase in the level of cholesterol total lipid, decrease the level of total protein and hemoglobin and P.C.V. Moreover the liver exhibited some clinicopathological changes and decreased body weight. Both Ginseng extract and Nigella sativa Oil 1% reduced the development of hepatotoxicity by Aflatoxin. Nigella sativa showed more improvement of all enzymes of kidney and liver, and also total lipid and cholesterol were reduced and dody weight increased. [Mona S. Zaki, Olfat M. Fawzi and Iman M. Zytuun. Reduction of Alfatoxin in Clarious lazara Catfish By Ginseng Extract and Nigella sativa Oil. Journal of American Science 2011; 7(2):591-596]. (ISSN: 1545-1003). http://www.americanscience.org	<u>Full Text</u>	61
62	 Influence Of Gender And Self-Esteem On The Organisational Commitment Of Civil Servants In Ekiti-State, Nigeria Adebayo Sulaiman Olanrewaju¹, Olowookere Funmilola Kansola¹ ¹ Department of Psychology, University of Ado-Ekiti, Ekiti State, 5363, Nigeria lanryadd@yahoo.com, sadebayo@unad.edu.ng, Abstract: This study examined the influence of gender and self-esteem on the organizational commitment of civil servants in Ekiti State. Two hundred civil servants drawn from five ministries in Ekiti State responded to a battery of instruments (Self-Esteem Scale, Index of Self-Esteem and Organizational Commitment Scale). Four hypotheses were tested in the study. Results showed that there was no significant gender difference in employees' level of self-esteem in ministries in Ekiti State [t (198) = 0.41; p>.05], significant gender difference in employees' level of perceived organizational commitment was also observed [t (198) = 2.18; p<.05]. Further revealed was a significant main effect of gender [F (1, 199) = 3.99; p <.05] and self-esteem [F (1, 199) = 101.96; p <.05] on organizational commitment amog civil servants in Ekiti State. Findings from hypothesis four showed that gender [B = 0.18, t = 2.78; p<.05] and self-esteem [B = 0.60, t = 10.49; p <.05] had significant independent prediction on perceived organizational commitment of civil servants. The implications of these findings were discussed in light of the literature. [Adebayo Sulaiman Olanrewaju¹, Olowookere Funmilola Kansola. Influence Of Gender And Self-Esteem On The Organisational Commitment Of Civil Servants In Ekiti-State, Nigeria. Journal of 	<u>Full Text</u>	62

	American Science 2011; 7(2):597-603]. (ISSN: 1545-1003). <u>http://www.americanscience.org</u> Keywords: Gender, Self-esteem, Organisational commitment, Civil Servants, Ekiti State, Nigeria		
	Cultivation and detection of sulfate reducing bacteria (SRB) in sea water	Full Text	
63	 E. A. Ghazy¹, M.G. Mahmoud¹, M. S. Asker¹, M. N. Mahmoud¹, M. M. Abo elsoud¹ and M. E. Abdel Samie¹ ¹Microbial Biotechnology Department, National Research center, Dokki, Cairo, Egypt Corresponding author: Eman A. Ghazy Microbial Biotechnology Department, National Research center, Dokki, Cairo, Egypt Telephone: +20233335982, Fax: +20233370931, E-mail marnoryas@hotmail.com Abstract: Sulfate-reducing bacteria (SRB) represent a class of anaerobic microorganisms that conduct dissimulatory sulfate reduction to obtain energy. This study is an attempt to isolate SRB from sea water by rapid and sensitive culture media and to control their effect using eight commercial biocides (Aldehydes and quaternaries). The present work studies the effective of composition of four recommended culture media (Postgate medium B, Starkey's, Bars' and API media), besides, the presence of metal coupons in these media to enhance the growth of sessile SRB. Furthermore, the present study evaluates the efficiency of filtration of these culture media on the growth of SRB. The results revealed that modified Postgate medium B was the recommended medium for SRB growth. In addition, the results showed that rapid and abundant growth of SRB when the metal coupons were immersed in the culture media which were deficient in iron. Furthermore, the unfiltered culture media improved the SRB growth. The growth of SRB was depressed by 15 ppm of the commercial quaternaries rather than 20 ppm of the aldehydes. The present study aimed to detect and control SRB activities using a very rapid detectable culture medium .Besides reduction of their the economic loss in the petroleum sector. [E. A. Ghazy, M.G. Mahmoud, M. S. Asker, M. N. Mahmoud, M. M. Abo elsoud and M. E. Abdel Samie. Cultivation and detection of sulfate reducing bacteria (SRB) in sea water. Journal of American Science 2011; 7(2):604-608]. (ISSN: 1545-1003). htttp://www.americanscience.org Key words: SRB, Cult		63
64	Identification of <i>Cryptosporidium species</i> infecting camels (<i>Camelus dromedarius</i>) in Egypt. Abdel- Wahab, A., Abdel -Maogood, S. Parasitology Department, Faculty of Veterinary Medicine, Cairo University, 12211 Giza, Egypt dr.azza1973@yahoo.com Abstract: Cryptosporidium sp was investigated among 145 camels (5-8 years old) from Egypt. The prevalence of infection was 19.3%. The detected oocysts were ellipsoidal in shape with a mean length and width 7.5 × 5.6 um. Ten Cryptosporidium free mice were orally inoculated each with 350.000 oocysts (camel isolate). The prepatent period in mice was 2 days and the patent period could not be determined since they were still shedding oocysts until day 100 post- infection. The camel isolate of Cryptosporidium and the same isolate propagated in mice was non infective for lambs during an examination period of 3 months. Molecular characterization of the camel isolate indicated that the target gene (18SrRNA) gave positive result for C. muris at 435bp. [Abdel- Wahab, A., Abdel -Maogood, S. Identification of <i>Cryptosporidium species</i> infecting camels (<i>Camelus dromedarius</i>) in Egypt. Journal of American Science 2011; 7(2):609-612]. (ISSN: 1545-1003). http://www.americanscience.org. Key words: Camels, <i>Cryptosporidium muris</i> , prevalence, morphology, PCR.	<u>Full Text</u>	64
65	Effect of Ultrasound Radiation on the Aqueous Humor of Rabbits' Eye EMAN S. ELABRAK* AND EMAN M. ALY Basic Science Department, Biophysics Unit, Research Institute of Ophthalmology, Giza, Egypt.	Full Text	65

	frequency 10.8MHz for 10, 20 and 40 minutes exposure time. Estimation of protein content, gel filtration chromatography and Sodium Dodecyl Sulfate-Polyacrylamide gel electrophoresis (SDS-PAGE) were carried out to aqueous humor for all the studied groups. The results showed a significant decrease of protein content of rabbits aqueous humor of all groups reached to maximum decrease (-41.3%) at 40 minutes of exposure. A change in the molecular structure of aqueous humor protein was observed in the shift of the protein fractions to high molecular weight and decrease in the mobility of all peaks in the electrophoretic pattern. It is concluded that aqueous humor protein is sensitive to the ultrasound exposure as a function of time of exposure and may lead to denaturation of proteins. [EMAN S. ELABRAK AND EMAN M. ALY. Effect of Ultrasound Radiation on the Aqueous Humor of Rabbits' Eye. Journal of American Science 2011; 7(2):613-619]. (ISSN: 1545-1003). http://www.americanscience.org Key words: Ultrasound, Aqueous humor, Column chromatography, Electrophoresis, rabbits		
	A New Pool Market Method for Generation Expansion Planning in Restructured Power System	Full Text	
66	Morteza Thaerkhani ¹ , Mohammad Sadegh Javadi ² , Amin Javadinasab ² ^{1.} Islamic Azad University, Karaj Branch, Karaj, 2139643711, Iran ^{2.} Islamic Azad University, Shoushtar Branch, Shoushtar, 6138663849, Iran <u>msjavadi@gmail.com</u> Abstract: The issue of generation expansion planning (GEP) is more complicated in the restructured and modern power systems rather than traditional and monopoly systems. In Modern power systems, each Generation Company (Genco) invests in the section of generation in order to get to his own maximum profit. This paper presents a new mixed method to solve the GEP problem in Power Pool Market. This method is formed of two levels: local level and national level. In local level, each of Gencos declares his own generation level to Independent System Operator (ISO) aiming maximize the profit just with respect to local constraints. In national level, first the competition between Gencos will be modeled by game theory and Nash-Cournot equilibrium. Then, due to the generation level of each of Gencos, the system national constraints will be checked. If these constraints would be satisfied, problem-solving would be completed but if each of these constraints won't be satisfied, their relevant coefficients will be changed in problem and this procedure would be repeated again and again until problem was converged to accepted solutions which satisfy local and national constraints. [Morteza Taherkhani, Mohammad Sadegh Javadi, Amin Javadinasab, A New Pool Market Method for Generation Expansion Planning in Restructured Power System. Journal of American Science 2011; 7(2):620-624]. (ISSN: 1545-1003). http://www.americanscience.org. Keywords: Generation Expansion Planning, Pool Market, Game Theory, Uncertainty		66
67	An Empirical Study of Awareness in Web Based Cooperative Writing Applications Aslam Muhammad ¹ , Muhammad Yasir ² , Martinez Enriquez A. M. ³ , G. Escalada-Imaz ⁴ ^{1.2} Department of CS & E, U. E. T. Lahore, Pakistan ³ Department of CS, CINVESTAV-IPN, D.F. Mexico ⁴ Artificial Intelligence Research Institute, IIIA-CSIC, Spain ¹ maslam@uet.edu.pk, ² yasir.muhammad1983@gmail.com, ³ ammartic@cinvesta.mx, ⁴ gonzalo@iiia.csic.es Abstract: As part of any groupware that supports people achieving a common goal, it is required to provide information about structured shared objects and the activities of participants, as well as an efficient communication service and effective coordination mechanism. Thus, this paper presents an empirical study of the trade-off concerning awareness functionality with in fifteenth well know cooperative writing applications (CWAs), evaluated on the basis of present and past awareness elements, these applications are classified taking into account time and work place parameters. [Aslam Muhammad, Muhammad Yasir, Martinez Enriquez A. M., G. Escalada-Imaz. An Empirical Study of Awareness in Web Based Cooperative Writing Applications. Journal of American Science 2011; 7(2):625-633]. (ISSN: 1545-1003). http://www.americanscience.org. Keywords: Computer Supported Cooperative Work; Groupware; Cooperative Writing Applications; Awareness	<u>Full Text</u>	67

	Preventive Measures to Reduce Post - spinal Anesthesia Hypotension for Elective Cesarean Delivery	Full Text	
68	Nahed F., Khedr. Maternity and Gynecology Nursing, Faculty of Nursing, Mansoura University drnahed2010@yahoo.com Abstract: Aim: To explore the effect of wrapping and/or raising of the legs as a Preventive Measures to Reduce Post - spinal Anesthesia Hypotension for Elective Cesarean Delivery.Setting: The study was conducted in the operating room (cesarean section) at Ain Shams maternity hospital .Study design: An experimental design. Type of Sample: - purposive sample. Methods: 120 parturients were undergoing elective Caesarean section randomly scheduled to four groups: Group (1) (n=30) parturients legs wrapped immediately before injection anaesthesia and elevated immediately after anesthesia administration. Group (II) (n=30) parturients legs wrapped , tightly wrapping was achieved after leg elevated to 45 degree for 2 minutes, with an elastic bandage applied from ankle to mid – thigh, immediately before anesthesia administration. Group (III) (n=30) parturients legs elevated to 20 degree immediately after anesthesia administration. Group (IV) (n=30) no intervention. Tools of data collection consisted of 1) Demographic data,2)Automated monitors for measurement of blood pressure,3)Graphic flow sheet to record blood pressure, and 4) neonate assessment sheet to record Apgar score at 1and 5minutes.Results : The findings revealed that, This study showed that, there is no inter group's differences regarding their age, body mass index & baseline. Mean systolic arterial pressure MSAP. Meanwhile, a significant difference was noticed among the groups, whereas GI (wrapping & elevation) had a higher MSAP, lower percent of hypotension women late onset time of hypotension and a lower percent of babies with bad outcome the Neonatal outcome was excellent and similar in both groups. Conclusion: wrapping and elevation of the legs for parturients at spinal block for Cs had more effective measures to prevent hypotension. Recommendations: wrapping and elevation of the legs should be used in addition to traditiona		68
69	 Preliminary Horticultural Studies To Describe And Identify Of Two New Egyptian Mango Strains Using DNA Fingerprint. M. T. Wahdan¹, A. Z. Abdelsalam², A. A. El-Naggar³ and M. A. Hussein³ ¹Department of horticulture, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. ²Department of Genetics, Faculty of Agriculture, Suez Canal University, Cairo, Egypt. ³Department of Botany, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. wahdan2011@gmail.com Abstract: Selection of some strains is considered the first step in improving mango production. So, this study was done for a three successive seasons (2003 – 2005) on two Egyptian mango strains "Hania" and "Aml" to describe them, horticulturally and identification those genetically utilizing DNA fingerprint. The vegetative characters (leaf shape, length, width, etc) and histological characteristics (number of stomata per mm (stomatal density), stomata length and width) showed great variation between the two studied strains. The fruit weight was 581 gm for Hania strain and 1020 gm for Aml strain. The two strains fruits had good characters as shape, net weight ratio, firmness, SSC, TA, Vit. C and total sugars. In generally, physical and chemical properties of Aml strain fruits were better than Hania strain. These differences of horticultural aspects due to genetic variances, which were determined by using SSR markers, of the 42 primers screened, 36 primers gave reproducible polymorphic DNA amplification patterns. 60.7 % of the scored fragments are considered putative genotypes-specific markers in both strains. The polymorphic information content (PIC values) ranged from 0.25 to 0.75, with a mean value 0.51 for all loci. The heterozygosity level was 0.68 and 0.53 for Hania and Aml strains, respectively. By banding patterns obtained from these 36 primers was an efficient method for genotype identi	<u>Full Text</u>	69

	[M. T. Wahdan, A. Z. Abdelsalam, A. A. El-Naggar and M. A. Hussein. Preliminary Horticultural Studies To Describe And Identify Of Two New Egyptian Mango Strains Using DNA Fingerprint. Journal of American Science 2011; 7(2):641-650]. (ISSN: 1545-1003). <u>http://www.americanscience.org</u> . Key words: Mango, Histological, Morphological, fruit, DNA, Fingerprint, SSR		
	Effect of some chemicals on growth, fruiting, yield and fruit quality of "Succary Abiad" mango cv. Wahdan, M. T., Habib, S. E., Bassal, M. A. And Qaoud, E. M. Department of horticulture, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt.	Full Text	
70	Abstract : The present investigation was carried out in two successive seasons of 2007 and 2008 on mango cv. "Succary Abiad", at Abou Swear region, Ismailia Governorate, Egypt in a sandy soil and irrigated with immerged irrigation system, to study the effect of some chemicals and growth regulators on growth, leaf mineral contents, flowering, fruiting, yield and fruit quality. The trees were subjected to eleven treatments using urea 2%, NAA 40 and 60 ppm, Ca Cl ₂ 2%, GA ₃ 20 and 40 ppm and water spraying as control. The results revealed that, spraying with urea, NAA and GA ₃ at all concentrations significantly increased shoot length, number of leaves per shoot and leaf area higher than control while urea showed the superiority effect. Nitrogen and Potassium content in leaves significantly increased within urea, NAA and GA ₃ higher than control. Calcium content in the leaves showed fluctuated values during the two seasons within the different treatments although Ca Cl_2 2% sprayed at two months after full bloom showed the highest values in the two seasons of study. All treatments had significantly higher yield than control in the two seasons. The fruit weight and volume were the highest within all treatments compared with control. Fruit firmness and SSC were increased within all treatments with significantly increments than control. Vitamin C was significantly increased in fruits harvested from trees sprayed with GA ₃ 40 ppm at two months after full bloom. Total sugars in the fruits significantly increased higher than control within all treatments except GA ₃ 20 ppm added at one month after full bloom. [Wahdan, M. T., Habib, S. E., Bassal, M. A. And Qaoud, E. M. Effect of some chemicals on growth, fruiting, yield and fruit quality of "Succary Abiad" mango ev. Journal of American Science 2011; 7(2):651-658]. (ISSN: 1545-1003). <u>http://www.americanscience.org</u> Key words: Mango, Urea, NAA, CaCl ₂ , GA ₃ , yield, fruit quality		70
71	Economic effects of rural women's financial self-reliance 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: Since, village is suitable place for farming and additional activities, so it can be said that women's role at villages, has been toward this point and by developing agriculture sector and possibility to institutionalized appropriate infrastructure, we would have suitable attitude toward development process. Agriculture sector has critical responsibility, as one of the productive part of country for supplying needed food security, that it can assist this sector to access this main goal up to proper level, in accordance with workforce efficiency. To achieve this goal, women play main role, too. In spite of that, they couldn't represent their abilities in this field, because of limitations that they face. [Mohammad Abedi and Sharareh Khodamoradi. Economic effects of rural women's financial self- reliance. Journal of American Science 2011; 7(2):659-663]. (ISSN: 1545-1003). http://www.americanscience.org Keywords: rural women, financial self-reliance	<u>Full Text</u>	71
72	Evaluation of radio protective effects of wheat germ oil in male rats ¹ Ibrahim A.H. Barakat, ² Osama A. Abbas, ² Samia Ayad and ³ Aziza M. Hassan ¹ Zoology Department, College of Science, King Saud University, Riyadh, Saudi Arabia, ² Radiation Research Department, Atomic Energy Authority, Cairo, Egypt, ³ Cell Biology Department, National Research Center, Dokki, Giza, Egypt,	Full Text	72

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	Corresponding author: e-mail: <u>azizayahassan@yahoo.com</u> ABSTRACT: Wheat germ oil posses various biological properties as an anticancer and antioxidant agent. Present study was undertaken to evaluate the radio protective ability of wheat germ (WG) oil against whole body irradiation rat. Wheat germ oil was given to rats by oral injection in a concentration of 1 ml/kg and 3 ml/kg body weight/dose for 3 successive days, last dose administered 24 h pre-irradiation exposure with an acute single dose level of 2 Gry delivered at a dose rate of 0.564 Gry/ min at the time of experiment. With regard to cellular system, the results clearly indicated that pre-treatment with 3ml oil is more potent than 1ml and there are no significant differences between control group and groups that received oil only at either 1ml or 3ml in comparison to the control. Prior administration of WG oil to rats, significantly countered radiation induced biochemical disorder (liver enzymes and kidney function analysis, as well as, cholesterol level in the serum) and DNA damage (evaluated by DNA fragmentation assay and chromosomal aberration in bone marrow) in a dose dependent manner maximally at a concentration of 3 ml/kg. The results clearly indicated that wheat germ oil has significant potential to protect cellular system from radiation induced damage and ability to scavenge free radicals might be playing an important role in its radio protective manifestation without any toxicity [Ibrahim A.H. Barakat, Osama A. Abbas, Samia Ayad and Aziza M. Hassan. Evaluation of radio protective effects of wheat germ oil in male rats. Journal of American Science 2011; 7(2):664-673]. (ISSN: 1545-1003). <u>http://www.americanscience.org</u> . Key words: wheat germ oil, DNA fragmentation, radioprotection, chromosome aberrations		
	Replacement age of agricultural tractor (MF285) in Varamin region (case study)	Full Text	
73	 Replacement age of agricultural tractor (MF285) in Varamin region (case study) Hossein Ahmadi Chenarbon¹, Saeid Minaei², Akbar Arabhosseini³ ¹Department of Agriculture, Varamin Pishva Branch, Islamic Azad University – Varamin Pishva - Iran ²Associate professor, Agricultural Machinery Eng. Dept., Tarbiat Modarres university, Tehran, Iran ³ Associate professor, Agrotechnology Dept., College of Abouraihan, University of Tehran, Tehran, Iran <u>h.ahmadi@iauvaramin.ac.ir</u> Abstract: One of the main aims in the management of farm equipment and tractors is deciding about their replacement, based on technical and economic conditions. The objective of this research was to determine the economic life time for common used tractor in Varamin region, Iran, namely Massey- Fergusen 285. First the annual depreciation and interest were calculated considering the initial purchase price of tractor, and then the economic life was calculated based on repair and maintenance costs. The results showed that the most suitable replacement age is nine years for Massey- Fergusen 285. [Hossein Ahmadi Chenarbon, Saeid Minaei, Akbar Arabhosseini. Replacement age of agricultural tractor (MF285) in Varamin region (case study). Journal of American Science 2011; 7(2):674-679]. (ISSN: 1545-1003). http://www.americanscience.org Key words: Economic life; replacement age; MF285 		73
	A Weighted Usability Measure for E-learning Systems	<u>Full Text</u>	
74	Aslam Muhammad ¹ , Aksam M. Iftikhar ² , Saqib Ubaid ³ , Martinez-Enriquez A. M. ⁴ ¹ Department of CS & E, U. E. T. Lahore, Pakistan ^{2.3} Department of CS & IT, University of Gujrat, Pakistan ⁴ Department of CS, CINVESTAV-IPN, D.F. Mexico ¹ maslam@uet.edu.pk, ² aksam.iftikhar@uog.edu.pk, ³ saqib.ubaid@yahoo.com, ⁴ ammartic@cinvesta.mx Abstract: Currently learning paradigms have been overcome, using information and communication technologies (ICT) to give rise to e-learning domain. Thus, classical classrooms based training has been substituted by online systems working on Internet. The aim of an e-learning system is to fulfill requirements of instructors as well as learners. However, institutions offering courses online have a lack of applying efficient evaluation methods to both teachers and students. Frequent preoccupation concerns with functionalities and interface that a system must satisfy for users needs. In our studied case, learners need to face up to functionality of e-learning infrastructure rather than to acquire knowledge. When users spend more time, resources (software, hardware) unnecessarily, consequently they spend more costs, instead quenching academic thirst. Thus, this research aims to evaluate the usability of e-learning systems, a		74
	pondered measure of usability evaluation is proposed as a result of the analysis of the inquiry applied to the system users. We study, evaluate, and compare the usability of two applications, to highlight recommendations for improvement. [Aslam Muhammad, Aksam M. Iftikhar, Saqib Ubaid, Martinez-Enriquez A. M. A Weighted Usability Measure for E-learning Systems. Journal of American Science 2011; 7(2):680-686]. (ISSN: 1545-1003). http://www.americanscience.org Keywords: e-learning; Usability; Evaluation; Assessment		
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75	Relationship between the Quality of Work Life and Employees' Aggression ¹ Masoud Porkiani, ² Mehdi Yadollahi, ³ Zahra Sardini, ⁴ Atefeh Ghayoomi ¹ Islamic Azad University, Kerman Branch, Iran E-mail: pourkiani@yahoo.com , ² Dept of Management, Payame Noor University, (PNU), Sirjan, I, R., Iran E-mail <u>mfma155@yahoo.com</u> ³ M.A. of Governmental Management, Islamic Azad University, Kerman Branch, Iran ⁴ M.A. of Business Administration, Islamic Azad University Abstract: People working in organizations have a lot of needs, all of which are regularly in competition to guide their behaviors; if these needs aren't met, they result in frustration, and failure in meeting the needs doesn't necessarily cause the quick death of organizations. One of the most common reactions against failure is aggression which is harmful and affects soul and spirit, working relations and performance of employees even though it doesn't cause physical damages. Thus, conditions of working environment which result in meeting the material and spiritual needs of people, represents the quality of work life; it is in such an environment that employees can feel possession, self-direction, responsibility and self-respect. The aim of the present research is to study the relationship between the quality of work life and employees' aggression. This research was carried out using correlation method in statistical universe of employees in 2007); the sample volume was 384 people. To gather and collect information, two closed-ended questionnaires of "quality of work life and aggression" were used. Data was analyzed using Kendal's Tau to rest and linear logarithm by means of SPSS Software. Results revealed that there is a relationship between quality of work life (and components of job security, justi	Full Text	75

A Theoretical Study of Family Resource Management

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Abstract: Family resource management has a fundamental role in helping meet and alter the increasing complexities faced by the families. In this way, this article attempts to describe the theories of family economic management. To solve the economic problems we need to improve the economic status of families who fail to manage their budget, which results in high debt levels and a lack of personal savings. The implication of this study arises from the fact that there has been little research carried out on the family economic status. Theoretically, the findings of this study enrich the knowledge concerning family economic and management functions.

[**Mehdi Yadollahi & Laily Hj Paim.** A Theoretical Study of Family Resource Management. Journal of American Science 2011;7(2):1-6]. (ISSN: 1545-1003). <u>http://www.americanscience.org</u>.

Key Words: Family resource, Economic function, Economic organization

Introduction

Family economics is now a honourable and growing field (Becker, 1965). Economic status and family are among the most widely used terms in the field of family economics and household management. Family resource management has a fundamental role in helping meet and alter the increasing complexities faced by the families. Household management is the process of using the resources to attain its goals through planning and taking the steps necessary to meet these goals. A crucial part of the management process is the allocation of resources for the appropriate goals (Deacon & Firebaugh, 1988). In other words, management is the process of using what one has to get what one wants. Resource recognition is the realization of the skills, talents, and materials that the household possesses (Goldsmith, 1996; Hallman, 1990). The management process involves thinking, action, and results. Although household management is practical, it is not necessarily simple. It becomes complex because the choices of the individuals and the family are constrained by limited resources. Each individual has his or her own resource, attitudes, talents, and skills that are brought to bear on situations. Management, therefore, has to be viewed within the context of the greater life environment, which is constantly changing (Goldsmith, 1996). Household management consists more than merely the economic management of resources to produce a high standard of living through consumption. The management process begins with a problem, need, want, or goal, which has to be identified. Once identified, the individual or family moves to the second step, which is the clarification of values. The third step involves identifying the available resources. Deciding, planning,

and implementing are in the fourth step of the process. In the fifth step, the goals are accomplished or fulfilled and the process as a whole is evaluated. Then, the information returns to the system and enables the individual's overall management knowledge and ability to grow (Goldsmith, 1996). Each person has his or her own management style, or way of making decisions and acting. Various factors including history, biology, culture, personality, and technology influence the individual's management style. But the field of resource management is even broader than these influences suggest (Goldsmith, 1996). Life management, on the other hand, encompasses all the decisions a person or family will make, and the way their values, goals, and resources affects their decisionmaking. It includes all the goals, events, situations, and decisions that make up their lifestyle. Life management, thus, is a holistic approach that looks at management as a process that evolves over an entire life span (Goldsmith 1996). The study of household management is a combination of theory, concepts, technique, research, and practice. There is not only one management theory or framework; instead. management is an interdisciplinary field that borrows concepts and theories from related disciplines (Goldsmith 1996). Much of a household's decisionmaking shaped by the environmental settings in which the family functions. These environments either constrain this decision-making or offer opportunities for the family. Because the physiological and the psychological makeup of the family members differ, so do as the environments in which they interact, it becomes essential to view decision-making from an ecological perspective (Paolucci et al., 1977).

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Family Resource Management

Family resources are the means for satisfying our needs and reaching our goals.

-Money, salary, rent, interests from savings bank account etc.

-House for living and working.

-Time, like an hour a day, month etc.

-Energy to do work.

-Knowledge, skills and abilities for doing our work, like sewing, driving,

swimming, etc.

-Material goods like household equipments, car etc.

-Community facilities like park, hospitals, roads, bus etc.

The resources possessed and utilized by persons are called human resources. Non-human resources are external to individuals, but they can be possessed and utilized by them (Home Science 2010).



Figure 1: The family resources

Family structural-functional theory

This theory provides a foundation for understanding relationships between family and health care systems (Friedman, 1998; Pratt, 1976). The family is a basic social unit that meets both individual and societal needs and interacts with other societal institutions. Family structure includes familv composition, size, and roles. Function refers to why the family exists, which is to meet the needs of both individuals and society. It is through the family structure that family functions occur. Within the context of family structural and functional theory, a more specific understanding of the relationships among family economics, quality of life, and changes in the health care system is provided by an economic approach to families. Becker's treatise on the family (1991/1993) is a seminal work on the economic perspective of the modern family. Higher divorce rates, lower birth-rates, single-parent households, working mothers, and increased life span led Becker to reformulate traditional economic theories regarding the 2

function of the family in society. Traditional economic theories typically focused on men as income-earners and women as homemakers and non-participants in the labour force. Becker analyzed the effects of women's participation in the labour force on the division of labour in both the market and the household.

One model in Becker's treatise concerns the allocation of energy (or effort) among various household and market activities. According to Becker, "firms buy a package of time and effort from each employee, with payment tied to the package rather than rendered separately for units of time and effort" (p.65). This premise explains lower earnings by women who are usually the primary persons responsible for childrearing and household duties. For example women earn less when less energy is available for the labour force because of sick children require mothers' care at home, or the inability to work required hours due to childcare needs. This premise also explains the affect of illness on family economics (Backer 1991/1993).

Economic Organization of the Household

The economic organization of the household is the main approach of the economic family. It has described by Bryant in 1990. Bryant synthesized various threads of research and scholarly works regarding economics of the family into a single text on the subject. According to Bryant the family is considered a household, a smaller unit of the larger society, that forms to pursue the goals of "satisfaction seeking" (p.1) and "increasing the well-being of their members" (p.2). Bryant uses the concepts of well-being and satisfaction interchangeably with utility, which he defined as preferences a household has for goods and services for meeting goals. He states, "The household's preferences reflect its likes and dislikes, its views as to what will increase and what will decrease its wellbeing, its goals if you will" (p.17). The economic organization of the family refers to the size, structure, and composition of the household as well as the patterns of resource use and of activities pursued by the household "(pp.5-6).

Circumstances in the broader social environment also affect the available resources of a household to achieve the goal of well-being. According to Bryant (1990) conditions of both inside and outside the household determine the amounts and kinds of resources possessed by the household. The productivities of each resource in the activities pursued and the satisfaction the household receives from the activities. The conditions inside and outside the household change, thus the patterns of resource use and of activities pursued by the household change (Bryant, 1990). In this model and in Becker's model

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management practice is viewed as an investment in human capital. Income and earning potential is increased when people practice management and getting better result than the past. On the other hand, a person who has less management knowledge tend to have lower financial resources comparing to those with higher knowledge. To summarize, Becker's and Bryant's family economic theories are based on the assumptions that human behaviour is goal directed, and humans make rational choices concerning how goals are met. Families form to pursue the goal of satisfaction of its members. Income is a resource available to use on goods and services needed by a family to achieve its goals, including the health, educate and economic objectives of family members.

Family Financial Management

Effective financial management as defined by Schnittgrund and Baker (1983) combines financial management practices and outcome results such as the type of budget used, the frequency of saving, and the frequency of financial management problems in the family. Research shows that consumers believe financial management practices like budgeting and saving is valuable (Godwin & Carroll, 1986; Mullis & Schnittgrund, 1982; Schnittgrund & Baker, 1983). However, most studies relating to financial management practices identify the audience using recommended financial management practices rather than the results of using the practice.

Characteristics of those who adopt recommended management practices have been the topic of previous research. Beutler & Mason (1987) studied factors associated with using formal budget planning. They found that young, married, and welleducated households with high demand on available resources were more likely to adopt the practice of written budgets. Level of income did not significantly affect the practice of written budgets. Level of income did not significantly affect the practice of budgeting. Most families who budgeted their money, compared to families who did not budget, believed that they could increase their satisfaction with financial management by planning expenditures (Mullis & Schnittgrund, 1982). Rosenfield and Neese-Todd (1993) showed that most aspects of the quality of satisfaction with financial status are related to the individual's perception of their control over finances (Rosenfield & Neese-Todd, 1993). Women, more often than men, view themselves as powerless and lacking essential resources to be able to make changes in their lives (Burman, 1994). Even though financial management practices have been proven to increase net worth and satisfaction with financial resources, there is evidence of resistance and failure of consumers to adopt such practices (Beutler & Mason, 1987; Davis, 1988; Godwin & Carroll, 1986; Schnittgrund & Baker, 1983) suggests that even affluent households do not see the balance sheet as a useful financial tool. Davis (1987) found that lack of time and knowledge were the two reasons most often given for not using recommended practices of budgeting, record keeping, comparing records to the budget, and preparing a balance sheet. The need for budgeting financial resources and wise use of credit are most often felt by those with low incomes or who are in debt (Davis, 1987). To encourage adoption of financial management practices, Walker, Tremblay and Parkhurst (1984, p.429) recommend that educational programming be inexpensive, uncomplicated, and readily accessible (Walker et al., 1984).

Steps in the management process

According to the literature review from previous studies, there are four steps of management. To achieve our goals with limited resources, we have to follow a systematic method. Management involves the following steps:

- Planning
- Organizing
- Controlling
- Evaluating

Step 1: Planning

The first step in management consists of thinking in advance of what needs to be done i.e., planning. A simple way to plan is to make a list of all the things that need to be done (Resource Management 2010). It means both to assess the future and make provision for it" (Fayol, 1949, p. 43)".

Step 2: Organizing

Organizing involves assembling resources and fixing responsibilities Fayol (1949) enumerates the managerial duties of organizations that must be realized through personnel. Fayol considers the functional components of organizations along with the constituent personnel, and discusses the ideal conditions required of each in considerable detail.

Step 3: Controlling

It has consists of the ongoing, routine verification of plan implementation, instructions issued, and principles. Controlling applies to all processes. Its purpose is to identify weaknesses and problems such that they can rectify and recurrences prevented.

Step 4: Evaluating

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The evaluation helps to understand the weaknesses and mistakes so that it is checked and will not be repeated in future. This is also called looking back or "feedback" (Home Science 2010).

Engel Curve Analysis

In Engel curve analysis, the choice of a functional form is based on both economic and statistical considerations (Prais & Houthakker, 1971). The simplest of these is linear. Theoretically, this is a plausible relationship because in a complete system of demand equations linearity satisfies the adding up criterion (Phlips, 1983) that the sum of the one at all income levels (Phlips, 1983; Prais & Houthakker, 1971) In statistical analysis, the fit of linear Engel curves has been found to be poor (Phlips, 1983), leaking economists to use other functional forms. From a theoretical point of view, this functional form is not desirable because it violates the adding-up criterion (Deaton & Muellbauer, 1980). However, the tradition of household budget analysis has been to choose functional forms on the basis of statistical fit (Deaton and Muellbauer, 1980). The double- logarithmic form is useful in expenditure studies because the income elasticity of expenditures for the good in question can be read directly from the income coefficient.

Estimation of this functional form is appropriate for broadly defined expenditure categories like clothing (Dardis, Derrick, & Lehfeld, 1981a, 1981b; Wagner & Hanna, 1983), in which all or most households report expenditures during the survey period. Consequently, by utilizing the concept of wants, Engel devised a classification method that enabled him to empirically measuring the impact that particular wants have on consumption over a range of observed income. He did so by classifying expenditures into the want for nourishment, clothing, accommodation, heating and light, household goods, spiritual education (education and entertainment), public safety, health and recreation and personal services (Engel 1857:6). Engel did not create a separate category for travel and trade, reasoning that these types of expenditures are not end purposes in themselves, but that they were done for other purposes, e.g. expenditure on travel contributed to either work or pleasure (Engel, 1857).

Therefore, those wants whose expenditure is left at the lowest level of observable income can be understood to be the most urgent. In turn, he reasoned that a rough approximation for public welfare can be attained by investigating how much of the consumer budget is dedicated the want for nourishment, which appeared to be the most basic want (Engel 1857:50).



Figure 2: The processes of family management (Home Science, 2010)

4

Wants	Relevant expenditures
Nourishment	Daily nourishment from meals and beverages, spices, stimulants
Clothing, linen,	Clothing and shoes of all kinds
Housing	Shelter, furniture, household appliances
Heating and lighting	Wood, heating, lighting via candles, oil and gas
Appliances for work	Tools, machines, mechanical instruments; crockery and vessels etc;
Intellectual education	Tuition,; worship; scientific equipment, literary and artistic production
Public safety	Legal protection; administration; police; state defence; care for the poor etc.
Health, recreation,	Medical treatment and pharmaceutical expenses, bathing;
Personal service	Personal services attained from use of domestic servants of all kinds.

Source: (Engel, 1857)

Conclusion

One of the most important reasons for researchers to study family economic status is that any community development in all dimensions starts with the family. Theoretically, the findings of this study will enrich the knowledge concerning family economic and management functions. The most important implications of this study is that researchers and practitioners can able to develop effective programmes for families, especially in economic aspects.

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Biological Investigation of Persian Gulf blue swimmer crab (portunus pelagicus) In Khuzestan coasts

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Abstract

A bout 361 Million square kilometers (70.8 percent) of the totol area of the earth is covered by seas and oceans. Although this huge ecosystems always make the human curious about them, but the need for advanced equipments for approaching deep and semi-deep areas is the reason for shortage of information about these ecosystems and their inhabitants comparing to the lands. So during recent years extensive researches have performed about marine ecosystems with emphasis on recognition of biological variations and make use of aquatics in several new applications such as nutrition, health, medical and industry. Although Persian Gulf have unique specifications such as different ecosystems and different varieties but it is less investigated accurately. Certainly for protecting such a valuable ecosystem, at first we must have comprehensive information of its structure. This emphasizes the necessity of exact study in all the parts. Therefore current study, investigates blue swimmer crab (one of the valuable Persian Gulf crustaceans) in hunting zones of Khuzestan province. This variety which is spreader globally has a high economic value and is considered in growing aquatics in many countries. The results of this research show that in width parts of some carapace, male blue swimmer crabs are always heavier than female ones. Also there is a positive and meaningful correlation between weight and the parameters of carapace's length, carapace's width, propodus length of male's claws and the width of 6th band of abdomen. Sex ratio during a year of study was F: M = 56%: 44% which should be the relative frequency of females. In the investigation of females maturation during a years it is found that although there were mature female crabs at all of the month in a year but the climax of their maturation in a year and in the place of study, were march and April. Indeed according to the factor of mature carapace width, the mature width was 88 mm carapace width in females. In male crabs, the mean gonad weight in immature was 0.15g and in matures was 1.2g. Also gonad index (GSI) in male crabs was observed with minimum amount of 0.480 during Feb and maximum of 0.807 in Dec.

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Keywords: Biology, blue swimmer crab, Persian Gulf, portunus pelagicus

1. Introduction

Persian Gulf is one of the rare ecosystems which have ecological, geographical specifications and also physicochemical, biologic specifications etc... It includes considerable numbers of known marine sensitive ecological zones such as estuary, gulfs, coral reef and stone coasts. Variation of environment in the Persian gulf area cause the presence of various species in plant and animal communities, which include phytoplankton, algae, grasses and marine plants and also animal communities including various groups of invertebrates such as sponges, corals, molluscans, crustaceans and marine vertebrates such as fish, reptiles, birds and mammals. It should be noticed that some of the mentioned species of organisms are under the protection of global protection and support and some are native and only belong to this zone. The others have migrated to this zone and choose this environment for living all their lives or they spend a part of their life

cycle in this place for food, generation, spawning and growing and passing the winter.

In this study, blue crab as one of the marine crustaceans which is spreader in northern areas of Indian Ocean (Persian Gulf and Oman Sea) is investigated. Although in other countries, this variety has a special place in nutrition and includes a huge part of industrial hunting, but in our country it is treated like a incident hunting and it is eaten as a food only in limited areas of south of the country such as Khuzestan, Hormozgan and Boushehr provinces. This variety is one of the biggest crabs of tide zones of Indian and Pacific Ocean. Males of this species are blue with white dots on the shells and females are green- brown. In spite of the other crabs, this species can not survive a long time out of the water and its food, shelter and growth are dependant to estuaries. Growth speed, high reproductive power, and strength against PH and salinity changes make the blue crab a proper species for aquaculture in the world. Indeed its marketable

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meat has expanded its hunting and growing. Such that the price of a kilogram of blue crab is 5-8 dollars of America per fresh hunting and 8-12 dollars per alive.

2. Material and Methods

Sampling was performed in Persian Gulf coast								
provin	ce) ii	ıcluc	ling	easter	rn co	oasts		
(Bahrekan hunting zone) with following specifications:								
2957/639			El	ND LAT	4928/06	5		
2954/712			El	ND LAT	4928/05	9		
ern coasts	(Boseaf	and	lifea	hunting	zones)	with		
following specifications:								
2955/184			El	ND LAT	4906/85	5		
2952/199			El	ND LAT	4903/86	7		
	mpling w provin 2957/639 2954/712 ern coasts ifications: 2955/184 2952/199	mpling was perfo province) in unting zone) wit 2957/639 2954/712 ern coasts (Boseaf ifications: 2955/184 2952/199	mpling was performe province) incluc unting zone) with fo 2957/639 2954/712 ern coasts (Boseaf and ifications: 2955/184 2952/199	mpling was performed in province) including unting zone) with followi 2957/639 El 2954/712 El ern coasts (Boseaf and lifea ifications: 2955/184 El 2952/199 El	mpling was performed in Persian province) including easter unting zone) with following spec 2957/639 END LAT 2954/712 END LAT ern coasts (Boseaf and lifea hunting ifications: 2955/184 END LAT 2952/199 END LAT	mpling was performed in Persian Gulf c province) including eastern cc unting zone) with following specification2957/639END LAT 4928/062954/712END LAT 4928/052954/712END LAT 4928/05rn coasts (Boseaf and lifea hunting zones)ifications:2955/184END LAT 4906/852952/199END LAT 4903/86		

It was performed monthly during a year from Oct 2007 to the end of Sep 2008. Sampling was performed with several methods such as research ship and local hunters (hunting boats) with fishing net and sampling. After hunting, samples were incubated in ice and were transferred to laboratory at maximum 24 hours. Then the crabs are washed for removing the mud and algae's and barnacles sticked to the external skeleton. After that they were dried with drying paper at first crabs were separated to male and female type according to their morphological characteristics. Male crabs were bright blue and their womb area were narrow and in a form of spear and female crabs were green – brown and their womb, area were round. Then the following parameters were measured in both groups:

- Wet weight (ww)
- Gonad weight in males (GW)
- Carapace length (CL)
- Carapace width(CW)
- Propodus length of claw in males (PL)
- Wide of sixth band of womb in females (All of the items were measured by a coils with accuracy of 1 millimeter)

In mature females (from maturation stage 4 to next) the eggs were investigated for calculating the fecundity .for determining the gonad maturation, an index key is used. Different sex stages in female crabs include:



Fig1.Male and female blue crab (24)

- Immature stage (1), white narrow ovary is observed in young and immature crabs
- Rudimentary stage (11), light yellow ovary which shows a considerable volume
- Developing stage (111), light orange ovary. From this stage, ovary is considered to be mature.
- Intermediate stage (IV), The size of ovary increases and occupies 25% of carapace
- Advanced stage (V), Bright dark orange ovary which is dotted and besides carapace it occupies the front side of the belly
- Resting stage (VI), Eggs are settled below the womb feet and ovary shows one of the 2-5 stages

After identifying the gonad developing stages in female crabs, they were classified in groups of 10 millimeter width and the percent of the samples of each stage were calculated. Based on the percent of maturation during the seasons of a year (different months of each season), the maturation peak and hatch were identified. Female crabs which has the maturation stage of more than 4, were selected and their fecundity were calculated in this way (sukumaran 1996).

At first the ovary was separated by a scalpel after drying with drying paper it was weighted with a sensitive scale (with accuracy of 0/001 grams) (G) Then a small part of ovary were separated and weighted again (about 1 gram) (g) The small sample of ovary were kept in dish containing distilled water with detergent and eggs were isolative slowly by shaker .Then the eggs were counted with a binuclear with magnification of 16x. (For decreasing the error a colony counter was used) After that fecundity was calculated with this formula:

F=n×G/g



Fig 2. Mature female blue crab Samples of blue crab eggs

For calculating the gonad index in male crabs, gonads were removed carefully after analyzing the womb plate and immediately were weighted with sensitive scale then gonad index was calculated with a

formula: $GSI = \frac{Gonal \text{ weight}}{body \text{ weight}} \times 100$

In this study for determining maturation in males of blue crab, after identifying the male crabs and their weighting, the parameters of length and width of the carapace and length of propodus were measured carefully. Then besides analyzing the womb protective plate, gonads were separated and weighted (by an electric scale 0.001 gr). considering that gonad weights in matures are 10 times more than gonad weight in young and immature males (kogous and ettal 2001), based on the mean weight of gonad, male crabs were divided to two groups of mature (mean weight of gonad 1.2 gr) and immature (mean weight of gonad 0.15 gr). Then width classification was performed based on carapace width. At each of the classes, which include minimum 50 percent of mature samples, that class was introduced as the maturation class of male crabs (carapace width of maturation).

Then with identifying the minimum of maturation carapace width, the minimum of propodus

length in males were identified. During sampling in each month, beside counting the female and male crabs and calculating the sex percentage, the sex proportion (M: F) for each month, each season and finally each year of sampling for that zone, were calculated. Also minimum width of carapace in maturation (LM 50) was calculated and stated for female and male mature crabs. In such a way that after dividation of crab to width groups of 10mm in each width group, if 50 percent of the sample were mature, that width became the maturation width. In male crabs, after measuring the propodus length of the claw, the relation between propodus length and carapace width and length were determined with statistical methods (determining equations and correlation coefficient).

3. Results

Statistical test results for comparing mean weights of female and male blue crab showed that there was a meaningful difference between 2 groups at the level of %1 and males were heavier than females. Indeed males were always heavier than females in width classes of carapace. The highest weight difference was observed in carapace width (170-179 mm) and the least weigh difference (100-109mm) was observed in carapace width.

Table 1. Statistical relation of weight with Morphmetric in weight classes (1)

Females weight	X	Y	\mathbf{R}^2	Correlation equation
group				
	Cl	ww	0.96	Y=1.278 x - 29.93
ww <50 gr	CW	WW	0.85	Y=0.529 x - 63.15
	AW6	ww	0.91	Y=1.1 x + 150.09
	CL	WW	0.90	Y=2.565 x - 77.5
ww 50- 100gr	CW	WW	0.73	Y=0.640 x - 59.30
	AW6	WW	0.91	Y=3.28 x - 32.70
	CL	ww	0.97	Y=3.423 x - 62.54
ww>100gr	CW	ww	0.94	Y=3.249 x - 297.3
	AW6	WW	0.74	Y=0.749 x +134.7

males	X	У	R ²	Correlation
weight				equation
group				
	Cl	WW	0.94	Y=1.095 x +
				9.088
ww	CW	ww	0.85	Y=0.157 x +
<50 gr				28.52
	PL	ww	0.93	$Y=1.474 \ x +$
				19.36
	CL	ww	0.97	Y=1.237 x +
				33.56
ww 50-	CW	ww	0.97	Y=0.716 x +
100gr				1.225
	PL	WW	0.94	Y=4.197 x +
				2.135
	CL	ww	0.75	Y=4.433 x -
				88.32
www.100an	CW	ww	0.95	Y=3.948 x -
ww>100gr				386.8
	PL	ww	0.96	Y=14.80 x +
				238.8

 Table 2. Statistical relation of weight with Morph metric in weight classes (2)

Table 3.	Freq	percent	of	gonad	weight	in	carapace
weight							

/Freq percent	(g	ght	
carapace width	< 0.75	0.75 - 1.5	> .5
80 -89	90	10	
90 -99	32	40	28
100 -109	10	44	46
110 -119	0	35	65
120 -129	0	13	87
130 - 139	0	5	95
140 -149	0	1	99
150 -159	0	0	100
160 -169	0	0	100
170 -179	0	0	100

The results of the relationship of weight with Morphometric factors in blue crab in two sex classes and three weight classes, showed the statistical relation of carapace length, carapace width, and width of sixth womb band with weight, and the equation and correlation coefficient were obtained between weight and all other factors.

Based on sex frequency percentage of blue crab in Persian gulf coasts (Khuzestan province) the total sex proportion during a year of sampling were estimated as F: M= 56%: 44% Which showed that

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female blue swimmer crab has the larger part of the population Results show that in the Oct and Sep months, sex proportion was balance, in Nov and Dec, the percentage of males was higher. And in other Months of the year females has the higher percentage of the population. Indeed, the maximum frequencies of males were observed in Nov and maximum frequencies of females were observed in Mar.

Considering that blue crabs in stages 1 and 2 are immature and in other stages are mature, results showed that in all of the months of a year, there were mature female crabs in addition, during the seasons of fall and summer, the frequency percent of immature crabs were more than immature, and the frequency of maturation stage 5 was observed in Mar and Apr. Stage 5 existing in all of months in the year (except summer months) showed the possibility of spawning of this type during the year.

Determining the percentage of female crabs mutation stages with width classes dividation showed that females with carapace width less than 80 mm were immature considering that in 80-89 mm width class, minimum of 50 percent of the samples were mature, physiologically so the minimum physiological maturation with for females of this type, were calculated 85 mm. indeed the smallest mature female blue crab had 82 mm carapace width, in other words minimum size if the maturation carapace width in the females of this type was 82 mm.

Table 4. Frequency percent of female crabs sexmaturation with width group's dividation of percentof maturation stages

(mm) width groups	Ι	Π	III	IV	V	VI
80-89	35	10	45	10		
90-99	25	20	40	10	5	
100-109	7	43	13	7	30	
110-119	13	30	13	10	30	4
120-129	4	44	15	11	18	8
130-139		17	38	7	37	1
140-149	6	37		48	6	1
150-159			38	15	42	5
160-169			33	23	8	36
170-179			25	25	50	

Results of statistical comparison for determining crabs morphological maturation in two groups of mature and immature (isolated based on the size of the width of womb 6th band) showed that, there was always a meaningful relationship between weight

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and width of womb 6th band at the level of %1 and with increasing the weight, the width of womb 6th bund also increased. Minimum width of womb 6th band in mature females (with carapace width more than 85 mm) was 22 mm and maximum width of womb 6th band in female crabs was 46 mm. investigating the gonad weight in groups of mature and immature male crabs showed that mean weight of gonad in mature group was 1.2 gr and mean weight of gonad in young and immature male group was 0.15 gr. Regression analysis of gonad weight and total weight factors, carapace length and carapace width showed a meaningful relationship between then at the level of %1 which this relationship was observable in triple weight groups.

Gonad weight less than %75gr was considered immature and more than that was mature. So because in the 90-99 mm width class, more than 50 percent of male samples were mature considering gonad, so 95 mm carapace width (above class width mean) was stated as male crab maturation carapace width. With regard to minimum width of maturation carapace in male crabs (95 mm), minimum length of maturation propodus was specified as 20mm. in other words all of the male crabs whose propodus length were less than 20 mm, were immature. The maximum length of observed propodus in male pelagian crabs was 29mm.

Results of gonad index calculations in male types of blue crab showed that minimum GSI in Feb was 0.420 and maximum GSI was 0.801 in Dec. (Table) Also it have been observed that maximum GSI was in Nov, Dec, May and Jun. Investigating absolute generation in female crabs showed that minimum amount of generation was 150496 and its maximum was 1106215 generation in blue crab was observed as 815249. the results of variance analysis showed that there was a meaningful correlation between weight and generation, carapace width and generation and carapace length and generation at the level of %1 Based on that, with increasing the weight, generation increased and also with increasing carapace length and width, generation amount increased.

Discussion

Growth is caused by consumption, assimilation and food participation in constructing the body of organisms (vastenso 1979). Growth is a special adapting property which is supplied by the continuity of the species with its environment. Strategy of r.selection property shows the adaptation of this species with its environment. (Considering the changeable and in consistency of ecologic situations of the zones under tide (main ecosystem of swimmer crab) this species has short life time (3-5 years), rapid growth, early maturation and abundant generation, as a seizing species. Based on the results of this study in Persian Gulf coasts (Khuzestan province), the mean weight of male blue crabs were always more than females. Performed studies in Boshehr, and Bandar abas coast had the same results (sharafy 1377, Ghorbani 1380) with changing the latitude and longitude and in various ecological situations in other places in the world, changing some parameters such as temperature, salinity, food diet and available food, etc, caves that in some zones, mean weight in male and female blue crab be in favor of males. In Australian coasts, mean weight of males, was reported considerably more than females potter and co-workers 1993).

Investigating the relations of carapace length and width with weight showed that there is a linear relation between those parameters which has a positive and high correlation coefficient. Also results of this study showed that there is a positive correlation between weight and carapace length and width of blue crab and in same carapace width, males are always heavier than females, in biological and ecological studies of populations, one of the considerable characteristics Is the sex ration in the population of that species, which a sex ratio of 50 :50 is expected in the normal situation and without any external stresses such as private hunting, sudden change of temperature and decreasing available food sources. Such a situation cause consisting and constancy of this species. Because it increases the chance and possibility of successful mating and generation. If any factor causes this ratio to change considerably in favor of one of theses sexes (dominance of males or females in a population), so the chance of finding proper mate and successful mating decreases, and also it endanger the constancy and survival of this population. (Navbaken 2005). The results of current study showed that during a year of research in investigated zone, the sex ratio of blue crab population was as F: M = 56% : 44% which this ratio shows the female proportional frequency to males in the population of this species. Same researches in Australian coasts showed a double ratio of females to males of this species. (Kangas and ettal 2000). Generally, hunting method (e.g. hunting with cage) season situation and sudden changes of the weather and migration patterns can affect the sex ratio in the population of this species (nicolsky 1997). Among blue crabs when female crabs migrate to estuary areas for spawning (for reaching to food and enough oxygen for larvas) male crabs prefer living in sweet waters. Vice versa in spring migration, mature males of this type migrate to upper areas of estuary. (Sukumaran 1992).

Like other aquatics we can use either of two methods for determining the morphological maturation,

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size of 50 percent of matures and mean size of twin matures group. in all of the studied the mean size of twin matures group in female blue crab was large than 50 percent of matures size. (Korgus and ettal 2006). Because in first width class of carapace width (20-29 mm) of 50 percent of samples were mature, so maturation carapace width in blue crab was stated as 850mm. indeed minimum of maturation width was 82mm it means that all of the female crabs with carapace width larger than 22 mm were mature. Same researches in other areas show the changes of maturation width in this species which it proves the dependency of growth and generation of the species on the ecologic situation and position. For example in Indian coasts, maturation width of this species is stated as 110 mm (Kumar 2003). In Southern Australia estuaries, the minimum maturation carapace in blue crabs is states 94 mm (lestang 2001). We expect that in same ecological situation and ecosystem adjacency, the maturation size of the variety shows a quiet same result. (Filder 1999) in a same research which is performed in Hormozgan coasts, maturation carapace width of blue crab was stated as 90mm. (valinasab 1380). Where as this parameter shows 108 mm in Boushehr coasts. (Ghorbani 1380).

Results showed that there was a meaningful linear correlation between width of womb 6th band and carapace width in female pelagian crabs (at the %1 level). It means that with increasing the weigh of female crab, the width of womb 6^{th} band increased so the parameter of womb 6^{th} band with can be used as an index for determining maturation or maturation stage of the female types with regard to physiologic and morphologic changes of female crab after maturation, mating and specially during spawning. in same researches in Malaysia (kampel and ettal 2001) beside studying reproductive biology of blue swimmer crab, they use the parameter of womb 6^{th} band width for determining the sex maturation in female types. Those researchers stated that, female crabs with mean womb 6th band width of (21 mm) are considered as mature. Considering that aquatic generation cycles are affected by several ecological, biological, chemical and physical factors, increasing the surface temperature of water or increasing the available food will accelerate the generation activity and maturation process. Indeed, photo periods or light- dark cycles has an important role on regulating seasonal reproductive activities in aquatics with direct effect on pineal gonads and hypothalamus. (Bon 1996).

In the family of portunidae and blue crab the situation of along day, accelerates the maturation process and as a result, gonad weight and index increases. (Shimizo 1995). Results of this research

showed that gonad index (GSI) of blue crab has little fluctuations during the seasons of a year. The maximum gonad index was observed in the middle to the end of fall and from the middle to the end of spring. Which is not consistent with the season of spawning peak of female crabs (the time of maturation peak)? This shows a time gap about 3 months between mating to Spawning in this variety. Indeed mean gonad index during the seasons of the year .shows the male readiness for repeated mating. in spite of female types which mate only one time in their life cycle (Alono 1998), same studies in the Mexican gulf zone on the reproductive biology of blue crab showed that males of this rarely have considerable gonad index at the most months of the year so they can mate several times (kampel 1994)

Considering the reproductive strategy of blue crab this species has high fecundity like other members of portunidae family. This amount of power is always affected by biological, chemical and physical parameters. (Shield 1998). Based on that under the environmental situations, in the seasons which there is enough available food, generation shows higher amounts. (potter 1996): same results in Australian estuaries showed that when nutrition stress have impact on variety population of blue crab, energy reserves will decrease in the animals liver and after that fecundity of females decreases severely. Considering this reality that increasing the body size (increasing the carapace width and length) provide a proper and enough space for length and volume growth of ovary and as a result cause more eggs settles in the womb plate area, (sukumaran 1995), results of this study showed that there is a positive and meaningful relation and correlation between reproductive power of female blue crabs with wet weight and carapace width and length. So increasing the body dimensions (carapace width and length) and increasing the weight of female crab, fecundity increases. Researches in the north of Australia have same results, so they observed a high correlation between fecundity and carapace width in female blue crab at the level of 5% (potter and ettal).

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Biotechnological and phytochemical studies on Sabal yapa Becc. growing in Egypt

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Abstract: This study aim to production of the biologically active lipoidal fraction from *Sabal yapa* Becc., Family Palmae, using tissue culture techniques, which previously proved its remarkable and significant antiandrogenic activity. Immature embryos of *S*. *yapa* unripe fruits were cultured on fortified MS media with different type of growth regulators, and incubated under light or dark conditions. Calli growth and lipoidal content were evaluated. MS medium augmented with 10 mg/l 2,4-D and 3mg/l of 2iP and BAp showed the best modified medium for calli production under light or dark conditions. The highest mass of calli production was recorded after the 5th week of cultivation. However, the significant growth rate (mg/day) was recorded during the 3rd week of cultivation. Moreover, fractionation of lipoidal calli, was analyzed by GLC and compared with that of the *in vivo* fruit. The obtained results revealed that the lipoidal content was 0.85% in *in vivo* fruit while it recorded 0.64 and 0.68 (%) with calli resulted under light or dark conditions, respectively. The maximum percentage of unsaponifiable fraction (59.6%) was recorded in fruit, however, it recorded 20.83 and 17.53 (%) with calli produced under light or dark conditions, respectively, it was 32.3 (%) in *in vivo* fruit. Further, we recommend the exploitation of plant tissue culture techniques for the preparation and production of the biologically active non-polar fraction of *S. yapa* fruit at semi-industrial scale using bioreactors.

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Keywords: Sabal yapa, Palmae, non-polar extract, callus, lipoidal fraction.

1. Introduction

The difficulties of obtaining the source plants led to an increasing interest in developing alternative for the production of plant secondary metabolites. There has been a considerable interest in plant cell cultures as a potential alternative to traditional agriculture for the industrial production of secondary products (Dicosmo and Misawa, 1995). Moreover, plant tissue cultures have long been regarded as a source of commercially important steroids, alkaloids, and terpenes for pharmaceutical industry (Taha 1999; Zaho and Verpoorte 2007; Zarate and Verpoorte 2007).

The genus sabal was reported to be rich in saturated and unsaturated fatty acids, mainly lauric, myristic, palmitic; oleic acids and contain fatty alcohols and sterols (Cristoni *et al.* 1997). The lipidosterolic extract from some species of sabal was reported to possess as anti-androgenic activity through the inhibition of 5α -reductase isoenzymes (Iehle *et al.* 1995; Weisser *et al.* 1997; Bayne *et al.* 2000). Furthermore this extract proved to be effective in treatment of problems of micturation caused by Benign Prostate Hyperplasia (BPH), which is a condition experienced by a majority of aging men

(Bach and Ebeling 1996; Gerber et al. 1998; Boyle et al. 2000).

2. Materials and Methods Plant material:

Unripe fruits of *S. yapa* were secured from the available tree in the Orman garden, Giza, Egypt.

1- Establishment of calli cultures from immature embryos of *S. yapa*:

The immature embryos were excised from sterilized unripe fruits and aseptically cultured on 150 ml glass jars containing 25 ml modified MS medium (Murashige and Skoog, 1962).

The following MS media were used as follows:

- MS₁) MS-basal medium free growth regulators
- MS_2) MS + 10 mg/l 2,4-D
- MS₃) MS + 10 mg/l 2,4-D +3 mg/l 2iP
- MS₄) MS + 10 mg/l 2,4-D +3 mg/l BAp
- MS₅) MS + 10 mg/l 2,4-D +3 mg/l 2iP+3mg/l BAp

Activated charcoal was added to all culture media at 3gm/l as antioxidant substance. One immature embryo was cultured /jar, and cultures of all treatments were divided into two groups, the first group was maintained under light condition (16h/day

photoperiod at intensity of 3000 lux from white cool light of fluorescent lamps) while the second group was maintained under dark condition, these jars were incubated in growth chamber at $26\pm1^{\circ}$ C for 4 weeks.

The following data were recorded after four weeks of cultivation:

- 1- Percentage of survival.
- 2- Percentage of calli formation.
- 3- Fresh weight (mg/embryo).
- 4- Dry weight (mg/embryo).
- 5- Dry matter content (%).

2- Mass calli production:

An equal weight (250 mg/jar) of calli cultures derived from immature embryos were re-cultured onto the same MS medium contained the optimum concentration of growth regulators (MS₅). The comparative study was done under light or dark conditions and incubated at $26 \pm 1^{\circ}$ C. The following parameters were recorded during five weeks of cultivation:

- 1- Fresh weight (mg/jar).
- 2- Dry weight (mg/jar).
- 3- Dry matter content (%).
- 4- Determination of growth dynamics for embryo calli cultures during five weeks of cultivation under light or dark conditions:

The following parameters of growth dynamics were recorded during the five weeks of cultivation:

Growth value (G_V) of immature embryo calli cultures was calculated during the experiment time according to Szoke *et al.*(1979).

Growth rate (Gr) of calli cultures (mg/day) was determined weekly as described by Dung *et al.* (1981).

All comparative studies were applied under light or dark conditions.

The obtained results were statistically analyzed according to Snedcor and Cochran (1972).

3- Chemical analysis:

The obtained calli under light and dark conditions were collected individually, lyophilized, pulverized and extracted with petroleum ether, the obtained extract was subjected to saponification as previously described, and both the unsaponifiable matter and the fatty acid methyl esters were analyzed using GLC.

3. Results

1- Calli initiation and mass production:

There no callus and survival embryos were formed on free growth regulators of MS medium (MS_1) . However fortified MS medium with 2,4-D in concentration of 10 mg/l showed highly significant

embryos survival and calli formation under light (13.2, 9.4 %) and dark (20.8, 12.9 %) conditions, respectively. These previous percentages were enhanced by the addition of either 2iP or BAp in concentration of 3 mg/l which gave 31.2, 21.6 (%) or 31.4, 25.2 (%) under light condition, respectively and 43.2, 31.9(%) or 50.6, 46.3 (%) under dark condition, respectively. Moreover, augmented of MS medium with a combination of 10 mg/l 2,4 D and 3mg/l each of 2iP and Bap gave highest value of embryos survival which gave 51.8 % under light and 64.8 % under dark conditions. In addition, the percentage of calli formation 42.4 and 71.5 was recorded under light and under dark condition, respectively. Furthermore, the previous combination of growth regulators showed the best results of mass calli production in terms of calli fresh and dry weights (mg/embryo) and the percentage of calli dry matter content recorded 79.8, 0.65, 0.809 (%) and 91.7, 0.82, 0.895 (%) in case of light and dark incubation condition, respectively. In a view glance for the previous data, it can be concluded that, culturing of immature embryos of Sabal yapa on MS medium fortified with a 10 mg/l 2,4 D and 3 mg/l each of 2iP and BAp and the incubation under dark condition gave the best results of calli production as compared with incubation under light condition, at 26±1°C (Table 1).

2- Callus growth dynamics:

Calli derived from immature embryos were gradually increased to the 5^{th} week of cultivation. The highest significant and promising growth values of immature embryos calli production 0.14 and 7.21 were recorded after the 5^{th} week of cultivation under dark and light condition, respectively (Fig. 3). However, the highest value of growth rate was recorded during the 3^{rd} week of cultivation. They were 102 and 177.7 (mg/day) under light and dark conditions, respectively (Fig. 4).











Fig (1). Effect of fortified MS-medium with different growth regulators and incubation under light and dark incubation conditions on establishment of calli culture from immature embryos of *Sabal yapa*



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3- Investigation of calli lipoidal content :

Data presentes in Table (2) show the quantitative and qualitative determinations of lipoidal mater of *S.yapa* derived from either *in vivo* whole fruits or *in vitro* calli. Data revealed that the yield of the lipoidal matter of *in vivo* whole fruits (0.85%) was slightly higher than that of calli produced under either light or dark conditions (0.64 and 0.68%), respectively. Moreover, the percentage of unsaponifiable fraction was higher in *in vivo* whole fruits (59.6%) than in the calli produced under light or dark conditions (20.83 and 17.53%), respectively. While, the percentage of fatty acids fraction was much higher in calli produced under light or dark conditions (70.8 and 74.1%), respectively than in *in vivo* whole fruits (32.3%).

In addition, as shown in Table (3) the percentage of identified hydrocarbons in USM of *in vivo* whole fruits of *S.yapa* 94.98 (%) it was 81.6 and 95.4 (%) in calli derived under light or dark conditions, respectively. Sitosterol, campesterol and stigmasterol were identified; however, heneicosane (C21) was the

main identified hydrocarbon in the USM of *in vivo* whole fruits. In contrast, the sterols cholesterol, β -cholesterol was absent in the USM of the calli derived under dark condition while stigmasterol was the only sterol which identified in the USM of the calli derived under light condition.

The percentage of the identified unsaturated fatty acids in in vivo whole fruits was 36.87, and it was 22.6 and 39.8 (%) in calli incubated under light dark conditions, respectively. Oleic acid or represented the main unsaturated fatty acid in in vivo whole fruits (33.8%), and in calli derived from light or dark conditions (13.27 and 48.19%), respectively. While, the percentage of the identified saturated fatty acids in in vivo whole fruits recorded 58.54 %; however, it was 41.47 and 27.5 (%) in calli incubated under light or dark conditions, respectively. Lauric acid represented the main saturated fatty acid in the whole fruits (31.1%) while, arachidic acid was the main saturated fatty acid in calli incubated under light condition. However, palmitic acid was the main saturated fatty acid in calli incubated under dark condition.

Peak	Retention	In-vivo	In vitr	Comparable	
number	time	(Whole fruits)	Dark	Light	with
1	7.007	0.25	-	-	Caprylic acid
2	9.137	0.603	-	-	Capric acid
3	11.878	29.2	2.272	6.975	Lauric acid
4	15.293	15.01	3.55	6.975	Myristic acid
5	15.986	-	0.1562	7.998	Myrisoleic acid
6	19.110	9.19	24.495	5.0375	Palmitic acid
7	20.04	0.32	1.1502	7.2695	Palmitoleic acid
8	21.207	0.07	0.2982	6.5875	Stearic acid
9	23.284	33.8	48.1948	13.268	Oleic acid
10	24.090	7.05	7.2136	7.812	Linoleic acid
11	24.470	-	0.2982	6.3085	Linolenic acid
12	26.324	0.67	3.0814	15.562	Arachidic acid
13	30.06	0.53	1.9596	2.5265	
14	34.06	0.27	0.3124	4.3245	Lignoceric acid

 Table (2). GLC analysis of FAME of S.yapa in vivo whole fruits and in vitro derived calli under light or dark conditions.

		Ar			
Peak	Retention	In vivo	In v	itro (calli)	Comparable
number	time	(whole fruit)	Light	Dark	with
1	2.335	0.21	-	-	-
2	4.434	0.26	-	0.163809	n-decane
3	5.704	0.17	-	0.083105	-
4	7.206	0.45	-	0.055495	n-dodecane
6	9.116	3.5	0.751	0.073955	tetradecane
7	11.197	20.7	0.168	0.251886	Hexadecane
8	13.380	12.3	1.376	2.493242	Heptadecane
9	14.409	2.5	1.433	0.129232	Octadecane
10	15.567	8.1	32.7	19.35281	Nonadecane
11	16.517	9.9	1.502	1.782878	Eicosane
12	17.880	31.9	32.76	59.66856	Heneicosane
13	18.001	-	6.468	-	-
14	18.310	-	7.765	-	-
16	19.216	1.8	1.436	1.631449	Tetracosane
17	20.960	0.6	2.352	1.438461	Pentacosane
18	21.175	_	2.917	1.305722	-
19	21.293	0.36	-	-	-
20	22.071	1.1	4.451	2.813	-Hexacosane
21	24.410	0.7	1.608	0.686839	Heptacosane
22	25.844	0.37	0.219	1.006208	Octacosane
23	27.913	0.8	0.859	2.58838	Nonacosane
27	29.416	0.18	-	-	Cholesterol
28	30.161	0.29	-	0.755591	Campesterol
29	31.031	0.31	0.753	1.483642	stigmasterol
30	33.380	0.27	-	0.998	β-sitosterol

Table (3).	GLC analysis of	USM of S.yapa	in vivo who	e fruits and <i>i</i>	<i>n vitro</i> derived	calli under	light or dark
	conditions.						

4. Discussion:

Callus is basically a more or less non organized tumor tissue which usually arises on wounds of differentiated tissues and organs. The initiation of calli formation is referred to as calli induction. Monocotyledons are less likely to form callus tissue than dicotyledons and auxins are often needed as a hormonal stimulant for callus induction (Tisserat 1979; Ibrahim 1999). An exogenous supply of regulators is often recommended to initiate callus formation on an explants, the exogenous regulator requirements regarding the type of regulator, its concentration and auxin/cytokinin ratio depends strongly on the type and endogenous hormones content (Pierik 1987).

The obtained results showed that, supplementation of MS-medium with different combinations of growth regulators had a promising effect on callus production from immature embryos of *S. yapa* and the best results were achieved when MS medium was augmented with 10 mg/l 2,4-D+3 mg/l 2iP + 3 mg/l Bap, so it can be concluded that supplementation of culture medium with auxins and cytokines achieved of mass callus production.

The obtained results seem to be in accordance with the published data by Shatnawi *et al.*,(1997) how reported that MS medium supplemented with 0.1 mg/l NAA and 5 mg/l 2iP showed the best results of calli production from auxiliary buds of *Phoenix dactylifera* and it was superior of MS medium supplemented with 2,4-D alone at different concentrations. In addition, Sharma *et al.*,(1984) reported that best results of callus formation from shoot tips of date palm were obtained when MS medium supplemented with 100 mg/l 2,4-D and 5mg/l BA was used. Further, Al-Ghamdi (1993) mentioned that good calli initiation and growth was obtained on MS medium supplemented

with 100 mg/l 2,4-D and 3 mg/l 2iP. However, Saker *et al.*, (1998) initiated and proliferated calli from immature embryos of *Phoenix dactylifera* on MS medium supplemented with 10 mg/l 2,4-D. In addition , Zaid (1987) found that MS medium fortified with 100 mg/l 2,4-D and 3 mg/l 2iP promoted calli initiation from embryos of date palm during 4-8 weeks.

Regarding the effect of culture conditions on callus formation, the incubation under dark condition showed the best results compared the incubation under light condition. In contrast, the growth value and growth rates were higher than in case of incubation under light conditions. These results are in harmony with data have been published by Tisserat (1981), Zaid (1987), Bawa (1993) and Veramendi and Navarro (1996).

Analysis of the non-polar extract of in vitro obtained calli revealed that the yield of the lipoidal matter of calli incubated under light and dark conditions was 0.64 and 0.69 (%), respectively, which was significant compared to that of whole fruits (0.85%). While, the total percentage of the fatty acids in the lipoidal matter of calli incubated under light (70.8 %) or dark (74.4 %) conditions, it was 32.2 (%) in whole fruits. Moreover, oleic acid (which may be responsible for the antiandrogenic activity) was present in the lipoidal matter of the obtained calli in a reasonable concentration. It present in higher concentration in the fatty acid mixture of calli incubated under dark condition compared with incubated calli under light condition. Further, it can be conclude that, the exploitation of tissue culture techniques for the preparation of the biologically active non-polar extract of S. yapa fruits proved to be a successful alternative for the extraction of the whole fruit with the advantages of overcoming the difficulties of obtaining the source plants.

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The Role of Natural Antioxidants and Snacks on the Weanling Rats Health

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Abstract: The effect of processed snacks, commercial snacks and commercial snacks + orange juice on the lipid profile, haematogenic characteristics, liver and kidney functions, glucose and malonlialdehyde (MDA) in weanling rats were evaluated. Weight gain and histological examination of liver and kidney tissues were also evaluated. The results showed that there were no significant (P>0.05) in hemoglobin, haematocrit, red blood cell, glucose and creatinine between control and other snacks groups. Rats fed commercial snacks resulted in a significant (P \leq 0.05) increase in total cholesterol, triglyceride, low density lipoprotein (LDL), very low lipoprotein (VLDL), alanine amino transferase (ALT), aspartate amino transferase (AST), urea, creatinine and malonlialdehyde compared to rats fed basal diet. However, supplementation commercial snacks diet with orange juice as a source of antioxidant resulted in significant (P \leq 0.05) decrease in the previous parameters and improves the liver and kidney tissues as compared with commercial snacks diet.

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Keywords: Snacks foods, hemoglobin, lipid profile, liver function and malonlialdehyde.

1. Introduction:

We live in society where it is easy and convenient to eat unhealthy food and difficult to eat healthy food (1). Any food that has poor nutritional value, lack in micronutrients such as vitamins. minerals, amino acids, fibers and high content level of calories is considered unhealthy and may be called a junk food such as some snacks and breakfast cereals. Snacks and breakfast cereals are essentially produced from starchy substances such as corn, rice, wheat (2). Snacks food is easy to carry, purchase and consume. Junk food is given a very attractive appearance by adding food ingredients. The major role of these ingredients is to give structure, texture, mouth feel, bulk, and many other characteristics desired for specific finished products (3 and 4). Fast food consumption has been associated with higher total energy intake and higher intake of fat, saturated fat, carbohydrates, sugar, and carbonated soft drinks and lower intake of micronutrients and fruit and vegetables (5-11). In addition to the high consumption of fast foods contributes to passive overeating, weight gain and obesity in humans (12.13.14). Obesity is a major risk factor for many chronic conditions, including hypertension. cardiovascular disease, type-2 diabetes, as well as certain types of cancer (15). An increased consumption of fruit and vegetables has been associated with beneficial effects on the risk of disease (16,17) The beneficial effect could be related to minor components, especially flavonoids, which are proposed to exert their action by inhibiting LDL oxidation(18) and vitamins C and E and betacarotene, which are thought to act mainly as antioxidants (19). Citrus juices, especially orange juice and grapefruit juice, are rich sources of flavonoids, folate, and vitamin C (20). Commercial fresh orange juice has high content of vitamin C (54mg/100 ml juice), flavonoids (10.7 mg²/100 ml juice) and carotenoids (423.9 μ g²/100 ml juice) (21). In this study snack form corn flour was produced. The processed snacks, commercial snacks and commercial snacks + orange juice were evaluated in rats to ascertain effects on lipid profile, haematogenic characteristics, liver and kidney functions, glucose, malonlialdehyde, weight gain and histological examination of liver and kidney tissues.

2. Materials and methods

Commercial karate (jellio) was purchased from El-Gawhara Company for Food Industries. Corn flour was purchased from National Company for Corn Products, El-Asher min Ramadan, Egypt. Orange was purchased from the local market, Shibin El-Kom, Egypt.

2.1 Preparation of processed snacks

Corn flour was used to prepare the corn based snacks by using twin screw extruder (Wenger TX52) with the following extrusion information: Feed screw speed 13 rpm, preconditioner speed, 150rpm; extruder shaft speed, 340 rpm; Head temperature, 133°C; Head pressure, 1600 psi. The products were sprayed using corn oil (15%) on the extruded product after it was dried at 125 °C for 4-5 min.

2.2 Experimental Design

Twenty four weanling male albino rats, Sprague drawly strain, weighing $45-50 \pm 5$ g were purchased from Helwan farm. The rats were housed individually in cages and fed basal diet for one week for adaptation. The basal diet consisted of 100 g/kg corn oil; 140 g/kg casein; 40 g/kg mineral mixture, USP XIV; 10 g/kg vitamin mixture; 3 g/kg DLmethionine and 2 g/kg choline chloride and 50 g/kg fiber and corn starch 505 g/kg (22). The rats were randomly divided into four groups, 6 rats per group. Control group fed basal diet, the other three groups were fed processed snacks, commercial snacks and commercial snacks + 2 ml dally of orange juice (15%) of snacks was incorporated into the basal diet at the expense of corn starch content). Body weight was recorded at the beginning and at the end of experimental period. At the end of experimental period (8 weeks), the rats fasted overnight and anaesthetized. Blood sample were collected and aliquots were analyzed to measure the hematological parameters. The remaining blood was centrifuged to obtain serum for determination serum glucose, serum lipid profile (total cholesterol, triglyceride and LDL, HDL, VLDL), kidney functions (urea and creatinine), liver functions (ALT and AST) and malonlialdehyde (MDA).

2.3 Analytical methods:

Total nitrogen content, fat, moisture, and ash were determined according to (23). The carbohydrate was calculated by difference. Serum glucose was estimated according to Rojas et al., (1999). Alanine amino transferase (ALT), aspartate amino transferase (AST) and malonlialdehvde (MDA) were assaved by the methods of (25,26) respectively. Hemoglobin (Hb) red blood cell (RBC) and haematocrit (Ht) in heparinized blood samples were measured using automated hematology analyzer (Sysmex, Kobe, Japan). Urea and creatinine levels were determined according to the method described by (27). Serum total cholesterol, triglyceride (TG) and high density lipoprotein (HDL) were determined by using methods of (28,29,30) respectively. The determination of low density lipoprotein (LDL), very low density lipoprotein (VLDL) and mean corpuscular volume (MCV) were carried out according to the methods of (31) as follows:

TG $VLDL = \frac{5}{5}$ LDL = Total cholesterol - (HDL + VLDL) HT $MCV = \frac{1}{RBC}$ $X \ 10$

2.4 Histopathology examinations

Small specimens of the organs liver and kidney were taken from each experimental group, fixed in neutral buffered formalin, dehydrated in ascending concentration of ethanol (70, 80 and 90%), cleared in zylene and embedded in paraffin. Sections of 4–6 μ m thickness were prepared and stained with hematoxylin and eosin according to (32).

2.5 Statistical Analysis

The experimental data were subjected to an analysis of variance (ANOVA) for a completely randomized design using a statistical analysis system (33). Duncan's multiple range tests were used to determine the differences among means at the level of 95%

3. 3. Results and Discussion

Table (1) showed the proximate chemical composition of commercial and processed snacks. There were significant (P \leq 0.05) differences in fat and carbohydrate between commercial and processed snacks. While no significant (p>0.05) differences were observed among protein, moisture and ash. The commercial snacks had higher content of fat (38.16%) than the processed snacks (15.57%). These results are in agreement with reported by (34) who found that the chemical composition of commercial snacks were 8.8, 35.56, 3.28 and 47.78% for protein, fat, moisture and carbohydrate respectively.

Body weight gain of rats fed basal diet and snacks diets were presented in Table (2). Rats fed commercial snacks diet had the highest ($P \le 0.05$) final weight as compared to rats fed basal diet, processed snacks diet and commercial snacks diet + orange juice. This effect may be due to the high content of fat in commercial snacks diet. There was no significantly (P>0.05) difference in weight gain between rats fed basal diet and rats fed processed snacks. Rats fed commercial snacks diet and commercial snacks diet + orange juice had higher $(P \le 0.05)$ weight gain than rat fed processed snacks. These results are in agreement with those reported by (6,35,10) they found that there were significant associations between snacks food consumption and increased BMI, increased body weight and a higher probability of being overweight.

Serum lipids profile of rats fed basal and snacks diets were shown in Table (3). There were no significant (P>0.05) differences in cholesterol, high density lipoprotein (HDL) and low density lipoprotein (LDL) between rats fed basal diet and those fed processed snacks. However, cholesterol and LDL were significantly (P \leq 0.05) increased in rats fed commercial snacks and commercial snacks + orange juice as compared with rats fed basal and processed snacks diets. Rats fed processed snacks, commercial snacks and commercial snacks + orange juice had higher (P \leq 0.05) triglyceride than those fed basal diet.

Cholesterol, triglyceride, LDL and VLDLc were reduced ($P \le 0.05$) by supplementation commercial snacks diet with orange juice as source of natural antioxidant. These results are in agreement with those reported by (36) they found that snacks food consumption increases concentration of cholesterol, triglycerides and lipoproteins of low and very low density. The serum LDL and cholesterol decreased by 43% and 32%, respectively after supplementation of hypercholesterolemia rabbits diets with orange juice, (37).

Data presented in Table (4) showed the hemoglobin (Hb), haematocrit (Ht), red blood cell (RBC) and mean corpuscular volume (MCV) of rats fed basal and snacks diets. There were no significant (P>0.05) differences in hemoglobin, haematocrit, red blood cell and mean corpuscular volume in rats fed basal diet and rats fed all type of snacks diets except mean corpuscular volume in rats fed commercial snacks diet which significant (P \leq 0.05) decrease. Similar results were reported by (34) who found that there was no significant difference in Hb, Ht, RBc and MCV in children after had commercial snacks and commercial snacks + lemon juice for two months.

Liver and kidney functions of rat fed basal and snack diets were shown in Table (5). Rats fed commercial snacks and commercial snacks + orange juice diets had a higher (P≤0.05) serum aspirate amino transfrease (AST) activity than those fed basal diet, processed snacks diets. However, rats fed commercial snacks diet had a higher (P<0.05) serum alanine amino transferase (ALT) activity than those fed basal diet, processed snacks diet and commercial snacks + orange juice diet . There were no significant (P>0.05) differences in ALT and AST enzymes between rats fed basal diet and those fed processed snacks diet. Also, there were no significant (P>0.05) differences in ALT enzyme among rats fed basal diet, processed snacks diet and commercial snacks + orange juice diet. These results are in agreement with (38) who reported that after consumption three meals a day of junk food restaurant for 1 month liver ALT enzyme peaked at 290 u/l from baseline value 20 u/l.

On the other hand, there was no significantly (P> 0.05) difference in creatinine between rats fed basal and all snacks diets. Rats fed commercial snacks diet had a higher (P \leq 0.05) urea than those fed

basal diet, processed snacks diet and commercial snacks diet + orange juice. Supplementation of commercial snacks diet with orange juice resulted in a significant (P \leq 0.05) decrease in ALT enzyme and urea however, AST enzyme and creatinine was not affected.

Malonlialdehyde (MDA) and glucose of rats fed basal and snacks diets were presented in Table (6). There was no significant (P > 0.05) difference in glucose between rats fed basal diet and other snacks diets. There was no significant (P> 0.05) difference in malonlialdehyde between rats fed basal diet and processed snacks diet. However, malonlialdehyde was affected (P≤0.05) by commercial snacks diet and commercial snacks diet + orange juice. Rats fed commercial snacks diet and commercial snacks diet + orange juice had a higher value of malonlialdehyde than those fed basal diet and processed snacks diet. Supplementation of commercial snacks diet with orange juice resulted in a significant ($P \le 0.05$) reduction in malonlialdehyde concentration. Adequate dietary antioxidant supplementation may be effective in lowering oxidative stress (39). Similar results were reported by (40) who found that ascorbic acid significantly decrease the adverse effect of reactive species such as reactive oxygen and nitrogen species that can cause oxidative damage to macro molecules such as lipids, DNA and proteins which are implicated in chronic diseases.

Figure (1) showed the histological examination of liver tissues of rats fed basal and snacks diets. The liver tissues of rats fed basal diet, processed snacks diet and commercial snacks diet + orange juice showed no histopathological change. However, liver tissue of rats fed commercial snacks diet showed activation of epithelial lining bile duct, portal infiltration with mononuclear leucocytic cells. As well as necrosis and atrophy of some hepatocytes were found.

Figure (2) showed the histological examination of kidney tissues of rats fed basal and snacks diets. The kidney tissues of rats fed basal diet, processed snacks diet and commercial snacks diet + orange juice revealed the normal histology of renal parenchyma. While, kidney tissues of rats fed commercial snacks diet showed granularity of epithelial lining renal tubules, presence of eosinophilic proteinaceous cast in the lumen of some renal tubules associated with atrophy of glomerulus tuft.

	Protein	Moisture	Fat	Carbohydrate	Ash
	(%)	(%)	(%)	(%)	(%)
Processed snack	$8.53^{a} \pm 0.15$	3.73 ^a ±0 .21	$15.57^{b} \pm 0.3$	$68.96^{a} \pm .45$	3.53 ^a ± 0.15
Commercial snack	8.37 ^a ± .15	$4.13^{a} \pm 0.15$	38.16 ^a ± 1.25	46.2 ^b ± 1.25	$3.13^{a} \pm 0.21$
LSD	0.35	0.41	2.1	2.1	0.41

Table1: Proximate chemical composition of processed and commercial snacks.

Means in the same column for each variable with different letters are significantly different ($p \le 0.05$)

Table 2: Body weight gain of rats fed basal and snacks diets.

Groups		Snacks groups			
variables	Control	Processed Snacks	Commercial Snacks	Commercial snacks $+ O J^1$	LSD
Initial weight (g)	46 ^a <u>+</u> 1.7	47.3 ^a <u>+</u> 1.5	47.67 ^a <u>+</u> 2.5	47 ^a <u>+</u> 2.6	4
Final weight (g)	$98^{d} + 12$	104 ° <u>+</u> 2.6	119.67 ^a <u>+</u> 4.9	112.67 ^b <u>+</u> 4.9	5.5
Weight gain (%)	52.39 ^b <u>+</u> 1.57	54.5 ^b <u>+</u> 0.64	60.2 ^a <u>+</u> 1.26	58.3 ^a <u>+</u> 0.51	2.8

¹ orange juice

Means in the same row for each variable with different letters are significantly different ($p \le 0.05$)

Table 3: Serum lipid profile of rats fed basal and snacks diets.

Groups		Snacks groups			
	control	Processed	Commercial	Commercial	LSD
variables		snacks	snacks	snacks + O J^1	
Cholesterol (mg/dl)	$102.99^{\circ} \pm 1.3$	104.33 ^c \pm 1.5	$124.17^{a} \pm 1.3$	121 ^b ± 2	2.9
Triglyceride (mg/dl)	105.13 ^c \pm 1.8	$110^{b} \pm 3$	$119.83^{a} \pm 1.3$	$112.7^{b} \pm 2.5$	4.23
HDL (mg/dl)	$41.53^{a} \pm 1.6$	$41.1^{a} \pm 0.92$	32.63 ± 2.5	$37.4^{b} \pm 1.4$	3.18
LDL (mg/dl)	$40.9^{\circ} \pm 1.1$	$40.9^{\circ} \pm 1.5$	$67.6^{a} \pm 2.1$	$61.1^{b} \pm 0.3$	2.65
VLDL (mg/dl)	$21.0^{\circ} \pm 0.36$	$22.3^{b} \pm 0.83$	$23.96^{a} \pm 0.25$	$22.53^{b} \pm 0.5$	1.0
1					

¹ orange juice

Means in the same row for each variable with different letters are significantly different ($p \le 0.05$)

Table 4: Hemoglobin, haematocrit, red blood cell and mean corpuscular volume of rats fed basal and snacks diets.

Groups			Snacks groups		
	control	Processed	Commercial	Commercial	LSD
Variables		Snacks	Snacks	snacks + O J^1	
Hemoglobin (g/dl)	$13.1^{a} \pm 0.6$	13.3 ^a ±0.5	$13.5^{a} \pm 0.5$	13.5 ^a ±0.3	0.92
Haematocrit (%)	$47^{a} \pm 2$	$50^{a} \pm 2$	$50.7^{a} \pm 06$	$52^{a} \pm 3$	3.92
Red blood cell (mil/cmm)	$6.5^{a} \pm 0.3$	7 ^a ±0.2	$7.3^{a} \pm 0.2$	$7.2^{a} \pm 0.3$	0.48
Mean corpuscular volume (fl)	$72.32^{a} \pm 0.3$	$71.4^{a} \pm 0.82$	$69.9^{b} \pm 0.54$	$72.2^{a} \pm 1.6$	1.45

¹ orange juice

Means in the same row for each variable with different letters are significantly different ($p \le 0.05$)

Table 5: Liver and kidney functions of rats fed basal and snacks diets.

Groups		Snacks groups			
	control	Processed	Commercial	Commercial	LSD
Variables		snacks	snacks	snacks + O J^1	
ALT (u/l)	95 ^b ± 2	95.33 ^b ± 3.5	$122^{a} \pm 2.6$	$98.33^{b} \pm 1.52$	4.8
AST (u/l)	44.73 ^b ± 1.3	43.77 ^b ± 1.5	59.67 ^a ± 3	$45.63^{a} \pm 0.98$	3.6
Urea (mg/dl)	$19.03^{b} \pm 0.42$	$19.43^{b} \pm 0.75$	$25.16^{a} \pm 0.76$	$20.16^{b} \pm 0.76$	1.3
Creatinine (mg/dl)	$0.73^{a} + 0.03$	$0.72^{a} + 0.02$	$0.77^{a} + 0.03$	$0.75^{a} + 0.02$	0.04

Means in the same row for each variable with different letters are significantly different ($p \le 0.05$)

Groups		Snacks groups			
variables	control	Processed snacks	Commercial snacks	Commercial snacks + $O J^1$	LSD
MDA ² (n mol/ml)	$6.67^{\circ} \pm 0.42$	$7.3^{\circ} \pm 0.1$	$13.47^{a} \pm 0.55$	$10.57^{\rm b} \pm 0.3$	0.72
Glucose (mg/ dl)	110.67 ^a ± 3.96	$112.33^{a} \pm 4.16$	$117.33^{a} \pm 2.5$	$113.67^{a} \pm 3.2$	6.67

Table 6: Malonlialdehyde and glucose in rats fed basal and snacks diets.

¹ orange juice, ²Malonlialdehyde

Means in the same row for each variable with different letters are significantly different ($p \le 0.05$)



Rats fed basal diet



Rats fed commercial snacks diet



Rats fed processed snacks diet



Rats fed commercial snacks diet + O J

Fig (1). Histological examination of liver tissues of weanling rats



Rats fed basal diet





Rats fed processed snacks diet



Ratsfed commercial snacks dietRatsfed commercial snacks diet + O JFig(2). Histological examination of kidney tissues of weanling rats

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Devising an instrument to assess human resources productivity in an Iranian context

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ABSTRACT: There are various factors affecting human resources productivity. Moreover, it is a necessity to identify the most important and efficient ones. Therefore, there should be an appropriate instrument to determine the factors in the most comprehensive way. Accordingly, this study has employed Factor Analysis to devise a locally-based instrument to assess human resources productivity in Guilan University of Medical sciences. To achieve the mentioned goal, two stages have been taken during the fall of 2009. One was qualitative and the other was cross-sectional. In the former step 45 expert managers were included as the sample of the research to determine productivity factors and in the latter 321 staff members of scientific society, training and human resources departments of Guilan University were selected to establish the productivity variables. To enclose, One questionnaire with 5 headings and 42 questions has been obtained as follows: Organizational culture with 18 questions / Environmental conditions with 7 questions. The invented device, regarding to it reliability, validity, relevance and indigenousness in assessing of human resource productivity, could be useful for all the universities of medical science. Using of this device could improve the effectiveness of educational activities which are performed for the faculty members and experts of education.

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Key words: human resources productivity, Factor Analysis, locally-based instrument, productivity factors, productivity variables

INTRODUCTION

Productivity issues have attracted increasing interest amongst researchers during the last decade (Sahay, 2005, Parasuaman, 2002) in today's world; one of the main issues that assure consistency for organization is productivity. Implementing culture of productivity will lead organizations to make the best use of human and material resources and in doing so, competencies and potentials of organization will flourish. (Soltani, 2007). Manpower is the most important factor to improve productivity (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). Also manpower could be considered the most valuable asset and resource for organizations. Organizations that located manpower on top of their list of agenda as prime importance factor succeeded to a desirable level (Abdolahi and Navehebrahim; 1999). Identifying influenced factors in order to upgrade manpower productivity is the main objective of many researchers. Moreover, 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, because of 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 together with the countries in the east of Asia.(Taheri, 2007). Therefore, proposing a strategy that could result in improving manpower productivity, systematic methods are required for measurement and evaluation (Soltani, 2007). One of the main factors for manpower productivity evaluation and assessment is to employ

suitable tools (Sarmad and bazargan and hejazi, 1997). Additionally, the tools are expected to be reliable and valid (Tabibi, Maleki, Delgoshaee, 2009).

According to what has been mentioned, this study concerns devising an instrument by which manpower productivity may be assessed. In other words, present study has employed exploratory and confirmatory factor analyses, considered as suitable methods for measuring instrument validity and reliability, to assess the reliability and validity of the researchers' composed questionnaire. It is worth mentioning that in the composed questionnaire almost all characteristics of research situation such as cultural, social and local aspects are expected to be involved. The researchers hope that all the results achieved in this study will provide sufficient local and native tools for Iranian researchers to facilitate their tasks in assessing human resources productivity.

Review of the related literature:

Alvani and Ahmadi (2001) tried to devise an instrument in assessing manpower productivity by establishing an expert panel from which the proposed comments were regarded as a basis to set up the content validity of the instrument.

Gigans and Oerman(2001), Gibons,Adaam and Padden (2002), Zimeren andWestfall (1998) made an effort to achieve the reliability of their self-developed instrument through a group of experts.

Amini, Vanaki and Emamzadehghasemi (2005) made use of a group of specialists and experts to obtain the reliability and validity of an assessment instrument for nursing management practical learning. That is to say, the researchers achieved the face and content validity of the devised instrument by experts' confirmation and the reliability of the mentioned instrument was accomplished by internal reliability.

Song, Joo, and Chermack (2009) made an attempt to accomplish a validation study for learning organization questionnaire in Korean context.

To recapitulate, it is necessary to mention that there are lots of studies conducted on instrument devising in Iran and other countries, but these studies do not concentrate on devising an instrument assessment for human resources productivity in Universities and Higher education departments of Iran. Moreover, in the present study, confirmatory and exploratory factor analyses were employed to achieve the reliability of the devised instrument.

MATERIAL AND METHODS

Descriptive, analytical and cross-sectional studies were carried out during three months of September, October and November of 2009 in two stages (crosssectional and qualitative). At quality stage, 45 experts in manpower productivity were included in the research sample population to determine manpower productivity dimensions. Data collection instruments at qualitative stage were interview and questionnaire. Issues that appear through research include empowerment of staff, method of leadership, organizational support, clarifying and documenting services, staff intention and motivation, Likert scale was used (Andaleeb,2004), completely agree (5), agree (4), no comment (3), disagree 2) and completely disagree (1). 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 common 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 lead to a total agreement.

At cross-sectional stage, the research samples were consisted of education department employees and faculty members of medical sciences university's such as dentistry, health care, nursing, midwifery, medical laboratories and international school of medical sciences in Guilan. Data collection mechanism at this stage was a questionnaire consisted of two sections. Section one comprises 8 questions regarding personal information such as sex, age, marital status, employment status, work experience, level of education, management experience and scientific group membership. Section two contains 42 questions on the subject of manpower productivity variables. As before for each question, Likert scale has been used for scoring as follows: 5 indicates very much, 4 indicates a lot, 3 indicates average, 2 indicates little and 1 indicates very little. The validity of manpower assessment instrument has been performed by library research and item analysis. Furthermore, the content validity has been obtained by an expert panel of manpower productivity. To put it into a simple language, the 12 specialists' comments were considered as a basis to modify some parts of the questionnaire. To determine construct validity, to identify the most effective components on manpower productivity and to recognize the loading level of each component on the main components, exploratory factor analysis with Varimax rotation method has been accomplished. Additionally, Kaiser_ Mayer_ Olkin (KMO) has been used for volume sufficiency (Dixon, 2001). To attain the data suitability, Bartlet test was employed.

To assess the reliability of the questionnaire, a test retest technique was carried out.

To verify internal consistency, Alpha Cronbach method has been used. The questionnaire distribution of cross-sectional stage was done during September November. After explaining necessary information about the research objective of the questionnaires to 347 people, 321 out of those completed the questionnaire accordingly.

RESULTS

A- QUALITATIVE RESULTS

1) After revising related studies conducted both in Iran and overseas, a primary model for manpower productivity was designed by the researchers. This model has been consisted of six components (factors) such as staff empowerment, method of leadership, organizational support, clarifying giving service, employees' tendency and motivation and validity of decisions.

2) The proposed primary model was given to the experts in order to obtain their comments and views (N = 45). The approved factors were rated as follows:

- Staff empowerment = 100 %
- leadership method = 100 %
- Organizational support = 91.1 %
- Clarifying giving servicing = 82.2 %
- Employees' tendency and motivation = 97.7 %
- Creditability of decisions = 86.7 %

3) At the previous stage some of the experts believed other factors like organizational culture, environmental condition, organizational structure, innovation and creativity also to be discussed in order to upgrade manpower productivity efficiency .Therefore, the following issues also went through rating process by experts and the following compromised results revealed as:

- Organizational culture = 91.1 %
- Organizational structure = 86.7 %
- Innovation and creativity = 73. 3 %
- Environmental condition = 71.1 %

4) At the end of the given stages, 10 components were confirmed by the experts.

B- CROSS SECTIONAL RESULTS

The model devised from the mentioned stages(the qualitative stage) and the researchers' proposed questionnaire consisting of 8 questions in relation to personal and demographic characteristics and 42 questions in connection to efficient manpower productivity variables(the variables were selected based on the literature review) were submitted to 347. Out of those 347 persons from scientific and training group members, medical university experts of human resources, dentistry , nursing , health care , medical laboratories and international unit, 321 persons completed and returned these questionnaires. After analysis, the following results in two sections obtained.

1) PERSONAL AND DEMOGRAPHIC CHARACTRISTICS RESULTS

1-1) AGE: 58. 6 % of the sample population was between 40 - 49 years of age. Minimum age was 28

years, maximum was 68 years and average age found to be 43. 87 \pm 7.10 years.

1-2) **Sex**: 62. 9 % (202 persons) of the sample population was male and 37. 1 % (119 persons) was female.

1-3) **marital status**: 88. 2 % (283 persons) was married and 11. 8 % (38 persons) was single.

1-4) **Employment status**: 58.9 % (189 persons) was official and the rest were non-official employees

1-5) **Work experiences:** 11.8 % (38 persons) had less than five years of experience, 20.6 % (66 persons) had 5 - 10 years of experience, 48. 6 % (156 persons) had 11- 20 years of experience and 19% (61 persons) had more than 20 years of work experiences.

1-6) **Level of education**: 35. 8 % (115 persons) were experts and at PhD level, 22. 1 % (71 persons) was educated up to post-PhD degree, 19 % (61 persons) had B. Sc. degree, 15. 3 % (44 persons) was educated up to M. Sc. degree, 2. 5 % (8 persons) was general physicians (GP), 3. 7 % (12 persons) had above-diploma degree and 1. 6 % (5 persons) had diploma.

1-7) **scientific group members**: 62 % (199 persons) was the faculty members and 38 % was not.

1-8) **Managerial work experiences**: 49. 2 % (158 persons) who carried out related researches had management experiences and the rest didn't.

2) EXPLORATORY FACTOR ANALYSIS RESULTS

Reliability of this questionnaire calculated by testretest method was 0.98 and internal consistency was 0.89 using Alpha Cronbach method. Content validity conducted by expert panel. 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 (Dixon, 2001). 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.

In exploratory analysis the 5 main issues with 42 questions were identified and they are as follows in the order of importance:

1. Organizational culture with 18 questions; 29.26 % variance and Eigen value of 21. 62

2. Environmental conditions with 7 questions, 12. 96 variance and 2. 63 Eigen value.

3. Motivation factors with 10 variable, 12. 84 % variance and 1. 58 Eigen value

4) Empowerment with 4 variables, 7. 47 % Variance and 1.34 Eigen value.

5) Leadership method with 5 questions, 5.05% variance and 1.21 variance.

It is necessary to mention that these 5 issues with 67. 60 % variance could explain manpower productivity changes (Tables 1).

ROW	MAIN TOPICS	VARIABLES (OUESTIONS)	VARIANCE (%)	SPECIAL(ADDED) VALUE
1	ORGANIZATIONAL CULTURE	18	29.265 %	21.622
2	ENVIROMENTAL CONDITIONS	7	12.961 %	2.634
3	MOTIVATION FACTORS	10	12.849 %	1.584
4	EMPOWERMENT	4	7.47 %	1.341
5	LEADERSHIP METHOD	3	5.05 %	1.211
6	TOTAL	42	67.595 %	28.392

Table 1. Identifying 5 main issues using exploratory factor analysis

To achieve the purpose of identifying the loading degree of each component based on their main components, exploratory Factor analysis with Varimax rotation (Table 2)

Table 2. Main issues matrix after	Varimax rotary with loading	; level from all variables (Questions)
		/ .	

RO W	VARIABLES	ORGANIZATIONAL CULTURE	ENVIROMENTAL CONDITIONS	MOTIVATION FACTORS	EMPOWER MENT	LEADERSHIP METHOD
32	Supporting new ideas by managers	0.823				
29	Financial and spiritual support for new ideas	0.807				
۳١	Allocating budget for innovation	0.795				
38	Award and recognition for doing difficult tasks	0.790				
28	Suitable working environment	0.765				
33	Benchmarking-using innovation & creatability of other organizations	0.760				
37	Supporting staff by managers at difficult situations	0.758				
39	Promotion of capable staff for other positions	0.758				
30	Innovation by staff	0.735				
34	Creativity training courses for staff	0.720				
36	Delegation by managers	0.710				
40	Permission of decision making by staff from managers	0.702				
27	Using updated technology and technical knowledge	0.701				
26	Safety and health care in working environment	0.687				
24	Promoting staff to higher positions	0.637				

r						
	(Capable staff)					
35	Documenting clear job	0.635				
	descriptions					
25	relation manual and	0 227				
25	code of conduct	0.327				
	Award and recognition					
16	of staff in group	0.582				
10	gathering	0.002				
	Human resources					
12	forecasting in relation to		0.680			
	different tasks					
	Equipment and tools					
13	forecasting in relation to		0.657			
	different tasks					
	Exact definition of					
8	responsibilities and		0.636			
	authorities					
7	Systematic activities		0.611			
_	Having related					
5	knowledge to carry out		0.598			
	tasks(job)					
11	Suitable equipment and		0.552			
11	actifices for customer		0.555			
	Knowledge and					
	education in					
1	correspondence with		0.451			
	related job					
	Time boundaries to do			0.504		
21	particular tasks			0.694		
	Written or verbal					
18	warnings on basis of			0.692		
	customer complaints					
20	Title register and names			0.691		
14	Recommendation and			0.670		
14	complaint box			0.070		
17	Customer evaluation			0.647		
	appraisal for staff					
15	Job holder participation			0.538		
	in decision making					
19	clarifying different kind			0.504		
	Desolving stoff					
22	complaints by			0.502		
22	management			0.502		
	Departments inspection					
23	and providing reports			0.450		
	Maintenance and					
	commissioning of					
10	internet site and answer			0.437		
	call system					
4	Holding courses and				0.701	
4	seminars				0.781	
2	Holding training courses				0.721	
3	for managers				0.721	
2	Holding training courses				0.690	

	for staff			
6	Establishing quality		0.442	
0	improvement committee		0.445	
42	Exact staff response to			0.000
42	rules and regulation			0.098
41	Exact manager response			0.(10
41	to rules and regulation			0.019
	Emphasis on carrying			
9	out work instructions			0.474
	and procedures			0.474
	accordingly			

3) CONFIRMATORY FACTOR ANALYSIS

In order to confirm and fit obtained issues in exploratory factor analysis and the loaded variables described under each issue (fig. 1), LISREL 8.80 was used (Schumacher, 2004).



Figure 1. "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, therefore the model is approved accordingly (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 was (NFI) was equal to 0.96

7. Relative fit index (RFI) was equal to 0.96

DISCUSSION AND CONCLUSIONS

The present study concerns devising an assessment instrument for manpower productivity. One of the most important steps for this purpose is validity and reliability. As far as it is considered, one of the most common methods for finding validity is gaining ideas from a group of experts (Gibbons, 2002). Accordingly in this research, the content validity was carried out by the help of management and productivity specialists.

Construct validity was confirmed using exploratory and confirmatory factor analysis.

Even though all components and questions of the questionnaire were the experts' comments and previous researchers' views, but factor analysis has been employed for the following reasons:

1. regarding the fact that questionnaires are affected by cultural and social features

2. determining the different important factors in composing questionnaires content

3. simplifying the extracted factors

Studies that carried out by kline indicated that factor analysis is often suitable for defining content layout (Kline, 1986). Comparing factor analysis results in qualitative research stage proved that 10 components confirmed by experts as the most efficient ones for manpower productivity. In contrast, in exploratory and confirmatory factor analysis 5 components including organizational culture with 18 questions, motivation factors with 10 questions, empowerment with 4 questions and leadership method with 3 questions listed as the most resourceful ones. It is also needed to state that 5 issues were eliminated because of having a factor load of less than 0.5 or overlapping with other factors. Correspondingly, Organizational culture was identified as the prime importance to upgrade manpower productivity. Similarly, schermerhorn studies specified that organizational culture may influence the whole

grounds of organization (1999). Also in exploratory factor analysis, the following variables (questions) such as enthusiastically accepting new ideas and keenly supporting those who proposed the ideas, supplying financial and non-financial support for staff creativity and innovation and at last allocating budget for the creativity advised from sub-divisions of organizational culture were regarded respectively as the most important factors to promote manpower productivity. In conjunction to this matter, Robbins believed 7 issues are the main criteria for organizational culture. He also believes creativity and innovation are the most central entities for organizational culture (Robbins, 1998). Alvani and Ahmadi carried out research with the title of total productivity management model and considering qualitative result, they came up with 8 issues including motivation factors, leadership method, and competitiveness, physical and spiritual status, on the job and off the job training. These 8 issues considered being main issues and other 47 issues called organizational sub-groups (Alvani&Ahmadi, 2001).Some of the issues that carried out in both researches could be visualized like leadership method and motivation factor. Also in total productivity management model, researches by revising different models and using personal management experiences, may seem like some important issues were not mentioned, like cultural values, training and environment.

In this study, research environments were medical universities, training and medical training contents, research society included scientific group, trainers and manpower. This research carried out at 2 stages considering cultural values, social and training.

One of the important actions in tool making process is to obtain creditability of tools.

Burns says that reliability with ratio of one indicates a complete reliability and zero ratios shows that there is no reliability. Also he says that 0.7 ratio reliability is a suitable tool (Burns and Groves, 2003).

This research made use of test re-test reliability of tools at a high level of 0.98 what is more the designed instrument in this study could also be used for the development of manpower productivity in medical sciences universities, medical training centers and governmental hospital all over the country.

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Production and application of Spirulina platensis rich in fatty acids, and vitamins

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Abstract: *Spirulina platensis* is a microscopic blue-green <u>alga</u> in the shape of a spiral coil, living both in sea and fresh water .It is widely used as health food due to its protein content, vitamins and active substances for immune system. Polyunsaturated fatty acids amount to 46.548 %(w/w) of total lipids.Among the essential fatty acids detected in El Khadra lake water body in Waadi El Natroun micro- alga, cholesterol decreasing -linolenic acid with 0.986%(w/w) .Vitamin A amounts to 120.13 µg/100g, vitamin C amounts to 540.34 µg/100g and vitamin D amounts to 105.6 µg/100g were found . Vivo studies revealed *Spirulina* effectiveness on Triglycerides(TG) ,Total cholesterol(TC),High density lipoprotein-chloestero(HDL-ch), body weight ,serum calcium., serum iron, and serum ferritin after treatment of the experimental rabbits for 30 days.

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Keywords: *Spirulina platensis*, -linolenic acid, vitamins, hypercholesterolemia, serum calcium, prothrombin., serum iron, serum ferritin.

1. Introduction:

Spirulina is a natural health food as a blue-green algae. It contains beneficial nutrients that are readily digested and absorbed by the body, so none of its nutritional benefits are lost. It is excellent in combating imbalances arising from lifestyle habits and it is effective in overcoming and preventing various disorders arising from a poorly balanced diet, including insufficient intake of vegetables as it supplies several of the vitamins that all living beings need to carry on processes or prevent some serious metabolic diseases ;Cingi et. al .(2008): This cyanobacterium is important for its content of polyunsaturated fatty acids as it is frequently rich in gamma-linolenic acid (GLA), and also provides alpha-linolenic acid (ALA), linoleic acid (LA), stearidonic acid (SDA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (AA(;Babadzhanov et. al.(2004), betacarotene and other antioxidants pigments;Ciferri (1983) ; Gemma et. al. (2002); anti-virals sulphated polysaccharides; Noaman et. al. (2004), antimicrobials sterols. Spirulina contains a number of vitamins: B1, B2, B3 B6 ,B9, vitamin C, vitamin D, vitamin E; Babadzhanov, et. al. (2004) and B12, Watanabe et. al. (2002).

Spirulina is accepted as functional foods, which are defined as products derived from natural sources,

whose consumption is likely to benefit human health and enhance positive medical properties These foods are used as a supplement/ingredient or as a complete food to enhance the performance and state of the human body, or improve a specific bodily function. It is being widely studied for its possible antioxidant, antibacterial, and antiparasitic properties, and for several medical conditions such as allergies; Mao *et. al.* (2005) ulcers, anemia, heavy-metal poisoning, Barmejo-Bescós *et. al.* (2008), and radiation poisoning, Misbahuddin *et. al.*(2006); Raj *et. al.* (2008). *Spirulina* or its extracts can prevent or inhibit cancer in humans and animals and has other medical effects; Wang *et. al.* (2005)

Functional foods are used mainly as products to nourish the human body after physical exertion or as a preventive measure against ailments. *Spirulina* contains unusually high levels of gamma-linolenic acid,(GLA),an essential polyunsaturated fatty acid; <u>Otle and Pire</u>.(2001); Babadzhanov, *et. al.*(2004). The aim of this study is to evaluate fatty acids and vitamins contents of *S. platensis* isolated from Waadi El Natroun and studying its effect on cholesterol TG,TC ,HDL levels , body weight , and serum calcium ,serum iron and serum ferritin in the blood of experimental animals.

2. Materials and Methods:

Micro-organisms:

Spirulina platensis used in this study were obtained from El-Khadra lake at Wadi El Natroun, Egypt, characterized by extreme conditions of pH 10.5 and salt concentration of 0.55 M,,(Aly,2000).

Maintenance stock media:

Zarrouk's synthetic medium was used for maintaining and batch culture preparation of *S.platensis*(SP) as described according to Ali and Amber (2010).

Harvesting the biomass:

After seven days incubation , the algal biomass were harvested with the 40 μm mesh size cloth , filtered and washed with distilled water to remove the salts from the algal surface then, the washed slurry was divided into tow sets ,first for determination of vitamins and fatty acids which stored at -20 C while the second set was dried at 50 C for I h and milled ;Ali and Amber (2010). The dried samples were milled and stored in plastic container to be used in feeding experimental animals.

Extraction and Identification of vitamins; Qian and Sheng (1998): Ten grams of SP tissue fresh weight were homogenized with methanol for extraction of water soluble vitamins while acetone-chloroform (30:70 v/v) was used for extraction of fat soluble vitamins The mixtures were shaken on a vortex mixer for 5 min, centrifuged at 4000 rpm for 5 min and filtered through a Millipore filter (45 im). The filtrates were evaporated under nitrogen and the residues were re-dissolved in 1ml water for water soluble vitamins and in 1 ml butanol for fat soluble vitamins were quantified by HPLC Vitamin C was analyzed by AOAC(1995), -tocoferol (vitamin E) by HPLC;Manz Vuilleumier(1988). and carotene bv spectrophotometric method, AOAC(1995).

Lipids were obtained from lyophilized biomass sample according to Folch *et. al.* (1957) lipids were extracted with chloroform/ methanl (2+1 v/v) purified in methanol/ water (2:1 v/v), containing 9 g Na Cl to remove sugar, salts, protein and concentrated in a rotary evaporator residual solvents were evaporated. Lipids were gravimetrically estimated.

Extraction and Identification of fatty acids (FA): (Isik *et. al. 2006;* Diarman *et. al. 2009*).

FA were analysed by IUPAC (1982) method with Thermoquest Trace GC. FID detector (250 °C) and SP-2330 Fused silica capillary column 30 m.-0.25 mm ID-0.20 μ m (film thickness)of cyanopropyl were also used. Air was adjusted 350 ml/min. 35 ml/min H₂ and 30 ml/min He were used. The range, carrier ratio, split flow and split ratio were 1, 0.5 ml/ min, 75 ml/min and 1/150, respectively. Oven temperature was 120 °C (up to 220 °C with the adding of 5°C. The sample injection was 0.5 μ L. The FA was identified by comparing them in their retention time with standards obtained from Sigma.

Vivo studies.

Healthy adult(1-1.5 kg)t white Newzeland rabbits were kindly provided by NRC, Egypt, housed and maintained under a constant temperature of 30±1°C.i,e. animals were acclimatized to laboratory conditions before the experiment with a week .Rabbits were given food and water ad libitum along the period of the treatment. . Animals were randomly divided into two groups (n=10 per group) and treated for a period of a months as follows: 1) Group 1 (SP-treated group): animals were fed on a standard diet as 100 mg Spirulina /k weight) (100mg powder dissolved in 10 ml sterilized water) by a gavage daily for one month and given water for 30 days ; 2) Group 2 (untreated control group): animals were fed on a standard diet until the termination of the experiment; Body weight of the animal was recorded every 10days.

Biochemical analysis

After 15 days fasting following the end of the experimental time, the animals were cut in ears, blood samples were collected from the marginal vein of the ear every 10 days for one month in clean dry test tube. The samples were kept for 30 min at room temp to clot then centrifuged at 3000 rpm for 10 min. Clear serum was divided into aliquots and was used for the biochemical determinations.

Aliquot of serum samples were kept at -20° C until used for determinations.

Total cholesterol (TC) was evaluated according to Richmond (1973).

Triglcerides(TG)) were evaluated according to Trinder (1969).

High density lipoprotein cholesterol (HDL-CH) was evaluated according to Lopez-Virella *et. al.* (1977).

Calcium serum was evaluated according to Gindler and King (1977).

Ferritin serum was evaluated according to White *et. al.* (1986).

Iron serum. was evaluated according to Dreux (1977).

Atherogenic index (AI)=TC-HDL-ch/HDl-ch.AI when increased than 2 lead to atherosclerosis.

Statistical analysis:

Data are expressed in mean \pm SE of three replicates, results were considered significant when p<001.

3. Results and Discussion

Fatty acids content in the local strain of S.platensis:

A concentrated source of nutrients such as Spirulina is loaded with fats, starches and calories. Spirulina contains 7 % lipid, and most of that is in the form of essential fatty acids that promote cholesterol normalization. The essential fatty acids sometimes called vitamin F, include linoleic, linolenic and arachidonic acid. They are used by the body to manufacture prostaglandins, the hormonal regulators of blood pressure and capillary resilience ;Tomaselli et. al. (1988) .Otle and Pire (2001) determined the composition of Spirulina platensis fatty acid composition. The essential fatty acids are involved in respiration in all the cells, and are especially important to oxygen transport. They affect the health of the hair, skin and nails, and help break up cholesterol in the blood stream. Isik et. al.(2006) found that chemical composition of Spirulina related to the environmental cultivation conditions, salinity, light intensity, pH which determines the solubility of CO₂ and minerals in the medium. Data presented in table 1 indicated that PA amounted to 33.452%(w/w). Quoc and Dubacq (1997); Romano et. al. (2000); Mühling et. al. (2000) and Tomaselli et. al. (1988) studied the temperature influence on S. platensis M2 and determined the fatty acids contents of S. platensis at the different temperatures, They observed that the lessening temperature led to the decrease of the C16:0 content. Similarly, Tomaselli et. al. (1988) and Romano et. al. (2000)reported the increase in C16:1 level by the low temperatures 26 °C. On the contrary, Quoc and Dubacq (1997) reported the increase in the level of C18:2 with the lowering of the temperature. It was shown that cyanobacteria responded to a decrease in ambient growth temperature by de-saturating the fatty acids of membrane lipids to compensate for the decrease in membrane fluidity at low temperatures ;Tomaselli et. al. (1988). In addition, the proportion of de-saturated fatty acids increases by the decrease in temperature . Oliveira et. al. (1999) reported the increase of the C18:3 with lowering temperature .The percentage of C18:2 decreased at the 26 °C. In agreement with these studies, table 1 indicated the level of C16:0 was found to be 33.452% (w/w), palmitolleic fatty acid (16:1) 1.262, (w/w)% of total lipids which is used to fight weight gain., while C18:2.yield was 5.523% (w/w). Table 1, showed also, the content of C18:3: was small yields 0.986% (w/w)..;oleic acid (C18:1) 37.988%(w/w).; linoleic acid (C18:2) represents 5.523 %(w/w)., LA content was similar to a previous study 10-37% (w/w).;Diraman et. al.(2009).

GLA (-linolenic) acid (C18:3) that is associated with pharmaceuticals and nutraceuticals amount to 0.986% (w/w) of lipids . However, S. platensis is a very rich source in -linolenic acid. It has been found that contents and composition of fatty acids are temperature dependent in S.platensis; an increase in temp reduce the composition of fatty acids in membrane lipids, Colla et. al. (2008). GLA yield depends on dark and light cycles .indoor or outdoor cultivation, harvest time, age of the culture, This low yield in fatty acids in Wadi El Natroun S.platensis may be due to the fact stated by ;Diraman et. al. (2009) who confirmed that, the mechanism of fatty acids composition are not fully understood ,variation in percentage concentrations may be due to temp.or sodium nitrate concentration in the synthetic media used .Table 1 showed that PA and OA were the most abundant.

Caprylic acid (C8:0) yield is 1.578 (w/w) % known with its strong anti-fungal properties, Capric acid (C10:0) yield is 6.154% w/w)used in making artificial fruit flavors. Lauric acid (C12:0) helps in curing skin infections and dandruff,; myristic acid (C14:0) represents 2.761%, palmitic acid (C16:0) yield is 33.4(w/w) %,; stearic acid (C18:0)represents 5.08 (w/w) %, and arcachedic acid (C20:0) yield is 2.761%. -linolenic acid (GLA and),linoleic fatty acid (LA) are poly and mono- unsaturated fatty acids. Table2 indicated the amount of -linolenic acid was only 0.986% (w/w)). Total saturated fatty acids amount to 53.452.% (w/w). GLA in particular has a role in lowering the decrease blood cholesterol level; Ishikwa et. al. (1989)used it in treatment of hypercholesterolemia.

Fatty acid	Concentration%(w/w)	Fatty acid	Concentration% (w/w)
Short chain (CA)C8:0	1.578	Unkown	0.789
(CA) C10:0	6.154	(SA)C18:0	5.089
Long chain (IA)C12:0	1.657	(OA)C18:1	37.988
(MA)C14:0	2.761	(LA)C18:2	5.523
(PA)C16:0	33.452	(GLA)C18:3	0.986
(POL)C16:1	1.262	Very long chain (AA) (C20:0)	2.761

Table (1). (%) Fatty acid in S. platensis biomass.

Table 2. Concentration % of -linolenic poly unsaturated fatty acid in S.platensis biomass

GLA% (w/w)	UFA% w/w)	L A% (w/w)	OA% (w/w)	SF% w/w)
0.986	46.548	5.523	37.9	53.452

Total fatty avidsTFA, linoleic fatty acid(LA), oleic fatty acid(OA)- -linolenic fatty acids(GLA)-Unsaturated fatty a cids(USf).

Vitamins content in the local strain of S. platensis :

S. platensis biomass could be obtained under the optimum laboratory cultivation conditions for the quantification of -carotene, vitamin A and tocopherol. Spirulina contains a number of vitamins: B1 (thiamine), B6 (pyridoxine), B9 (folic acid), vitamin C, vitamin D, vitamin E and B12. (cobalamin), , biotin, pantothenic acid, beta carotene (source of vitamin A, Elizabeth and and Lillian (1968); Watanabe et. al. (2002); García-Martínez et. al.((2007), Spirulina is rich in vitamin B12 Vitamin B12 (vitamin (cobalamin), ascorbic acid. C),tocopherols(vitamin E),phylloquinone (vitamin K 1) and menaquinones (vitamin K 2), vitamins: A (Bcarotene); B1(thiamine), B2 (riboflavin), B3 (niacin), were also detected in Spirulina extract in a satisfactory amounts; García-Martínez et. al.((2007).

S.platensis and vitamin A: Table (3) indicated that *Spirulina* contains 120.13 μ g / 100g wet weight. Vitamin A helps form and maintains healthy teeth, skeletal and soft tissue, mucous membranes, and skin. It is also known as retinol because it produces the pigments in the retina of the eye; Duester(2008).

S.platensis and vitamin C (ascorbic acid) :Table (3) indicated that *Spirulina* contains 540.34 μ g / 100g wet weight .Ascorbic acid is required for healing of wounds, the production of digestive enzymes and connective tissue, brain and nerve function, formation of teeth and bones, glandular activity. Also vitamin C aids in the absorption of iron and protection of cells, B complex vitamins, vitamin E and vitamin A from oxidation . *Spirulina* helps in curing all immune system problems as well; Quereshi *et. al.* (1994).

S.platensis and vitamin E: Table (3) indicated that *Spirulina* contains Tocopherol or vitamin E (105.6 μ g/100gwet weight). This nutrient protects heart and vascular health, promotes oxygenation of cells; Zingg ,and Azzi(2004), and retards aging. Vitamin E deficiency *in* humans results in ataxia (poor muscle coordination with shaky movements), decreased sensation to vibration, lack of reflexes, and paralysis of eye muscles. One particularly severe symptom of vitamin E deficiency is the inability to walk.

Table (3).	Vitamins	in <i>S</i> .	platensis	biomass.
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Vitamin	Concentration (µg/100g) wet biomass
(C)Ascorbic	540.34
-carotein(A)	120.13
- tocopherol (E)	105.6

In vivo studies

Effect of *S.platensis* on experimental animals body weight:

Table 4 shows the change recorded in rabbits that had been fed Spirulina diet for 4 weeks ,as the body weight of each animal along the experimental period (one month) is given. Kumar et. al. (2010) stated that S.platensis has diverse biological effect due to high content of highly digestible protein, vitamins, betacarotene ,phycocyanin and other pigment. Table 4 shows increasing percentage of change in the animals weight with time in all the groups under treatment as compared to control groups, this comes in agreement with Yin, et.al. (2008). As the body weight of each animal was recorded every 10 days, the recorded increase in the 3rd period in the experimental animals is $2.10 \pm .30$ (Kg) refers to the "free-feeding" weight of an animal, "The rabbit's ad libitum weight was about 300 grams.

Table (4). Mean weight of rabbits over the test period ±SD

Weight elevation (%)	Test (Kg)	Control (Kg)	Period(10 days)
7.462	1.40±0.11	1.34±0.05	1
6.211	±0.2011.66	1.61±0.10	2
5.376	2.10±0.30	1.86±0.12	3

Effect of *S.platensis* on the cholesterol titres:

Early interest in *S.platensis* focused mainly on its potential as a source of protein and vitamins but recently more attention has been made to study its therapeutic use and number of reports suggested its beneficial effect in acute allergic rhinitis, anticardiotoxic ,anti-hepatotoxic, anti-nephrotoxic; Kumar *et. al.* (2010). Table 5 indicated that after 30 days feeding with *Spirulina*, TG reached 48.23 ± 2.11 mg % compared to the control, TC $45.61\pm3.21\%$ mg % reached **a** decrease to 18.27 ± 0.28 mg % compared to the control 25.24 ± 0.42 mg % , HDL-Ch recorded 17.91 ± 1.50 mg % increase as compared to the control 12.23 ± 1.15 mg % under the same laboratory conditions, as total blood cholesterol below 200 mg/dL indicating the relatively low risk of coronary heart disease , even with a low risk this comes in agreement with , Edlin *et. al.* (2009).

Serum levels of total glycerides and free glycerol are important indices of lipid metabolism and cardiovascular disease risk. Khan *et. al.*(2005). Triglycerides(TG) are a type of fat in the bloodstream and fat tissue. Too much of this type of fat, as tabl; 5 indicated this value reached 48.23 ± 2.11 mg % can contribute to the hardening and narrowing of arteries. This puts risk of having a heart attack or strok; John *et. al.*(2008) .Diseases such as diabetes, obesity, kidney failure or alcoholism can cause high triglycerides. Often, high triglycerides occur along with high levels of cholesterol, which is an important component for the <u>manufacture</u> of fat-soluble vitamins including <u>vitamin</u> <u>A</u>, <u>vitamin D</u>, <u>vitamin E</u>, and <u>vitamin K</u>. HDL particles are able to remove cholesterol from <u>atheroma</u> within <u>arteries</u> and transport it back to the liver for excretion or re-utilization, which is the main reason why the cholesterol carried within HDL particles, termed HDL-C, is sometimes called "good cholesterol". higher levels of HDL-C recorded 17.91±1.50 mg % as data presented in table 5 seem to indicate fewer problems with <u>cardiovascular diseases</u>, while low HDL-cholesterol levels (less than 40 mg/dl or about 1 mmol/L) have indicated rates for heart disease .

There are several types of cholesterol, each made up of lipoproteins and fats. Each type of lipoprotein contains a mixture of cholesterol, protein and a type of fat (triglyceride), but in varying amounts. Nayaka *et. al.*(1988). Of the lipoprotein types, VLDL contains the highest amount of triglyceride. Because it contains a high level of triglyceride, having a high VLDL level means the increased risk of coronary artery disease, which can lead to a heart attack or stroke. A normal VLDL cholesterol level is between 5 and 40 milligrams per deciliter. Since higher levels of LDL particles promote health problems and <u>cardiovascular disease</u>, they are often called the bad cholesterol particles, (as

opposed to <u>HDL</u> particles, which are frequently referred to as good cholesterol or healthy cholesterol particles). VLDL-cholesterol is a minor lipid component of very low-density lipoprotein (VLDL) particles of VLDL particle; Ren *et. al.* (2010). A study involving geriatric patients determined that *Spirulina* helped to significantly reduce the <u>LDL-to-HDL</u> ratio after four months of supplementation; Park *et. al.* (2008). Treatment with over a six week period, exhibited significant changes in <u>cholesterol</u> and <u>blood</u> pressure as it lowered total cholesterol, increase <u>HDL</u> <u>cholesterol</u>, lower <u>triglycerides</u>; and lower <u>systolic</u>; Torres-Duran *et. al.* (2007).

Table 5 showed that AI 0.71 ± 0.13 for the control and 0.27 ± 0.11 for the treatment .i.e. not the least evidence for atherosclerosis. Arteriosclerosis is hardening of the arteries. This condition not only thickens the wall of arteries, but also causes stiffness and a loss of elasticity. Over time, the arteries become harder and harder as they are slowly damaged by high blood pressure. Atherosclerosis is the most common type of arteriosclerosis, or hardening of the arteries, and caused by plaque building up in the vessel. Over time the plaque causes thickening of the walls of the artery, when AI increased than 2 lead to atherosclerosis.

Table (5).Effect of S. platensis tablets(100mg/Kg) in lipid profile of the rabbits ±SE.

Period	group	TGmg%	TC mg%	HDL-ch mg%	Al
1	control	48.93±5.84	20.63±3.06	14.43±2.45	0.726±0.124
	test	58.8±3.60	26.5±2.82	11.22±1.42	1.74±0.266**
2	control	46.8±5.96	14.93±1.15	9.22±0.99	0.93±0.223
	test	49.58±3.83	19.48±0.43	18.08±1.74**	0.37±0.23*
3	control	45.61±3.21	25.24±0.42	12.23±1.15	0.71±0.13
	test	48.23±2.11	18.27±0.28	17.91±1.50	0.27±0.11*

*p<0.1 corresponding to control

**p<0/001 corresponding to control.

**p<0/001 corresponding to control

Effect of S.platensis on serum calcium level:

Serum calcium is a test that measures how much calcium is in blood. The presence of free calcium was a necessary condition of co- agulation as certain salts of citrate and oxalates electrolytes have low solubility product, so it can be used as anti-coagulation. However, in combination with prothrombin, calcium acts not as free element but as a complex that could interfere in blood clotting; Levelock and Porterfield (1952). Fig .1 shows the increase in the serum calcium level of the treated than the control sample in the three periods, which is prominent in the 2nd period, as it amounts to 11.3mg/dl compared to 5.43mg/dl of the control test. Also, the parameter is still high in the 3rd period as it measures 9.6 mg/dl compared to 7.1 mg/dl of the control. This decrease may be due to the use of this calcium by the body. the body uses vitamin D to help transport calcium to the bones When blood calcium levels drop too low, the vital mineral is "borrowed" from the bones. It is returned to the bones from calcium supplied through the diet. . Calcium is the most abundant mineral in the body; the bones and teeth accounting for about 99% of the total body stores.



Fig. (1) S. platensis effect on serum calcium.

Effect of S.platensis on serum iron and serum ferritin:

Free iron is toxic to cells as it acts as a catalyst in the formation of free radicals from reactive oxygen species via the Fenton Reaction. Orino et. al.(2001). Hence body uses an elaborate set of protective mechanisms to bind iron in various tissue compartments. Within cells, iron is stored in a protein complex as ferritin. The amount of ferritin in blood (serum ferritin level) is directly related to the amount of iron stored in the body, i.e. under steady state

conditions, the serum ferritin level correlates with total body iron stores; thus, the serum ferritin is the most convenient laboratory test to estimate iron stores as it serves to store iron in a non-toxic form, to deposit it in a safe form, and to transport it to areas where it is required, Seckback (1982). The serum ferritin concentration is a clinical parameter measured widely for the differential diagnosis of anemia. Ferritin is a ubiquitous intracellular protein that stores iron and releases it in a controlled fashion. In humans, it acts as a buffer against iron deficiency and iron overload Seckback (1982). The function and structure of the expressed ferritin protein varies in different cell types. Fig (2,3) illustrates the effect of 100 mg Spirulina/ kg daily administration to the rabbits . Control and treated samples showed a significant decrease in serum ferritin in the third period due to the use of its iron in hemoglobin formation for example as high ferritin is correlated to iron in excess. Serum iron increase significantly in the 3rd period as it reached 5.5mg/dl compared to 2mg/dl of the control. Ferritin decreased in the 3rd period 12 mg/dl because of iron consumption in hemoglobin but it still in a higher concentration than control. 9.4 mg/dl.



wina platensis for one month

Fig. (2) S. platensis effect on serum iron.



Fig. (3) S. platensis effect on serum ferritin.

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Efficacy of Inspiratory Muscle Training on Ventilatory Functions in Postmenopausal Asthmatic Women

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Abstract: This study was conducted to determine the effect of inspiratory muscles training in improving ventilatory functions in postmenopausal asthmatic women. Fourty postmenopausal women, complaining from bronchial asthma, one year ago, were participated in this study. They were divided randomly into two equal groups (A and B).Both groups received traditional medical treatment which was consisted of theophyline, salbutamol sulphate(bronchodilator), dexamethasone, carbocisteine (muco-regulator) & antibiotic for six weeks. In addition to traditional medical treatment, Group "A" received the inspiratory muscles training by using inspiratory muscle trainer. Assessment was performed by measuring weight and height then the body mass index was calculated for each woman in both groups (A&B) before treatment. Also, Electronic Spirometer was used to measure ventilatory functions (Forced Vital Capacity, Forced Expiratory Volume, Forced Vital Capacity /Forced Expiratory Volume and Maximum Voluntary Ventilation) before and after 6 weeks of treatment. Results showed a statistically highly significant improvement (P<0.001) in all ventilatory functions in group (A) than group (B) after end of treatment. So, it could be concluded that inspiratory muscle training with traditional medical treatment were more effective than traditional medical treatment only for these cases as it was effective, safe, easy to perform and led to reducing symptoms of asthma.

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Key words: Inspiratory Muscle Trainer - Ventilatory Functions - Postmenopausal Asthmatic women.

1. Introduction:

Bronchial asthma is a major health problem throughout the world which reached the epidemic proportions (Kellett and Mullan, 2002) as its prevalence has increased over the last decades (Aly and Essa, 2006). Bronchial asthma is defined as a complex respiratory disease characterized by airways inflammation and hyperresponsiveness of bronchial smooth muscles leading to reversible bronchospasm (Bellia and Augugliaro, 2007). Bronchial asthma leads to impairment of ventilatory function that results in deterioration in functional capacity and quality of life and this impairment is influenced by age, duration and severity of the disease (Cibella et al., 2002). Menopause is associated with decreased lung function and increased respiratory symptoms (Real et al., 2008). The female hormone estrogen is an important factor in the regulation of airway function and inflammation, and sex differences in the prevalence of asthma (Matsubara et al., 2008). Bronchial asthma that begins around the time of menopause is frequently characterized by marked clinical severity and poor response to treatment

(Grady, 2006). The airway inflammation present in women with menopausal asthma is poorly responsive to anti-inflammatory treatment with corticosteroids and predisposes to frequent severe exacerbations. Airway inflammation should be monitored in women with menopausal asthma (Balzano et al., 2007).Longterm use of inhaled corticosteroids is associated with significant bone loss in asthmatic women and is especially related to the duration of therapy (Kemp et al., 2010). Therefore, it is necessary to give prophylactic treatment to those who are likely to develop osteoporosis from inhaled corticosteroid treatment (Sivri and Coplu, 2001).In moderate to severe chronic obstructive pulmonary disease there is a generalized loss of muscle bulk including the respiratory muscles. Similar loss of respiratory muscle strength can occur particularly in more severe asthma due to the effects of steroid therapy (Ram et al., 2003).Asthmatic patients suffer from increased airway resistance as well as air trapping and lung hyperinflation which lead to changes in the thoracoabdominal mechanics and inspiratory muscles impairment. It was found that the use of inspiratory muscles training as a complement to pharmacological treatment provided clinical benefits to asthmatic patients (Veruska et al., 2008). Inspiratory muscle training has been used as an adjunct treatment for various chest diseases. So, It is very important to study the effect of inspiratory muscles training to know its effect on postmenopausal asthma to improve the inspiratory muscles endurance and ventilatory functions as there is no previous study assessed the effect of inspiratory muscles training after menopause. This study was conducted to determine the effectiveness of inspiratory muscles training in improving ventilatory functions in postmenopausal asthmatic women.

2. Subjects, Materials and Methods Subjects:

Forty

postmenopausal women. complaining of bronchial asthma, one year ago were participated in this study. They were selected from Abbasia Chest Hospital. They were divided randomly into two equal groups (A and B). Group A (Study group) consisted of twenty patients. They received the inspiratory muscles training in addition to traditional medical treatment. Group B (Control group) consisted of twenty patients. They received traditional medical treatment only. Informed consent form was signed by each patient before starting this study. Their age ranged from 50 - 60 years old .All patients were non-smokers, had no cardiac ,chest diseases, diabetes, hypertension and did not receive hormonal replacement therapy. Their Body Mass Index didn't exceed 30 kg/m² to exclude obese women from the study.

2) Material

(A)Evaluative instruments

1-Electronic Spirometer

It was used for ventilatory functions measurements which are:-

(Forced vital capacity) is one of the most useful tests to assess the overall ability to move air in and out of the lungs (ventilation). This is the maximum amount of air that can be forcefully and rapidly exhaled after a deep breath (maximal inspiration). (Forced expiratory volume) is the volume of air forcibly exhaled in one second during the FVC test. It decreased in obstructive lung disease as bronchial asthma.

(Maximum voluntary ventilation) is the maximum air, which can be expired in a minute by deepest and fastest breathing. It typically decreased in subjects with moderate or severe obstructive disease.

2-Weight and height scale:

It was used for measuring the weight and height of each patient to calculate the body mass index.

(B)Treatment instruments:

Inspiratory muscles trainer

It is an inspiratory muscle trainer that helps to increase respiratory muscles strength and endurance through conditioning. This is similar to muscle conditioning used in weight training. It works by placing a specific constant resistance on respiratory muscles regardless of how quickly or slowly the patient breathes. This resistance is provided by a spring loaded valve which exercises the respiratory muscles when inhalation occurred (Lima et al., 2008). The goal is that the patient should be able to inhale with enough force to open the valve. He will know that he is training correctly and using the proper force when the air is heard flowing through the device. This may be difficult to do at first but will be easier as he progresses (Deane, 2005).. Inspiratory Muscle Trainer device consisted of a portable handheld device through which patients would inspire only when they overcome the threshold resistance of the device. Inspiratory muscle training appeared to consistently improve ventilatory muscle strength, endurance, and dyspnea (Deturk and Cahalin, 2004).

3) Methods:

(A)Methods for Evaluation:

These methods for patient's evaluation were preformed for both groups A &B. Each patient participated in this study was primarily diagnosed and referred by physician. Before engagement into the study, a detailed medical history was taken and recorded including name, age, address, occupation, parity, any chest diseases before menopause. As well as, the vital signs were measured. Then, weight and height were measured for each patient in both groups before starting the study and body mass index was calculated to exclude obese women.

Ventilatory Function Test, was performed before and after the end of treatment period (6 weeks).In which the patient was instructed to assume the erect standing position carrying the breathing tube that was connected to the spirometry and in its other end there was a disposable mouthpiece to prevent infection. The patient's age, weight and height were introduced into the screen of the apparatus. Then, each patient was instructed to perform the test while wearing nasal clip (Kevin, 2006). These procedures were repeated 3-5 times with rest in between and the maximum value was recorded for evaluation of ventilatory functions (Forced Vital Capacity test, Forced Expiatory Volume, Forced Expiatory Volume / Forced Vital Capacity and Maximum Voluntary Ventilation).

(B)Treatment procedure:

Both groups(A&B) received traditional medical treatment which was consisted of theophyline, salbutamol sulphate (bronchodilator), dexamethasone, carbocisteine (muco-regulator) & antibiotic for six weeks. In addition to traditional medical treatment, Group "A" received the inspiratory muscles training by using inspiratory muscle trainer. The maximum training load was set, the user identified the load at which she could successfully execute ten breaths at maximum resistance depending on the patient's rate of perceived exertion (Alison, 2005). It is recommended to start training with a load equal to 30% of the maximum inspiratory patient's effort. This individualized load increased progressively as the inspiratory muscles became more stronger. The recommended pressure load determined by the 30% maximum 10-repetition method by using the device.

Duration:

Each session was divided into six work sets, separated by rest intervals lasting for two minutes. Each set consisted of five breathing in and out and rest for three seconds this repeated for three minutes.

Frequency:

Three sessions/week for six weeks.

Precautions during inspiratory muscles training:

*Breath in should be deep and forced with a long and slow expiration.

*If patient started to feel headache, she will ask to slow down pause before the next breath.

*Patient was instructed not to eat at least 2 hours before training.

*Patient was instructed to keep her mouth tight around the mouthpiece for the total time.

*Using nasal clip to close the nose.

The patient was asked to sit in a comfortable position and put the nose clip on her nose and to breathe through the mouth only. Relax, place her lips around the mouthpiece, and inhale as deeply as she can with enough force to open the valve. Exhale through mouthpiece; continue inhaling and exhaling without removing the device from her mouth.

Data Processing (Statistical Analysis):

The collected data were statistically analyzed and interpreted using Mann-Witney U test, Friedman test, Wilcoxn Signed Ranks test and Chi square test to compare between both groups to obtain Mean, Standard deviation, Minimum as well as maximum values & Percentages. The level of significance (P value) was considered as follow: P>0.05 considered not significant, P<0.05 considered significant, P>0.01 considered highly significant.

3. Results

Forced Expiratory Volume:

In group "A", it showed a highly significant (P< 0.0001) difference between before and after treatment where the mean values were (1.38 ± 0.16) and (1.73 ± 0.17) respectively with mean difference equal 0.35 and percentage of improvement equal 25.36% While in group "B", there was non significant (P< 0.78) differences between before and after treatment where the mean values were (1.37 ± 0.28) and (1.38 ± 0.23) respectively with mean difference equal 0.36%. When comparing both groups (A&B) together there was non significant (P<0.87) difference before treatment. While, there was a highly significant (P<0.0001) difference after treatment.

Table	1.	Mean	values	of	Forced	Expiratory
Volum	e be	fore an	d after	treat	ment of g	group (A).

Group A	FEV ₁			
(Study group)	Before After			
	treatment	treatment		
Mean	1.38	1.73		
±SD	±0.16	±0.17		
Mean difference	0.35			
Percentage of improvement	25.36 %			
DF	19			
t-value	10.97			
P-value	0.0001			
S	HS			

*SD: standard deviation, P: probability, S: significance, HS: highly significant, DF: degree of freedom.

Table	2.	Mean	values	of	Forced	Expiratory
Volum	e be	fore an	d after t	reat	ment of g	group (B).

Group B	FEV ₁	
(Control group)	Before	After
	treatment	treatment
Mean	1.37	1.38
±SD	±0.28	±0.23
Mean difference	0.005	
Percentage of improvement	0.36%	
DF	19	
t-value	0.27	
P-value	0.78	
S	NS	

*SD: standard deviation, P: probability, S: significance, NS: non-significant, DF: degree of freedom.

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Figure 1. Total percentage of improvement in Forced Expiratory Volume from before to after treatment for both groups (A&B).

Forced Vital Capacity:

In group "A", It showed a highly significant (P< 0.0001) difference between before and after treatment where the mean values were (2.26 ± 0.28) and (2.57 ± 0.28) respectively with mean difference equal 0.31 and percentage of improvement equal 13.71%. While in group "B", there was non significant (P< 0.75). differences between before and after treatment where the mean values were (2.2 ± 0.33) and (2.21 ± 0.34) respectively with mean difference equal 0.006 and percentage of improvement equal 0.27 % .When comparing both groups (A&B) together there was non significant (P< 0.57) difference before treatment. While, there was a highly significant (P< 0.001) difference after treatment.

Table 3. Mean values of Forced Vital Capacitybefore and after treatment of group (A).

Group A	FVC		
(Study group)	Before After		
	treatment	treatment	
Mean	2.26	2.57	
±SD	±0.28	±0.28	
Mean difference	0.31		
Percentage of improvement	13.71 %		
DF	19		
t-value	9.9		
P-value	0.0001		
S	HS		

*SD: standard deviation, P: probability, S: significance, HS: highly significant, DF: degree of freedom. http://www.americanscience.org

Group B	FVC			
(Control group)	Before After			
	treatment	treatment		
Mean	2.2	2.21		
±SD	±0.33	±0.34		
Mean difference	0.006			
Percentage of improvement	0.27%			
DF	19			
t-value	0.31			
P-value	0.75			
S	NS			

*SD: standard deviation, P: probability, S: significance, NS: non-significant,

DF: degree of freedom.



Figure 2. Total percentage of improvement in Forced Vital Capacity from before to after treatment for both groups (A&B)

Forced Expiratory Volume / Forced Vital Capacity:

In group "A", it showed a highly significant (P<0.0001) difference between before and after treatment where the mean values were (61.57 ± 6.19) and (67.73 ± 5.49) respectively with mean difference equal 6.16 and percentage of improvement equal 10.0%. While in group "B", there was non significant (P<0.69) difference between before and after treatment where the mean values were (62.18 ± 6.89) and (62.61 ± 6.81) respectively with mean difference equal 0.42 and percentage of improvement equal 0.67%. When comparing both groups (A&B) together there was non significant (P<0.76) difference before treatment While, there was a highly significant (P<0.01) difference after treatment

Table 5. Mean values of Forced ExpiratoryVolume / Forced Vital Capacity before and aftertreatment of group (A).

Group A	FEV ₁ /FVC	
(Study group)	Before	After
	treatment	treatment
Mean	61.57	67.73
±SD	±6.19	±5.49
Mean difference	6.16	
Percentage of improvement	10.0 %	
DF	19	
t-value	4.42	
P-value	0.0001	
S	HS	

*SD: standard deviation, P: probability, S: significance, HS: highly significant, DF: degree of freedom.

Table 6. Mean values of Forced ExpiratoryVolume / Forced Vital Capacity before and aftertreatment of group (B).

<u> </u>		
Group B	FEV ₁ /FVC	
(Control group)	Before	After
	treatment	treatment
Mean	62.18	62.61
±SD	±6.89	±6.81
Mean difference	0.42	
Percentage of improvement	0.67%	
DF	19	
t-value	0.39	
P-value	0.69	
S	NS	

*SD: standard deviation, P: probability,

S: significance, NS: non-significant,

DF: degree of freedom.



Figure 3. Total percentage of improvement in Forced Expiratory Volume / Forced Vital Capacity from before to after treatment for both groups (A&B).

Maximum Voluntary Ventilation:

In group "Å", It showed a highly significant (P< 0.0001) difference between before and after treatment where the mean values were (55.5 ± 6.53) and (69.56 ± 6.8) respectively with mean difference equal 14.04 and percentage of improvement equal 25.29 %. While in group "B", there was non significant (P< 0.62) difference between before and after treatment where the mean values were (54.84 ± 11.09) and (55.24 ± 9.39) respectively with mean difference equal 0.4 and percentage of improvement equal 0.72 %. When comparing both groups (A&B) together there was non significant (P< 0.82) difference before treatment. While, there was a highly significant (P< 0.0001) difference after treatment.

Table 7. Mean values of Maximum Voluntary Ventilation before and after treatment of group (A).

Group A	M	VV
(Study group)	Before	After
	treatment	treatment
Mean	55.5	69.56
±SD	±6.53	±6.8
Mean difference	14.04	
Percentage of improvement	25.29 %	
DF	19	
t-value	10.97	
P-value	0.0001	
S	HS	

*SD: standard deviation, P: probability, S: significance, HS: highly significant, DF: degree of freedom.

Table 8. M	ean values of Ma	aximum V	oluntary
Ventilation	before and after	r treatme	nt of group
(B).			

Group B	MVV	
(Control group)	Before	After
	treatment	treatment
Mean	54.84	55.24
±SD	±11.09	±9.39
Mean difference	0.4	
Percentage of	0.72%	
niprovement	10	
DF	19	
t-value	0.49	
P-value	0.62	
S	NS	

*SD: standard deviation, P: probability, S: significance, NS: non-significant, DF: degree of freedom.



Figure 4. Total percentage of improvement in Maximum Voluntary Ventilation from before to after treatment for both groups (A&B).

4. Discussion:

Severe bronchial asthma is more predominant in women. Asthma in women is associated with excess mortality risk and women with asthma were found to visit the emergency department for asthma more frequently than men. Subsequent hospitalization rates for asthma reflect the difference in asthma prevalence and severity as observed between men and women (Melgert et al., 2007). Asthma that begins around the time of menopause is frequently characterized by marked clinical severity and poor response to treatment (Carey et al., 2007). The airway inflammation presented in women with menopausal asthma was poorly responsive to antiinflammatory treatment with corticosteroids and predisposed to frequent severe exacerbations. Airway inflammation should be monitored in women with menopausal asthma (Balzano et al., 2007). Causes of asthma at menopause are not clear until now. It was suggested that declining estrogen levels increased insulin resistance, which in turn increased risk of lung inflammation (Denoon, 2007). In patients with pulmonary diseases, several studies have shown that inspiratory muscle training improves inspiratory muscle strength as well as exercise endurance. Also, it may prevent or delay the onset of ventilatory muscle fatigue and failure, and has been shown to decrease dyspnea over time (Jones et al., 2003). Some studies have shown that the respiratory muscles can be trained if an adequate training stimulus is applied, and that exercise performance and dyspnea may improve as result of such training by resistive breathing or threshold loading (Thomas, 2000). (Koessler et al., 2001) found an increase in the strength of inspiratory muscles, increase VC and MVV in patients with neuromuscular disorders and obstructive lung disease after program of inspiratory muscle training. Another improvement in MVV had been reported by (Winker et al., 2000) after 9 months

of inspiratory muscle training. The thorax is a complex assembly of muscles and bony structures. Like other skeletal muscles, the rational for inspiratory muscle training is that increasing the strength or endurance of the respiratory muscles can improve clinical outcomes, reduce the severity of dyspnea) and enhance the ability of individuals to perform daily activities (Murray and Mahler, 2009). This study was designed to determine the effect of inspiratory muscles training in improving ventilatory functions in postmenopausal asthma. Fourty postmenopausal asthmatic women were selected from Abbasia Chest Hospital & they were divided randomly into two groups (A and B) equal in numbers with non significant differences (p > 0.05) in age, weight, height and body mass index. Assessment of FEV₁, FVC, FEV₁/FVC and MVV were performed to each woman before starting the study and after 6 weeks of treatment. The current study showed a statistacally significant improvement after treatment (FEV1,FVC,FEV1/FVC and MVV) in group "A". The ventilatory muscle training in individuals with respiratory disease (asthma) leads to increasing the strength and/or endurance of the respiratory muscles as well as reducing the severity of dyspnea and improve exercise capacity which was supported by Mahler, 2000. The results of this study showed that inspiratory muscles training not only significantly increased inspiratory muscle strength and endurance but also improved ventilatory functions. In addition, a significant clinical decrease in dyspnea sensation at rest and during exercise was observed after its usage and this was supported by the work of Weiner et al., 2004. These results were supported with the results obtained by Keene (2007) who stated that Inspiratory Muscle Training offered a basis for pulmonary rehabilitation to patients with obstructive lung diseases as they were able to increase their Inspiratory muscle strength. With the strengthening of these muscles. There was increasing of the FVC of a patient, also their overall lung volume increased. This produced a direct decrease in their airway resistance and presumably a decrease in their levels of dyspnea. In conclusion, inspiratory muscles training are a proven way to increase an asthma patient's FVC. This aided in reducing their levels of dyspnea and in turn can be used as an alternative physiologic form of therapy to reduce their intake of systemic corticosteroids and inhaled beta -2 agonists. Also, its effects were noted in improving inspiratory muscle strength and endurance, improved functional exercise capacity, and decreased dyspnea, during exercise and at rest. As well as, it was suggested that inspiratory muscle training is a very essential in pulmonary rehabilitation programs (Hanneke et al., 2001). The results of the basic

training program parallel other studies in which increased inspiratory significantly muscle performance was associated with improved exercise tolerance, decreased dyspnea and improvement of ventilatory functions. The training response of respiratory muscles is similar to that of skeletal muscles as it produced a hypertrophy of muscle fibers & increased the vascularity of muscle fibers (number of capillaries in each fiber) (Minoguchi and Shibuya, 2002). Finally, from statistical point of view, it could be concluded that inspiratory muscles training with medical treatment appeared to be an effective, safe, easy to perform and improve quality of life.

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Analyzing Efficiency of Agricultural Extension Programs by Participatory Rural Appraisal (PRA) (Illustrate: Wheat Farmers of Khouzestan Province, Iran)

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Abstract: The purpose of research is analyzing efficiency of agricultural extension programs by Participatory Rural Appraisal (PRA). The method of research was qualitative. The research carried out by 4 analytical loops in rural area of Shoushtar township of Khouzestan province, Iran. Each analytical loop consist 6 to 9 rural people and one outsider as facilitator. According to results extension programs based on efficiency was ranked. This ranking respectively include: Farmer Filed School (FFS), Meeting in Farm (MF), Results Farm Demonstration (RFD), Method Farm Demonstration (MFD), Extension classes, Bulletin and Posters, Radio and TV program. Also ranking of educational needs respectively include: productivity indicators, sustainability, farm management, water management, pest and disease, west management.

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Keywords: PRA, Extension program, Shoushtar

1. Introduction

Agricultural extension is a significant social innovation, an important force in agricultural change, which has been created and recreated, adapted and developed over the all centuries. Its evolution extends over nearly four thousand years, although its modern forms are largely a product of the past two centuries (Jones and Garforth, 1997).

Van den Ban & Hawkins (1996) defined agricultural extension services (AES) as "transferring information from the global knowledge base and from local research to farmers, enabling them to clarify their own goals and possibilities, teaching them to be better decision-makers, and stimulating desirable agricultural development". Change in agriculture has brought about new challenges for farmers in relation to production and technology. As a result, more attention should be paid to agricultural extension. Van den Ban (1996) iterated the necessity of a progressive agricultural extension system. He pointed out that in many countries agriculture is in a process of rapid change and stressed that the demand for food is growing, as is international competition, labor productivity, and the rate of agricultural research. However, Van den Ban (1996) also pointed out, that employment opportunities and governmental supports for agricultural products are decreasing.

According to Rivera and Gustafson (1991), agriculture and farming, informational technology, and governments are all in the process of changing. These socio-economic, political, and technical changes inevitably affect the institution of agricultural extension and exert pressure on it to change. Considering the changes and challenges in agricultural extension today, one of the roles of an extension organization should be to contribute to the development of agriculture by helping villagers to become aware of the changes in their environment. While these changes do offer new opportunities for farm development they can also threaten development because it is no longer possible to earn a decent income from the present farming methods (Van den Ban, 1996).

Agricultural extension has now become recognized as an essential mechanism for delivering information and advice as an input into modern farming. Since commercial farmers can derive direct financial benefits from these inputs, there is a trend towards the privatization of the extension organizations, often as quasigovernmental agencies, with farmers being required to pay for services which they had previously received free of charge (Jones and Garforth, 1997).

Therefore, a major role of agricultural extension is to help farmers with the knowledge construction process and to support them to learn from their own experiences (Van den Ban & Hawkins, 1996). There are many different definitions and interpretations of extension from various extension specialists. Most definitions support previous statements and assume extension officers and personnel as: supporters of farmers, facilitators for knowledge exchange between researchers and farmers, introducers of new techniques and information to farmers, supporters of innovation, and of farmers, creativity, self-confidence relationship-builders between government and farmers, etc. (Campbell & Barker, 1997; Prawl,

Medlin & Gross, 1984; Rathore et al., 2001; Sulaiman & Hall, 2003).

Different approaches (often used in combination with other approaches) have been applied by extension policy makers of different countries. Some of most important approaches are ministry-based or general, commodity-based, university-based, training and visit (T&V), integrated or project-based, animation rural, client-based and client-controlled, extension as a commercial service, participatory or privatized extension (Baxter, Slade & Howell, 1989; Nagel et al., 1992; Rauch, 1993; Umali & Schwartz, 1994). However, in Iran, like many other Middle Eastern countries, a mixed approach is used with a focus on governmental or common extension approaches. In sum, it could be said that agricultural extension, as a whole, aims at improving the competencies (knowledge, skills and perceptions) of farmers in order to improve their career performance.

Some of the most promising recent developments in extension methodology have occurred where the key agenda is environmental or is concerned with equity, for example in the need for the joint management of forests by professionals and local forest users and in integrated pest management. A consistent theme running through the innovative approaches being used, such as participatory rural appraisal (Chambers, 1993), is a fundamental change in what are the respective roles of extension agent and clients. The agent is no longer seen as the expert who has all the useful information and technical solution; the clients own knowledge and ingenuity, individually and collectivity, are recognized as a major resource; solutions to local problems are to be developed in partnership between agent and clients. Since the scale at which extension support is required is thus often larger than the individual farm, extension workers need new skills of negotiation. conflict resolution, and the nurturing of emerging community organizations (Smith, 1994).

Participatory Rural Appraisal (PRA) is a research method that use visualization techniques and interviews to create information for the design of effective communication programs, materials, media and methods for development purposes to ensure relevance and ownership by the farmers. PRA facilitates conversation among the rural people themselves and between them and the extension agents in order for all parties to reach mutual understanding and plan for action. PRA is therefore used to promote the participation of rural people in decision-making affects that their living (Anyaegbunam et al, 2004). PRA are tool to increase participation by local people organizing for rural and agricultural development (Toness, 2001). PRA is 'a growing family of approaches and methods to enable local people to share, enhance and analyze their knowledge of life and conditions, to plan and to act' (Chambers, 1994: 1).

Bhandari (2003) pointed, in PRA information is shared by local people. Researchers go to rural areas, but they facilitate rural people in collection, presentation and analysis of information by themselves.

In last decades Participatory Rural Appraisal (PRA) and later Participatory Learning and Action (PLA) methods emerged. PRA and PLA recognized that there were many things that researchers and subject matter specialists did not know and the only way to know them was by listening to the rural people. Similarly rural people were lacking some of the technical knowledge that the experts had to solve some of their problems. Thus, knowledge sharing became an essential component of PRA (Noorivandi and Ommani, 2009., Anyaegbunam et al, 2004).

PRA has been used extensively in agriculture, forestry and a number of other areas. PRCA belongs to the same family as PRA, PLA and the other participatory methods, but it is unique because it focuses specifically on rural communication systems and how to improve information sharing among all rural people in a development effort. From the time it was conceptualized in 1994, PRCA has undergone changes to better adapt it to field realities. (Anyaegbunam et al, 2004).

2. Material and Methods

The method of research was qualitative research. At this research used techniques of PRA for analyzing of agricultural extension efficiency. PRA is techniques and methods innovated for field data collection and analysis. The data is collected and analyzed using a group of tools" which help representation of realities in unusual form. The tools help to observe facts on the diagrams lead to best estimates; indicate people's preferences and priorities etc (Adebo, 2000). The research carries out by 4 analytical loops in rural area of Shoushtar township of Khouzestan province, Iran. Each analytical loop consist 6 to 9 rural people and one outsider as facilitator.

3. Results

Efficiency of Extension Program: For analyzing of efficiency of extension program was used matrix ranking technique. In matrix ranking criteria's (indicators of efficiency) are used for the rows in a matrix and items (extension programs) for columns, people fill in the boxes for each row. The items are ordered for each of the criteria and people put seeds for scoring relative values. According to results calculated efficiency of extension programs by summing of seeds in each column. Base on the results extension programs was ranked. This ranking respectively include: Farmer Filed School (FFS), Meeting in Farm (MF), Results Farm Demonstration (RFD), Method Farm Demonstration (MFD), Extension classes, Bulletin and Posters, Radio and TV program (Figure 1, Table 1).

Educational Need Assessment: For analyzing of educational need assessment of farmers was used another one matrix ranking. In this matrix

ranking criteria's (indicators of importance) are used for the rows in a matrix and items (educational needs) for columns, people fill in the boxes for each row. The items are ordered for each of the criteria and people put seeds for scoring relative values. According to results calculated importance of educational needs by summing of seeds in each column. Base on the results educational needs was ranked. This ranking respectively include: productivity indicators, sustainability, farm management, water management, pest and disease, west management (Table 2).



Figure 1: Matrix ranking for analyzing of efficiency of extension programs

						1 0		
Extension	Meeting	Extension	Results Farm	Research	Radio, TV	Bulletin	FFS	Method Farm
program	in Farm	Classes	Demonstration	Centers		Posters		Demonstratio
Indicators								n
Increasing	9*	8	8	3	5	4	7	6
Knowledge								
Increasing skills	8	8	9	6	5	6	8	7
Optimization of	9	5	8	5	6	5	7	6
attitude								
Increasing crop	7	6	6	7	8	7	8	7
yield								
Optimization use of	7	5	7	5	6	7	9	8
inputs (how)								
Optimization use of	5	6	5	6	5	8	9	7
inputs (how much)								
Sum	45	38	43	32	35	37	48	41
Priorities	2	5	3	8	7	6	1	4
* Minimum (without effect)= 0 Maximum (High effect)=10								

Table1: Matrix ranking for analyzing of efficiency of extension programs

needs	productivity	water	sustainability	farm	pest and	west
Indicators	indicators	management		management	disease	management
Crop yield	9^*	8	8	8	5	4
Income	9	8	9	6	6	6
Reduce cost	9	5	8	7	6	5
Knowledge	7	6	7	7	8	6
Skill	7	6	7	8	6	7
Food security	5	6	6	6	6	7
Sum	46	38	45	42	37	35
Priorities	1	4	2	3	5	6
	*: Minimum (v	without effect)	= 0, Maximun	n (High effect)	=10	

Table 2: Matrix ranking for educational need assessment of farmers

Conclusion:

Major role of extension programs is to help farmers with the knowledge construction process and to support them to learn from their own experiences (Van den Ban & Hawkins, 1996).

Agricultural extension and education is considered an essential factor in development of agricultural programs (Shahbazi, 1996). Agricultural extension and education has economic impact and sustainability in agriculture by providing information to induce the following sequence:

"A: Farmer's awareness

K: Farmer's knowledge, through testing and experimenting

A: Farmer's adoption of technology or practices

P: Changes in farmers productivity" (Evenson, 1997 p. 29).

Agricultural extension is a public service for human resource development (HRD) in the agricultural sector (van den Ban and Hawkins, 1996). Multiple studies in Iran showed that, although extension services has played a positive role in agricultural development of Iran, but there are difficulties, barriers, misunderstandings, and weaknesses in the transfer of new technology and information to farmers (Ommani and Chizari, 2002)

Participatory Rural Appraisal (PRA) is a suitable research method that use visualization techniques and interviews to create information for the design of effective communication programs. PRA facilitates conversation among the rural people themselves and between them and the extension agents in order for all parties to reach mutual understanding and plan for action. PRA is therefore used to promote the participation of rural people in decision-making that affects their living (Anyaegbunam et al, 2004). PRA is a adopted method for analyzing of extension programs and assessment of educational needs of farmers.

According to results extension programs based on efficiency was ranked. This ranking

respectively include: Farmer Filed School (FFS), Meeting in Farm (MF), Results Farm Demonstration (RFD), Method Farm Demonstration (MFD), Extension classes, Bulletin and Posters, Radio and TV program. Also ranking of educational needs respectively include: productivity indicators, sustainability, farm management, water management, pest and disease, west management.

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Bio-Oil From Rice Straw By Pyrolysis: Experimental And Techno -Economic Investigations.

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Abstract: The use of biomass as a source of energy is gaining increasing interest in both developed and developing countries as a renewable source of energy and to avoid the environmental hazards associated with open burning of ligno-cellulosic materials. This work is concerned with the fast pyrolysis of rice straw which is generated seasonally in enormous quantities to produce biooil which could replace fuel oil #2. Experimental investigations on a pilot-scale in an entrained flow reactor demonstrated that the biooil produced is of acceptable characteristics and has a calorific value of about 29kJ/kg. Process design has been developed for a 200 ton/day commercial facility. Material and energy balance and basic engineering have been accomplished using ASPEN PLUS. Techno-economic investigations have been conducted and financial analysis has been performed using ASPEN ICARUS. Results indicated that for a Base Case, the Fixed Capital Cost is about US \$ Million 7.6 and for a pessimistic selling price of US \$ 0.13/kg, the Internal Return of Return (IRR), exceeds 43%. Sensitivity analysis indicates that even for increase of the Fixed Capital to about US \$ Million 10.9 the IRR still exceeds 30%. Further work is needed for technological development and for ensuring processing over the year round using other ligno-cellulosic materials.

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Keywords: biomass, fast pyrolysis, bio-oil, entrained reactor, process design, techno-economics

1. INTRODUCTION

Enormous quantities of agricultural wastes are generated annually worldwide. Some useful applications of biomass such as animal fodder, fertilizer, paper making, source of energy in rural stoves and panel boards are widely adopted. However, large quantities are disposed of sporadically causing severe environmental problems. One of the most problematic agriculture wastes is rice straw which is generated in huge amounts over a limited harvesting period. In Egypt the quantity of rice straw generated annually exceeds 3 million tons. Its high content of silica limits its direct application as animal fodder, in paper making and as a solid fuel. Thus, farmers proceed towards its immediate disposal, even through open burning, despite the stringent regulations banning this practice, for its serious health hazards, to prepare the land for the next crop. On the other hand, the depletion of petroleum, natural gas and other conventional fuel sources is motivating researchers to develop new renewable sources of energy. The use of biomass as a source of energy is being widely addressed as reported by EERE^[1], Bridgewater^[2] and Mullaney^[3]. Of specific concern is the production of biooil by fast pyrolysis as addressed by Ringer^[4] and Dynamotive ^[5]. Investigations on the preparation of biooil from rice straw have been reported by Putun ^[6], Islam^[7], Wang^[8] and Tu^[9] Several fast pyrolysis reactor configurations exist today including ablative reactors, entrained flow reactors, rotating core reactors, vacuum pyrolysis reactors, circulating fluidized bed reactors, and deep bubbling fluidized bed reactors Mullaney ^[10]. Fast pyrolysis on a near-commercial status has been reported in Canada and UK Bridgewater ^[11], Finland Oasmaa ^[12] and the Netherlands Venderbosch ^[13]

Various techno-economic analyses of fast pyrolysis plants are available in the literature. Prior investigators estimated bio-oil production costs to range between US\$ 0.11 and \$0.32 per litre Ringer ^[4]. Other investigators Mullaney ^[10] developed economic model for bio-oil plants for 100, 200 and 400 ton /day wet wood plant. Cost per litre was estimated to be about US\$ 0.34, US\$ 0.26 and US\$ 0.22 for the above mentioned capacities respectively. Also, Brigdwater (2009) has carried an analysis of several plants around the world out and updated their data to 2008 to develop generalized short-cut equation for the capital cost including all design, equipment, construction, civil work and commissioning.

The analysis in this work is based on experimental and published work, in particular; the National Renewable Energy Laboratory (NREL) report by Ringer ^[4] for a 550 dry ton/day plant producing 28 million gallons of bio-oil per year at a cost of US\$ 0.17 per litre. This analysis includes the detailed techno-economic study of pyrolysis technology from wood.

This paper is concerned with the production of bio-oil from rice straw by fast pyrolysis. Experimental studies on bench-scale have been conducted, oil characteristics have been identified and engineering data for the design and techno-economic assessment of a full-scale facility of 200 ton / day biomass (rice straw) dry basis have been developed.

2. EXPERIMENTAL 2.1 Material and Methods

The reactor used is the entrained flow type in which heat is transferred to the pyrolysing biomass particles from the hot gases by convection. A bench scale set-up has been designed, constructed and tested. The set-up comprises the followings:

A feed vessel, a screw conveyor for feeding the straw to the bottom of an entrained flash pyrolyser flow reactor operating at atmospheric conditions and using nitrogen gas as a carrier medium .The gas is preheated before being introduced into the bottom of the reactor. The reactor is made of stainless steel with about 50 mm diameter and 3500 mm height. Eight temperature controlled electric heaters of 0.2 kW each provided the heat required for biomass pyrolysis. The pyrolysis products pass through a cyclone to collect the char before being quenched in a double pipe- cooler to condense the bio-oil while the non-condensable volatiles are released. The set-up is provided with the necessary instruments to follow- up temperatures and rate of flow. The experimental set-up is depicted in Figure 1.

Fast pyrolysis experiments have been conducted using milled rice straw of particle size 1-2 mm at temperatures in the range 450 to 600°C. The capacity of the experimental set-up is about 1 kg/hr (batch fed with about 25 gm every about 2 minutes). Nitrogen flow has been in the range of 15 to 30litres/min. The residence time of the volatile matter is around 1-5 s.



1-cyclone cooler/condenser, 2- reactor/ cyclone connection, 3-cyclone, 4, 5, 6-1st, 2nd, 3rd 4th cooler/condensers,

7, 8-condensate collectors and accessories, 9- reactor, 12-sleeve, 14-feeding hopper, 17-screw,

Figure 1. Experimental set-up for fast pyrolysis of rice straw

2.2 Characterization

a) Rice Straw Characterization

A sample of newly harvested rice straw was washed, dried and milled and laboratory analyzes for moisture content and water soluble materials. The weight percentage composition as determined according to standard procedures is provided in Table (1).

b) Bio-oil Characterization

The collected bio-oil has been characterized through measurement of the following:

- a) Elemental composition using Vario El Elementar Analyzer
- b) Calorific value using IKA-C 2000.
- c) Types of functional groups by Fourier Transfer Infra Red FTIR using JASCO FT/IR 300 Fourier Transfer Spectrometer N
- d) Structure of organic compounds by Proton Magnetic Resonance Spectroscopy 1H-NMR using Jeol-Ex-270 NMR Spectrometer

2.3 Results

2.3.1 Rice Straw characteristics

Typical composition of rice straw samples used in this work is presented in Table (1).

Table (1) Analysis of Egyptian rice straw (on dry basis)

Rice Straw Component	%
Pectic substances	2.3
Holocellulose	71
a) α-cellulose	34.94
b) hemicellulose	36.06
Lignin	12.3
Soluble materials	14.17
Total	100
Ash	18.5

2.3.2 Bio-oil Characteristics

The elemental composition of the bio-oil as collected at different pyrolysis temperatures using Vario El Elementar Analyzer is presented in Table 1. Average values are almost in agreement with reported data. Highest carbon content and H/C molecular ration is at pyrolysis temperature 550 C.

Table (2) Elemental Analysis	of Bio-oil Samples at Var	rious Pvrolvsis Temperatures
	or bio on Sumples ut vu	ious i jioijsis i emperatures

Temperature C	С%	H%	N%	O% (by difference)
450	49.939	3.877	2.473	43.711
500	47.419	3.344	2.556	46.681
550	54.122	9.55	1.761	34.567
600	47.41	3.744	1.791	46.29
Average	49.723	5.129	2.337	42.812
Reported Average Bridgewater (1999)	44-47	6.50	0-0.2	46-48

The calorific value has been found to have an average value of about 29kJ/kg. This is in agreement with data for bio-oil from rice straw published by Putun ^[6] which is relatively higher than average reported data by Bridgewater ^[3] for bio-oil from other biomass (22kJ/kg). Results of the H- NMR and FTIR are depicted in Figures (2) and (3) and Table (3). These results indicate the existence of following:

- Hydroxides at wave length 3150-3600 cm-1
- Esters and carboxylic acid derivatives at wave length 1720-1780 cm-1
- Methyls and methylene groups at wave length 2930-2980 cm -1
- Aromatic compounds at wave length 700-900 cm -1
- Amines at wave length 2361cm-1 for biooil prepared at 500-600 but not at 450oC indicating that amines are not formed at lower temperatures.
- The H- NMR confirmed the presence of the phenyl, phenolic, ethanol, methyl and methylene groups



Figure (2) Results of H- NMR for biooil produced at 550 $^{\rm o}\,{\rm C}$



Figure (3) FTIR for bio-oil produced at 550 ° C

3. PROCESS DESIGN OF PROPOSED COMMERCIAL FACILITY

The process design of a production unit processing 200 ton/day rice straw has been developed. This capacity has been selected guided by a demonstration commercial facility for the production of biooil from ligno-cellulosic materials (Dynamotive ^[5]. Design indicators have been based principally on the results of undertaken experimental investigations as outlined in section 2 and complemented by published data reported by Ringer ^[4] and others. Since bubbling fluidized bed is the recommended reactor for implemented facilities, it has been adopted in this work. The process design for the fast pyrolysis process essentially comprises the following stages:

3.1 Rice Straw Handling and Preparation

Bales of rice straw are opened and transferred to the milling area using a set of appropriate conveyors. The straw is then passed below a magnetic separator before it is directed to knife mills. The milled straw is then sieved using mechanically vibrating screens with 2mm openings. The milled straw of size <2mm is then fed to a storage bin equipped with rotary air locked feeder to ensure the regular and steady feed to the pyrolyzer.

3.2 Fast Pyrolysis in a Fluidized Bed Reactor

The bubbling fluidized bed reactor contains a bed of silica sand which is fluidized using clean gases produced from the pyrolysis as well as inert nitrogen gas. The milled rice straw is introduced at the bottom of the reactor where it is mixed with the hot agitated sand particles and transformed to three basic phases:

§ A solid phase comprising small carbon particles and ash (char)

§ Condensable vapours which essentially constitute the biooil product

§ Non- condensable gases

The fluidized silica sand, which withstands a temperature of about 1500 $^{\circ}$ C and is in a state of continuous turbulence, promotes fast and homogeneous heat transfer to the rice straw particles ensuring high efficiency of the pyrolysis process and the continuous and rapid evacuation of the produced vapours. The reactor is indirectly heated using the gases produced from the char burning, as described below, as well as the non- condensable gases which have been cleaned using electrostatic precipitator and are burnt in the pyrolyzer to get rid of all combustible matters. The fast pyrolysis process is completed in about 1-5 seconds at 450-550 $^{\circ}$ C.

3.3. Solid Phase Separation and Combustion

The char is separated from condensable vapours and gases in high efficiency cyclones and is temporarily stored before being directed to a combustor using pneumatic conveyor. The char is combusted at about 1600-1800 °C and the hot gases are used for heating the pyrolyzer.

3.4 Bio-oil Cooling and Storage

After separating the char, the condensable vapours and non-condensable gases are cooled to about 100 $^{\circ}$ C by a condenser using chilled water at 4 $^{\circ}$ C. The condensed vapours are further cooled to about 40 $^{\circ}$ C to form the bio-oil which is then stored.

3.5 Cleaning and Reusing the Non-Condensable Gases

After condensing the bio-oil, the resulting gases and residual vapours are directed to electrostatic precipitators before being reused in the fluidized bed reactor.

The proposed fast pyrolysis process is depicted in Figure (4).

4. SIMULATION SOFTWARE

The process has been simulated using ASPEN PLUS 2006.5 from ASPENTECH. Detailed material end energy balance has been developed. The physical data for the components was obtained either from the ASPEN inhouse databank or from literature. Equipment sizing and basic engineering have been developed manually for capital cost evaluation. Figure (3) presents process flow sheet as depicted by the program for selected processing areas while table (2) presents basic equipment blocks utilized in the simulator.

4.1 Design Basis

The production of bio-oil from rice straw was modelled using various assumptions. Table 1 summarises key design parameters.

5. TECHNO-ECONOMIC INDICATORS

Techno-economic indicators have been developed under Egyptian conditions as a case study.

5.1 Basis of Cost Estimates

Equipment costs have been obtained from ASPEN ICARUS whenever available. Other equipment costs have been obtained from other published sources, such as Peter's et.al ^[15], as updated to 2007 using Marshal and Swift cost index. ASPEN ICARUS was also used for estimating material cost as well as installation cost for nearly all equipment used in the process. ASPEN ICARUS was also used to for estimating material and installation cost for auxiliary

equipment such as piping, insulation, electrical... etc. Manpower, utilities and raw material costs have been estimated using current Egyptian market prices. Biooil sales price has been assumed to be about US\$ 0.13/kg which is 30-40% of the price of fuel oil #2 on international market as of August 2008.

5.2 Results

Financial Analysis has been conducted using ASPEN ICARUS 2006.5.Table (4) shows the distribution of equipment costs among the processing stages while Table (5) presents the breakdown of capital costs.. Elements of fixed capital costs have been analyzed to take into consideration the possibility of probable variations. Accordingly, four scenarios have been developed. Table (6) shows rice straw fast pyrolysis annual operating cost based on 200 ton / day plant size for Scenario I. Estimates for the four Scenarios as presented in Table (7) and Figure (5). The sales price of biooil which provides an Internal Rate of Return (IRR) of 10% which is the current prevailing discount factor has been estimated as presented in Table (7).

Results indicate that the IRR for the Base Case exceeds 43% for Fixed Capital Cost of about US \$ Million 7.6. However, the IRR remains about 30% if the Fixed Capital Cost increases to about US \$ Million 10.9 as depicted in Scenario IV.



Figure (4) Process Flowsheet of the Proposed Pyrolysis Facility

Parameter	Value			
1. Feedstock				
Туре	Rice straw			
Moisture content	8-10%			
Throughput	200 ton / day			
Particle size	2 mm			
2. Pyrc	olysis design			
2.1	Reactor			
Pyrolysis type	Bubbling fluidized bed			
Pyrolysis temperature	550 C			
Pressure drop across the reactor	5 KPa			
Input pressure	30 KPa			
Retention time	1-6 seconds			
Feed	833 kg / hr			
Ground Particle size	2 mm			
2.2	2 Gases			
Carrier gas	2.75 kg gas / kg feed *			
2.3 Be	ed material			
Туре	Silica sand			
Particle size diameter	1 mm			
Density	3.4 g /cm3			
3. Yield %				
Oil	27			
Char and ash	27			
Gas	26			
Water and others	20			

Table (3) Key Design Parameters for Bio-oil Production

*Nitrogen gas is used for process start up, for steady state operation recycled clean gases are used in fluidized bed pyrolyser

Table (4) Distribution of equipment cost among processing stages

Stage	%
Rice straw handling and preparation	19
Pyrolysis reactor	25
Product recovery and storage	21
Heat recovery	35
Total	100

Table (5) Distribution of Capital Costs components

Item	%	Remarks			
Purchased Equipment	100	Of Purchased Equipment			
Equipment Installation	30	Of Purchased Equipment			
Piping	15	Of Purchased Equipment			
Civil	15	Of Purchased Equipment			
Steel	15	Of Purchased Equipment			
Instrumentation	10	Of Purchased Equipment			
Electrical	10	Of Purchased Equipment			
Insulation	8	Of Purchased Equipment			
Paint	8	Of Purchased Equipment			
Other	10	Of Purchased Equipment			
Engineering	10	Of Total Project Costs			
Contract Fee	5	Of Total Project Costs			
Contingencies	10	Of Total Project Costs			
Total Project Costs					

Item		Cost (\$)	
Operating Costs \$		1,068	
1	Raw Materials	390	
2	Utilities	470	
3	Operating Labor and Supervision	55	
4	Maintenance Labor	154	
Fixed Costs – Depreciation		427	
Plant Overhead		10	
General and Administrative Costs		120	
Total Annual Production Costs		1,626	

Table (6) Rice Straw Fast Pyrolysis Annual Operating Cost \$ (Scenario I)

Table (7) Financial Indicators for Proposed Scenarios

Item	Ι	II		IV
Capital Costs (US \$ Million)	7.64	8.55	9.27	10.91
Annual Production Cost (US \$ Million)	1.63	1.71	1.75	1.87
IRR % @ US\$ 0.13/kg biooil	43.5	39.4	35.7	30
Net Present Value (US\$ Million)	17.84	16.85	15.99	14.16
Price of Biooil for IRR 10 %	0.058	0.061	0.064	0.069



Figure (5) Results of Financial Analysis for Proposed Scenarios

5. CONCLUSIONS

Bio-oil with adequate characteristics has been produced by fast pyrolysis of rice straw which is currently an environmental nuisance. Experimental results indicate that bio-oil with relatively high yield could be could be obtained at 550° C. The produced bio-oil has composition of 54% C, 9.55% H₂, 1.76% N₂ and 34.56% O₂ with calorific value of 29 kJ/ kg. Based on process design and basic engineering for an integrated scheme, techno-economic appraisal indicated that the process is viable. However, production facilities have to take into consideration storage and transport requirements. Also, it is necessary to ensure steady production using other ligno-cellulosic materials along the whole year.

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Effect of Stitch Geometry on Particle Bypass in Air Filter Bags

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Abstract: Fabrics used as a filter media in dust control may have satisfactory efficiency. However, the sewn areas of the filter fabric sleeves may cause a bypass of fine particle size dust through needles holes or bent areas of fabric layers. The present study focuses on the filtration and cleaning efficiency of the sewn areas of filter fabrics using different particle size of solid material. Four types of stitch formation type EFa-1, SSa-1, LSc-1 and BSa-1 according to British Standard BS 3870 were used.

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Keyword: Filter Bag – sewing stitch – sewing machines- sewing threads

1. Introduction:

Filter fabrics are tested and examined for physical and mechanical properties subsequent to usage and application in a typical industry. Nevertheless, it is more important to measure the filter performance in terms of air permeability, filtration efficiency, cleaning efficiency and pressure drop. From the practical point of view, the fabrics having particular air permeability and filtration efficiency may be used in a particular type of industry. Early investigations[1-4] show that air permeability of different materials and fabrics used with gaseous and liquid media were measured using different methods, e.g., by drawing air through filter fabrics via suction fans or compressed air, and then measuring the pressure drop using water manometers or gas meters.

Different filtration apparatus were described in previous literature by Lamb[5], Igwe[6], Chatterjee[7] and Saad [8-10]. However, the main parameters influencing the filtration efficiency of the filter media are deduced from different variables, which are as follows:

1. Yarn variables: Count, twist and napping.

2. Woven fabric variables: Woven staple fibers: weave – set, woven monofilament: weave - filament denier and woven multi filament: weaveintermingling – number of filaments per crosssections.

3. Nonwoven fabric variables: Mechanical web weight, needling density, needling penetration, number of barbs, needle arrangement, shape of holes in bed plate and needle gauge and type.

4. Field variables: Pressure drop and flow rate, rate of jet-pulse, duration of jet-pulse, quality and size of dust particles and dust moisture content.

The aim of the present work is to optimize the factors affecting seam efficiency of different parts of the filter bag. During filtration and cleaning process such parts are subjected to stresses and dust bleed through the sewing lines might happen.

Sewing machines:

Nowadays, industrial sewing machines are much diversified and packed with mechanical and electronic high-technology. Nevertheless, all of the basic mechanisms of stitch formation remain similar to those created during the second half of the 19th century.

Stitch and seam type classification:

Stitch formation is the actual process by which threads are interlaced in or around a material resulting in a stitch. When a stitch is used with a defined geometry for material layer positioning, a seam is formed. Stitch and seam types are classified in specific international and national standards [11-17].

Seams perform many different functions, and all of the standards regarding their classification are quite extensive. Fig. 1 shows examples of seams and their representation, classified according to the British Standard 3870 [11].

Stitch types are chosen for a seam depending on the functional or aesthetical requirements of the seam. All of the referenced standards are very similar in the way stitch types are classified.

Stitch Formation:

The over-edge, or over-lock stitches, class 500, are performed according to Portuguese standards [11].

Filter Bag Sewn Parts:

The following options can be manufactured as part of the standard bag design or used to compensate for minor equipment or system problems [15].





Fig. 2 Snap band 3-notch designs eliminates the separate tool needed to lock and release the collar, making removal and installation easier



Fig. 3 Discs (metal rings) are sewn to the filter bag with an over lock stitch



Fig. 4 Anti-collapse rings into filter Bags using lock stitching



Fig. 5. Woven fiber glass bags are sewn with three needle chain stitch in vertical seam

Sewing Threads:

Factual information about sewing threads and sewing needles suitable for various kinds of fabrics is provided by YLI Corporation [19].

The proper sewing needles selection is determined by two key factors:

1) the thread to be used and 2) the fabric to be sewn. Thread/ Machine Needle Charts are a good guide in the industry in order to minimize thread breaks caused by a number of variables including:

- Using the wrong thread for the application.
- Incorrect needle or damaged needle.
- Thread defects.
- Too much elongation.
- Being sewn with too much tension.
- Worn machine parts.
- Machine out of adjustment.
- Operator handling.

Fabrication and description of filtration apparatus:

Due to non-availability of a standard and reliable apparatus for measuring filtration efficiency of filter fabrics, an attempt was made to build up a simple apparatus with facilities to allow direct evaluation of filtration efficiency, flow rate, pressure drop and cleaning efficiency. The main purpose behind the design and development of the concerned apparatus is to ensure the efficiency and durability of filter fabrics to avoid risking failure when a proposed filter is introduced. Inefficient filter means production interruption as well as higher dust emissions. Using such apparatus may well lead to less cost and to reliable decisions in selecting filter fabrics, Figures (7 and 8) show the photographs of the apparatus, it consists of the following parts:

- 1. Particle separator unit air sampler
- 2. Master filter.
- 3. Test filter sample
- 4. Glass dust collection chamber.



Fig.6. Nonwoven filter bags are sewn with an over lock stitch consisting of approximately 16" of thread per linear inch

- 5. Flexible joint.
- 6. Electronic control unit for jet pulse system.
- 7. Differential pressure gauge.
- 8. Dust vacuum chamber
- 9. Dust feeder.
- 10. Air compressor
- 11. Magnihelic (differential pressure device).
- 12. Low pressure inlet (downstream).
- 13. Nozzles for air circulation
- 14. High pressure inlet (upstream).
- 15. Clean air inlet to jet pulsing.
- 16. Residual Collected dust.
- 17. Tested Sample.

A) Particle Separator Unit Air Sampler:

This unit is based on inertial impact system adapted to be used in work sites of different dust concentration and suction flow rate up to 500 l/ min. The unit also has the facility to use a range of pressure drop between 2 and 18 cm WG.

b) Dust Feeder and Dust Chamber:

Dust is fed through a conical tube at controlled rates into a glass chamber whose the dimensions are $75 \times 50 \times 50$ cm. It has the facility to clean the dust via compressed air, so as to obtain uniform scattering inside it and also to prevent sticking of dust against chamber walls.

c) Fabric Sample Holder:

The fabric sample holder is designed so that a wide range of fabrics of different thickness could be accommodated and tested conveniently. They range between the extremely thin and the very thick nonwoven fabrics. The area of fabric under test has a circular diameter of 11.3 cm; it is cut with a standard sample cutter to give a total cut area equal to 100 cm² to facilitate clamping into the fabric holder.



Fig. 7 Photograph of the filtration efficiency apparatus



Fig. 8 Close up photo of test zone

d) Jet-pulse unit:

An electronic control unit is used to apply different rates of jet-pulses and changeable duration of pulses similar to field conditions. Applicable pulse range is between 0.5 and 60 pulse/min.

e) Heat sensor:

Hot gases and heated dust are used to test filter fabrics used in some industrial sites, particularly cement plants. A heat sensor is provided in order to obtain different temperatures of the dust particles and gases inside the vacuum chamber. Temperature up to 90° C could be reached.

f) Pressure Gauge:

The air sampler unit is provided with a pressure gauge to adjust the flow rate of gas through the tested fabric.

2. Materials and Methods: Test method
The fabric sample to be tested is placed in contact with a vacuum chamber fed with different concentrations of dust having a certain quality and characteristics. The particle separator evacuates air from the dust chamber through an outlet port for emitting air. The vacuum is indicated on a scale. The separating unit is provided with a master filter made of glass fibers, housed at the front face of the air sampler to prevent micro dust particles that may escape through the tested filter fabric sample. During the evacuation of dusty air through the tested sample, the cleaning action is automatically performed, and could be adjusted to normal and field conditions. The pressure drop is automatically monitored and measured in the filtration experiments. Mass efficiency is obtained by weighing the amount of fly dust on the surface of the sample filter. The filteringcleaning cycles are run for each filter sample and the efficiency is measured after time intervals of 15 minutes. And thus filtration efficiency (E %) is given as:

$$E\% = \frac{\left\{ \text{ (Mass of dust fed)} \cdot \text{(Mass of dust deposited on master filter)} \right\}}{\text{Mass of dust fed}}$$

Materials

All fabrics tested using the present apparatus were successfully used as bag filters in a number of filtration applications, where woven fabrics and conventional scrim supported needle felts are used in application. These include cement, aluminum, iron and steel, fertilizers and other industries. The article is the outcome of an experiment in which needle punched nonwoven fabrics were produced with the following specifications:

- Fabric quality: Polyester (3 denier), Nomix (3 denier).
- Nominal fabric weight 200, 300, 400, 500, 600 (g/sq.m).
- Needle penetration 3/8 inch.
- Scrim Support Fabric: 2/2 twill polyester woven scrim fabric, 22 warp / inch, 19 weft / inch, 130 g / sq m weight.
- Surface finish: Heat set at 220° C on one side and raised on the other side.

Sewing Variables: Stitches: SSw- LSc- LSb Threads: Nomix and Polyester.

Sewing Machine:

JUKI LH-3100 with 2-needles, Lock-stitch Machine.

Experimental

Both kinds of nonwoven fabrics (Polyester & Nomex) were sewn using the seam types shown in fig. 1 and nomex thread for nomex fabric and polyester thread for polyester fabric. Lock-stitch, class 500 was used according to BS. Thread count and needle number were selected according to charts provided by YLI Corporation [18].

Fig. (9) Shows all samples of the fabrics represented in this study: Snap band cuff (see#1), Flange Top (see#2), Ring Top (see#3), Sleeve Top (see#4), Hem Top (see#5), Raw Edge (see#6).



Fig.9 Samples of filter fabrics represented in study

3. Results and Discussions:

Typical bag-houses have cylindrical bags hanging vertically in the unit, representing a critical component of operation. Bag fabric and design should be designed for maximum filtration efficiency, cake release, and durability.

Pulse-jet and plenum pulse bag-houses collect dust on the outside of the filter. Dust laden gas floods the bag-houses, and clean air exits through the inside of the bag while the dust particles collect on the outside filter surface. A support cage prevents bag collapse during filtration and aids in the redistribution and cleaning of the dust-cake. A wide variety of filter bag can be manufactured to meet specific application needs.

Results of measuring filtration efficiency of sewn areas for both styles of nonwoven fabrics are given in table (1).

Table (1) Optimum sewing factor for both Polyester and Nomex fabric weights.

Nominal weight gm/m2 sewing	200	300	400	500	600
Seam Type	SSw	SSw	LSc	LSc	LSb
Thread NO. "Tex"	16	16	24	24	35
Needle Count	70/11	70/11	80/11	80/11	90/14
Filtration Efficiency"%"	99.9	99.9	99.9	99.9	99.9

Filtration Efficiency of sewn areas:

Different seam stitches shown in fig. (1) were tried for both kinds fabric samples. The filtration efficiency test using the apparatus shown in fig. (7) was run for all sewn samples. Table (1) show the suitable stitch, needle and thread to achieve an efficiency value of 99,9%. The criterion for assessing optimized factors of sewing is not to experience any distortion or wear away of the sewn part.

4. Conclusions:

- 1-The most suitable stitch for the fabric weighed $200,300 \text{ g/cm}^2$ is SSw with needle count 90/14.
- 2-The most suitable stitch for the fabric weighed $400,500 \text{ g/cm}^2$ is LSc needle count 80/11.
- 3-The most suitable stitch for the fabric weighed 600 g/cm^2 is LSb needle count 70/11.

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Diagnostic Value of Serum Cystatin C as an Early Indicator of Renal Impairment in Chronic HCV Egyptian Patients with Liver Cirrhosis

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Abstract: Background and aim: Diagnosis of moderately impaired renal function is of great importance in patients with liver cirrhosis. Patients with a markedly impaired glomerular filtration rate can be diagnosed easily by elevated serum creatinine concentrations but, moderately reduced renal function may be missed. Cystatin C (CysC) has been suggested as a sensitive marker of renal function, independent of sex or muscle mass. Therefore, the aim of this study was to investigate the value of serum cystatin C concentrations for the detection of moderately impaired renal function in chronic HCV Egyptian patients with liver cirrhosis as well as its correlation with Child-Pugh score and renal resistive index (RRI). Patients and Methods: This study was conducted on seventy subjects; group I fifty non azotemic chronic HCV patients with liver cirrhosis (furtherly subdivided according to the Child-Pugh score into group I^a, I^b, I^c) and group II twenty healthy subjects with matching age and sex as control group. Liver function tests, renal function tests, CysC levels and RRI were measured on the same day for all patients. CysC levels were measured using the automated latex-enhanced immunonephelometric method. Results: Mean serum levels of serum Cystatin C were 0.66±0.05, 1.02±0.28, 1.17±0.32 and 0.65±0.10 mg/dl in groups I^a, I^b, I^c and II respectively. Serum cystatin C was significantly higher in cirrhotic patients than in controls. Moreover, It was significantly higher in Child C cirrhotic patients than in those with Child B and A (F=19.14 and P=0.001). Significant positive correlations were found between serum cystatin C and each of blood urea (BU), serum creatinine, RRI and Child-Pugh score in patients with HCV induced liver cirrhosis.(r=0.454.0.781,0.508 and 0.412respectively) (p≤0.01). On the other hand, significant negative correlation was found between serum cystatin C and creatinine clearance. (r= -0.746 and $p \le 0.01$). Conclusion: Determination of serum cystatin C is advantagous over serum creatinine particularly in early detection of mild renal impairment in patients with liver cirrhosis.

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Keywords: Chronic Hepatitis C, Liver cirrhosis, serum markers of GFR and serum cystatin C

1. Introduction:

Impairment of renal function in patients with liver cirrhosis usually progresses in parallel with severity of liver cirrhosis and portal hypertension (1). Diuretic abuse, gastrointestinal bleeding and infection are common predisposing factors of renal dysfunction(2).

Patients with liver cirrhosis and functional renal failure are particularly sensitive to decreased plasma volume. (3) Therefore, parameters of moderately impaired renal function are valuable and close monitoring of renal function is of great clinical importance (1).

In patients with liver cirrhosis, malnutrition and reduced muscle mass can accentuate the difference between serum creatinine level and actual renal performance. Moreover, measurement of Creatinine might be influenced by high serum bilirubin. Although, inulin clearance is considered the gold standard for measurement of glomerular filtration rate (GFR), its cost makes it difficult to use in clinical practice. (4)

Cystatin C is a non-glycosylated low molecular weight protease inhibitor produced by

nucleated cells. It is considered as a sensitive indicator of early renal impairment and glomerular filtration rate over serum creatinine. (5) Furthermore, its value is not affected by sex, muscle mass, hyperbilirubinaemia or haemolysis. (5,6) The rate of production of cystatin C is stable regardless of age or any inflammatory process. (6)

Aim of the work

The aim of the present work was to verify the value of serum cystatin C concentrations in chronic HCV Egyptian patients with liver cirrhosis as well as its correlation with Child-Pugh score and renal resistive index (RRI).

2. Patients and Methods:

Patients

This study was conducted on seventy subjects; fifty non azotemic chronic HCV patients with liver cirrhosis (further subdivided according to the Child-Pugh score) and twenty healthy subjects with matching age and sex as control group. Diabetic, renal and hypertensive patients were excluded from the study. All patients were chosen from Tropical Medicine Department, Alexandria University. Patients gave their informed consent to participate. The protocol was approved by the committee of ethics medical research, Faculty of Medicine, University of Alexandria .Proper and detailed history taking and thorough clinical examination were done.

Laboratory analysis

Routine laboratory investigations including; complete blood picture, liver function tests, renal function tests, fasting and 2hr-postprandial blood sugar and lipid profile (triglycerides, cholesterol, VLDL, LDL, HDL) and complete urine analysis.

Serum samples were obtained on the day of urine collection for creatinine clearance and measurement of creatinine, urea, and cystatin C concentrations. Creatinine was analyzed by a rate blanked modified Jaffé method. (7) Urea was determined using a kinetic urease method followed by a GLDH-UV test where the decrease in NADH adsorbance is determined photometrically. (8) Both assays (Roche Diagnostic Systems, Mannheim, Germany) were implemented on an Hitachi multianalyzer system. The between run coefficient of variance of these methods was constantly below 3%. Cystatin was determined with the Dade Behring N Latex Cystatin C assay (Dade Behring Diagnostics, Marburg, Germany), (9) a particle enhanced nephelometric immunoassay implemented on the Dade Behring Nephelometer II. Intra and interassay coefficients of variation were always below 5% in accordance with earlier reports. (10) Creatinine clearance (CrCl) was calculated as a product of urinary Cr and 24-h urine volume divided by serum Cr (mg/dl) and multiplied by 1440.

Renal resistive index

Each individual was assessed using renal Doppler ultrasonography. The radiologist used the same ultrasound machine (Acuson X300, Siemens, Germany) and a convex transducer with a 3.5 - 5MHz probe to assess renal hemodynamics in all subjects. To avoid confounding effects on renal hemodynamics caused by food ingestion, all examinations were performed in the morning after overnight fasting. The intrarenal arteries were evaluated bilaterally using color Doppler images of the distal arcuate branches. At least three peak systolic flow rates and three peak diastolic flow rates were noted for each individual and average values were recorded. RRI was then calculated using the formula: peak systolic flow - end-diastolic flow/peak systolic flow.

Statistical analysis:

Differences between groups were analyzed with the unpaired t test or the Mann-Whitney U test, where appropriate. Data are presented as mean diagnostic (SD).Sensitivity, specificity, and efficiency were calculated for each value of cystatin C, creatinine, and RRI (11). The cut off value was then determined at the maximum efficiency derived from analyzing all patients. The significance of differences in sensitivity, specificity, or efficiency between the parameters was evaluated by the McNemar test with a two tailed probability. A p value <0.05 was considered statistically significant for all tests applied.

Receiver-operator characteristic (ROC) curves were calculated by standard procedures. (12) The area under the curve (AUC) and 95% confidence interval (CI) were calculated for each plot. The statistical significance of differences between AUC values was determined as proposed recently. (13) Accordingly, a z value was calculated for each comparison and values of z above 1.96 were taken as evidence that ROC areas were different, assuming a two tailed probability.

3. Results

The characteristics of the 50 enrolled patients and 20 controls are presented in Table I.

The mean value of serum cystatin C was significantly higher in patients with liver cirrhosis than controls $(1.05\pm 0.32 \text{ and } 0.65\pm 0.10 \text{mg/dl} \text{ respectively})$ (t=5.29, P= 0.001). Moreover, serum cystatin C was significantly higher in Child C cirrhotic patients than in those with Child B and A (F=19.14 and P=0.00)

Significant positive correlations were found between serum cystatin C and each of BUN, serum creatinine, RRI and Child-Pugh score in patients with HCV induced liver cirrhosis.(r=0.454,0.781,0.508 and 0.412respectively) (p \leq 0.01) (Table II). On the other hand, significant negative correlation was found between serum cystatin C and creatinine clearance. (r= -0.746 and p \leq 0.01) (Table II).

In patients with liver cirrhosis Receiveroperator characteristic (ROC) (Figure 1) curve analysis showed that the diagnostic potential of cystatin C and RRI to detect patients with a creatinine clearance of less than 80 ml/min was superior to that of creatinine. Cys.C at a cut off value of 1.14mg/dl had 85% specificity and 66% sensitivity. RRI at a cut off value of 0.665sec had 74% specificity and 79% sensitivity. Serum creatinine at a cut off value of 1.0500mg/dl had 70% specificity and 62% sensitivity to detect patients with a creatinine clearance of less than 80 ml/min.

Table (III) shows positive predictive value, negative predictive value and efficacy of serum Cystatin C, RRI and serum creatinine in the prediction of decreased creatinine clearance of less than 80 ml/min.

Table I: The characteristics in the four studied gr	oups.
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	Group I ^a	GroupI ^b	GroupI ^c	Group II	F	Significance
	Child A	Child B	Child C	Controls		between
	(n=7)	(n=20)	(n=23)	(n=20)		Groups
Sex, M/F	2/5	13/7	13/10	11/9	-	-
Age, years	43.5±1.61	42.±7.73	44.5±4.68	44±2.17	0.822	-
Bilirubin (up to	0.54±0.05	1.05 ± 0.58	4.27±1.50	0.29±0.11	50.999	I ^a -I ^c , I ^b -I ^c , I ^b - II,
1.00 mg/dl)						I ^c - II
Prothrombin time	14.71±0.26	15.34±2.05	20.89±2.57	11.70±1.41	37.933	I ^a -I ^c , I ^a - II, I ^b -
(11-15 sec)						I ^c , I ^b -IV, I ^c - II
Albumin (3.4-5	3.05 ± 0.05	2.42±0.52	1.91±0.22	4.17±0.17	24.898	I ^a -I ^b , I ^a -I ^c , I ^a -
g/dl)						IV, I ^b -I ^c , I ^b - II,
						I ^c -IV
BU(7-18 mg/dl	9.57±0.53	14.25±12.94	15.86±6.41	11.50±5.68	1.450	-
Creatinine (0.6-1.3	0.71±0.01	1.03±0.33	1.10±0.23	0.62±0.07	4.966	$I^{a} - I^{b}, I^{a} - I^{c}, I^{b} -$
mg/dl)						II, I ^c - II
Creatinine	121.14±1.06	81.94±14.98	79.63±33.06	151.75±16.11	6.448	I^{a} - I^{b} , I^{a} - I^{c} , I^{a} –
Clearance (80-						II, I ^b - II, I ^c - II
130ml/min						
Cystatin C (mg/dl)	0.66±0.05	1.02±0.28	1.17±0.32	0.65±0.10	7.857	$I^{a} - I^{b}, I^{a} - I^{c}, I^{b} - I^{c},$
						I ^b - II, I ^c - II
RRI (sec)	0.57±0.01	0.63±0.03	0.73±0.08	0.53±0.02	22.892	$I^{a} - I^{c}, I^{b} - I^{c}, I^{b} -$
						II, I ^c - II
Child Pugh score	6±0.00	8.05±0.68	12.26±1.78		-	-

* Significant at $P \le 0.05$ Data are given as mean±SD

Table II: Co	orrelations	between	serum	Cystatin	С
and studied	parameters	S			

	Serum cystatin C
BUN	0.454**
Cr	0.781**
CrCl	-0.746**
RRI	0.508**
Child-Pugh score	0.412**

**. Correlation is significant at the 0.01 level (2tailed).



Figure 1: Receiver-operator characteristic curves for detection of patients with a creatinine clearance less than 80 ml/min. Sensitivity and specificity are displayed at various discrimination levels for serum concentration of cystatin C, RRI and serum creatinine.

Cr Cystatin RRI Reference Line

Table (III): Positive predictive value, negativepredictive value and efficacy of serum Cystatin C,RRI and serum creatinine

	Positive predictive value	Negative predictive value	Efficacy
Cystatin C	80%	73%	76%
RRI	70%	78%	74%
Serum creatinine	60%	66%	64%



Figure 2: US picture showing cirrhotic shrunken liver with caudate lobe hypertrophy.



Figure 3: US picture showing cirrhotic liver and ascites.



Figure 4: Renal Doppler Ultrasonography

4. Discussion:

Development of hepatorenal syndrome in patients with liver cirrhosis can be detected by measurement of serum creatinine. (14) On the other hand, serum creatinine levels can be influenced by multiple factors and it can miss cases with mild renal impairment. (15)

Therefore, the aim of this study was to investigate the value of serum cystatin C concentrations for the detection of moderately impaired renal function in chronic HCV Egyptian patients with liver cirrhosis as well as its correlation with Child-Pugh score and renal resistive index.

In the current work, Serum cystatin C was significantly higher in Child C cirrhotic patients than in those with Child B and A. Moreover, it was significantly higher in Child B cases than in Child A cases. Our results were in accordance with Randers et al. (16) and El-Agroudy et al. (17)

Gerbes et al. (18) evaluated serum cystatin C concentrations as a marker of renal function in patients with cirrhosis of the liver and found that Serum cystatin C concentrations are significantly increased in patients with cirrhosis and moderately impaired renal function. Similar results were obtained by Ustundag et al. (19)

In our study, the significant positive correlation noticed between serum cystatin C and Child Pugh score might indirectly reflect the degree of liver dysfunction which may limit the export of protein synthesized by the hepatocytes

On the other hand, Woitas et al. (20) reported that although, cystatin C was significantly higher in patients with Child B and C than in those with Child A, no differences were noted between patients with Child B and C.

In our study, 24 of 50 patients with a normal serum creatinine concentration had a decreased creatinine clearance below 80 ml/min. Furthermore, cystatin C showed higher sensitivity than creatinine in detection of reduced creatinine clearance and hence GFR in patients with hepatitis C liver cirrhosis. ROC curve showed more diagnostic accuracy of cystatin C than serum creatinine for evaluating renal function.

Gerbes et al. (18) and Coll et al. (21) found that ROC curve support an advantage of cystatin C over serum concentrations of urea and creatinine and concluded that cystatin C tended to be more sensitive and specific than creatinine throughout almost the whole range of possible cut off values.

Woitas et al, (20) showed that the sensitivity of serum cystatin C for detecting reduced GFR is higher than that of creatinine in patients with liver cirrhosis.

Randers et al, (16) studied 36 patients with liver cirrhosis with normal to severely impaired kidney function and revealed a significant difference between cystatin C and plasma levels of creatinine in detection of reduced CrCl and hence GFR .These results are similar to those of Randers et al (22) and Price et al. (23)

El-Agroudy et al, (17) found that Serum cystatin C showed higher sensitivity and specificity than serum creatinine in the studied subjects and that Cystatin C showed more significant correlation than serum creatinine with GFR by 99mTc-DTPA techniques. Moreover, he reported that a mild degree of renal dysfunction may develop unnoticed as creatinine level may remain in the normal range despite a major decline in GFR, and the use of serum creatinine may inaccurately estimate GFR due to dietary intake, tubular secretion of creatinine.

These results confirm those noticed by Coll et al. (21) They reported that serum cystatin C levels started to increase when GFR was 88 ml/min/1.73 2m, while serum creatinine level began to increase when GFR was 75ml/min 1.73 2m. These data indicate that serum cystatin C may detect mild reduction in GFR than serum creatinine. Wang et al, (24) reported close correlation between creatinine clearance and 99m Tc-DTPA.

Similarly Page et al. (25) reported that the serum creatinine level failed to rise above normal even when the GFR (Tc-DTPA) was very low (less than 25 ml/min) in their study on 23 non-azotemic cirrhotic patients whose mean GFR (inulin) was 32 ml/min,

Meanwhile, Orlando et al. (26) showed similar diagnostic accuracies in cirrhotics for serum creatinine and cystatin C. This may be attributable to methodological reasons as they measured creatinine by the enzymatic PAP method, which is far less sensitive to interferences than the routinely used Jaffe reaction. Demirtas et al. (27) and Risch et al, (28) found that compared to creatinine-based tests, serum cystatin C is a more sensitive indicator of early decreases in GFR in kidney recipients with transplant rejection and in all cirrhosis patients with ascites, regardless of the risk level for hepatorenal syndrome.

Cystatin C is synthesized by all nucleated cells at stable rate. It is completely metabolized within the proximal renal tubules. Accordingly, its low molecular weight with its steady production may clearly reflect GFR (29,30). Furthermore, Barret et al. (29) observed that cystatin C is not an acute phase reactant and is less expensive than the GFR (Tc-DTPA) test. Thus, it seems to be a reliable, fast and easy to use marker of renal failure.

Changes in renal hemodynamics are correlated with the stage of liver failure. Doppler ultrasonography allows non-invasive evaluation of intrarenal arterial resistance, as it can easily reveal renal vasoconstriction due to substantial splanchnic vasodilatation in cirrhosis patients (31).

Sacerdoti et al. (31) suggested that renal resistive index (RRI) measurement is also a sensitive marker of intrarenal hemodynamics and it has been reported to increase even in nonazotemic patients with cirrhosis. (31,32)

In the present work RRI was significantly higher in Child C patients than those with Child B or A. Moreover, it was significantly higher in child B patients than Child A cases. Our results was similar to those of Celebi et al. (33)

Ustundag Y et al (19) observed a trend towards higher RRI in cirrhotic patients with ascites than in those without ascites; however, the difference was not statistically significant. Another study that involved 44 cirrhosis patients revealed that only the subgroup that was azotemic and decompensated had significantly higher RRI compared to non-azotemic decompensated patients. (34)

Further work is needed to identify whether the high level of serum cystatin C in cirrhotic patients results from liver fibrosis or renal dysfunction in consequence of reduced GFR. Takeuchi et al (35) suggested that cystatin C may participate in the progression of fibrosis by inactivating cathepsins.

5. Conclusion:

Determination of serum cystatin C is advantagous over serum creatinine particularly in early detection of mild renal impairment in patients with liver cirrhosis.

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6. References:

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Breast Cancer Gene 1 (BRCA 1) Mutation in Female Patients with or without Family History in Qalubia Governorate

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Abstract: Breast cancer is the most common cancer in women and its impact on morbidity and mortality is significant and well documented. BRCA genes mutation account for most of the cases of familial breast cancer. Female BRCA1 mutation carriers have an 80% to 85% risk of developing breast cancer over their life-time. This study aims to detect 5382insC ,185delAG and C61G mutations in BRCA1 gene in healthy females and breast cancer female patients in Qalubia Governorate and correlate them with the presence or absence of family history of breast &/ or ovarian cancer to allow identification of individuals at high risk. Materials and methods: 50 females divided into 20 healthy females and 30 breast cancer patients with or without family history of breast &/or ovarian cancers were included in the study.185delAG and 5382insC mutation were detected by multiplex mutagenically separated PCR (MS - PCR) and C61G mutation was detected using the RFLP method. Results: It was found that the incidence of BRCA1 gene mutation in the breast cancer group was higher than its incidence in the control group Also the incidence of BRCA1 gene mutation in the groups with family history was higher than in the groups without family history. In addition, multiple exons mutation frequency was higher than one exon mutation in the breast cancer group with family history .Moreover, 5382insC mutation was found to be the most frequent BRCA 1 gene mutation among the females of Qalubia governorate followed by C61G mutation and 185 delAG mutation. Conclusion: In conclusion, BRCA1 gene mutation and multiple BRCA1 exons mutations play an important role in the pathogenesis of familial breast cancer in Qalubia Governorate, Egypt.

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Key words: familial breast cancer, BRCA1 gene, 5382insC mutation, 185delAG mutation, C61G mutation

1. Introduction:

Breast cancer is the most common cancer in women and its impact on morbidity and mortality is significant and well documented (Jemal et al., 2008).Epidemiological studies have revealed several risk factors associated with increased susceptibility to breast cancer including genetic, family history, reproductive history and environmental factors (Lacey et al., 2009). About 5% to 10% of breast cancer patients carry germ line mutations that predispose them to inherited disease (Malone et al., 1998). Several genes have been involved in the pathogenesis of hereditary breast/ovarian cancer, but mutations in the BRCA1 gene are by far the most recurrent (Baudi et al., 2001). Germ line mutations of the BRCA1 gene account for 40% to 45% of hereditary breast cancers and 80% of the families whose members have a high incidence of both breast and ovarian cancers

BRCA1 (breast cancer 1, early onset) was cloned in 1994 based on its linkage to early-onset breast and ovarian cancer and is one of the most important tumor suppressor genes associated with

breast cancer (Zhang and Powell, 2005).. It is located on the long (q) arm of chromosome 17 at band 21 and contains 24 exons and 5592 nucleotides encoding a large protein of 1863 amino acids (Malone et al.,1998; Antoniou et al., 2003 and Chen and Parmigiani ,2007). This protein is called breast cancer type 1 susceptibility protein (Jaworowska, 2009). It is present in many tissues including normal breast and ovarian epithelium .It is either altered, reduced, or absent in some breast and ovarian tumors (Miki et al., 1994). BRCA1 interacts with numerous proteins that are involved in many important biological processes/pathways which may contribute to its tumor suppressor activity. These processes affect cell cvcle checkpoints. transcription. protein ubiquitination, apoptosis, and DNA repair. BRCA1 deficiency causes abnormalities in the S-phase checkpoint, the G₂/M checkpoint, the spindle checkpoint and centrosome duplication since it is involved in all phases of the cell cycle. The genetic instability caused by BRCA1 deficiency also triggers cellular responses to DNA damage that blocks cell proliferation and induces apoptosis. Thus BRCA1

mutant cells cannot develop further into full-grown tumors unless this cellular defense is broken. On the other hand, the absence of BRCA1 allows further genetic alterations, including further tumor suppressor mutations and activation of oncogenes, which overcomes growth defects and ultimately results in breast cancer formation (Zhang and Powell, 2005 and Deng, 2006).

Researchers have identified hundreds of mutations in the BRCA1 gene, many of which are associated with an increased risk of cancer (Breastcancer.org, 2008). Female BRCA1 mutation carriers have an 80% to 85% risk of developing breast cancer over their life-time (Burga et al., 2009). In addition to breast cancer, mutations in the BRCA1 gene also increase the risk of ovarian, uterine, cervical, fallopian tube, pancreatic, colon and liver cancers (Thompson et al., 2002).

In a study of polish population on females with family history of breast & /or ovarian cancer, three important mutations in BRCA1 gene were detected including 5382insC, 185delAG and C61G on exons 20, 2 and 5 respectively (Grzybowska et al.,2002).In addition, exons 2 and 5 of BRCA1 gene were found to have a significant role in protein function .Moreover, other significant studies showed that exons 2, 5 are most likely to harbor germ line BRCA1 mutations (Yassaee et al., 2002).Also 5382insC mutation accounted for 80% of mutations found in both BRCA1 and BRCA 2 genes in the polish population (Górski et al., 2000). The aim of this study is to detect 5382insC, 185delAG and C61G mutations in BRCA1 gene in female patients in Qalubia Governorate and correlate them with the presence or absence of family history of breast or ovarian cancer to allow identification of individuals at high risk.

2. Subjects and Methods:

This study was performed on 50 females who were attending general surgery department -Benha University Hospitals Qalubia governorate – Egypt after taking written informed consent from them. They were divided into two groups: 1- Control group: including 20 healthy females, 10 of them were with a family history of breast &/or ovarian cancers .2- Breast cancer group: including 30 female patients, 15 of them were with a family history of breast &/or ovarian cancers..

∨ Detection of Mutation in BRCA1 gene:

- 1-185delAG mutation on exon 2 was detected by *multiplex mutagenically separated PCR* (*MS - PCR*)
- 2-5382insC mutation on exon 20 was also detected by *multiplex MS PCR*

3-C61G mutation on exon 5 using the RFLP method.

I- Sampling:

3 ml of venous blood was collected on vaccutainer tube containing EDTA. Each sample was mixed and divided into 2eppendorf tubes then stored at -80 for further processing.

- Genomic DNA extraction:

Genomic DNA was extracted from 400 ul of whole blood using Genomix Easy Quick Blood DNA Extraction Kits-USA, according to standard protocols (Sambrock et al., 2001). 50 ul of DNA was eluted. The DNA yields were determined from the concentration of DNA in the elute measured bv absorbance at 260 nm. On the other hand the DNA purity was determined by calculating the ratio of the absorbance at 260nm to the absorbance at 280 nm. Pure DNA had an A260/A280 ratio of 1.7 -2.0. If there is a contamination with protein or phenol, the A260/A280 is significantly less than 1.7. On the other hand if there is a contamination with RNA, the A260/A280 is significantly more than 2.0 (Haque et al., 2003). The extracted DNA was then stored at -20°C until further processing.

-Detection of 185delAG and 5382insC BRCA1 gene mutations by multiplex MS -PCR: 1-DNA Amplification:

Primers were designed for amplification of 185delAG and 5382insC BRCA1 gene mutations in exons 2 and 20 respectively to be suitable for PCR for these genes (Chan et al., 1999). Table (1) shows the sequence of these primers.

Amplification was done using Dream Taq Green PCR Master Mix (2x) supplied by Fermentas, Germany. The PCR mix for each exon contained 25 ul of Taq PCR master Mix 2x, 2.5 ul of each of the three primers, 5 ul of the template DNA and 12.5ul of nuclease free water to reach a final volume of 50 ul. *G storm* thermal cycler Uk was used for amplification according to the following program: initial denaturation at 95 °C for 3 mins, 35 cycles of denaturation at 95 °C for 1 min, annealing at 50 °C for 1 min and extension at 72 °C for 2 mins, followed by final extension at 72 °C for 10 mins then hold at 4 °C.

2- Agarose gel electrophoresis:

10 μ l of each amplified DNA & 1000 bp ladder (molecular weight marker) were separated on 2% agarose gel containing 0.3 μ g/ml of ethidium bromide .The bands were visualized using UV transilluminator (254nm), photographed & analyzed table (2).

BRCA 1 gene	Mutation detected by primers	Primers	Primer sequence 5' 3'	Alleles amplified using these primers
		Common forward	GGT TGG CAG CAA TAT GTG AA	
Evon 2	185delAG mutation	wild type reverse	GCT GAC TTA CCA GAT GGG ACT CTC	Wild type allele
L'AUT 2	105den10 mututon	Common forward	GGT TGG CAG CAA TAT GTG AA	
		mutant reverse	tant reverse CCC AAA TTA ATA CAC TCT TGT CGT GAC TTA CCA GAT GGG ACA	
		Common reverse	GAC GGG AAT CCA AAT TAC ACA G	Wild type allele
	5382insC mutation	wild type forward	AAA GCG AGC AAG AGA ATC GCA	
Exon 20		Common reverse	GAC GGG AAT CCA AAT TAC ACA G	
		Mutant forward	AAT CGA AGA AAC CAC CAA AGT CCT TAG CGA GCA AGA GAA TCA CC	Mutant allele

Table	(1): Sequenc	e of the primer	s used for detection	of 185delAG and	d 5382insC l	BRCA1 gene	mutations
	() · · · · · · · · · ·					- 8	

Table (2): Expected results on gel electrophoresis for detection of 185delAG & 5382insC BRCA1 gene mutations.

BRCA1 gene	Expected results on gel	Conclusion
Exon 2	One band of 533 bp size	Wild type allele
(BRCA1 185delAG	One band of 638 bp size	Homozygous mutant allele
mutation)	two bands of 533 & 638 bp size	Heterozygous mutant allele
Exon 11	One band of 227 bp size	Wild type allele
(BRCA1 5382insC	One band of 354 bp size	Homozygous mutant allele
mutation)	Two bands of 227& 354 bp size	Heterozygous mutant allele

IV- Detection of C61G BRCA1 mutation in Exon 5 using the RFLP method: 1-DNA Amplification:

Primers were designed for amplification of C61G BRCA1 gene mutation in exon 5 to be suitable for PCR for this gene (Grzybowska et al., 2002). The sequence of forward primer was CTC TTA AGG GCA GTT GTG AG and that of reverse primer was TTC CTA CTG TGG TTG CTT CC.

Amplification was done using (Dream Taq green PCR master Mix 2x) supplied by (Fermentas, Germany). The PCR mix contained 25 ul of Taq PCR master Mix 2x, 2.5 ul of each of the two primers, 5 ul of the template DNA and 15 ul of nuclease free water to reach a final volume of 50 ul.*G* storm thermal cycler UK was used for amplification

according to the following program : initial denaturation at 95 °C for 3 mins ,35 cycles of denaturation at 95 °C for 1 min, annealing at 44°C for 1 min and extension at 72 °C for 2 mins , followed by final extension at 72 °C for 10 mins then hold at 4 °C.

2- Restriction endonuclease digestion:

The amplified products of exon 5 were digested by AvaII restriction endonuclease to detect C61G mutation, using Eco 471_AvaII kits (Fermentas) according to the following protocol: Preparation of a mixture of 10 ul of PCR reaction mixture, 18ul of nuclease free water, 2ul of 10xbuffer R and 4ul of Eco471. The product was mixed gently and spinned down for few seconds, incubated at 37 for 4 hours. Then thermal inactivation was done at 65 °C for 20 min .This protocol was done in Thermo cycler (Hybaid, USA).

3-Agarose gel electrophoresis:

After AvaII digestion gel electrophoresis of the amplified products was done and one band was expected to be seen if no mutation and 3 bands if heterozygous mutation was present.

3. Results:

Three germ line mutations in BRCA1 gene were analyzed including 185delAG, C61G & 5382insC mutations in exons 2, 5 & 20 respectively. .Some clinical data of the subjects were taken and analyzed (tables 3, figures 1&2).Gel electrophoresis of the PCR products is shown in figures .3, 4 & 5.

The incidence of BRCA1 gene mutation in the breast cancer group (86.7%) was higher than its incidence in the control group (55%). Also the incidence of BRCA1 gene mutation in the groups with family history (73.35%) was higher than in the groups without family history (68.35%) (table 4). Besides that, the frequency of 2 or 3 exons mutation as compared to one exon mutation and normal gene is shown in table 4. Multiple exons mutation frequency was higher than single exon mutation frequency in the breast cancer group with family history (60% compared to 26.7%) and in the control and breast cancer groups with family history (45% compared to 28.35%). On the other hand one exon mutation in the breast cancer group without family history (46.7% compared to 40%) and in the control group without family history (50% compared to 0%).

The genotype frequency of 185delAG, 5382insC and C61G mutations was estimated in all studied groups (Table 5). The 5382insC mutation was the most frequent BRCA 1 mutation among the females of Qalubia governorate (56 %). It was followed by C61G mutation (40%) and 185 delAG mutation (22%).The same order of frequency of exons mutation occurred among the study groups with + ve family history, with 5382insC mutation being the most frequent (55%) followed by C61G mutation (50%) then 185 delAG mutation (26.665%). Also the most frequent mutations among the breast cancer group with + ve family history were 5382insC mutation and C61G mutation with the same frequency level each being (60%), while the least frequent mutation was 185 delAG mutation (3.33%). In addition, the absence of 185 delAG mutations in control women who had no family history of breast cancer was observed.

The allelic frequency of 185delAG, 5382insC and C61G mutations was also evaluated in all studied groups (Table 6). The evaluation revealed that the 5382insC homozygous mutation was more frequent than the heterozygous mutation in the studied groups but the heterozygous mutation was more frequent in the control group without family history..Also 185delAG homozygous mutation was more frequent than the heterozygous mutation in the studied groups except for the control group with absence of this type of mutation.

According to the current result, the age of females with BRCA1 gene mutations ranged between 15 &30 years was 3/37(8.1%), above 30 to 40 was 23/37 (62.6%) and above 40 to 50 years was 11/37 (29.7%) Beside that the age of onset of breast cancer in the breast cancer patients with BRCA1 gene mutation was between 30 to 40 years in 17/26 of cases (65.4%) and above 40 to 50 years in 9/26 of cases (34.6%). The age of onset of breast cancer in the breast cancer patients with family history and BRCA1 gene mutation was between 30 to 40 years in 9/13 of cases (69.2%) and above 40 to 50 years in 4/13 of cases (30.8%).

groups	Controls		Breast canc			
Clinical items	Without family history N =10	With family history N=10	Without family history N=15	With family history N=15	X ²	Р
		Num	ber (%)			
Marital status Married	10/10	8/10 (80)	14/15	14/15		
Single	0/10 (0)	2/10 (20)	1/15 (6.7)	1/15 (6.7)	2.8	>0.05
Lactation +ve	10/10 (100)	6/10 (60)	12/15 (80)	12/15 (80)	5	>0.05
-ve	0/10 (0)	4/10 (40)	3/15 (20)	3/15 (20)	5	20.05
<u>Parity</u> +ve	10/10 (100)	6/10 60	13/15 (87.7)	12/15 (80)	5 74	. 0.05
-ve	0/10 (0)	4/10 (40)	2/15 (13.3)	3/15 (20)	5.74	>0.05
Hormonal Contraception +ve	3/10 (30)	3/10 (30)	3/15 (20)	5/15 (33.3)	4 93	>0.05
-ve	7/10 (70)	710 (70)	12/15 (80)	10/15 (66.6)	4.95	20.05
History of DM +ve	0/10 (0)	0/10 (0)	3/15 (20)	2/15 (13.3)	4.07	> 0.05
-ve	10/10 (100)	10/10 (100)	12/15 (80)	13/15 (86.7)	4.07	>0.05

 Table (3):
 clinical data of the study group

Clinical items		F	р			
Age	34 ± 6.1	28 ±10.7	40.9 ±13.3	39.7 ± 6.9	4.14	>0.05
Onset of menarche	12.3 ±0.66	11.7 ±0.95	11.5 ±0.99	11.5 ±0.64	2.52	>0.05

p>0.05 : non significant







Figure (2): Percentage of the family history of breast and ovarian cancers in breast cancer patients with family history of breast&/or ovarian cancer



Figure (3): gel electrophoresis of amplified products of BRCA1 gene of exon 2 and exon 20 .Lane 1 shows DNA ladder 1000bp .Lane 4 and7 show exon 2 amplification products ;with lane 4 showing heterozygous mutation and lane 7 showing homozygous mutation. Lanes 2, 3.5 and 6 show exon 20 amplification products; with lanes 2, 5 showing heterozygous mutation and lanes 3, 6 showing homozygous mutation.



Figure (4): gel electrophoresis of the amplified products of BRCA1 gene of exon 5 before avaII digestion. Lane 1 shows DNA ladder 1000bp, Lanes 3, 5 and 7 show one band.



Figure (5): gel electrophoresis of amplified products of BRCA1 gene of exon 5 after avaII digestion. Lane 1 shows DNA ladder 1000bp.Lanes 2, 3 and 4 show three bands indicating heterozygous mutation.

Table (4): Exons mutation frequencies in BRCA1 Gene among control and breast cancer groups

	G	control		Breast cano			
Exons Mutation frequency	Groups	Without family history N =10	With family history N=10	Without family history N =15	With family history N=15	\mathbf{X}^2	р
			Number (%)			
Normal gene		5/10 (50)	4/10 (40)	2/15 (13.3)	2/15 (13.3)	12.3	>0.05
Mutant gene		5/10 (50)	6/10 (60)	13/15 (86.7)	13/15 (86.7)	23.3	>0.05
One exon mutation		5/10 (50)	3/10 (30)	7/15 (46.7)	4/15 (26.7)	12.3	>0.05
Two or three exons mutation		0/10 (0)	3/10 (30)	6/15 (40)	9/15 (60)	12.3	>0.05

Table (5): Genotype frequency of 185delAG, 5382insC and C61G mutations in exons 2. 20 and 5 respectively among the control and breast cancer groups

Groups	control group Breast cancer group			er group			
	Without family history N =10	With family history N=10	Without family history N =15	With family history N=15	Total	X ²	р
Genotype frequency		Nui (9	mber %)				
185delAG (Exon 2) Mutation	0/10 (0)	2/10 (20)	4/15 (26.7)	5/15 (33.33)	11/50 (22)	4.16	>0.05
5382insC (Exon20) Mutation	3/10 (30)	5/10 (50)	11/15 (73.3)	9/15 (60)	28/50 (56)	4.8	>0.05
C61G (Exon 5) Mutation	2/10 (20)	4/10 (40)	5/15 (33)	9/15 (60)	20/50 (40)	4.44	>0.05

Table (6): Allelic frequency of 185delAG, 5382insC and C61G mutations in exons 2. 20 and 5 respectively among the control and breast cancer groups.

Groups	Control	group	Breast cancer groups				
	Without family history N =10	With family history N=10	Without family history N=15	With family history N=15	Total	X ²	р
Allelic frequency		Num (%	ber)				
185delAG (Exon 2):	10/10 (100)	8/10 (80)	11/15 (73)	10/15 (66.7)	39/50		
WildHeterozygous	0/10 (0)	0/10 0	0/15 (0)	2/15 (13)	2/50	8.06	>0.05
• Homozygous	0/10 0	2/10 (20)	4/15 (26.7)	3/15 (20)	9/50		
5382insC (Exon 20): • Wild	7/10 (70)	5/10 (50)	4/15 (26.7)	6/15 (40)	22/50		
• Heterozygous	3/10 (30)	0/10 (0)	2/15 (13)	2/15 (13)	7/50	11.52	>0.05
• Homozygous	0/10 0	5/10 (50)	9/15 (60)	7/15 (46.7)	21/50		
<u>C61G (Exon 5):</u>							
• Wild	8/10 80	6/10 60	10/15 66.7	6/15 40	30/50	4.44	>0.05
• Heterozygous	2/10 20	4/10 40	5/15 33	9/15 60	20/50		

4. Discussion:

Breast cancer is the most common female malignancy and a major cause of death in middleaged women (Lacey et al., 2009). Germ-line BRCA1 mutations confer a substantial lifetime risk of breast and ovarian cancer. The absolute risk of cancer by the age of 70 years conferred by a BRCA1 mutation is reported to be between 45% and 87% for breast cancer and between 36% and 66% for ovarian cancer (Thompson et al., 2002). Germ-line mutations of the BRCA1 gene are responsible for a substantial proportion of families with multiple cases of early-onset breast cancer (Haffty et al., 2009).

The magnitude of the risk of breast cancer in carriers of mutations in BRCA1 is critical for guiding decisions concerning cancer prevention options. Some women found to carry such mutations undergo prophylactic mastectomy and/or oophorectomy, because their cancer risk is extremely high. However, although it is very clear that mutations in these genes, segregating within these types of families, confer a substantial risk of both breast and ovarian cancer, the same may not apply to mutations detected in other settings, such as in families with less-extreme cancer histories or in incident cases, even those of early onset (Antoniou et al., 2003).

The study was carried on 50 females to detect different mutations in BRCA1 gene in female patients in Qalubia Governorate and correlate them with the presence or absence of family history of breast or ovarian cancer to allow identification of individuals at high risk.

Multiplex mutagenically separated PCR was used to detect 5382insC mutation (exon20) and 185del AG mutation (exon2) in the BRCA1 gene as this method is easy, simple and rapid for detection of mutation (Chan et al., 1999). But for detection of C61G substitution mutation in exon 5 we used the RFLP method (Grzybowska et al., 2002).

In the current study, the percentage of the first degree family relative (mother, sister, or daughter) with breast and/or ovarian cancer (93.3%) was extremely higher than that of the second degree family relative (6.7%) in the breast cancer group with family history.

Couto and Hemminki (2007) reported that women with a first- and second-degree relative with breast cancer had a higher risk of breast cancer than women without such a family history. This risk was higher when the affected relative is a mother and/or a sister(s) compared to a grandmother and/or an aunt(s).

A family represents a group of individuals sharing a common environment and genes. Hence, the higher incidence of breast cancer in the first and second degree relatives and the mechanisms leading to this higher risk of breast cancer could be environmental, genetic, or a combination of the two. The familial risk among first-degree relatives is so high that a substantial part of it must be caused by heritable factors. As environmental sharing between second-degree relatives is probably low, the breast cancer risk associated with having affected seconddegree relatives is assumed to be due to heritable causes (Couto and Hemminki, 2007). So since first degree relatives share genes and environment more than second degree relatives, it may explain the higher incidence of breast cancer in the first degree relatives.

This study showed that the percentage of family history of breast cancer (93.3%) was much more than the percentage of family history of ovarian cancer (6.7%) in the breast cancer group with +ve family history. In support of this, other studies have

indicated that the incidence of breast cancer and ovarian cancer in families is correlated with the location of the BRCA1 mutation. When mutations that result in a truncated BRCA1 protein occur in the first two-thirds of the gene, the risk of ovarian cancer relative to breast cancer in the family is significantly higher than when truncating mutations occur in the last one-third of the gene (Gayther et al., 1995). More recently, other researchers have found that mutations in a central region of BRCA1 were associated with a lower risk of breast cancer (Thompson and Easton, 2002). Also another study has reported that the risk of breast cancer increases with mutation position, from 5 to 3 (Risch et al., 2001). Researchers categorized BRCA1 mutations into three groups as: nucleotides 1-2400, 2401-4184, and 4185 onward. The relative risk of breast cancer for mutations in the central region as compared to that for mutations in the 5 region was estimated to be 0.93, and that for mutations in the 3 region was estimated to be 1.4; the corresponding risks for ovarian cancer were 1.8 and 1.1 respectively (Antoniou et al., 2003).Note that in this study, the most frequent exon mutation was in exon 20 (5382insC mutation) .this was followed by exon 5 and the least frequent mutation was in exon 2.

The incidence of BRCA1 gene mutation was higher in the breast cancer group than in the control group and in the groups with family history than in the groups without family history. In addition, multiple exons mutation frequency was higher than single exon mutation frequency in the control and breast cancer groups with family history. On the other hand one exon mutation was more frequent than multiple exons mutation in the control and breast cancer groups without family history. These results indicate that BRCA1 gene mutation plays an important role in the process of carcinogenesis in the breast cancer patients especially in those with family history and that multiple exons mutation plays a role in the carcinogenesis in the breast cancer patients with family history of breast cancer than in those without family history.

The higher frequency of BRCA1 gene mutation in the breast cancer patients is in agreement with the previous studies which reported that the prevalence of BRCA1 mutations in Korean women with breast cancer at a young age was high. They stated that certain variations of the BRCA1 gene lead to an increased risk for breast cancer and that researcher have identified hundreds of mutations in the BRCA1 gene, many of which are associated with an increased risk of cancer (Choi et al., 2004).

The higher frequency of the multiple BRCA1 gene mutations in the breast cancer patients and in the groups with family history is supported by a study which reported that most inherited breast cancer risk results from the interaction of several mutated genes. Families with this pattern of inheritance will contain only a few members with breast cancer. These mutated genes by themselves are associated with only a small increase in breast cancer risk, but when several of these genes are inherited together, they can lead to significant increase in breast cancer results from multiple gene mutations. The initial mutation can be inherited from one's parents.

According to the current study, the 5382insC mutation (exon 20) was the most frequent BRCA 1 mutation among the females of Oalubia governorate (56 %) .It was followed by C61G mutation (exon 5) (40%) and 185 delAG mutation (exon 2) (22%). The same order of frequency of exons mutation occurred among the study groups with + ve family history, with 5382insC mutation being the most frequent (56%) followed by C61G mutation (52%) and 185 delAG mutation (28%). Also the most frequent mutations among the breast cancer group with + ve family history were 5382insC and C61G mutation with the same mutation frequency level each being (60%), followed by 185 delAG mutation (33.33%).

It was reported that in the Upper Silesia population that the most frequent germ line mutation was 5382ins C mutation (73%), while mutations 185delAG and C61G were less frequent (Grzybowska et al., 2002). Also, it was found that there were 3 founder mutations in the BRCA1 gene in Polish families with breast-ovarian cancer including 5382insC, C61G, and 4153delA. They accounted for 51%, 20%, and 11% of all identified mutations respectively (Górski et al., 2000). Other ethnic groups with high frequencies of founder mutations include Ashkenazi Jews, Icelanders, French, Canadians, the Dutch, Norwegians, Swedes, and, possibly other populations of central and Eastern Europe (Streuwing et al., 1995; Szabo and King, 1997 and Dorum et al., 1999).

5382insC BRCA1 mutation was reported as the most frequent mutation (51%) detected in polish families with breast or ovarian cancer (Górski et al.,2000).Also it was reported that this mutation accounts for about 80% of the mutations found in the BRCA 1 and BRCA 2 genes in the polish populations (Grzybowska et al.,2000).This mutation is also common in Ashkenazi Jews, and it constitutes 25% of the mutations found in Jewish women with a high genetic risk of breast and ovarian cancers (Streuwing, 1997). The findings from small case series suggest that the 5382insC mutation also occurs frequently in families from Hungary and Latvia who have breastovarian cancer (Ramus et al., 1997 and Csokay et al.,

1999). Also, the 5382insC mutation has been identified in other several countries, as Russia, Czech Republic and Lithuania, where it accounts, respectively for 94%, 33% and 50% of the BRCA1/2 gene mutations. In subjects with a family history of breast /ovarian cancer, the frequency in Russia is of 11%, in Greece of 8%, in Germany of 4%, in Italy of 3% and in Canada of 13% .On the contrary, a low frequency has been found in the Scandinavian countries, in Belgium and in Holland. It is thought that this mutation probably originated in the Baltic area 38 generations ago, with a gradual decrease going from east to west. A haplotype analysis indicated the likelihood of a single founder both in Europe and in North America for 5382insC mutations (Ferla et al., 2007).

The second most commonly observed mutation in the present study was the BRCA1 C61G mutation in exon 5. This is in accordance to a previous study which reported that the second most commonly observed mutation in the polish families with breast or ovarian cancers was the BRCA1 C61G missense mutation in exon 5 which accounted for 20% of families with mutations (Górski et al., 2000).

The least observed mutation in the present study was the BRCA1 185delAG mutation .This mutation was also found in 1% of Ashkenazi Jews and contributed to 16%–20% of breast cancer diagnosed before age 50. A second founder mutation in the BRCA1 gene, 5382insC, was found in 0.13% of this population. A combined analysis of several studies based on 22 different Ashkenazi populations had shown that breast cancer lifetime risk is similar in carriers of the 185delAG and 5382insC mutations (respectively of 64% and 67%), (Ferla et al., 2007).

In the current study, the 5382insC and 185delAG homozygous mutations were more frequent than the heterozygous mutation in the studied groups. On the contrary. the heterozygous5382insC mutation was more frequent in the control group without family history and the 185delAG mutation was absent in the control group without family history .This may be explained by that both alleles of the tumour suppressor gene BRCA1 must be affected before breast cancer to develop. This is due to the fact that if only one allele for the gene is damaged, the second can still produce the correct protein (Parkin et al., 2002). Also the low frequency of 185delAG mutation explains its absence in the control group without family history

According to the current result, the age of females with BRCA1 gene mutations between 15 &30 years was 8.1%, above 30 to 40 was 62.6% and above 40 to 50 years was 29.7% .Also, the age of onset of breast cancer in the breast cancer patients with BRCA1 gene mutation was between 30 to 40 years in 65.4% of cases and above 40 to 50 years in 34.6% of cases. The age of onset of breast cancer in the breast cancer patients with family history and BRCA1 gene mutation was between 30 to 40 years in 69.2% of cases and above 40 to 50 years in 30.8% of cases.

Antoniou et al.(2003) reported that the breast cancer incidence in *BRCA1*-mutation carriers increased with age up to age 45–49 years but remained roughly constant thereafter. Women who carry BRCA1 mutations are particularly susceptible to the development of breast cancer before age 35–40. The relative risk of breast cancer in *BRCA1*-mutation carriers, relative to general population rates, declined with age from >30-fold at <40 years of age to 14-fold at >60 years of age. As a consequence of this, the incidence in *BRCA1*-mutation carriers raised to a plateau of 3%–4% per annum in the 40–49-years age group and were roughly constant thereafter.

Ottini et al. (2000) reported that BRCA1 mutations were mostly found in breast cancer patients with disease diagnosis before the age of 50 years. Moreover, in cases with familial clustering of site-specific breast cancer, BRCA1 mostly accounted for tumours diagnosed before age 40 years.

Ford et al. (1995) estimated that the proportion of breast cancer in the general population due to BRCAl was 5.3% in women younger than the age of 40 years, 2.2% in women between the ages of 40 and 49 years, and 1.1% in women between the ages of 50 and 70 years. The proportion of breast cancer cases predicted to be attributable to BRCAl gene decreases markedly with age; approximately 33% of cases age 20-29 years compared with approximately 2% of cases age 70-79 years.

In conclusion, the 5382insC mutation was the most frequent BRCA 1 mutation among the females of Qalubia governorate followed by C61G mutation and 185 delAG mutation and multiple BRCA1 exons mutations play an important role in the pathogenesis of familial breast cancer in Egypt.

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Serum Visfatin in patients with chronic hepatitis C

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Abstract: Background: The role of visfatin in non alcoholic fatty liver diseases (NAFLD) is now well known accordingly, the aim of this work was to study the serum level of Visfatin in patients with chronic hepatitis C (HCV) and their relations to the nutritional state of patients as well as the biochemical markers of liver disease. Subjects: This study was carried out on 75 male subjects classified into five groups all of them were subjected to measurement of body mass index (BMI), lipid profile, liver function tests, PCR for HCV, serum visfatin level & liver biopsy when ever possible was done. Results: Mean serum Visfatin level was significantly elevated in group II (HCV & cirrhosis) and group IV (HCV & steatosis) than in group V. (P < 0.05). Significant positive correlation was found between serum visfatin & apolipoprotein A1. (P<0.05). Conclusion: High levels of visfatin in patients with HCV-induced cirrhosis and schistosomiasis suggest its role in liver fibrogenesis.

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Keywords: Serum; Visfatin; patient; chronic hepatitis C

1. Introduction:

The growing interest in the biology of adipose tissue derived from the understanding that fat is not only a passive energy depot, but functions as a hormonally active tissue, capable of producing numerous molecules, including cytokines, (1) chemokines and adipokines. The term "adipokines" comprises a group of polypeptide hormones which are expressed predominantly, although not exclusively, by adipose tissue in a regulated manner. ⁽³⁾ These molecules are secreted into the circulation and regulate the functions of different tissues through local, central and/or peripheral actions.⁽²⁾

Visfatin is a recently discovered adipokine that exerts insulin-mimicking effects, by activating the insulin receptor in a manner distinct from that of insulin.^(3, 4) Visfatin is an insulin-mimetic adipokine that was originally discovered in liver, skeletal muscle and bone marrow as a growth factor for B lymphocyte precursors (whence its alternative name, pre-B-colony enhancing factor, or PBEF).⁽⁵⁾ It is upmodulated in models of acute lung injury and sepsis. ⁽⁶⁾

Dahl et al ⁽⁷⁾ have suggested that visfatin is an inflammatory mediator in cardiovascular pathologies based on its localization and actions in macrophages within atherosclerotic lesions. Moreover, it has been reported that visfatin up regulates endothelial matrix metalloproteinases (MMP-2/-9). ⁽⁸⁾

Similarly to insulin, visfatin enhances glucose uptake by myocytes and adipocytes, and inhibited hepatocyte glucose release, resulting into insulin resistance in hepatocytes which distorts directly glucose metabolism, especially the control over glucose output into the circulation and interferes with cell survival and proliferation, while hepatic fatty acid synthesis remains stimulated by compensatory hyperinsulinaemia, resulting in steatosis .⁽⁹⁾ Visfatin is secreted by activated lymphocytes, monocytes, and neutrophils. It induces the cellular expression of inflammatory cytokines such as TNF-α, IL-1and IL-6; visfatin-induced IL-6 expression might be involved in the pathogenesis of IR (insulin resistance) associated with visceral obesity. IL-6 has been demonstrated to promote IR via induction of SOCS (suppressor of cytokine signaling) proteins. ⁽¹⁰⁾ Since visceral adipose tissue is strongly associated to insulin resistance and nonalcoholic fatty liver disease (NAFLD), visfatin represent a possible link between visceral fat, insulin resistance and the development of NAFLD. (11,12)

Although circulating visfatin was found to be higher in NAFLD than in normal individuals, its lower expression in adipose tissue of NAFLD patients suggests that such difference is not due to adipose tissue overproduction. Taken together these findings suggest a possible role for visfatin in the pathophysiology of NAFLD. ⁽¹²⁾ Fibrosis and cirrhosis are the final outcomes of all chronic liver disease; however, some morphological and biological differences distinguish fibrosis due to NASH from the one secondary to other causes of liver damage. The main cell type responsible for extracellular matrix deposition is represented by HSCs, which undergo activation in conditions of liver injury, acquiring a phenotype that enables them to participate in the liver wound healing process. ⁽¹³⁾

The profibrogenic mechanisms operating in NASH are partly in common with those observed in other chronic liver diseases. However, the increase in circulating adipokines, oxidative stress generated by accumulation of fat in hepatocytes and the hormonal profile associated with the metabolic syndrome might have a specific role for the induction of fibrogenesis in this condition. ⁽¹³⁾ Taken together, these data identify a role for visfatin in obesity-related diseases, including insulin resistance and metabolic syndrome, and suggest possible implications in liver pathophysiology, especially in the context of NAFLD. ⁽¹⁴⁾

Aim of the work

The aim of this work was to study the serum level of Visfatin in patients with chronic hepatitis C (HCV) and their relations to BMI of patients as well as the biochemical markers of liver disease.

2. Subjects and Methods

This study was carried out on seventy-five male subjects classified into five groups:

- Group I: Fifteen patients with Chronic Hepatitis C (CHC).
- Group II: Fifteen patients with CHC and liver cirrhosis. (Diagnosis of liver cirrhosis was based on clinical, laboratory and ultrasonographic findings).
- Group III: Fifteen patients with mixed CHC and Schistosomal hepatic fibrosis.
- Group IV: Fifteen patients with mixed CHC and steatosis.

Group V: Fifteen control healthy subjects

Male subjects were selected to exclude gender differences in visfatin hormone levels. Patients with history of the following were excluded from our study: Hepatitis B, autoimmune hepatitis, any alcohol intake, patients receiving hepatotoxic drugs. Other conditions associated with high visfatin values also were excluded including; type 2 diabetes, rheumatoid arthritis, and cardiovascular disease, the use of antiepileptic drugs, inflammatory bowel disease, renal failure, and renal transplant recipients. Patients gave consent to participate in the study. The protocol was approved by the committee of ethics, faculty of medicine, Alexandria University.

All groups were subjected to the following: Thorough history taking, clinical examination, calculation of BMI. Investigations which include: routine laboratory investigations; urine and stool examination, CBC, blood urea and serum creatinine, fasting and 2hr-postprandial blood sugar and lipid profile (triglycerides, cholesterol, VLDL, LDL, HDL and Apolipoprotein). Liver function tests; ALT, AST, prothrombin activity, serum albumin, serum bilirubin (total and direct), serum alkaline phosphatase, and gamma glutamyl transferase (GGT) were measured.Viral markers: serum HCV-antibodies using third generation ELISA and HCV-RNA using PCR-RNA quantitative were performed. Ultrasound examination of the abdomen and liver biopsies whenever possible (all cases in group I, 4 cases in group II, 4 cases in group III and 10 cases in group IV). In addition, sigmoidoscopy was performed to diagnose cases with mixed schistosomal affection.

Visfatin plasma concentrations was conducted by using enzyme immunosorbent assay, Visfatin, C-terminal, (Human) EIA Kit, catalog No.: EK-003-80, Phoenix Pharmaceuticals, Belmont, USA.

Statistical methods:

All analyses were performed using ANOVA test. All values are expressed as the mean \pm standard deviation. Variables significantly deviating from normal distribution were logarithmically transformed. (F = ANOVA test, P = probability)

3. Results:

Significant differences were found between the studied groups and controls regarding hemoglobin level, red blood cells, white blood cells and platelets count (Table I). In addition, there were also a statistically significant difference between all groups and group IV as regards; serum cholesterol level, triglycerides, HDL, LDL, VLDL and apolipoprotein A1 levels (Table II).

Statistical difference between the studied groups regarding AST, ALT, total bilirubin, serum albumin and serum visfatin levels was summarized in (table III, IV and V).

		Mean	Standard Deviation	F	Sig.
Hb	I	11.40	2.051	9.488	.0001*
	II	12.23	2.210		(groups II,III,IV)
	III	11.22	2.234		
	IV	14.33	1.764		
	V	14.53	1.324		
RBCs	I	3.78	.702	4.489	.003*
	II	3.84	.774		
	III	3.71	.981		(groups II,III,IV)
	IV	4.34	.748		
	V	4.69	.637		
Platelets	Ι	201.07	160.298		.002*
	II	156.13	71.592	4.485	(groups II,III,IV)
	III	154.07	86.315		
	IV	179.07	67.646		
	V	212.67	55.053		
WBCs	Ι	8.01	2.611	3.677	.009*
	II	4.52	2.077		(groups II, III,IV)
	III	7.93	5.077		
	IV	7.51	1.899		
	V	7.31	1.820		

Table (1): Comparison between the different studied groups regarding blood picture

Table (II): Comparison between the different studied groups regarding lipid profile

		Mean	Standard Deviation	F	Sig.	
cholesterol	I	124.07	42.516	8.039	.0001*	
	п	123.67	62.314	IV # other groups.		
	III	138.40	64.293			
	IV	231.13	76.800			
	V	139.40	58.058			
TGs	Ι	75.93	22.327	12.113	.0001*	
	II	187.20	47.644			
	III	87.53	36.424	W # other groups		
	IV	97.93	84.218	IV # other groups.		
	V	93.73	35.101			
HDL	I	72.20	15.907	11.638	.0001*	
	п	72.73	13.609			
	III	43.47	24.118	IV # other groups.		
	IV	44.07	14.733			
	V	71.53	17.960			
LDL	I	127.13	8.861	7.224	.0001*	
	п	126.13	12.609			
	III	107.27	8.216			
	IV	147.67	18.027	IV # other	r groups.	
	V	124.33	38.814			
VLDL	I	22.47	7.060	13.057	.0001*	
	п	28.00	3.891			
	III	24.33	7.880			
	IV	44.67	16.762	IV # other groups		
	V	27.13	6.534			
Apolipoprotein	Ι	115.87	4.794	6.840	.0001*	
A1	II	114.80	3.098	IV #	other groups.	
	III	115.67	7.277			
	IV	104.67	12.344			
	V	115.13	3.889			

		Mean	Standard Deviation	F	Sig.
AST	Ι	40.53	14.372	3.979	.006*
	II	53.40	35.874		(group II,III)
	III	45.27	23.423		
	IV	67.93	57.528		
	V	22.00	5.477		
ALT	Ι	66.40	19.145	17.881	.0001*
	II	43.07	8.972		
	III	36.93	13.074		(group IV,I)
	IV	123.40	67.232		
	V	46.07	11.361	11.361	
Total Bilirubin	Ι	1.65	1.65	8.65	0.001*
	II	3.21	2.69		(In group II,III,IV)
	III	2.95	1.41		
	IV	1.75	1.23		
	V	0.90	1.42		

Table (III): comparison between the different studied groups regarding liver function

Table (IV): Comparison between the different studied groups regarding serum albumin

	Mean	Standard Deviation	F	Sig.
Ι	3.80	.705	15.949	.000
п	2.09	.623		
III	2.96	.593		
IV	3.00	.615	II, III, IV # c	other groups.
V	3.85	.341		

	Table (V): Con	parison	between	the	different	studied	group	os reg	garding	g plasma	Visfati
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		Mean	Standard Deviation	Р	Sig.
S Visfatin(ng/ml)	I	612.00	13.638		
	п	631.87	15.811	.001*	(group II, group IV)
	III	608.20	16.476		
	IV	638.80	15.086	.001*	(group IV, group V)
	V	602.33	7.480		

Mean serum Visfatin level ranged from 590 ng /ml to 665 ng/ml among different studied groups with highest mean level of Visfatin found in group IV (638.80 ng /ml) followed by group II (631.87 ng/ml) showing the highest significant values.

A significant positive correlation was noted between BMI and serum visfatin. Also significant positive correlations were noted between serum levels of Visfatin and postprandial and fasting blood sugar. Significant positive correlations were found between levels of Visfatin and serum cholesterol and serum triglycerides and VLDL. Furthermore, significant positive correlation was also noted between serum levels of Visfatin and each of AST and ALT level. Moreover, a significant positive correlation was noted between the degree of inflammation, fibrosis and serum visfatin.

On the other hand, significant negative correlation was noted between serum levels of Visfatin and HDL, serum albumin and apoA1. In addition, there was a significant negative correlation

between prothrombin activity and serum visfatin. (TableVI)(Figures 1-4)

Table (VI): Correlation between S. visfatin and some other variables.

S Visfatin	R	Р
Cholesterol	.347(**)	0.002
TGs	.264(*)	0.022
AST	.278(*)	0.016
ALT	.375(**)	0.001
BMI	.279(*)	0.015
HDL	259(*)	0.026
LDL	0.16	0.17
VLDL	.399(**)	0
Apolipoprotein	258(*)	0.025
Serum albumin	-0.406(*)	0.0001
HCV PCR	0.134	0.260
Degree of portal inflammation.	0.36(*)	0.0004
Degree of fibrosis	0.58(*)	0.0002

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).



Figure (1): Correlation between visfatin & BMI



Figure (3): Correlation between visfatin & AST



Serum visfatin

Figure (2): Correlation between visfatin & albumin



Figure (4): Correlation between visfatin & ALT



Fig 5: Histopathological image showing chronic hepatitis C with steatosis. The fat replaces less than one third of the liver tissue. A portal area with chronic inflammation is at the bottom of the field.



Fig.6: Histopathological image of schistosomiasis with deposition of calcified eggs in the hepatic portal tract.



Fig7: Histopathological image showing Cirrhosis with chronic hepatitis

4. Discussion:

In view of the possible association of PBEF with adipose tissue, steatosis and insulin resistance, a question is being raised: if the circulating level of PBEF could be related to several pathological processes including HCV, post hepatitis cirrhosis, steatosis in humans. Accordingly, the aim of the present work was to study the serum level of visfatin in patients with some chronic liver diseases (CLD) and their relations to the biochemical markers of liver disease. The mean age of subjects was around 47 years. The mean BMI among groups in the present study was around 27.2 kg/m^2 . In the present study no significant difference was found regarding BMI among the studied groups but there was a significant positive correlation between BMI and serum visfatin. In obesityassociated insulin resistance, circulating visfatin levels increases during the development of obesity, apparently due solely to secretion by abdominal white adipose tissue (WAT).⁽¹⁵⁾

Regarding serum albumin level, chronic hepatitis C patients had a normal range of albumin level as liver functions were maintained as they were discovered early in the course of the diseases. There was a significant difference in group II (cirrhotics), III (mixed shistosomal hepatic fibrosis) and IV (mixed steatosis) regarding the serum albumin level, Also a significant negative correlation between serum visfatin and albumin level. This can be explained by high serum visfatin in patients disease with chronic liver in which hypoalbuminemia is already present as a result of decreased synthetic functions of the liver. (16)

In the present study, significant positive correlation was found between total serum cholesterol, serum triglycerides, LDL, VLDL and serum visfatin. This finding might be attributed to visceral adiposity that is present in conditions associated with high cholesterol, triglycerides, LDL and VLDL as a result of insulin resistance that causes elevation of serum visfatin.⁽¹⁷⁾

As regards serum HDL, apolipoprotien A1 in the present study there was a negative correlation between serum visfatin and HDL and apolipoprotien A1. The present study was conducted on patients with chronic liver diseases. Lipoproteins play an important role in the absorption of dietary cholesterol, long chain fatty acids and fat soluble vitamins. All patients suffered from hepatitis C virus infection in the present work, Hypolipidemia is more marked in cases suffering from hepatitis C virus and this abnormality is directly related to viral load and viral response.⁽¹⁸⁾ In the present study a

significant positive correlation was found between serum ALT, AST and serum visfatin. The same correlation was also observed by Carlson ⁽¹⁹⁾ this could be explained by the fact that visfatin is a proinflammatory cytokine so naturally it correlates with the degree of hepatic inflammation.

Prothrombin was found to have a significant negative correlation with serum visfatin. High serum visfatin associated with a low prothrombin activity is due to associated chronic liver disease causing both high serum visfatin and low Prothrombin concentration. Also a significant positive correlation was found between serum visfatin and the degree of portal inflammation as well as fibrosis this is in consistent with Aller R. et al (20) who showed a relation of visfatin with portal inflammation in This could be explained by the his study. insulin mimetic effect of visfatin which may have a role in hepatic inflammation. Secondly, visfatin could play a direct inflammatory role. The inflammatory relations of visfatin have molecular explanations. For example, clear visfatin induces the production of IL-6 in human monocytes whereas IL6 negatively regulates visfatin gene expression in adipocytes.

Regarding difference in the serum visfatin levels among the studied groups. Significant differences among groups were found, with the highest levels in group IV (mixed CHC and steatosis) followed by group II (CHC and liver cirrhosis). Visfatin could play a role such as an insulin mimetic molecule producing inflammation in the liver; this may explain why it was higher in cirrhotic patients as well. Inflammation in the liver triggers repair responses that involve activation of hepatic stellate cells to myofibroblasts, a process that ends in cirrhosis. ⁽²¹⁾ In obese patients, the primary abnormality may be genetically induced insulin resistance, with a secondary increase of serum triglyceride levels due to enhance of peripheral lipolysis. The resulting hepatic supply of fatty acids and insulin may increase triglyceride deposition in the liver. (22)

Moreover, visfatin levels could predict the presence of the portal inflammation; this involve molecule could а non-invasive technique to determine this pathological change. This question overlaps with the more general issue of how the various adipokines interact with each other because the net effect of the simultaneous release of several agents with diverse biological properties is not readily predictable. Addressing such problems will require the development of new

pharmacological tools that target specific adipokine systems. As a consequence, we anticipate that new therapeutic targets will be identified to realize control of these systems.

5. Conclusions:

From the previous results we can conclude that high levels of visfatin in patients with HCV and steatosis than other patients' groups suggest its involvement in the process of steatosis and its progression. High levels of visfatin in patients with HCV-induced cirrhosis and schistosomiasis suggest its role in liver fibrogenesis.

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Characterization of Poly-isoprene Rubber Layer Backed with Porous Material as Sound Absorber and Vibration Damper

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Abstract: There are several methods to decrease acoustic noise. Sound absorption is one of the noise control methods, commonly, multi-layer sound absorbers are applied to absorb broad band noise. , the sound absorption coefficient of different materials are measured in impedance tube using two-microphone transfer-function method according to ISO 10534-2 and ASTM E1050-98 international standards. Multi-layers sound absorbers effectiveness depends on their construction. The effects of different porous materials (R, S &F), and layers backing on the sound absorption and of poly isoprene rubber will be studied. The treatment for Multi-layered with sponge and rubber increasing the sound absorption coefficient value at about 0.9 and shifted towards a lower frequency range 315-800 Hz.

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Keywords: Characterization; Poly-isoprene; Rubber Porous; Material; Vibration Damper

1. Introduction:

With the increase in public awareness and concern for noise pollution, many kinds of sound absorbing or isolating materials, such as glass wool, polymeric fibrous materials, and various types of foams, alone or with viscoelastic materials. These materials are used to absorb airborne noise and to optimize the transmission loss (TL) and damping coefficient in multi-layer systems, may be found in trim lining, under carpets, in seats, cavity interior, etc. It is interesting to measure the acoustical properties, and moreover to be able to predict the noise control impact of these materials in the design stage. Associated with the characteristic impedance and propagation constant, the absorption ratio and TL that represent sound reflection and penetrating capability of a sample material, are usually considered as the most important acoustical properties, and may be measured by a standing waveduct system. It is essentially an impedance tube with a loudspeaker at one end, a test specimen at a certain location inside and a specifically designed terminator at the other end. Several microphones are mounted along the wall of the tube. Based on the onedimensional wave equations, the measurement process may be performed by decomposing the standing wave in the tube, and frequency analysis or transfer function techniques may be applied to compute the normal incidence absorption coefficient, characteristic impedance, propagation constant, TL, and other acoustical properties of the noise control materials. According to the comparison results, a concept so-called experimental hybrid multi-layer

prediction was proposed and applied to predict absorption and transmission characteristics of a set of multi-layered treatments of different materials, and some useful results were obtained. In actual application, by adding the hybrid prediction function into standing wave-duct, one can measure any material sample, regardless of its consisting layers, thickness, etc.; moreover, optimize the acoustical properties of a multi-layer treatment by replacing one or several layers with some new materials with known transfer matrix information in absorption and transmission characteristic design. It should be mentioned that the work done in this paper may be extended to study other types of noise control configurations or equipment, such as measuring or predicting the acoustical properties of automotive muffler. [1]. the absorption coefficient () of metallic hollow sphere structures (MHSS) was measured in the range from 500 Hz to 3500 Hz. The detailed form and frequency dependence is influenced strongly by material parameters like sample thickness, pore size, porosity and sphere diameter. It is possible to reach high absorption over a wide range of frequencies by variation of different technological parameters. The ability of sound absorption can be adapted to different noise spectra. These parameters include thickness, sphere diameter, packing density, mixture of different sphere diameters and others. Thus the material can be used for an efficient and new design of noise control systems [2]. Multi-layer acoustic absorbers composed of perforated plates, airspaces or porous materials are commonly applied to absorb broadband noise. However, the acoustic absorption of

these multi-layer acoustic absorbers is strongly dependent on their constructions. It was suggested that the outer-layer porous material with appropriate impedance should encourage the incident sound to enter the composite structure, but the inner-layer porous material was selected to attenuate the sound energy and prevent the incident sound from recombining. The acoustic impedance and acoustic absorption coefficient for a realistic multi-layer acoustic absorber containing several compartments with perforated plates, airspaces and porous materials can be calculated. The drawback of the equivalent electrical circuit approach in analyzing multi-layer acoustic absorbers, (which always assumes the back surface acoustic impedance of airspaces or porous materials as that of the rigid wall), is compensated. Besides, several acoustic features of the multi-laver acoustic absorber are also discussed in detail. This provides a reliable guidance for the design of multilayer acoustic absorbers [3]. Both combinations of putting perforated plates (PP) before or after the fiber layer were eligible to improve the absorption. When coir fiber was backed by (PP) and air gap, porosity of the plate had small influence in adjusting the amount of low frequency absorption. In the condition that PP was in front of coir fiber, low porosity of plate caused the most incident sound to reflect from the plate. Therefore sound waves could not reach the fibers and medium and high frequency absorption were greatly reduced. Coir fiber is naturally a good acoustical absorption in medium and high frequency bands. Observation of different arrangements showed that utilization of (PP) may help to improve low frequency absorption of coir fiber while medium frequency values vanished [4]. This paper puts

forward a simple extended acoustic model that can be used to calculate the sound absorption coefficients of porous sintered fiber metals and then briefly discuss its characteristics. At last, the sound absorption coefficient and surface impedance of fiber metals and multi-layer-assembled sound absorbing structures are theoretically calculated using the extended model and Biot-Allard model and compared with the measured results. By comparisons, it is shown that the values calculated by using the extended model fit the measured ones better, in particular at the higher frequencies. This work would provide references and guides for the future studies of sound absorption in porous sintered fiber metal under the high temperature conditions as well as the optimization of sound absorption characteristics of grade-assembled sound absorbing structures. [5]. The tea-leaf-fibre (TLF) waste material is natural, renewable and is a waste produced during processing of tea leaves which does not pose harm to human health. The backing of TLF with a single layer of cotton cloth increases its sound absorption properties significantly. For the samples with 10 mm thickness, TLF exhibits a better sound absorption when compared with polyester and poly-propylene based non-woven fiber (PNF) material. When compared to PNF, samples of backed TLF with 20 and 30 mm thickness yield comparable sound absorption over the frequency ranges of 500-3200 and 500-2400 Hz, respectively. One should note that, TLF test samples utilized throughout this work were not pressurized as PNF materials, which may affect the sound attenuation properties.[6].

2. Materials and methods

viau	faterial properties of the tested samples							
	Material	Thickness (cm)	2	4	6			
	Foam (F)	Mass (Kg/m ²)	3.2	6.4	9.6			
		Density (Kg/m ³)	143					
	Rubber (R)	Mass (Kg/m ²)	21.15	42.3	63.6			
		Density (Kg/m ³)	1057					
	Sponge(S)	Mass (Kg/m ²)	3	5.7	8.6			
		Density (Kg/m ³)	150					
	Wood(W)	Mass (Kg/m ²)	103					
		Density (Kg/ m^3)	5098					

Table 1: M	laterial pr	operties	of the	tested	samp	les

Table 2: Multi-layered material treatment of the tested samples

Multi-layer	material	Explanation
Construction		
R2cm+W		2 layers formed from 2cm of rubber faced to incident sound and backed with wood layer
R2cm +S1cm +W		3 layers formed from 2cm of rubber faced to incident sound, backed with 1cm of sponge layer and the last layer is wood

R2cm +F1cm +W	3 layers formed from 2cm of rubber faced to incident sound, backed with 1cm of	
	foam layer and the last layer is wood	
R1cm +S2cm +W	3 layers formed from 1cm of rubber faced to incident sound, backed with 2cm of	
	sponge layer and the last layer is wood	
R1cm +F2cm +W	3 layers formed from 1cm of rubber faced to incident sound, backed with 2cm of	
	foam layer and the last layer is wood	
R2cm +S2cm +W	3 layers formed from 2cm of rubber faced to incident sound, backed with 2cm of	
	sponge layer and the last layer is wood	
R2cm+F2cm+W	3 layers formed from 2cm of rubber faced to incident sound, backed with 2cm of	
	foam layer and the last layer is wood	

In this section, the sound absorption coefficient of different materials is measured in impedance tube, using two-microphone transfer-function method according to ISO 10534-2 and ASTM E1050-98 international standards. The experimental apparatus (Fig. 1) include: B&K BZ 5050 & 5051 software, B&K3550 analyzer, B&K4260 impedance tube, 1/4 in. B&K4187 condenser microphones cartridge with B&K 2670 preamplifier, and B&K 2706 power

amplifier. The experimental frequency ranges between 50 and 6400 Hz, and the span is 2 Hz. [7]. The used samples are: one-layer of different materials (the parameters of samples are listed in Table1), twolayers assembled, and three-layer-assembled. During measurements, the single layered sample is backed by stiffness piston in the impedance tube and the multilayer-assembled are all backed by wood in the impedance tube.



Impdence tube system 4206

Figure. 1. Impedance tube setup for testing measurements.

The absorption coefficients for different materials (foam, rubber, wood and sponge) and combination layers (Multi-layered material treatment) with varying thickness were measured in the frequency range from 100 Hz to 2000 Hz.

3- Results and discussion

An optimal set of parameters is shown in Table 1&2. Porous absorptive materials used as sound absorbers are usually fuzzy. If the absorption efficiency of materials depending on the trapping and dissipating of sound energy in tiny pores, this can be seriously impaired if the surface pores are filled. For example, (foam, coarse concrete block), has many such pores and is a fair absorber of sound. If the pores of surface are filled, a painted surface can reduce porosity [8]. A greatly reducing in sound penetration occurs, and thus absorption is reduced. In figure 2, porosity of foam and rubber had small influence in absorption (the surface pores are filled). In wood had no porosity but for foam samples it has more porosity but filled so it has better sound absorption than wood, sponge has higher porosity than foam and wood, so it has higher sound absorption than foam and wood in frequency range higher than 1300Hz. For rubber samples it has a good sound absorption than wood, foam, rubber and sponge especially in the frequency range from 800Hz up to 2000Hz.

Greater sound absorption occurs from thicker material, but this absorption holds primary for the lower frequencies. Figures (3, 4 and 5), shows the effect of varying absorbent thickness. From Figs. 3 to 5 one can clearly see the effect of increasing samples thickness on its sound absorption coefficient of different materials F (foam), R(rubber) and S (sponge).

From figure 3, as increasing in foam thickness the maximum value of the sound absorption shifted toward the low frequency range. The sound absorption of 2cm foam reach its maximum value (0.25) at 800Hz. As foam thickness increased from 2cm to 4cm, the sound absorption coefficient of foam reaches its maximum value (0.48) at 630Hz. And for 6cm foam thickness, the sound absorption reaches its maximum value (0.38) at 500Hz.

For sponge samples in Figure 4, it is clear that, as increasing in the sponge thickness the maximum value of the sound absorption shifted toward the low frequency range. The sound absorption of 2cm sponge thickness reaches its maximum value (0.52) at 2000Hz. The sound absorption of 4cm sponge reaches its maximum value (0.85) at 1250Hz, and for the 6cm sponge thickness, the sound absorption reaches its maximum value (1.00) at 1000Hz. And for rubber samples in Figure 5, the maximum value of the sound absorption shifted toward the low frequency range. as increasing in the rubber thickness, the sound absorption of 2cm rubber reach its maximum value (0.68) at 1630Hz. When rubber thickness increases from 2cm to 4cm the sound absorption is shifted to a lower frequency 1000Hz and its value is 0.56. And for 6cm rubber the sound absorption reaches its maximum value (0.34)at 400Hz. Several acoustic features of the multi-layer acoustic absorber are also discussed in detail. This provides a reliable guidance for the design of multilayer acoustic absorbers.

Figure 6, appears the sound absorption of foam layer backed with wood and exhibits lower sound absorption at all frequencies from 100 to 2000 Hz. For the samples of (R1+F2+W) and (R2+F2+W), the sound absorption properties of them were comparable for the frequency range 100–1300 Hz, with a small deviation at 1300Hz between them. But if we compare between the samples with rubber and without rubber layer in Figure 6, the sample (F2cm+W) with foam of thickness 2cm faced the sound and backed with wood, appears the same behaviour of F2cm in Fig3. For the sample (R1cm+F2cm+W), a higher sound absorption in high frequency range especially at 1600Hz, it reaches 0.85. Increasing in the sound absorption of (R1cm+F2cm+W) is due to rubber layer addition (see fig.5). As rubber thickness increases its sound absorption values increases in the frequency range (100 -2000Hz), and due to foam effect as attenuator, an increasing in sound absorption values appears. For (R2cm+F2cm+W), an increasing in sound absorption values appears but in the frequency range (100 -1250Hz).

In Fig.7 it is clear that the sample (S2cm+W), sponge of thickness 2cm facing the sound and backed with wood, appears the same behaviour of S2cm in Fig4. The sample (R1cm+S2cm+W), has a higher sound absorption coefficient in low frequency range especially at 800Hz it reaches 0.98. An increasing in sound absorption values for (R2cm+S2cm+W) appears also, but shifting at a lower frequency at 400Hz., Figure 8 reveals that, the resonance frequencies of this multi-layers acoustic absorber are respectively higher than those of their individual compartments. It is noted that the lower porosity of the perforated plate has a lower acoustic resonance frequency and higher sound absorption [3].

Figure 8, appears a comparison between a multi-layers material treatment, a higher absorbing sample especially in frequency range higher than 1000Hz is (S4cm+W), and for frequency range lower than 1000Hz, the higher absorbing sample is (R2cm+S2cm+W).

Vibration damping

The system of vibration damping test consists of, an exciting vibration source, a compressor machine of mass 36Kg, to produce a vibration on the floor, this vibration transmitted directly from point to other [8]. To detect the vibration level, using a vibration meter type 2511 B&K with accelerometer type B&K 4173 to measure the transmitted vibration at a point. Firstly the vibration velocity transmitted from compressor directly to floor without any isolator (Comp.), see Fig.9. For damping this vibration, different materials construction layers were used.

Studying each damper under the compressor base and recording the results of each damper. These records are illustrated in fig.9, where the higher velocity level appears due to signal of compressor alone (Comp.), this vibration signal is transmitted directly to the floor under compressor without any isolated material. Using a wooden layer (2cm thickness) under compressor legs, appears a damping in vibration level of compressor signal especially at peaks of compressor vibration signal. Using a foam layer of 2cm thickness under the wooden layer, a little increasing in damping for the vibration signal occurs with the same behavior of wooden layer. But when inserting a sponge layer of 2cm thickness under wooden layer, a remarkable increasing in damping for the vibration signal occurs, in the interested frequency rang.

 Table 3. Multi-layer material
 Construction Explanation

Multi-layer material Construction	Explanation
Compressor without damper	Comp.
Compressor over Wood (W)	A wooden layer of 1cm thickness
Compressor over (W/F2)	A wooden layer over 2cm thickness foam
Compressor over (W/S2)	A wooden layer over 2cm thickness sponge







Figure 3; Effect of Foam thickness on sound absorption











Figure 6; Effect of Rubber and Foam materials as a multi-layers on sound absorption


Figure 7; Effect of Rubber and sponge materials as a multi-layers on sound absorption



Figure 8; Comparison between different materials of multi-layered at constant thickness



Figure 9; Effect of different materials as a damper for vibration (velocity)

4- Conclusion

The experimental data indicates that, porous (sponge), significantly increases sound layer absorption coefficient at low frequencies and improve it. 6cm thick layers of (R2cm +S2cm +W) exhibits peak value at frequencies between 315-800 Hz, with maximum value of 0.88 sound absorption coefficient. The experimental results of (S4+W) sample, gives a higher value for sound absorption coefficient at a higher frequency range from 1000-1600 Hz. The optimum values for Multi-layered sponge treatment is around 0.9 for sound absorption coefficient at frequencies between 315-800 Hz. Sound absorption coefficient of sponge layer was increased, and shifted to a lower frequency range when using Multi-layered sponge treatment. Using a rubber sample alone (R), appears a 0.67 of sound absorption coefficient at frequencies between 1150 - 1650 Hz. Using a sponge sample alone (S), appears a 0.35 of sound absorption at frequencies between 1150 - 1650 Hz

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The Effect of Boiling on Milk Microbial Contents and Quality

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Abstract: Though milk boiling is a widespread heat treatment in number of countries, the process was not thoroughly studied. In this study, the effect of boiling buffaloes' and cow's milk samples for different periods on their microbiological contents, keeping quality and bacterial ecology contents and chemical changes were determined. Lethality rate of 6.53, 6.77, 7.301 and 7.44 in buffaloes' and 6.76, 7.059, 7.012, 7.15 and 7.159 log₁₀ cfu/ml in cows' milk were obtained on boiling the samples for 0.5, 1,2 and 5min., respectively. Boiling milk for 0.5 and 1min decreased the bacterial count from 3.6×10^9 in cow's milk into 6.3×10^2 and 3.2×10^2 and from 7.8×10^9 in buffaloes 'milk into 2.26×10^3 and 1.3×10^3 cfu/ml, respectively. On cold storage, the microbial content of boiled milk, not only did not increase but also declined on the first week. Boiling destroyed bacterial vegetative cell leaving behind spores of the sporeformer which were dominated with *B.cereus* and *Micrococcus leteus*. Boiling affected milk quality far less than the effect occurred in UHT milk as determined by O.D- value measurements.

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Key words: Boiling period, *Bacillus cereus*, Buffaloes` and Cows` milk, UHT milk

1. Introduction:

In developed countries, only 2% of the milk produced is consumed in its raw form, most of the milk in underdeveloped countries is consumed raw. In number of African countries as well as in Egypt large percentage of milk is retailed directly to consumers by farmers and small-scale traders including hawkers. Most of the dairy farmers are small holders with 2 or 3 animals lacking the hygienic conditions of production and veterinarian services; therefore their milk is not of best quality. In many of these countries, there is no enough processing plants to coup up with the milk produced .Moreover, the industry processes mostly UHT milk, which is expensive for most consumers, a matter that encourages them to consume retailed raw milk.

There fore they have to rely on boiling to establish milk safety and a reasonable shelf life. Not only UHT milk is expensive but also the treatment produces number of undesirable changes and products such as maillard reaction products and lactulose (Seiquer, *et al.*2010). Boiling is a common practice in many countries and WHO (World Health Organization) recommended milk boiling for African countries and described the process in its training manual (Israel-Ballard and Chantry, 2010). Boiling milk is simple process and does not need temperature gauges or timing devices which limit the use of pasteurization.

However, period of boiling treatment, the effect of boiling milk on microbial contents, keeping quality and chemical changes were not thoroughly studied. Period of boiling varies between individuals, some turns off the heat as milk boils & foams and others boil milk for 5 or 10 min., however at such high temperature, period of heating has a decisive effect on milk quality. For example, furosine production in boiling milk was increased for different period of heating from 110.4 to 116.7 and 128.9 mg/100g proteins as the period increased from zero into 5 and 10min., respectively (Sun and Wang, 2009).

Therefore this research was carried out to determine the proper boiling period which results in the required safety through a proper shelf life with minimum quality deterioration. Moreover, chemical changes occurring in boiled milk and UHT-milk were compared using a UV- absorption method.

2. Materials and methods

Fresh buffaloes' and cow's milk samples from the herd of the faculty of Agriculture, Cairo univ., *Bacillus cereus* NRRL, B.3711, from Northern Regional research Laboratory, Ill.USA and *Listeria monocytogenes* type 1 from Hungarian National Collection of Medical Bacteria, OKI, Budapest, Hungary.

Bacillus cereus and *Listeria monocytogenes* were activated in Brain- heart infusion broth (oxoid) at 37°C for 24hr. After sufficient growth, the cultures were diluted in saline to about 7×10^6 cfu/ml measured by standard plate count (SPC).

Thermal resistance of *Bacillus cereus and Listeria monocytogenes:*

The test tube method of Donnelly and Briggs (1987) was used to determine heat resistance of both microorganisms. One tenth of ml of 24 old culture (after the come up period) was inoculated into 10 ml of sterilized cow's milk in screw- capped tubes and were heated in a thermostatically oil bath. Milk was heated at 100°C for 0.5, 1, 5 and 10 min, and then samples were cooled in ice bath. Aerobic plate counts using plate count agar (oxoid) at 37°C for 48h.were used to determine the survivals. Rates for thermal inactivation of each bacterium were determined graphically by plotting the log₁₀ cfu/ml of surviving cell population versus heating time. A line was drawn through the date points and D-values were obtained from the slope of the best lit line (El-Shenawy et al, 1989).

Effect of milk different heat treatments on bacterial contents:

A half liter milk samples in glass beakers were heated at 80°C, 90°C and 100°C for 15 second in thermostatically controlled oil bath followed by rapid cooling in ice-bath to 5°C. In another experiment buffaloes' and cow's samples (One liter) were boiled for different periods and followed by rapid cooling. Samples were tested microbiologically for viable bacteria using aerobic plate count in nutrient agar (oxoid) (37°C for 48hr) after the standard methods for the examination of dairy products. Lethality was calculated as the difference between the log of colony counts of the untreated (No.) and treated samples (N₁) (\log_{10} No- \log_{10} N₁) (Roig-Saguès *et al*, 2009).

Chemically, the samples were tested using a UV- method for evaluation of heat treatment of milk (Sun and Wang, 2009).

Boiled milk keeping quality:

Cow's and buffaloes' milk of one liter samples were boiled for different periods (1.0, 2.0., 3.0 and 5.0 min) and after cooling, the samples were kept in a household refrigerator (\sim 7-8°C) for 10 days. Total bacterial contents of the samples were determined after 5, 7 and 10days of cold storage using plate count agar (oxoid) at 37°C for 48h.

Bacterial ecology of boiled milk:

Buffaloes' milk was boiled at 100°C for 10 min and after cooling, milk was plated on nutrient agar at 37°C for 48hr. Colonies present were visually examined and colonies representative of each distinct morphology were counted, isolated and identified. Identification was carried out by observing colony shape and color, microscopic examination, reaction to gram stain, catalase test, and production of acid from glucose, mannitol and zylose.

3. Results and Discussion:

The effect of heating milk at different temperatures on its microbial content was determined, Fig (1).Maximum lethality values of 5.793, 6.09 and $6.60 \log_{10}$

cfu/ml for buffaloes' milk and 5.85, 6.18 and 6.959 \log_{10} cfu/ml for cow's milk were obtained in milk heated at 80, 90 and 100°C for 15s, respectively. The destruction effect was temperature dependent. The 80°C resembles ultra-pasteurization treatment.

Lethality values of above 6 \log_{10} cfu/ml which obtained by boiling treatment, was greater than the minimum microbial inactivation of 5 \log_{10} required for pasteurization. Boiling milk for 15s reduced microbial count from 2 or 3×10^9 cfu/ml into few hundreds about 900 cfu/ml. The 15s of heating resembles the boiling flow-over end point used by regular consumer at home.



Fig (1): Change in aerobic bacteria counts (ABC) of cow's & uffaloes` milk heated at different temperatures for 15 second.

Table (1) shows the effect of increasing the period of boiling into 0.5, 1, 2, 5 and 10min.on milk microbial contents. The lethality rate increased by increasing boiling period to reach 6.76, 7.059, 7.012, 7.15 and 7.59 in cow's milk and 6.53, 6.77, 7.301, 6.76 and 7.44 log₁₀ cfu/ml in buffaloes' milk at 0.5, 1, 2, 5 and 10min., respectively. Boiling for 0.5, 1.0 and 2min reduced total bacterial count from 7.8×10^9 to 2.26×10^3 , 1.3×10^3 , 3.9×10^2 in buffaloes' milk and from 3.6×10^9 to 6.3×10^2 and 3.2×10^2 to 3.5×10^2 in cows' milk. Increasing the holding period beyond the 0.5 min progressively reduced the numbers, but insignificantly. Lethality values of greater than 67 were obtained by boiling milk for 1 and 2 min for buffaloes' and cow's milk. Boiling periods beyond

2min did not show any increase in the lethality rate. Therefore, boiling milk need not to exceed than more 2min period.

Table (1) Total bact	erial count in for m	ilk boiled for	different period.
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Period of boiling, min ¹ .	Type of milk ²	Type of milk ²				
	Cow's	Buffaloes				
	Bacterial counts, $\times 10^9$ c	cfu/ml				
Raw milk (untreated)	3.6±26.46	7.8±31.22				
	Bacterial counts, $\times 10^3$	Bacterial counts, $\times 10^3$ cfu/ml				
0.5	0.63±0.03	2.26±0.26				
1.0	0.32±0.05	1.3±0.13				
2.0	0.35±0.07	0.39±0.04				
5.0	0.25±0.06	0.28±0.03				
10.0	0.25±0.07	0.29±0.04				
$1 - LSD_{(0.05)} = 2.5$ $2 - LSD(0.05) = 2$						

Microbial lethality at boiling temperature was higher in cow's milk than in buffaloes' milk. This is may be due to buffaloes' milk contains more fat and T.S than cow's milk, this high T.S reduces heat rate of exchange and the high fat content protects the microorganisms against heat. This is expected to increase bacterial heat resistance in buffaloes' than cow's milk. Some workers (Nasr, 2008) found that a temperature of 75°C for 25 seconds was required to kill *L.monocytogenes* in buffaloes' milk while the 72°C/15s was enough for cows' milk. But still boiling for both milks could be for 1 or 2 min at the most.

Therefore, regular boiling practices at home regardless the period used reduces the microbial load into a level considered to be safe for human consumption, particularly

that all pathogens are also destroyed. The bacterial counts were reduced by boiling to levels significantly lower than the 20.000 cfu/ml limit required for grade (A) pasteurized milk in pasteurized milk ordnance (Ranieri, *et al.*, 2009).

To determine the safety of boiled milk for human consumption, the survival of Listeria monocytogenes and Bacillus cereus, two pathogens usually found in milk, through boiling was studied. The D-value of both microorganisms at 100°C was determined. L. *momocytogenes* $(7.5 \times 10^6 \text{ cfu/ml})$ was completely destroyed at 100°C for all periods starting from the first instant of boiling, therefore, its D- value could not be determined. This means that non-spore former pathogens pose no danger in boiled milk. The Dvalue of *B.cereus* was determined in both milks to be 7.5min & 10.4 min for cow's and buffaloes` milk, respectively (Fig 2). These results are in the agreement with that obtained by El-kholy, 1993 who reported that D-value of L. momocytogenes was 1.4 sec. at 70°C. So, 100°C is more than enough for destroying the pathogen.

This means that *B.cereus* as spore formers would tolerate the boiling treatment; however, storage cold temperature would prevent its germination and growth.





Fig (2): D-value of Bacillus cereus determined in boiled milk.

Actually boiling destroys all vegetative cells leaving behind spores of sporeformers. This was found when the ecology of boiled milk was studied; results are presented in Table (2). Of the 15 grampositive spore forming bacteria isolated in the present study from boiled milk for 10min, 5 isolates were *Bacillus cereus* and the rest were characterized to be of the genus Micrococcus. Five isolates were *M*.

leteus, 7 isolates were *M. varians* and one was *M.roseus*.

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Table (2) Bacterial isolates	s obtained from	n milk boiled for	10min.
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Bacterial isolates	Number of isolates	Isolates (%)
Bacillus cereus	5	38.4
Micrococcus leteus	5	38.4
Micrococcus varians	2	15.3
Micrococcus roseus	1	7.6

Non –of these isolates were psycrotolerant endospore since after 10 days of cold storage the counts insignificantly increased as in Table(3) which presents the keeping quality of boiled milk. Milk samples boiled for 1, 2 and 5min were stored for 10 days in household refrigerator to determine the keeping quality.

Table	(3):	Boiled	milk	keeping	quality
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Cold	Cows' Milk	2		Buffaloes'		
Storage ² ,	Boiling ³ Pe	riod (min).				
days	1	2	5	1	2	5
			$\times 10^4$ cfu/m	l		
0	2.3±0.05	1.4±0.23	1.3±0.21	2.4±0.21	2.8±0.21	0.9±0.2
5	2.9±0.09	1.6±0.21	1.2±0.2	2.22 ± 0.26	3.6±0.44	0.20 ± 0.10
7	0.65 ± 0.02	0.65±0.02 0.65±0.02		0.8±0.09	0.2±0.03	0.2 ± 0.02
10	1±0.26	1.55 ± 0.03	0.75 ± 0.04	0.33 ± 0.03	0.2 ± 0.20	2.2 ± 0.02
1- LSD $_{(0.05) (overall)} = 2$ 2- L			$0_{(0.05)} = 1.2$	3-LSI		

There was insignificant count decrease after the fifth day of storage. This was followed by a significant decrease in the 7^{th} day and the counts insignificantly changed on the 10th day of cold storage. The counts were of a range between 2×10^3 and 7×10^3 cfu/ml on seventh day of storage, which means that boiling milk has a good keeping quality under refrigeration. Actually, the samples remained in good condition after 20 days of storage. It was found that spore formers are the major spoilage bacteria of heat treated milk .The bacterial ecology of high temperature short time pasteurized milk in the US for example was found to be gram positive endospore-forming bacteria (i.e Bacillus and Paenibacillus). During cold storage the predominant spoilage genera shifted from Bacillus spp to Paeinbacillus sp, some of these strains were psycrotolerant endospores and their growth caused milk spoilage, (Ranieri and Boor, 2009).

To compare the effect of heat treatment on milk quality a UV- method was use to discriminate between boiling & UHT treatments and the results are in Fig (3). Boiling up to 5 min developed less than half of 0.D- values of other heat treatments. The extended shelf life treatments was of intermediate 0.D-value between boiling & UHT-milk. These 0.Dvalues correlate with furosine contents and according to the above reference, furosine formation at 100°C is a straight line relationship with 0.D- values. This means that boiling is a more delicate treatment than all kinds of UHT treatment by forming far less amount of furosine.

A better flavor and causing less nutrition deterioration than UHT. The formation of furosine reduces the nutritive value of milk by decreasing protein availability and may behave as chelating agents for metal cations affecting their bioavailability. Over heating such as in bottle sterilization which sometimes is used in UHT production may result in decreasing of food intake (Seiquer, et al. 2010). It was found that UHT milk produce more furosine than boiling, boiling for 5min produced 116.7 while commercial UHT contained 142 mg furosine/100g protein (Sun and Wang, 2009). Also, UHT-milk was found to contain 0.181% free fatty acids, 0.453 mEq of O₂/kg of fat peroxides and thiobarbituric acid (TBA) values of 0.019 as compared to 0.118% Free fatty acids, 0.296 mEq of O₂/kg of fat peroxides and 0.018 TBA in 5min boiled milk (Meshref and Al-Rowaily,2008)

In conclusion, milk boiling for periods less than 2min whether boiling was carried out in an oil bath or on direct flame provides the consumer the required safety which lasts for a reasonable shelf life under at cold temperature life. The method though is simple and inexpensive for regular consumers preserves more of milk nutritive value and flavor compared to UHT treatments which is develops old or stale flavor. However, on boiling continuous stirring is essential particularly at boiling temperature to be sure that the formed foam is exposed to boiling temperature.



Fig (3): Discrimination between the effects of milk heat treatments using an UV-Absorption method

a: Boiling for zero period c: Boiling for 2min d: boiling for 1min d: boiling for 5min

e: Extended shelf life f&g: UHT of different brands

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Evaluation of the Effects of Bagasse on Tensile and Compressive Strength of Lightweight Concrete

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Abstract: Mechanical characteristics of lightweight concrete contained bagasse, including splitting tensile and compressive strength have been examined. Bagasse as an agricultural waste was obtaining in the form of small wood chips after extracting cane sugar in the Khouzestan state of Iran. In this research, at first a fixed mix design was considered according to ACI-21. Then some samples were prepared corresponding to the above mentioned design code and by inserting 20,30,40 and 50% bagasse as a replacement for aggregates in concrete mixture and consequently these samples were tested. The results showed that by increasing of the content percentage of bagasse, compressive strength decreased and this decrease for concrete containing 20% bagasse is about to 36%. The concrete containing 20% bagasse has more splitting tensile strength in compared to normal concrete approximately up to 13%. Finally, based on the obtained findings, it can be concluded that concrete with 20% bagasse could be introduced as an alternative lightweight concrete regarding to its lower unit weight and higher splitting tensile strength.

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Keywords: Bagasse; Lightweight concrete; Compressive strength; splitting tensile strength

1. Introduction

Using of lightweight concrete as an alternative to normal concrete in construction can decrease the building's dead load as well as the force exerted on the structure due to earthquake excitations and the resultant collapse weight of the building if it falls down (Liu, et al., 1995). The use of lightweight aggregate concrete (LWC) can lead to reduction in costs of the both superstructures and foundations. Furthermore, the better thermal insulation, the greater the fire resistance and the substantially equivalent sound-proofing properties make it preferable with respect to normal weight concrete (NWC) to use in building structures (Cavaleri, et al., 2003). In bridges and other precast construction, the lightweight concrete helps to reduce the costs of shipping and crane capacity, inclusive considering the higher cost of the aggregates (Tito, et al., 2010). Lightweight concrete provides high strength-to-weight ratio and is ideal for long span structures, super high-rise buildings and offshore floating structures. For example, in Norway (LWC) has been successfully used in offshore structures for oil drilling platforms, storage tanks and vessels (Koh. et al., 2008).

On the other hands, development of industries and stepping towards industrialization requires performing comprehensive research on consuming agricultural, mineral and industrial wastes in construction in order to decrease environmental pollutants. Bagasse is one of these agricultural wastes. It is a by-product of cane that is produced in the form of small wood chips after extracting cane sugar. At present, about one million tons of bagasse is produced in Iran annually which leads to environmental and retrieving management problems. So far, no significant research has been conducted on using bagasse in making concrete in Iran. Thus it is felt that such a study could be useful in this field.

Ganesan et al., (2007) studied on the effects of using bagasse ash as a replacement for cement in making concrete mixture on physical and mechanical characteristics of hardened concrete. They found that the bagasse ash was a useful mineral that its optimal replacement amount for cement in concrete mix was approximately 20%. Another research on using bagasse ash as a replacement for cement in concrete manufacturing has been conducted by Chusilp et al., (2009). He and his colleagues evaluated compressive strength, permeability and temperature characteristics of obtained lightweight concrete containing bagasse ash. They concluded that the optimal amount of bagasse ash which could be replaced with cement in preparing concrete would be about to 20% by weight of cement and increasing this ratio to 30% caused lower permeability and compressive strength. They also found that the maximum temperature rise of concrete containing 10-30% bagasse ash was less than that of control concrete so that an increase replacement ratio led to decrease in equivalent temperature rise.

Most of the researches on bagasse in concrete were associated with using it as a replacement for cement in order to improve concrete mortar. In this study, fibers of bagasse are used, in a new vision, as a replacement for aggregates so that its effects on mechanical characteristics of concrete such as compressive and tensile strength and relation between them could be investigated.

2. Material and Methods

2.1. Materials

2.1.1. Cement

Since today the most dominant type of cement used in Iran is type II, in this study cement of type II produced in cement factory of Doroud of Lorestan province located in the west of Iran has been used with density of 3.15 and specific area of about $3350 \text{ cm}^2/\text{gr}$.

2.1.2. Bagasse

Figure 1 show fibers of bagasse which used in this study. The needed bagasse has been supplied from Haft Tappeh Cane Sugar plant. The specific weight of bagasse can be found in table 1.



Figure 1. Bagasse fibers

Table 1. Unit weight of bagasse

	incompact	compact
Unit weight	64	129
$) kg / m^{3}($		

2.1.3. Water

Water which has been consumed for this study is drinking water of Ahvaz city of Iran.

2.1.4. Aggregates

Aggregates which used for this research are broken river materials from Dezful city of Iran. The coarse aggregates have two dimensions with combination of 50% 3/8in and 50% 3/4in, which makes well-graded materials. Table 2 shows physical characteristics of aggregates. In addition, according to Los Angles test on aggregates, percentage of lost materials has been determined as 16.2%.

Table 2. Physical characteristics	of	aggregates
-----------------------------------	----	------------

	Coarse	Sand
Humidity (%)	1.16	5.80
Unit weight(kg / m^3)	1526	1724
Void (%)	43	35
Density	2.68	2.64

2.2. Methods

2.2.1. Mix design, product and curing

Control concrete (CC) mix design has been provided according to ACI-211 and after creating experimental samples and making some modifications; final design obtained and has been illustrated in Table 3.It should be mentioned that mix design has been designed for 7 ± 1 cm slump and corresponding 28day cylindrical compressive strength which gained was equal to 300 kg/cm².

Since the purpose of this study is to examine the replacement of fibers of bagasse with aggregates in concrete mixture, we used bagasse as a replacement for aggregates with 0, 20, 30, 40, 50% volumetrically. It should be noted that because of 50% moisture absorbing of bagasse, water of the mixture has been increased as much as the half of the weight of bagasse to prevent decrease concrete humidity.

concrete)											
	Coarse	Coarse Sand Co		Water	Bagasse						
CC	1130	840	340	160	0						
CB20	904	672	340	176	32						
CB30	791	588	340	184	48						
CB40	678	504	340	192	63						
CB50	565	420	340	200	79						

Table 3. Mix design (kg per one cubic meter of

After being produced of mixtures, they are molded in 3 layers and each layer was compressed with 25 impacts by standard bar. Samples are then taken out from moulds after 24hours and put into curing basin for 7, 14, 28 days and then tested.

3. Results and discussions

3.1. Compressive strength

Figure 2 shows trend of growth of compressive strength of cylindrical samples according to curing time duration for each replacement percentage of bagasse.



Figure 2. Relation between compressive strength and curing time duration

As it can be observed, compressive strength of concrete samples reduces with increase of bagasse amount as a replacement for aggregates contents, which it can be attributed to weakening of concrete skeleton because of reduction of the amount of aggregates. Furthermore, the slope of growth of compressive strength of samples reduces with respect to the amount of baggase used in the mixture.

Figure 3 indicates the change in compressive strength of samples in relation to the percentage of bagasse used for different curing time duration. Loss of compressive strength of concrete with increase of bagasse percentage can be observed much better from this figure.



Figure 3. Relation between compressive strength and percentage of bagasse

Since the weight of the concrete samples is an important factor in surveying of mechanical properties of the samples and comparing with normal concrete, the variations of compressive strength in unit weight of the specimens i.e. C/W ratio at different curing time duration are given in Figure 4 for different percentage of bagasse, and Figure 5 indicates variations of (C/W) ratio with different percentages of bagasse for each curing time duration.



Figure 4. Relation between (C/W) ratio and curing duration



Figure 5. Relation between (C/W) ratio and percentage of bagasse

From the above figures it can be inferred that however unit weight of concrete decreases with increasing of percentage of bagasse, but loss of compressive strength is more and this ratio decreases. Therefore, it can be declared that compressive strength of concrete containing bagasse which replaced as a percentage of aggregates would decrease.

3.2. Splitting tensile strength

Figure 6 indicates the splitting tensile strength of concrete with respect to different curing time duration for each percentages of bagasse.



Figure 6. Relation between splitting tensile strength and curing duration

According to the above figure, behavior of tensile strength of lightweight concrete containing bagasse differs from that of compressive strength. Tensile strength of CB20 at all ages is more than that CC, which is considered as an advantage given to have an acceptable 28days compressive strength. Also 28days splitting tensile strength of CB30 is more than that of CC. For better observation of these variations, you can refer to figure 7 that show the relation between splitting tensile strength of concrete in different time curing and percentages of bagasse used in concrete.



Figure 7. Relation between splitting tensile strength and percentage of bagasse

Since the weight of concrete decreases with increasing of percentage of bagasse replaced for aggregates, this increase is more obvious. Figure 8 illustrates the change trend of tensile strength of lightweight concrete divided by its weight containing bagasse at different age of concrete specimens.



curing duration

As it can be understood, tensile strength to unit weight ratio (T/W) of CB20 and CB30 is more than CC, which can be attributed to fiber quality of bagasse. Figure 9 gives (T/W) ratio in relation to percentage of bagasse. By comparing this diagram with figure 5, it can be found that in contrast to C/W, the T/W ratio has an increasing trend at the first stages and after that we have decreasing curves. It shows that up to certain amount of added bagasse we can gain higher tensile strength of lightweight concrete. This can be related to the fiber structure of bagasse which improves the maximum resistant tensile stress of concrete. But after certain percentage of added bagasse i.e. 20% in this study, by increase the content of bagasse, the water in concrete which suctioned by fibers of bagasse also will be increased and consequently the w/c ratio (water to cement ratio) would be larger so both the tensile and compressive strength of concrete would decreased.



Figure 9. Relation between (T/W) ratio and Percentage of bagasse

3.3. Tensile/compressive strength ratio

Figure 10 and 11 displays the relation between tensile and compressive strength of lightweight concrete containing bagasse with respect to curing time duration and the percentage of bagasse replaced with aggregates, respectively.







Percentage of bagasse

As it can be indicated, tensile strength of CC is about 10% of its compressive strength that it has been predictable according to ACI standard. Also from the above figures it can be derived that (T/C) ratio of CB40 is maximum (0.43). This proofs that mechanical characteristic of splitting tensile strength of concrete containing bagasse is better compared with its compressive strength.

4. Conclusions

- All concretes containing 20% and more bagasse can be considered as lightweight concrete.
- By increasing of bagasse as a replacement for aggregates in concrete, compressive

strength decreases that it can be attributed to weakening of concrete skeleton, i.e aggregates.

- Although unit weight of concrete decreases by increasing of the percentage of bagasse, but loss of the compressive strength is more than its weight and this ratio also decreases. Therefore, it can be declared that compressive strength of concrete containing bagasse as a replacement for aggregates would be always decreased.
- Tensile strength of concrete containing 20% bagasse at all ages is more than that of normal concrete which can be considered as an advantage gained to have an acceptable 28days compressive strength and lower unit weight concrete when compared to normal concrete.
- Tensile strength divided by weight ratio of concrete containing 20% and 30% bagasse is more than normal concrete, that it can be attributed to fiber quality of bagasse.
- By increasing of the percentage of bagasse, tensile to compressive strength ratio increases up to 40%,that maximum of this ratio is for concrete containing 40%(0.43).

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Sulphur efficiency in rising of pollution soil by heavy metals qualification under conditions of lettuce plant cultivation

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Abstract: Pot experiment was established in green house at National Research Centre, Dokki, Egypt, in polluted soil from industrial wastes by heavy metals. Sulphur elemental was used at different rates (100, 200 and 300 ppm). Lettuce plant of class (lactuca sativa var. Capitata). Loamy sand soil type from Helwan region at south of Egypt. Some parameters in fresh plant were performed. From plant analysis showed existence positive relationships between sulphur concentrations were added and chlorophyll concentrations, (N,P and K) and (Fe, Mn, Zn and Co) while a negative contact between sulphur rates were added and (Cd, Ni, Pb and (Cu) contents by comparison with control. Water filtering from water irrigation was analyzed and shown that heavy metals were leached from soil. Soil was analyzed at experiment end shown that nonexistence heavy metals in soil except slight traces. All the differences between treatments were significantly.

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Key words: Sulphur. Loamy sand soil. Lettuce plant. Heavy metals. Macro-micro nutrients-chlorophyll.

Introduction

Pollution soils problems came great rate in modern time and dangerous as a result to several reasons as wastes human industries and heavy metals by different sources, it now scientists are trying in modern time finding solutions to this deteriorate through their researches until not happen decrease in foods, may occur world catastrophe as a result that and thus it give vegetable production is not healthy for human and animal to take extent, toxicity for them and may lead to death. Some modern researches, it found some solutions to this problem, such as cultivation of some plants species leaves plants specially to it high ability to absorption this heavy metals then using it at other purposes unlike the food, such as industries and vital fuel or using some amendments and conditioners or chemical materials to equal of charges this heavy metals and transformation to salts then leaching it from soil but this methods require to good drains to soil. Pollution and exces of salinity its main reason at decrease of soils fertilization in Egyptian soils. Accumulation of heavy material represents dangerous threat to vegetable production and animals.

These element important as pb. Zn, Cu, Ni, Hg and Cd six of the heavy metals, namely Ni, Zn, Cu, pb, Hg and Cd have been shown by many researchers to reach toxic levels in soils. their hazard extend to plants, animals and human being. In Egyptian soils the extent of pollution due to Cd and / or Hg accumulation is relatively limited (Hilal 1994). This study is important as only pb, Ni, Cd and Cu while others elements that find it with high concentrations in Egyptian soils are little ereas of soils.

Materials and Methods

Pot experiments were established under green house condition using pollution soil by industrial wastes from (Helwan) as Cairo south to evaluation effect of sulphur as amendment to improving soil properties under conditions lettuce plant cultivation and evaluation effect of sulphur on soil pollution and plants.

Soil textural was loamy sand. Table (1), shown some physical and chemical characteristics. Pot contents as 8kg of soil and pot diameter 40 cm, soil pot height 40 cm with position good drain system and receiving of filtering from pot after each irrigation to determination of heavy metals concentrations. Lettuce plant of class (lactuca sativa var capitata) were cultivated as indicator to treatments under study. Three replicates were taken to determination some macronutrients (N, P and K), some micronutrients (Fe, Mn, Zn and Co) and some heavy metals (Cd, Ni, pb. And Cu) that's after 60 days it growing period to lettuce plant. Nitrogen was added at a rate 100 ppm as a form ammonium suphate (NH4SO4) at one dose after plantation stage, di-hydrogen potassium phosphate KH2PO4 was added at a rate 200 ppm as a source for each of potassium and phosphours. There levels of sulphur were added as elemental sulphur, it (100,200 and 300) ppm uniformly mixed with soil surface layer. The moistur content of the pot was maintained 100% of saturation capacity along the experiment period plants were harvested on 60 days.

Site	Clay%	Tex	PH (1:2.5)	E.C (dSm ⁻¹)		Anions mq/L						
		ture	()		Ca ⁺⁺	\mathbf{Mg}^{++}	\mathbf{Na}^{+}	\mathbf{K}^{+}	HCO ₃	5	Cľ	SO4-
	17.3	Loamy sand	8.5	0.31	11.2	38.0	38.2	44.3	7.6		151	1.45
elwan	O.M%	CaCO ₃ %	0.1	w.	DTPA Available heavy metals (µg/g)			Total heavy metals (μg/g)				
Η					Cd	Ni	Pb	Cu	Cd	Ni	Pb	Cu
	0.39	1.35	1.3	37	0.47	13.90	3.92	0.31	0.7	25.2	10.2	0.35

Table (1): Some chemical and physical properties of the studied soil.

Fresh plants were divided to many parts to fresh weight, after that plant samples were dried to constant weight at 70°C in aventilated oven, dried plant material was anlysed for total nitrogen was determined by using microkjeldahel distillation (Jakson, 1985). Phosphours, potassium, heavy metals (Cd, Ni, pb) and micronutrients (Fe, Mn and Zn) as according (Jakson, 1985). (chlorophyll concentration fresh leaf was determined according to (Licheloem and weilburn, 1983).

Results and discussion

Sulphur of main amendments to different soil types clay, calcareous and sandy soils as decreasing of soil pH which increase available of nutrients to plants and thus increasing mobility of elements in soil of what contribute to get ride of high concentrations harmful elements by leaching during of soil irrigation. (Abd-Elfattah et al., 2005) indicated that a drop in soil pH as a function of applied sulphur which dependent upon soil type, rates of sulphurs application and inoculated period of soils with sulphur oxidizing bacteria. (Tyler and Mc Bride, 1982). They reported that the soil chemical properties had generally a larger effect on the mobility of metals than did the properties of the metal themselves. The least mobility of metals was observed in a mineral soil with a relatively high pH, CEC and exchangeable base content. The order of mobility of the metals in the used soils was: Cu $\leq Z \leq Ni$ $\leq Cd$. This work was used sulphur to evaluation effect on behavior of some heavy metals (Cd, Ni, pb and Cu under conditions of lettuce plants cultivation. Table (2) shown that some parameters of lettuce plant in harvest period end. Sulphur treatments were at a positive relation with the determination parameters by comparison control as increasing of sulphur concentration.

Increase of all parameters (Fresh plant weight, fresh roots weight and number of leaves). Fresh weight increased by rate (18.5, 29, and 68.3) % with sulphur concentrations (100.200 and 300) ppm respectively comparative control,

Table	(2):	Some	parameters	in lettuce	plant as	s affected	by	sulphur	additions	and	pollution	SOIL	by	heavy
		1	metals.											
Г														_

Sulphur treatments	Fresh plant weight gm	Fresh root weight gm	No of leaves
Control	109	24.3	46.0
100	131	28.7	58.0
200	149	39.6	68.0
300	194	45.7	77.0
L.S.D.0.05	12.6	5.43	7.32
L.S.D.0.01	16.9	7.66	9.15

Fresh roots weight took the same of previously trend increasing in the fresh roots weight comparative control (18.1, 63 and 88) % pursuant to sulphur concentrations respectively. Number of leaves were in the order (26, 48 and 67)% pursuant to sulphur concentrations respectively. In this results shows that sulphur increase plant ability in keeping of the water, and it increase of dry weight to plant.

(Abd-El Fattah et al, 2005). Found that the yield of garlic increased from 4 to 5.7 t by application of 2t per acre of suplphur alone.

Dry matter status:

Data in table (3) shows that dry plant weight was in a positive connection with sulphur concentrations addition as whenever increased of sulphur concentrations. Increased of dry plant weight pursuant to the following order head value of dry plant weight (36.4 gm) was with sulphur concentration (300 ppm) while among value (23.8 gm) was with (200ppm) of sulphur concentration, the least value (16.7 gm) was found with (100 ppm) of sulphur concentration. Increasing rates in comparison control as following (22, 74 and 166) % pursuant to sulphur concentrations respectively. All differences between the treatments were significantly almost to each of two significant levels.

Dry roots weight took the same previos trend as was found a positive contact between sulphur concentrations and dry matter weight to roots as whenever increased of sulphur concentration increased of dry roots weight. Increasing rates were estimated comparative control as following (61, 176 and 227)%, this results indicated that sulphur is play important role in life plants as that's increase plant uptake of macro micronutrients and its lead to structure of sulphureous amino acids which lead to structure of proteins which foundation plant growing, in addition to improving of soils physicals and chemicals properties. Some treatments were significantly under. L.S.D. 0.05 level and some the other were significantly under L.S.D. 0.01 level. (Graines and phatak 2002). Found that increases sulphus levels significantly increased the dry weight of corn, soybean, tomato, cowpea lops and soybean roots. They also stated that total N/S ratio in soybean were greatly reduced by increased sulphur rates, whereas protein N/S and (N/S): P ratio were constant and less affected. (Hilal and Abd El-Fattah. 1987) stated that the yield of barly dry matter per pot was shown to respond greatly to sulphur application in clay loam soil (Brazozowska et al., 1994). Reported that sulphur deficient plants of ground nut (Arachis hypogeal L.) had less protein N in all plant organs. There was an accumulation of arginine and a decrease in cyctine, cyctein and methonine contents.

Nitrogen contents status:

Data in table (3) showed that sulphur elemental treatments by different concentrations in a positive connection with nitrogen contents in lettuce plants. Whenever sulphur concentrations increased nitrogen contents is increase amounts of increasing of nitrogen contents comparative control as following (29, 107 and 134)% this mean's that the sulphur stimulate of plant uptake to nitrogen because of constitution sulphureous amino acids which lead to structure of the proteins. (Thomas et al., 1991; Frgle and Eaton 1994). Showed that (N/S) / P ratio of cotton leaves increased from 13 to 17 when sulphur was applied at rates varying between 1 to 10 ppm . (Spencer, 1990) found that sumptoms of sulphur deficiency did not appear until second growth period, when plants without added sulphur developed chlorotic leafiest. (McNaught and chrisloffels, 1991) indicated that sulphur addition enhanced nodulation, sulphur may have increased nodulation by increasing growth and nitrogen demand.

Phosphours contents status:

Phosphorus was found in a positive contact with sulphur concentrations, that's mean available phosphorus was continuing.

To plant a long the experiment period despite existence some bad properties in soil similar pH raising presence of calcium carbonate and bicarbonate, this properties contribute to phosphorus fixed as converted to unavailable form to plants, that's means the elemental sulphur was improved of soil properties. Increasing rates of phosphours contents compartive with control as following (17.5, 24.0 and 46.0)% with sulphur concentrations (100,200 and 300) ppm respectively. Previously results indicated that sulphur elemental increasing of phosphours available form and is protect's of soil phosphours from the fixation, it also improvement of soil properties.

(Khater, 1981) showed that application of sulphur generally increased available P in alluvial soil. (Shadfan and Hussein, 1985) found a significant increase in NaHCO3 extractable P from 10-13.5 ppm by applying 500 ppm S and 8 weeks of incubation in a loamy sand soil. (Heter, 1985) indicated that most of the added P to calcareous soil will be fixed as unavailable form for plants uptake due to the alkalinity reaction of the soil. He found that the addition of sulphur and H2SO4., through their effect on soil pH, are expected to increase the availability of P by increasing the solubility of the Ca compound he also found that the effect was more evident in the case of low CaCO3 content of soil and the available P was found to be correlated with the extracted SO4-S.

Sulphur treatments ppm	Dry plant weight gm	Dry root weight gm	N	Р	K
Control	13.7	3.42	1.76	0.63	0.13
100	16.7	5.52	2.27	0.74	0.17
200	23.8	9.44	3.65	0.78	0.21
300	36.4	11.2	4.11	0.92	0.26
L.S.D.0.05	5.56	1.05	0.82	0.092	0.026
L.S.D.0.01	5.63	1.78	1.11	0.128	0.038

Table (3): Dry weight and some macronutrients (%) content in lettuce plant as affected by sulphur additions and pollution soil by heavy metals.

Potassium contents status:

Potassium was found in a positive relation with sulphur concentrations (100, 200 and 300) ppm as whenever sulphur concentrations increasing potassium content in lettulce plant increases. Increasing rates of potassium contents comparative with control as following (31, 62 and 100)% respectively, this results showed that sulphur concentrations was contribute at potassium available to plants may be due to low of soil pH thus it protects of potassium of fixation between soil minerals layers and following turn unavailable to plants as a potassium element is needful for plants life also it enter in structure plant of dry matter. All differences between the treatments were significantly under each of two levels.

(Vijayapriya et al. 2009) showed that addition of 30 kg S h-1 in a clay loam soil at form gypsum in the presence bradyrhiobium inoculation with soybean resulted nutrient uptake and availability of nutrients were significantly by the addition of S % and Rhizobium compared to the control. The nutrient uptake and availability were significantly higher in plant inoculated with Rhizobium compared to uninoculated plants. The uptake of N, P, K and S by soybean and their availability in soil. increased with S levels and the highest values were recorded at 30 kg S h-1

Chlorophyll contents status:

Data in table (4) indicated that chlorophyll was found in a positive contact with sulphur concentrations (100,200 and 300) ppm. Also its in a positive contact with all micronutrients under study (Fe, Mn, Zn and Co) where as all this trace elements under study were found in a positive contact with sulphur concentrations under study also. Chlorophyll is nutrition factory to plants of during sunlight as perform light constructive process to industrialization carbohydrate matters especially. Chlorophyll is require to micro and macro nutrients at structure it is necessary, that's presence at available form in soil. Sulphur is play important role in available form abundance micro-macro nutrients in soil. Chlorophyll concentration increases with increasing of sulphur concentrations.

The increasing rates of chlorophyll concentrations comparative with control and due to sulphur concentrations were as (11.3, 17,8 and 58)% respectively. Head value of chlorophyll concentration was found with 300 ppm of sulphur concentration, among value with 200 ppm and least value with 100 ppm of sulphur concentrations from previously results show that sulphur is amendment conditions to polluted soils. (Rending and Mc comb, 1991) indicated that the chlorotic condition of leaves in sulphur deficient plants was visual evidence of disturbance in photosynthesis. They suggested that sever deficiency enough to disrupt normal photosynthesis would ultimately be reflected on the changes in the kinds and amounts of carbohydrates.

Sulphur treatments	Chlorophyll mg/g	Fe	Mn	Zn	Co
ррт					
Control	0.62	163	98	25	39
100	0.69	189	124	32	48
200	0.73	211	152	37	75
300	0.98	305	173	46	89
L.S.D.0.05	0.063	14.7	11.2	3.52	5.43
L.S.D.0.01	0.088	19.6	14.1	4.59	7.05

Table (4):	Chlorophyll	and some	micronutrients	(ppm)	contents	in	lettuce	plant	as	affected	by	sulphur
addition	s and polluti	on soil.										

Micronutrients contents status:

Table(4) contents shown that also some trace elements concentrations (Fe, Mn, Zn and Co) in lettuce plants as affected by polluted soil by heavy metals and different rates of elemental sulphur (100,200 and 300) ppm was added. Trace elements contents were found in appositive relationship with all off sulphur concentrations. Head values of (Fe, Mn, Zn and Co) were found with 300 ppm of sulphur concentrations while among values were found with 200 ppm and the least values with 100 ppm of sulphur concentrations increasing rates of trace elements contents comparison with control as to Fe (16, 29 and 87) %, as for Mn (27, 55 and 77)%, as to Zn (24, 48 and 84)% as for cobalt Co (23, 92 and 128)%. From previously results were recorded that sulphur of soil amendment under pollution conditions that's may be due to decreasing of soil pH and of rising of solubility rate to trace elements which lead to abundant available form for plant. (Abd El-Fattah and Hila., 1985) they suggested that the use of sulphur as a soil amendment would in case of Fe, Mn and Zn deficient soils, increase the availability of these elements and evok a plant response, if, however, the amount of applied sulphur exceeded the soils basicity, large quantities of Fe, Mn and Zn would be dissolved and may thus became toxic to both plants and animals and its probable that toxic level of Al would also occur under the conditions of high sulphur application.

Heavy metals contents status:

Data in Table (5) indicated of some heavy metals contents in lettuce plant as affected by different rates of elemental sulphur applications (100,200 and 300) ppm and pollution soil by heavy metals (Cd, Ni, Pb and Cu). Heavy metals contents were noticed in a negative relationship with sulphur concentrations applications. This's sulphur effect success in a slight of heavy metals concentrations in plants as its very harmful to human and animals health. Head value of heavy metals was found with sulphur concentration of 100 ppm, among value with 200 ppm and the least value with 300 ppm respectively.

Decreasing rates by comparison with control as following (34, 51 and 75.4)% as for Cd element respectively, as to Ni element (51, 65 and 89) % respectively, as to pb element (60, 63 and 65) % respectively and Cu element as (56,76 and 88)% respectively. This results may be due to the repetition leaches to soil in each once was irrigated soil as moisture reach to a rate 100% saturation capacity then was taken of the leaky as was determined heavy metals at every once as reached a times number of irrigation or leaches were twelve of time also it incourages aspossibility dilulion of to heavy metals from soil pollution to become useful to cultivation (Tyler and Bride, 2005). They reported that the soil chemical properties had generally a larger effect on the mobility of metals than did the properties of the metal themselves the least mobility of metals was observed in a mineral soil with a relatively high pH. CEC and exchangeable bas content. The order of mobility of the metals in the used soils was: $Cu \le Zn \le Ni \le Cd$. (Biddappa et al., 2002) found that the pb and Cu ions were less mobil than that of Zn and Cd. Nickel has exhibited greater mobility than other heavy metals under the leaching stress in two typical soils of Jappan.

The specific migration properties of each metal ion varied depending on the nature of the ion and of the leaching solution.

Heavy metals status in drain water:

Data in table (6) shown that heavy metals concentrations in irrigation water to evaluation heavy metals concentration in every leaching then added up to total at harvest season and under different rates (100, 200 and 300) ppm of elemental sulphur applications and lettuce plant cultivation. Results indicated that to presence a positive contact between amounts of heavy metals were leached in the filtrating water of the irrigation water and sulphur concentrations applications (100,200 and 300) ppm. Head values of heavy metals were found with sulphur rate 300 ppm, while among values were found 200 ppm and the least values were found with 100 ppm from sulphur rates were added, that's to all of heavy metals under study (Cd, Ni, pb and Cu) whenever increased of sulphur rate was added heavy metals concentrations in filtrating water were increasing. The increasing a mounts of heavy metals by comparison with control as (53, 67 and 93)% as for cadmium (Cd) element with sulphur rates were added (11,200 and 300) ppm respectively.

Sulphur	Cd	Cd Ni Pb							
Ppm		ppm							
Control	0.65	6.53	2.86	0.25					
100	0.43	3.17	1.15	0.11					
200	0.32	2.29	1.07	0.06					
300	0.16	0.75	1.00	0.03					
L.S.D.0.05	0.055	0.255	0.234	0.010					
L.S.D.0.01	0.072	0.331	0.304	0.024					

Table	(5): Some	heavy	metals	contents i	n lettuce	plant a	as affected	by s	sulphur	additions	and j	pollution :	soil by
heavy	metals.												

As to nickel element as (907, 2009 and 2973)% respectively.

As for lead (pb) element (1200, 1254 and 1319)% with sulphur rates were added (100,200 and 300) ppm respectively and copper element Cu (23, 154 and 169)% respectively.

From previous results shown that sulphur is play role very important in increasing of heavy metals elements mobility and it raising of solubility product. All differences between the treatments were significantly on both of two levels. Herms and Brümmer (2007) found that at pH 7 and 8 Zn and Cd show a very low solubility. Which increase strongly with decreasing pH. The concentration of Ni also rises with dropping pH. The concentrations of Cu and pb increases. When pH values decrease below 4 to 5. at pH 6 to 8 solubility of pb and Cu rises due to increasing solubility of complexing organic substances. The soil reaction influences the heavy metal solubility in the order Cd \geq Zn>Ni<pb. Compared with mineral soil components

Residua effect of heavy metals in soil:

Data in table (7) reveal that residual effect in soil of heavy metals under study after of yield harvest as found that the soil became devoid of the heavy metals pollution, that's due to elemental sulphur which confirmed of good efficiency at get rid of heavy metals from soil therefore necessary using it on wide range in soil pollution, through generally look on table (7) was found that not residual of heavy metals except slight traces, it not harmful.

Table (6): Heavy metals concentrations in irrigation water after each leaching under cultivation of lettuce plants and sulphur applications.

	ppm															
Sulphur		Co	ntrol			1	00		200				30	0		
treat ments	Cd	Ni	Pb	Cu	Cd	Ni	Pb	Cu	Cd	Ni	Pb	Cu	Cd	Ni	Pb	Cu
No of Irrigation																
1	0.00	0.01	0.01	0.01	0.02	0.03	0.01	0.00	0.01	0.02	0.03	0.01	0.02	0.03	0.03	0.02
2	0.00	0.02	0.01	0.00	0.03	0.02	0.01	0.02	0.01	0.02	0.05	0.03	0.02	0.05	0.02	0.02
3	0.00	0.04	0.02	0.01	0.03	0.05	0.01	0.01	0.02	0.03	0.05	0.01`	0.02	0.14`	0.07	0.03
4	0.01	0.03	0.05	0.00	0.02	0.05	0.02	0.01	0.02	1.15	0.11	0.02	0.02	1.87	0.61	0.03
5	0.02	0.07	0.03	0.00	0.02	0.17	0.04	0.01	0.02	1.12	0.12	0.05	0.02	1.19	0.55	0.03
6	0.01	0.06	0.05	0.02	0.04	0.22	0.03	0.01	0.02	2.14	0.09	0.07	0.02	2.47	0.45	0.03
7	0.02	0.02	0.07	0.01	0.02	0.15	0.15	0.01	0.02	1.22	0.13	0.06	0.02	1.33	0.76	0.03
8	0.01	0.05	0.01	0.00	0.01	0.21	0.18	0.01	0.03	1.15	0.15	0.03	0.03	2.36	0.53	0.03
9	0.02	0.10	0.01	0.02	0.01	1.15	0.20	0.02	0.02	1.24	1.12	0.01	0.02	2.35	0.67	0.03
10	0.02	0.07	0.06	0.03	0.01	1.10	2.06	0.02	0.02	1.28	1.06	0.02	0.03	1.47	0.58	0.04
11	0.02	0.03	0.02	0.02	0.01	1.17	1.03	0.02	0.03	1.11	1.03	0.01	0.04	1.36	0.03	0.03
12	0.02	0.05	0.03	0.01	0.01	1.22	1.07	0.02	0.03	1.14	1.07	0.01	0.03	2.24	0.95	0.03
Total	0.15	0.55	0.37	0.13	0.23	5.54	4.81	0.16	0.25	11.6	5.01	0.33	0.29	16.9	5.25	0.35
L.S.D.0.05	0.002	0.007	0.004	0.002	0.003	0.07	0.06	0.002	0.003	0.14	0.06	0.004	0.003	0.20	0.06	0.003
L.S.D.0.01	0.003	0.009	0.005	0.003	0.004	0.09	0.08	0.003	0.004	0.18	0.08	0.005	0.004	0.26	0.08	0.004

Table (7): Residual	effect of heavy	metals in soil	under study	after harvest	of lettuce plants.
	entered of means		and brandy		or returned promotion

Sulphur treatments ppm	ppm							
	Cd	Ni	Pb	Cu				
Control	0.02	0.01	0.22	0.01				
100	0.02	0.02	0.25	0.02				
200	0.03	0.01	0.43	0.03				
300	0.02	0.02	0.69	0.02				
L.S.D.0.05	0.003	0.002	0.04	0.002				
L.S.D.0.01	0.004	0.003	0.05	0.003				

Summary and Conclusion:

The aims of this work study and evaluate to elemental sulphur efficiency at rising fertilization of soil pollution by heavy metals (Cd, Ni, pb and Cu) under lettuce plant cultivation conditions. Sulphur was used at three rates (100, 200 and 300) ppm. All the parameters and determinations confirmed existence a positive effects to sulphur applications especially with rate 300 ppm of sulphur was added. Sulphur effects due to decreasing of soil pH, oxidation reduction reaction, availability of macro-micronutrients and increasing of mobility and solupility product to the elements, in the experiment end not reisual of heavy metals except slight traces therefore was recommended by using of sulphur especially of high concentration with soil leaching.

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Incorporation Jatropha Curcas Meal on Lambs Ration and It's Effect on Lambs Performance

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Absract: This study was conducted to determine the effect of heat (HJM), or biologically with lactobacillus bacteria (BJM), treatments of Jatropha *curcas* meal with on concentrate ion of anti-nutritive compounds. In order to replacement of costly imported soybean meal and find out their effects on rumen fermentation characteristics degradability and consequently lambs performance. Seven concentrate feed mixtures (CFM), contained soybean meal was replaced with untreated Jatropha meal (UJM) by 0%, JMU (CFM⁰), 25% JMU (CFM¹), 50% JMU (CFM²), or heated Jatropha meal (JMH) by 25% (CFM³) and 50% JMH (CFM⁴) or biological Jatropha meal (JMB) by 25% (CFM⁵) and 50% JMI (CFM⁶), were formulated to study their degradation kinetics in the rumen, concentration of anti-nutritive compounds and performance of lambs fed tested rations. Biological treated (BJM) was more effective in decreasing anti-nutritive compounds than heat treatment. These were reflecting on the degradation kinetics, where DM and OM and their effective degradability (ED) were higher in (BJM) than (HJM). No significant differences were detected for daily gain of lambs fed rations contained basel or that contained 50% BJM. Economic cash return was more profit for BJM ration than the basel ration. Under the conditions of the present experiment, could be concluded that the bacterial treated JCMB could be replaced up to 50% JMB with Soybean meal at CFM.

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Keywords: Jatropha curcas meal, biological treated heated treated, degradability and daily gain.

INTRODUCTION

In Egypt sheep and goats industry is the least developed compared to other livestock industries. Feeds costs in sheep production the highest cost of the production requirements and may account 70-80% of costs. Nutrition is an important factor in sheep development, and a variety of nutrients are involved in proper growth and reproductive maturation. On contrast, it is well known that in Egypt, there is a serious shortage in rations and many oil crops had by-product which represent a real problem. Jatropha is oil crops belonged to family was known for its toxicity. The toxicity of jatropha was related to contain anti- nutritional compounds, which can effect on animal performance. Several study found that addition of 5% detoxified of castor meal in the diet has not been caused adverse effects or nutritive problems on lactating dairy cows, beef cattle and sheep (Alexander, 2008). Moreover, these authors found that calves fed milk from test cows showed neither apparent muscle residue accumulation nor abnormality organs. The heat treatment in combination with the chemical treatment of sodium hydroxide and sodium hypochlorite has also been reported to decrease the anti- nutritional compounds level in jatropha to 75% (Hass and Mittelbach, 2000). Egypt was planted jatropha curcas in different areas (luxor, ismailia, suez and giza). The hectare is vield up to 5 tons seed given about 1.85 tons of oil in the year (El-Gamassy, 2008). The protein quality of the meal obtained from shelled jatropha seeds is high with 1-2% residual oil

has a crude protein (cp) content of between 58–64%. The available information on the toxic principles of jatropha is very scanty with feeding. The purpose of this study to investigate the effect of heat or biological treatments on degrading anti-nutritional compounds and their effects on lamb's performance.

MATERIALS AND METHODS

The present study was carried out at el-sero experimental station, belonging to the Animal Production Research Institute, Agricultural Research Center.

Detoxification methods Heat treatment:

Jatropha curcas meal sample which left after extraction of oil, was heated in boiling water for 15 min to inactivate the anti-nutritional.

Lactic acid bacteria (LAB) treatment:

Jatropha meal was treated with lactobacillus acidophilus, at rate of 1g/100kg JM, stored in plastic containers for 21 days at room temperature, then dried to reach about 6% moisture and was ground to pass a 2 mm screen.

Anti-nutritional compounds analysis:

Trypsin inhibitor activity was determined essentially in untreated and treated Jatropha meal samples, according to Smith et al., (1980). Analysis of Lectin content was conducted by haemagglutination assay described by Gordon and Marqardt (1974). Total saponin (triepennid and steroidal) content was determined using a spectrophotometric method described by Hiai et al., (1976). Phytate content was determined by a colorimetric procedure described by Vairtrash and Laptera (1988). Seven concentrate feed mixtures (CFM's) were formulated to be iso-nitrogenous iso-energetic, by replacing sovbean meal contained in the concentrate feed mixture (CFM*), with 25 or 50% of untreated Jatropha meal UJM, for CFMU¹, CFMU², respectively. Mixtures of (CFM*), where sovbean meal was replaced with 25, or 50% of heated HJM, for CFM³, CFM⁴ mixtures, respectively, or 25 or 50% of treated meal with lacto bacillus bacteria BJM, for CFM⁵ or CFM⁶ mixtures, respectively. Representative samples of different concentrate feed mixtures, were analyzed according to A.O.A.C, (`1999). Chemical composition of UJM and BJM are shown in Table (1).

Degradability of different nutrients

Nylon bags technique was applied to determine degradability of DM, OM and CP for CFM's as described by Orskov and McDonald (1979). The degradability kinetics of DM, OM and CP were estimated (in each bag) by fitting the disappearance values to be equation P=a+b (I-e[°]) as proposed by Orskov and McDonald (1979), were P represents the disappearance after time I least squares estimated of soluble fractions are defined as the rapidly degraded fraction (a), slowly degraded fraction (b) and the rate of degradation (c). The effective degradability (ED) for tested rations was estimated from equation of McDounald (1981).

Feeding trial was conducted by using twenty male growing male lambs, (18.9 + 1.20 kg and 5-6 months). Animal were divided into two similar groups (10 animals each). Feeding trials lasted 150 days and animals were fed according to NRC (1994). The control group (R1) received basel ration composed concentrate fed mixture (CFM)50% and fresh berseem (FB)40% and rice straw 10%, respectively. Meanwhile tested group (R2) received CFM where soybean meal was replaced by 50% of (BJM). Animals were weighed (biweekly). Economical evaluation was calculated for the tested rations according to the prevailing prices of feeds during the time of the experiment. The data were statistically analyzed to test the significant using one way analysis of variance according to SAS, 2004, and Duncan's multiple range test was applied to test significant among means (Duncan, 1955).

ESULTS AND DISCUSSION

Chemical analysis of untreated and treated Jatropha meal.

Treatment of JM with lactobacillus (Lac) was resulted in a decrease in CF content by about 18.8%, meanwhile other treatments had quite similar for CF content.

Ingredients	Untreated	Treated	
	JM	HJM	BJM
Chemical Composition (%)			
OM	92.76	92.87	92.48
СР	40.83	40.07	43.60
CF	10.77	11.24	8.25
EE	9.45	10.33	9.21
NFE	31.71	31.13	31.92
Ash	7.24	7.13	7.52
Anti-nutritional compounds			
Trypsin inhibitor mg/g	23.30	8.84	4.20
Lectin mg/ml ⁻¹	55.41	12.17	7.35
Phytate g/100g	6.50	3.40	2.75
Saponnin %	4.50	3.50	2.40

 Table (1): Chemical composition (%) of Jatropha meal and anti-nutritional compounds.

*JM :untreated Jatropha meal

*HJM : Treated Jatropha meal with heat

*BJM : Treated Jatropha meal with Bacteria

On the other hand CP content was increased by about 6.8%, while other treatment was resulted in a decrease in CP content by about 1.63% and 1.84% (with heat) (Table 1). Ash content was increased by about 4% with biological treatment. Data in Table (1), showed that both treatments had a positive effect on decreasing concentration of antinutritional compounds, which consider as inhibitors and negative had effect on animals appetite (Ahmed and Adam, 1979 and Hajos et al., 1995). Bacteria treatment with lactobacillus (LB) decreased concentration of Trypsin inhibitors and lectin by about 82% and 86.7%, respectively. Meanwhile, heat treatment decreased the concentration of Trypsin inhibitor and lectin by about 75.54% and 83%, respectively. These results are in agreement with Haas and Mittelbach (2000) and Harinder et al., (2008) who reported that heat treatment has a positive effect on reducing Trypsin inhibitor and lectin concentration in JCM. On the meantime, phytic acid concentration was decreased. Saponins concentration of JCM was less affected by different treatment methods, these results agreed with those of Rakshit et al., (2008) who have reported that saponins was the lowest anti-nutritional compound affected with different treatment methods. So, lactobacillus (LB) treatment had higher effect on reducing anti-nutritional compounds as compared with heat treatment, which had lower effect. On the meantime, Martinez-Herrera et al., (2008) and Belewu et al.,(2010) observed that biological treatment was more effective on decreasing anti-nutritional compounds than heat treatment.

Ruminal degradation kinetics contents (a,b and c) for DM, OM and CP of concentrate feed mixtures (CFM's) are presented in Table (2). It illustrated that washing loss fraction (a) degradable fraction (b) rate of degradation (c) and effective degradability (ED) of DM and OM were less (P < 0.05) for untreated (UJM with 25% & 50%) levels as compared with the control mixture (CFM).

Ingredients	Experien	Experienced concentrate feed mixtures								
	CFM ⁰	CFM ¹	CFM ²	CFM ³	CFM ⁴	CFM ⁵	CFM ⁶	+		
DM										
А	28.27 ^a	26.12 ^b	23.85 ^c	27.32 ^a	26.15 ^{ab}	28.13 ^a	27.32 ^a	1.07		
В	55.28 ^a	52.15 ^b	49.82 ^c	54.42 ^a	53.48 ^{ab}	55.20 ^a	54.62 ^{ab}	1.36		
С	0.045	0.042	0.038	0.041	0.038	0.040	0.038	0.004		
EDDM	54.46 ^a	50.39 ^b	45.52 ^c	52.78 ^{ab}	50.16 ^b	53.84 ^{ab}	52.71 ^b	6.58		
OM										
А	26.78 ^a	24.36 ^b	22.57 ^c	25.68 ^a	24.72 ^b	25.82 ^a	25.43 ^a	0.88		
В	56.72 ^a	52.63 ^b	50.65 ^c	53.72 ^b	52.67 ^b	56.16 ^a	55.44 ^a	0.67		
С	0.052	0.048	0.042	0.051	0.049	0.052	0.050	0.006		
EDOM	56.90 ^a	52.21 ^b	47.74 ^c	53.72 ^b	52.67 ^b	56.24 ^a	54.94 ^{ab}	7.62		
СР										
А	23.42 ^a	22.62 ^{ab}	21.53 ⁵	23.18 ^a	22.92 ^{ab}	23.28 ^a	23.12 ^a	0.53		
В	64.46 ^a	60.82 ^b	58.33 ^b	62.18 ^{ab}	60.18 ^b	64.32 ^a	63.63 ^a	0.65		
С	0.054	0.051	0.046	0.053	0.052	0.054	0.053	0.005		
EDCP	57.47 ^a	50.72 ^b	45.80 ^c	53.67 ^{ab}	52.35 ^b	55.83 ^a	54.68 ^a	1.43		
	1									

 TABLE (2):Degradation kinetics of DM, OM and CP for experimental concentrate feed mixtures

A,b and c means in the same raw for each parameters with different superscripts are significantly different (P<0.05).

Also, washing loss fraction (a) degradable fraction (b) rate of degradation (c) and effective degradability (ED) of DM and OM were higher (P<0.05) for biological treatment as compared with untreated one. Lower soluble fraction (%) and rate of degradation were noticed with untreated JM ration for DM and OM degradation compared to the control. The treatment with bacteria increased DMD and OMD slightly higher than treatment with heat treatment. The decrease of degradability of CFMs containing untreated UJM may be due to the negative effect of Trypsin inhibitor and lectin on ruminal microorganisms. Ahmed and Adam (1979) and Rakshit et al., (2008) concluded that Trypsin inhibitor content of JM as well as other anti-nutritional compounds are affecting digestibility. The digestibility of CP for CFMs contained untreated UJM was lower than digestibility of CP for CFMs contained treated JM as a result to the high content of Trypsin inhibitor on UJM. On the mean time, the degradability of CP with bacteria treatment was higher than heat treatment, may be as a result to the over protection with heat treatment.

Average daily feed intake, daily gain and economic return for lambs fed experimental rations are shown in Table (3). There were no significant differences between experimental rations concerning the average daily feed intake. There were no significant differences between experimental rations among average daily gain. These results could be due to the positive effect of the biological treatment. These results are in agreement with Belewu, et al, (2010) who reported that treated Jatropha meal has not a negative effect on both daily gain and feed intake.

Results of economical evaluation are shown in Table (3). As a result of replacement 50% Soybean meal with BJM, the average daily feed cost in D2 was decreased by 17.24% than the control group. At the same time, both economic return and economic efficiency was improved by 4.11 and 19.32%, respectively for D2 as compared with control ration.

Under conditions of the present experiment, could be concluded that bacterial treated BJM could be replaced up to 50% of soybean meal in CFM without any adverse effect on lambs performance.

 Table (3): Effect of experimental diets on feed intake,
 daily gain and economic efficiency

Items	Experimenta	1 Rations
	R1	R2
No of animals	10	10
Days of Experiment	150	150
Av. Initial B.W. kg	19.10	18.70
Av. Final B.W. kg	49.5	48.5
Total B.W. gain kg	30.4	29.8
Av. Daily gain g	203	199
Av. feed intake g	1234	1206
Av. Daily Feed cost	1.45	1.20
(LE)		
Price of daily gain LE	5.10	5.00
Economic Return	3.65	3.80
Economic Efficiency	3.52	4.20

LE= Egyptian

a, b ,c Means in the same raw having different significantly differ (P < 0.05)

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The Effect of Boiling on Milk Microbial Contents and Quality

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Abstract: Though milk boiling is a widespread heat treatment in number of countries, the process was not thoroughly studied. In this study, the effect of boiling buffaloes' and cow's milk samples for different periods on their microbiological contents, keeping quality and bacterial ecology contents and chemical changes were determined. Lethality rate of 6.53, 6.77, 7.301 and 7.44 in buffalo's and 6.76, 7.059, 7.012, 7.15 and 7.159 log₁₀ cfu/ml in cows' milk were obtained on boiling the samples for 0.5, 1,2 and 5min., respectively. Boiling milk for 0.5 and 1min decreased the bacterial count from 3.6×10^9 in cow's milk into 6.3×10^2 and 3.2×10^2 and from 7.8×10^9 in buffaloes 'milk into 2.26×10^3 and 1.3×10^3 cfu/ml, respectively. On cold storage, the microbial content of boiled milk, not only did not increase but also declined on the first week. Boiling destroyed bacterial vegetative cell leaving behind spores of the sporeformer which were dominated with *B.cereus* and *Micrococcus leteus*. Boiling affected milk quality far less than the effect occurred in UHT milk as determined by O.D- value measurements.

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Key words: Boiling period, Bacillus cereus, Buffaloes` and Cows` milk, UHT milk

Introduction

While in developed countries, only 2% of the milk produced is consumed in its raw form, most of the milk in underdeveloped countries is consumed raw. In number of African countries as well as in Egypt large percentage of milk is retailed directly to consumers by farmers and small-scale traders including hawkers. Most of the dairy farmers are small holders with 2 or 3 animals lacking the hygienic conditions of production and veterinarian services, therefore their milk is not of best quality. In many of these countries, there is no enough processing plants to coup up with the milk produced .Moreover, the industry processes mostly UHT milk, which is expensive for most consumers, a matter that encourages them to consume retailed raw milk.

There fore they have to rely on boiling to establish milk safety and a reasonable shelf life. Not only UHT milk is expensive but also the treatment produces number of undesirable changes and products such as maillard reaction products and lactulose (Seiquer, *et al.*2010). Boiling is a common practice in many countries and WHO recommended milk boiling for African countries and described the process in its training manual (Israel-Ballard and Chantry, 2010). Boiling milk is simple process and does not need temperature gauges or timing devices which limit the use of pasteurization.

However, period of boiling treatment, the effect of boiling milk on microbial contents, keeping quality and chemical changes were not thoroughly studied. Period of boiling varies between individuals, some turns off the heat as milk boils & foams and others boil milk for 5 or 10 min., however at such high temperature, period of heating has a decisive effect on milk quality. For example, furosine production in milk by boiling was increased by period of heating from 110.4 to 116.7 and 128.9 mg/100g proteins as the period increased from zero into 5 and 10min., respectively (Sun and Wang, 2009).

Therefore this research was carried out to determine the proper boiling period which results in the required safety through a proper shelf life with minimum quality deterioration. Moreover, chemical changes occurring in boiled milk and UHT-milk were compared using a UVabsorption method.

Materials and Methods

Fresh buffaloes' and cow's milk samples from the herd of the faculty of Agriculture, Cairo univ., *Bacillus cereus* NRRL, B.3711, from Northern Regional research Laboratory, Ill.USA and *Listeria monocytogenes* type 1 from Hungarian National Collection of Medical Bacteria, OKI, Budapest, Hungary.

Bacillus cereus and *Listeria monocytogenes* were activated in Brain- heart infusion broth (oxoid) at 37°C for 24hr. After sufficient growth, the cultures were diluted in saline to about 7×10^6 cfu/ml measured by standard plate count (SPC).

Thermal resistance of *Bacillus cereus* and *Listeria* monocytogenes:

The test tube method of Donnelly and Briggs (1987) was used to determine heat resistance of both microorganisms. One tenth of ml of 24 old culture (after the come up period) was inoculated into 10 ml of sterilized cow's milk in screw- capped tubes and were heated in a thermostatically oil bath. Milk was heated at 100°C for 0.5, 1, 5 and 10 min, and then samples were cooled in ice bath. Aerobic plate counts using plate count agar (oxoid) at 37°C for 48h.were used to determine the survivals. Rates for thermal inactivation of each bacterium were determined graphically by plotting the log₁₀ cfu/ml of surviving cell population versus heating time. A was line drawn through the date points and D-values were obtained from the slope of the best lit line (El-Shenawy *et al*, 1989).

Effect of milk different heat treatments on bacterial contents:

A half liter milk samples in glass beakers were heated at 80°C, 90°C and 100°C for 15 second in thermostatically controlled oil bath followed by rapid cooling in ice-bath to 5°C. In another experiment buffaloes' and cow's samples (0ne liter) were boiled for different periods and followed by rapid cooling. Samples were tested microbiologically for viable bacteria using aerobic plate count in nutrient agar (oxoid) (37°C for 48hr) after the standard methods for the examination of dairy products. Lethality was calculated as the difference between the log of colony counts of the untreated (No.) and treated samples (N₁) ($log_{10}No- log_{10} N_1$) (Roig-Saguès *et al*, 2009).

Chemically, the samples were tested using a UVmethod for evaluation of heat treatment of milk (Sun and Wang, 2009).

Boiled milk keeping quality:

Cow's and buffaloes' milk of one liter samples were boiled for different periods (1.0, 2.0., 3.0 and 5.0 min) and after cooling, the samples were kept in a household refrigerator (\sim 7-8°C) for 10 days. Total bacterial contents of the samples were determined after 5, 7 and 10days of cold storage using plate count agar (oxoid) at 37°C for 48h.

Bacterial ecology of boiled milk:

Buffaloes' milk was boiled at 100°C for 10 min and after cooling, milk was plated on nutrient agar at 37°C for 48hr. Colonies present were visually examined and colonies representative of each distinct morphology were counted, isolated and identified. Identification was carried out by observing colony shape and color, microscopic examination, reaction to gram stain, catalase test, and production of acid from glucose, mannitol and zylose.

Results and Discussion

The effect of heating milk at different temperatures on its microbial content was determined, Fig (1).Maximum lethality values of 5.793, 6.09 and 6. 60 log₁₀ cfu/ml for buffaloes' milk and 5.85, 6.18 and 6.959 log₁₀ cfu/ml for cow's milk were obtained in milk heated at 80, 90 and 100°C for 15s, respectively. The destruction effect was temperature dependent. The 80°C resembles ultra-pasteurization treatment. Lethality values of above 6 log₁₀ cfu/ml which obtained by boiling treatment, was greater than the minimum microbial inactivation of 5 log₁₀ required for pasteurization. Boiling milk for 15s reduced microbial count from 2 or 3×10^9 cfu/ml into few hundreds about 900 cfu/ml. The 15s of heating resembles the boiling flow-over end point used by regular consumer at home.



Fig (1): Aerobic plate counts for cow's and buffaloes` milk heated at different temperatures for 15 second.

Table 1 shows the effect of increasing the period of boiling into 0.5, 1, 2,5 and 10min.on milk microbial contents of milk. The lethality rate increased by boiling period to reach 6.76, 7.059, 7.012, 7.15 and 7.59 in cow's milk and 6.53, 6.77, 7.301, 6.76 and 7.44 \log_{10} cfu/ml in buffaloes' milk at 0.5, 1, 2, 5 and 10min., respectively. Boiling for 0.5, 1.0 and 2ml reduced total bacterial count from 7.8×10^9 into 2.26×10^3 , 1.3×10^3 , 3.9×10^3 in buffaloes' milk and from 3.6×10^9 into 6.3×10^2 and 3.2×10^2 and 3.5×10^2 in cows' milk. Increasing the holding period beyond the 0.5 min progressively reduced the numbers, but insignificantly. Lethality values of greater than 67 were obtained by boiling milk for 1 and 2 min for buffaloes' and cow's milk. Boiling periods beyond 2min did not show any increase in the lethality rate. Therefore, boiling milk need not to exceed the 2min period.

Table (1) Aerodic plate count for milk dolled at different per
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Period of boiling, min ¹ .	Type of milk ²			
	Cow's	Buffaloes		
	Bacterial counts, $cfu/ml \times 10^9$			
Raw (untreated)	3.6±26.46	7.8±31.22		
	Bacterial counts, $cfu/ml \times 10^3$			
0.5	0.63±0.03	2.26±0.26		
1.0	0.32±0.05	1.3±0.13		
2.0	0.35±0.07	0.39±0.04		
5.0	0.25±0.06	0.28±0.03		
10.0	0.25±0.07	0.29±0.04		

1- $LSD_{(0.05)} = 2.5$

2- LSD (0.05) = 2

Microbial lethality at boiling temperature was higher in cow's milk than in buffaloes' milk, which was expected. Buffaloes' milk contains more fat and T.S than cow's milk, this high T.S reduces heat rate of exchange and the high fat content protects the microorganisms against heat. This is expected to increase bacterial heat resistance in buffaloes' than cow's milk. Some workers (Nasr, 2008) found that a temperature of 75°C for 25 seconds was required to kill *L.monocytogenes* in buffaloes' milk while the 72°C/15s was enough for cows' milk. But still boiling for both milks could be for 1 or 2 min at the most.

Therefore, regular boiling practices at home regardless the period used reduces the microbial load into a level considered to be safe for human consumption, particularly that all pathogens are also destroyed. The bacterial counts were reduced by boiling to levels significantly lower than the 20.000 cfu/ml limit required for grade (A) pasteurized milk in pasteurized milk ordnance (Ranieri, *et al.*, 2009).

To determine the safety of boiled milk for human consumption, the survival of *Listeria monocytogenes* and *Bacillus cereus*, two pathogens usually found in milk, through boiling was studied. The D-value of both microorganisms at 100°C was determined. *L. momocytogenes* $(7.5 \times 10^6 \text{ cfu/ml})$ was completely destroyed at 100°C for all periods starting from the first instant of boiling, therefore, its D- value could not be determined. This means that non- spore former pathogens pose no danger in boiled milk. The D-value of *B. cereus* was determined in both milks to be 7.5min & 10.4 min for cow's and buffaloes` milk, respectively (Fig 2). This result is agreement with that by (El-kholy, 1993) who reported that D-value of *L. momocytogenes* was 1.4 sec. at 70°C. So, 100°C is more than enough for destroying the pathogen.

This means that *B.cereus* as spore formers would tolerate the boiling treatment; however, storage cold temperature would prevent its germination and growth.

Actually boiling destroys all vegetative cells leaving behind spores of sporeformers. This was found when the ecology of boiled milk was studied; results are presented in Table (2). Of the 15 gram-positive spore forming bacteria isolated in the present study from boiled milk for 10min, 5 isolates were *Bacillus cereus* and the rest were characterized to be of the genus Micrococcus. Five isolates were *M. leteus*, 7 isolates were *M. varians* and one was *M.roseus*.



Fig (2): D-value of Bacillus cereus determined in boiled milk.

Table (2) Bacterial isolates obtained from milk boiled for 10min.

Bacterial isolates	Number of isolates	Isolates (%)
Bacillus cereus	5	38.4
Micrococcus leteus	5	38.4
Micrococcus varians	2	15.3
Micrococcus roseus	1	7.6

Non –of these isolates were psycrotolerant endospore since after 10 days of cold storage the counts insignificantly increased as in **Table(3)** which presents the keeping quality of boiled milk. Milk samples boiled 1, 2 and 5min were stored for 10 days in household refrigerator to determine the keeping quality.

Cold	Cows' Milk			Buffaloes' Milk		
Storage ² , days	Boiling ³ Period	, min.				
	1	2	5	1	2	5
			$CFU/ml \times 10^4$			
0	2.3±0.05	1.4±0.23	1.3±0.21	2.4±0.21	2.8±0.21	0.9±0.2
5	2.9±0.09	1.6±0.21	1.2±0.2	2.22±0.26	3.6±0.44	0.20±0.10
7	0.65±0.02	0.65±0.02	0.6±0.18	0.8±0.09	0.2±0.03	0.2±0.02
10	1±0.26	1.55 ± 0.03	0.75 ± 0.04	0.33±0.03	0.2±0.20	2.2±0.02

Table (3): Boiled milk keeping quality¹

1- LSD (0.05) (overall) = 2

2- LSD $_{(0.05)} = 1.2$

3- LSD $_{(0.05)} = 2$

There was insignificant count decrease after the fifth day of storage. This was followed by a significant decrease on the 7th day and the counts insignificantly changed on the 10th day of cold storage. The counts were of a range between 2×10^3 and 7×10^3 cfu/ml on seventh day of storage, which means that boiling milk has a good keeping quality under refrigeration. Actually, the samples remained in good condition after 20 days of storage. It was found that spore formers are the major spoilage bacteria of heat treated milk .The bacterial ecology of high temperature short time pasteurized milk in the US for example was found to be gram positive endospore-forming bacteria (i.e Bacillus and Paenibacillus). During cold storage the predominant spoilage genera shifted from *Bacillus spp* to *Paeinbacillus sp*, some of these strains were psycrotolerant endospores and their growth caused milk spoilage, (Ranieri, *et al*, 2009 and Boor, 2009).

To compare the effect of heat treatment on milk quality a UV- method (was use to discriminate between boiling & UHT treatments and the results are in Fig (3). Boiling up to 5 min developed less than half of 0.D- values of other heat treatments. The extended shelf life treatments was of intermediate 0.D-value between boiling & UHT-milk. These 0.D- values correlate with furosine contents and according to the above reference, furosine formation at 100°C is a straight line relationship with 0.D- values. This means that boiling is a more delicate treatment than all kinds of UHT treatment by forming far less amount of furosine.



Fig (3): Discrimination between the effects of milk heat treatments using an UV-Absorption method

- a: Boiling for zero period
- c: Boiling for 2min
- e: Extended shelf life
- b: boiling for 1min d: boiling for 5min
- f: UHT of different brands

A better flavor and causing less nutrition deterioration than UHT. The formation of furosine reduces the nutritive value of milk by decreasing protein availability and may behave as chelating agents for metal cations affecting their bioavailability. Over heating such as in bottle sterilization which sometimes is used in UHT production may result in decreasing of food intake (Seiquer, et al. 2010). It was found that UHT milk produce more furosine than boiling, boiling for 5min produced 116.7 while commercial UHT contained 142mg furosine/ 100g protein(Sun and Wang, 2009). Also, UHT-milk was found to contain 0.181% free fatty acids, 0.453 mEq of O₂/kg of fat peroxides and thiobarbituric acid (TBA) values of 0.019 as compared to 0.118% Free fatty acids, 0.296 mEq of O₂/kg of fat peroxides and 0.018 TBA in 5min boiled milk (Meshref and Al-Rowaily,2008)

In conclusion, milk boiling for periods less than 2min whether boiling was carried out in an oil bath or on direct flame provides the consumer the required safety which lasts for a reasonable shelf life underat cold temperature life. The method though is simple and inexpensive for regular consumers preserves more of milk nutritive value and flavor compared to UHT treatments which is develops old or stale flavor. However, on boiling continuous stirring is essential particularly at boiling temperature to be sure that the formed foam is exposed to boiling temperature.

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Hormesis Influence of Glyphosate in Between Increasing Growth, Yield and Controlling Weeds in Faba Bean

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Abstract: This study investigates the response of faba bean and associated weeds to the low rates of glyphosate (1.4, 2.8, 5.6, 11.2, 22.4, 44.8, and 89.6 g a.i./feddan) corresponding to 0.3, 0.6, 1.2, 2.4, 4.8, 9.6, and 19.2% of the usage rate (0.467 kg/feddan), respectively. Two field experiments were conducted in this regard during two successive seasons (2008/2009-2009/2010). The plants were sprayed once and twice, 5 and 15 weeks after sowing. Generally, the one foliar application treatments were more effective in increasing faba bean growth than the two foliar application treatments. The best results obtained were for the concentration 11.2 g/feddan, especially sprayed as once. Plant height, fresh and dry weight as well as yield and its components were significantly increased in response. Total protein and carbohydrate contents were unaffected by the treatments. Weeds in converse were negatively affected recording the highest inhibition rate at the concentration 11.2 g sprayed also as once. It has been suggested that the low doses of glyphosate can induce faba bean growth, and this could indirectly affect weeds growth. [El-Shahawy, T.A. and Faida A.A. Sharara. Hormesis Influence of Glyphosate in Between Increasing Growth, Yield and Controlling Weeds in Faba Bean. Journal of American Science 2011;7(2):139-144]. (ISSN: 1545-1003).

Keywords: faba bean, glyphosate, herbicides, hormesis, weeds

Introduction

Searching for new uses of herbicides started very long time ago with discovering hormesis, the beneficial effect of low doses of toxicants against living organisms. The story is quite different than that, started earlier with using medicines in curing diseases. The chemist and surgeon scientist Aureolus Philippus Theophrastus who is known also as Paracelsus (1493-1541) is the one who set the broad lines of such science (Duke et al. 2006). Paracelsus who is regarded also as the father of toxicology is often paraphrased to have mentioned that the poison is in the dose ["Alle Ding sind Gifft nichts ohn Gifft. Allein die Dosis macht das ein Ding kein Gifft ist." (All things are poison and are not poison; only the dose makes a thing not a poison"]. On that basis, we can find many compounds toxic at higher doses and stimulatory or even beneficial at low doses as pharmaceuticals that are used for their beneficial effects and pesticides that are normally used as toxicants. The separating line is then the dose and every thing is turning around it. Although of this old historical background, discovering hormesis is even too older. According to Duke et al. (2006), the term hormesis was first used by Southam and Ehrliech (1943) to describe the effect of an oak park compound that promoted fungal growth at low doses but strongly inhibited it at higher doses. It is originated from the Latin word "Hormo" (to excite), the same used in hormone expression. Experimental data, which can be qualified as hormesis phenomenon should fulfill a number of requirements (Szarek 2005). Of which the most important is that the substances causing hormesis response should be potentially toxic or even very toxic. It is also important if researched materials are subjected to operation in at least 6 various doses.

Hormesis has been found within all groups of organisms from bacteria to fungi to even higher plants and animals (Calabrese 2005). Great concern has been forward in this phenomenon to animals and mammals (Cook and Calabrese 2006 and Sanders and Scott 2008). Slight concern, however, has been given to plants (Mushak 2007 and Calabrese 2009). Less documentation is found and approximately no information concerning the mechanisms of action is presented (Cedergreen *et al.* 2007).

Most researches on plants and herbicides have been done with the purpose of weed control (focus has been on the adverse effects) and hormesis is normally commented on as a non-target objective (Streibig 1980). Hormesis in the plants has therefore received little attention until recently (Calabrese 2005 and Calabrese and Blain 2005).

A survey of hormesis caused by herbicides in crops and aquatic plants demonstrated that hormesis can range from a few percentages up to 100% increase in the measured parameters, but with an average of 20-30% stimulation compared to the control (Cedergreen *et al.* 2005). Allender *et al.* (1997) found 2,4-D and metrbuzine as effective hormetization factors for increasing growth of cotton and corn plants when used at sublethal doses. On the other hand, the authors revealed that such increasing in growth can be stopped with using lithium and lanthanum chlorides. In an experiment involved 8 different herbicides and 10-15 doses of their effects on barely, the results showed that glyphosate together with the sulfonylurea metsufuron had caused aerial parts to increase by up to 25% more than the control only if used at 5 to 10% of the usual field rate. In contrast, the other 6 left herbicides failed to show a similar response on target plant (Cedergreen 2008a). With focusing research on glyphosate, the globally most widely used herbicide (Bradberry et al. 2004), the results showed a unique effect on increasing plant growth at rates less than toxic dose (Ellis and Griffin 2002, Ellis et al. 2003 and Velini et al. 2008). Similar action was noticed with gluphosinate. The authors revealed that the hormetic effect in glyphosate is related to the molecular target of action, since the effect was not seen in glyphosate- resistant crops e.g. soybean.

The present study evaluates the hormetic effect of glyphosate on faba bean growth as well as its associated weeds.

Materials and Methods

A field experiment was conducted during two successive seasons (2008/2009-2009/2010) to study the potential hormetic effect of glyphosate [(Roundup® 48%, N-(phosphonomethyl)glycine), Monsanto Co.] on the growth and development of faba bean (Vicia faba L., var. Aquadulce) plant as well as the effect on the associated weeds. The study was carried out at the experimental station of National Research Centre, El-Behera Governorate, Egypt. The soil texture was sandy soil with pH= 7.3, EC= 0.3%, OM= 0.3%, CaCl₃= 1.3%, N= 8.1 ppm, P= 3.2 ppm, and K (exchangeable)= 20 ppm (Chapman and Pratt 1978). Faba bean seeds were obtained from Agricultural Research Centre. Ministry of Agriculture and Land Reclamation, Egypt. The seeds were sown in the 1st week of November in the two seasons. After 5 and 15 weeks from sowing, the plants were sprayed once or twice with 0.3, 0.6, 1.2, 2.4, 4.8, 9.6, and 19.2% of the usage rate of 0.467 kg a.i./feddan glyphosate (1.4, 2.8, 5.6, 11.2, 22.4, 44.8, and 89.6 g/feddan, respectively). Three replicates were used for each treatment in a completely randomize block design. A preliminary trail was conducted under greenhouse conditions on faba bean without weeds infection to determine the appropriate time/concentrations to be used under field conditions, and to avoid confounding hormesis effect with weed control influence.

After two weeks of the treatment, a sample of one square meter of faba bean plants of those applied as once was taken. Plant height, number of branches per plant as well as fresh and dry weights were estimated. At the same time, weed samples were taken, and the fresh and dry weights were estimated for both broad and narrow-leaved weeds. Brassica nigra L., Melilotus indica L., Amaranthus cruentus L., Chenopodium ambrosioides L., Chenopodium murale L., Sisymbrium irio L., Eragrostis cilianensis All., Phalaris minor Retz. and Poa annua L. dominated the area.

At harvest, plant height, seed yield/feddan (feddan=4200m²) and yield components e.g. number of pods/plant, pods weight/plant, pod length, seeds number/pod, and 100- seeds weight were estimated. Total nitrogen and carbohydrates (%) were determined in the seeds (A.O.A.C., 1980). Nitrogen values were multiplied by 6.25 to determine total crude protein.

Results and Discussion

1-Growth

a-Faba bean plants:

Spraying faba bean with glyphosate at different concentrations varied between positive and negative effect to even no effect on increasing faba bean growth. No clear toxicity was observed at any of the examined concentrations (Table1). Applying the herbicide once was generally more effective than applying it twice (Data not estimated) at all traits had estimated (visual observation). Spraying glyphosate once at 11.2 g/feddan was the most effective treatment over all. Plant height, fresh and dry weights were all increased in response. Insignificant effect was noticed on no. of branches per plant. These results coincide with the results obtained by Cedergreen (2008b) who confirmed that glyphosate is able to increase plant growth if used at sub-toxic level (5-60 g a.i./ha). Velini et al. (2008) found that subinhibitory dosages of glyphosate (1.8-36 g a.i./ha) can induce hormesis growth in crops and plant species as different as sorghum, maize, convential soybean, Eucalyptus grandis. Pinus caribea. and Commelia benghalensis. The theories which explain hormetic growth are various based on the chemical being used and/or the plant species exposed to these chemicals. However, they all agree in one main point that is the plant escape to this phenomenon to overcome unfavorable growth conditions or chemical stresses (Cedergreen et al. 2007). Kovalchuk et al. (2003) reported that the induction in defense mechanisms induced by free radicals of oxygen can lead to increase growth in the presence of low doses of phytotoxic chemicals. On the other hand, some of the hormetic responses could stem from induction of plant hormonal systems as it was established with synthetic auxins (Morre 2000). The auxinic herbicides are a good example of these chemicals where they can induce growth at nontoxic concentrations through mimicking the growth hormone auxin, in the same time they are lethal at higher doses (Allender et al. 1997). Weyers and Paterson (2001) in this regard stated that if low doses of chemicals stimulated the production or activity of natural auxins or other plant hormone

systems, normetic responses courd men de exper	systems.	expected.
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Table (1): Effect of low doses of glyphosate applied once on the vegetative growth of faba bean plants at two weeks after treatment during the two successive seasons.

Clumbosata		Season 2	008/2009			Season	2009/2010	
rates (g a.i./feddan)	Plant height (cm)	Branches number /plant	Fresh weight (g/plant)	Dry weight (g/plant)	Plant height (cm)	Branches number /plant	Fresh weight (g/plant)	Dry weight (g/plant)
1.4	30.0	3.3	8.0	5.8	29.0	3.0	8.4	6.8
2.8	30.0	3.3	9.3	7.0	30.3	3.7	9.8	7.7
5.6	32.7	3.7	9.2	6.7	32.0	3.0	9.3	7.7
11.2	36.3	3.7	13.3	9.6	35.3	3.7	14.6	10.7
22.4	29.7	3.3	9.0	6.3	30.7	3.7	9.4	7.2
44.8	29.7	3.7	8.3	6.0	27.3	3.7	9.1	7.3
89.6	29.7	3.3	8.0	6.3	26.7	3.3	9.1	6.7
Control	32.0	3.7	9.0	6.0	31.3	30	9.5	8.0
LSD 0.05	2.5	N.S.	2.7	2.0	2.2	N.S.	2.3	1.9

b- Weeds:

Weeds were negatively affected in response of the different treatments (Table 2). Various activities were recorded in this regard. The three lowest concentrations (1.4, 2.8, and 5.6 g) had approximately no inhibitory effect on the associated weeds including broad and narrowleaved weeds. The greatest inhibition of weed growth was found with the concentration 11.2 g/feddan sprayed as once. This effect could be due to the greatest induction in faba bean growth which indirectly affected the growth of associated weeds (increasing competition upon weed plants). This result might be boosted by the results obtained by Cedergreen (2008b) who confirmed that glyphosate causes an actual biomass growth in plants and this growth can keep treated plants larger than untreated plants for up to six weeks which give them more opportunity to be more competitive than any others in the area including weeds. Moderate reductions were estimated at the concentrations 22.4, 44.8, and 89.6 g a.i./feddan, regardless if they applied once or twice (notes from visual seeing).

Table (2): Effect of low doses of glyphosate applied once	ce on the growth of associated weeds at two weeks after
treatment. (Combined analysis of two seasons)	

Glyphosate rates	Broad-1	eaved	Grasses		
(g a.i./feddan)	Fresh weight (g/m ²)	Dry weight (g/m^2)	Fresh weight (g/m^2)	Dry weight (g/m ²)	
1.4	13.4	9.7	6.5	3.9	
2.8	13.6	9.5	6.1	3.4	
5.6	10.8	8.3	4.3	3.1	
11.2	5.1	4.8	3.3	1.5	
22.4	7.2	7.7	4.2	2.2	
44.8	7.5	6.6	4.7	2.4	
89.6	6.7	6.1	4.6	2.4	
Control	14.0	11.7	7.3	4.4	
LSD 0.05	2.1	1.8	0.9	1.0	

2- Faba bean yield and its components:

Application of glyphosate (either once or twice) at the different non-toxicant rates resulted in increasing faba bean yield and its components. Applying the herbicide once was generally more effective than applying it twice at all traits investigated (Tables 3 and 4). This included the number of pods/plant, pods weight /plant, pod length, number of seeds/pod, 100- seeds weight, seed yield/plant and per feddan. Spraying faba bean plants with glyphosate at 11.2 g a.i./feddan after 5 weeks from sowing surpass all other treatments and cause an enhancement in seed yield per feddan by 58.93 and 65.23% over control in the first and second season, respectively (Tables 3 and 4). These increments may be due to the increasing occurred in number of pods per plant together with the weight of pods per plant not any of the other estimated characteristics. The decrement in enhancement of the subletheal dose of glyphosate at higher doses (more than 11.2 g a.i./feddan) could be explained by Wagner *et al.* (2003) who reported that total plant fresh weight presented a logistic response to glyphosate amounts, including a growth stimulant effect (hormesis), when corn plants absorbed less than $0.6 \ \mu g$, but when corn plants absorbed more than $0.6 \ \mu g$ they showed a decrease in growth.

Table (3): Effect of low doses of glyphosate on faba bean yield and its components in the first season (2008/2009).

Glyp ra a.i./f	bhosate ates (g eddan)	Plant height (cm)	Pods No. /plant	Pods weight /plant (g)	Pod length (cm)	Seeds No. /pod	100- seeds weight (g)	Seed yield/ plant (g)	Seed yield/ feddan (Kg)
	1.4	57.0	5.7	60.0	18.1	4.6	136.3	48.3	1158
	2.8	61.3	6.7	64.9	17.6	4.5	137.6	52.7	1266
ray	5.6	65.7	9.7	78.2	18.9	4.5	143.5	55.8	1338
ds	11.2	77.0	13.0	117.2	18.5	4.8	150.6	77.4	1858
)ne	22.4	62.0	11.0	72.3	18.2	4.5	133.8	56.5	1355
	44.8	53.3	7.3	59.2	18.4	4.7	126.2	43.6	1046
	89.6	49.7	5.3	48.2	16.3	3.8	126.3	40.3	968
	1.4	57.3	6.0	41.5	17.5	4.4	136.3	44.8	1074
>	2.8	53.7	6.3	45.6	18.0	3.6	135.7	46.5	1117
oray	5.6	52.0	5.3	44.7	18.6	3.7	142.8	53.4	1282
ls c	11.2	60.0	8.7	68.9	18.1	4.0	149.4	58.0	1392
[wc	22.4	58.3	6.3	55.3	17.0	4.4	128.8	40.4	969
	44.8	51.7	5.3	35.1	17.0	3.5	124.3	29.2	702
	89.6	50.3	5.0	32.9	16.6	3.9	122.6	26.2	629
Co	ntrol	59.0	6.3	62.1	16.7	4.0	137.2	48.7	1169
LSI	0.05	6.0	2.0	12.3	N.S	N.S	N.S	12.0	289

 Table (4): Effect of low doses of glyphosate on faba bean yield and its components in the second season (2009/2010).

(Glyp ra (g a.i./	phosate ates /feddan)	Plant height (cm)	Pods No. /plant	Pods weight /plant (g)	Pod length (cm)	Seeds No. /pod	100- seeds weight (g)	Seed yield /plant (g)	Seed yield /feddan (Kg)
	1.4	58.0	6.7	53.9	16.9	4.4	134.7	41.2	990
~	2.8	59.3	7.0	57.4	17.3	4.2	136.1	43.4	1042
oray	5.6	62.0	8.7	72.6	16.5	3.9	138.2	51.1	1226
e sb	11.2	79.3	11.7	101.4	16.3	4.1	148.6	70.5	1692
One	22.4	63.0	10.3	71.8	17.0	3.8	141.0	53.2	1276
Ŭ	44.8	59.7	9.3	67.7	17.5	4.1	128.9	45.4	1089
	89.6	49.7	5.3	41.1	16.9	4.2	125.6	27.1	650
	1.4	60.7	6.3	49.1	17.0	3.8	131.8	39.2	941
~	2.8	56.0	7.3	53.0	17.0	3.3	132.3	40.6	974
ora.	5.6	56.7	8.0	54.2	17.0	3.9	139.1	42.1	1010
ls c	11.2	64.3	8.7	66.8	18.0	4.4	145.0	49.8	1195
[we	22.4	58.3	7.3	48.5	16.8	3.9	132.2	33.8	811
	44.8	56.7	5.3	35.5	16.1	4.1	129.8	26.4	634
	89.6	47.3	5.0	33.1	15.7	4.8	124.8	25.7	618
Co	ntrol	61.0	7.0	55.8	17.9	4.1	134.5	42.7	1024
LSI	0.05	6.9	2.2	11.8	N.S.	N.S.	N.S.	11.8	283

More or less total carbohydrates and total protein slightly affected by the subletheal doses of glyphosate treatments (Table 5).

Glyphosate rates (g a.i./feddan)		Total Carbohydrates (%)	Total Protein (%)
	1.4	62.26	26.58
~	2.8	62.35	26.65
oray	5.6	62.43	26.44
e st	11.2	63.10	28.36
Jne	22.4	62.73	27.93
0	44.8	61.71	26.20
	89.6	61.63	25.95
	1.4	61.10	26.06
~	2.8	61.40	26.28
ora	5.6	61.80	26.59
ls c	11.2	61.60	26.73
Two	22.4	61.50	25.85
	44.8	59.66	25.64
	89.6	58.53	25.02
Control		62.30	26.66

Table (5): Effect of low doses of glyphosate on the protein and carbohydrate contents in faba bean seeds.

It could be suggested that the subinhibitory dosages of glyphosate can induce faba bean growth and gave more seed yield. It's worthy to mention that glyphosate is break down rapidly to amino acid glycine and it could be considered as safe herbicide (Cerdeira and Duke 2006).

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Neurological Disorders In Shoe-Makers And The Role Of Some Trace Elements

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Abstract: Workers in shoemaking industry are exposed to various risk factors which can result in many health hazards. They include CNS affection, other system abnormalities and biochemical derangements. The aim of this study is to investigate the possible neurological disorders among shoe makers, determinate the levels of Cu, Zn and Se in the workers, and correlate the environmental concentrations of respirable dust, heavy metals, and organic solvents contents in the workplaces with the detected neurological disorders. The study included 62 shoe makers and 72 control subjects who were matched for age, sex and socioeconomic status. Questionnaire and clinical neurological examination were done for all subjects. Serum trace elements (Cu, Se and Zn) were determined. Air samples were collected for environmental monitoring of volatile organic compounds (VOCs), respirable dust and its heavy metal content such as (Cu, Pb, Cr and Ni). Results showed that VOCs level in the ambient air of the workplaces were found to be lower than the OSHA standard. Respirable dust and heavy metal concentrations were found to be significantly lower than the Egyptian standard. The results of neurological examination revealed that 61% of the shoe makers had neurological disorders, while all the control subjects were normal. Nearly half the shoe makers with neurological abnormality had combined cranial and spinal neurological disorders (47.4%). Olfactory and auditory nerves recorded the highest frequency of affection. Among motor abnormalities, sensory abnormalities and disturbances in micturation reported in workers, muscular weakness had the highest frequency of occurrence. Duration of exposure in shoe makers with combined cranial and spinal abnormalities, and spinal alone were significantly longer than that in normal shoe makers and those with cranial problems while there was no significant effect of the age on the incidence of neurological abnormalities. On comparing levels of the trace elements between the shoe makers and controls, Se was significantly lower in the shoe makers, while levels of Cu and Zn levels were not significantly different between the two groups. No significant difference in levels of Cu, Se, and Zn between neurologically normal and abnormal shoe makers. In conclusion, Occupational exposure to organic solvents and other chemicals in shoemaking industry was found to have hazardous effects on nervous system both cranial and spinal. The protective role of trace elements has been suggested. Levels of Se were decreased in shoe workers, while, the exact role of Zn and Cu are not clarified in the development of neurological abnormalities and needs further study.

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Key Words: Cranial Neurological Disorders, Spinal Neurological Disorders, shoemakers, Organic Solvents, Zinc, Selenium.

Introduction

Shoemaking is one of the oldest professional industries. Various risk factors contribute to occurrence of health hazards in shoemakers. These include leather dust, noise, vibration, stress, ergonomic problems beside exposure to solvents, metals and other chemicals (Elci et al. 2007).

Although many processes are now completely automated, hand manufacture is still present in small scale industrial workshops (Hassan et al. 2001). Among the raw materials used in the manufacturing process and the most occupational hazardous materials are adhesives and dyes. These include natural solid and liquid adhesives and adhesive solutions based on organic solvents (Parmeggiani 1989). Polyurethane is one of the most commonly used adhesives in the studied workshops in the present work.

Synthetic adhesives used in shoemaking industry composed mainly of synthetic polymers (e.g. styrene, chloroprene, acrylonitrile) dissolved in a special mixture of organic solvents. These solvents are mainly n-hexan, benzene, xylene, ethylbenzene and toluene. Other constituents such as resins, catalysts, monomers, magnesium and zinc may be present as adhesives constituents (Hassan et al. 2001). Due to volatility of these organic solvents, inhalation is considered the main route of exposure, besides there is dermal absorption and may be ingestion. When solvents are used in mixture, the effects may be additive, synergetic or potential.

Adverse health effects which can occur in shoe workers include short term effects such as irritation of the eyes, nose and throat, headaches, dizziness, confusion, fatigue and nausea. Dermatitis and sensitization reactions can also occur in workers adhesives containing exposed to epoxyresins (wikipedia, 2009). The longer term effects include reproductive problems (ROM, 2007), neurological and psychological disorders (Elci et al., 2007). Hematological changes, lung, liver and kidney damage and increased risk of malignancy are also considered as long term hazardous problems in workers exposed to adhesives (Hassan et al., 2001).

The incidence of neurological disorders in shoe makers can be attributed to various factors including occupational exposures to organic solvents, metals and other chemicals. They include derangement of levels of neurotransmitters, enzymes, essential and trace elements inside the body (Alpay et al., 2004)

Copper, zinc and selenium and other trace elements are required in the formation of vital molecules and normal functions of many enzymes inside various body cells. Also they are essential for integrity and optimum function of the immune system. Cu is necessary for the manufacture of the neurotransmitter noradrenaline. Deficiency of Copper leads to anemia, liability to infections, osteoporosis, thyroid gland dysfunction, heart disease as well as nervous system problems (Clinical Neurophysiology, 2006).

Patients with amyotrophic lateral sclerosis are proved to have an essential trace element imbalance in the form of decrease CSF and serum Cu levels, increased serum manganese levels, while serum zinc and magnesium levels were unchanged (Kapaki, 1997). Moreover, Zn and Se are considered as essential antioxidant trace elements that have protective effects against the damaging effects of organic solvents in shoe makers (Hussein et al., 2008).

Therefore, a link between changes in serum levels of trace elements with neurological disorders caused in shoe makers is the hypothesis of this study. This relationship may be causal or an end result.

Usually, environmental monitoring of levels of pollutants in workplaces has been conducted in several studies to correlate between health risks and levels of these pollutants. Saad et al. (2008) found that levels of BTEX were measured in car spray painters' workshops to associate their levels with the neuropsychatric disorders among these workers. In another study, Sanni et al. (2002) correlated the levels of workplace organic solvents, airborne particles, SVOCs and noise in shoe repair shops with the self reported health effects such as musculoskeletal disorders, hearing loss, neurological disorders and allergic symptoms. Therefore, the current study aims to investigate the possible neurological disorders among shoe makers, determinate the levels of Cu, Zn and Se in the workers, and correlate the environmental concentrations of respirable dust, heavy metals, and organic solvents contents in the workplaces with the detected neurological disorders.

Subjects and methods

Study Design:

This study is a cross sectional comparative study between shoe makers and control subjects.

The principal steps of shoemaking are:

a- Making the uppers from natural leather or other materials. Leathers are prepared, arranged and cut according to prepared designs.

b- The leather parts are then sewn or glued together.

c- For making the bottom stock (soles or heels), leather is cutout, and then heels are made by compression of leather or wood strips. The stock is trimmed, shaped, scoured and stamped.

d- The uppers and bottom stock are assembled and then stitched or glued together.

e- Finally the shoe waxed, colored, sprayed, polished and packed.

Environmental Monitoring:

Air samples were collected for analysis of respirable particulate matter, heavy metal and organic solvents.

Determination of respirable particulate matter was done by using a vacuum pump with a rate of 2 liters/minute. Membrane filter paper was used for the collection of respirable particulates. On sampling, the membrane filter was weighted in the laboratory and then transported carefully to the field. It was mounted on open filter holder.

The air was a spirited on the weighted filter, and the reading of the gasmeter was recorded before and after sampling period. The membrane filter was reweighted after the sampling time (8 hours) and the difference in weight before and after sampling was recorded as the weight of respirable particulate matter. The volume of air was calculated from the gasmeter readings. The concentration of respirable particulates was calculated and expressed in μ g/m3.

Determination of heavy metals in respirable particulates, by using Atomic Absorption Spectroscopy (Jackson, 1973, Yasser, 2000).

Air samples were collected for volatile organic compounds (VOCs) (Activated Charcoal Tube Adsorption Method, ASTM Annual Book of ASTM Standards, Vol 11.03) aspirating air at a known rate through sampling tubes (Supelco ORBO 32 large) containing activated charcoal.

Subjects:

The present study included 62 shoe makers from six small scale workshops. The mean working daily hours ranged from 8 -10 hours. All steps of shoemaking were done at the same workplace in all the included workshops. A comparable group of 72 control subjects were also recruited in the study. They were matched for age, sex and socioeconomic status with the examined shoe makers. Non of the participants had a history of alcohol intake, nor a history of previous neurological problem.

Ethical approval was obtained from the National Research Center ethical committee prior to the clinical part of the study, and written consents were obtained from all examined subjects. Inclusion in the study was on volunteer bases.

Questionnaire and clinical examination:

All members of the study were interviewed and a full personal, occupational and medical histories were taken. A detailed neurological history and examination were carried out also for both the workers and their controls.

Laboratory:

A blood sample (5 ml) was obtained from all the examined subjects. The blood samples were

Table (1) Indoor Environmental Concentrations

centrifuged to separate sera which were kept at -20°C until analyzed. Serum trace elements (Cu, Se and Zn) were determined according to AOAC (1995), using Atomic Absorption Spectrophotometer (SOLAAR-UNICAM 989).

Statistical analysis:

The results were computerized. Statistical analysis was done through SPSS version 14.0. One sample t-test, Independent t-test, Pearson's Chi-square (χ^2) , Likelihood Ratio, and Analysis of Variance (ANOVA) and the least significant difference (LSD) as a post-hoc test were used in the analysis of the results. P-value 0.05 was considered significant.

Results

Table (1) revealed that environmental monitoring of total workshops' respirable particulate matter concentration was found to be significantly lower than the Egyptian standard set for indoor workplace environments. Again, heavy metals inside workplaces revealed that the concentration of copper (Cu), lead (Pb) chromium (Cr), and nickel (Ni) were significantly lower compared to the Egyptian standards. The total VOCs was in the range 0.074-0.082 mg/m³ (0.078 \pm 0.01 mg/m³).

Tuble (1) mabor Entrionmental etc				
	Mean	Egyptian standard	One sample t-test	P-value
Respirable particulates (µg/m ³)	1930±28.28	3000	53.5	P< 0.01
Heavy Metals				
$Cu (\mu g/m^3)$	10.5 ± 2.08	1000	950.7	P< 0.0001
$Cr (\mu g/m^3)$	17.75±3.09	500	311.6	P< 0.0001
Pb (μ g/m ³)	20.75 ± 4.65	50	12.59	P= 0.001
Ni (ug/m3)	9±1.83	1500	1633.3	P< 0.0001
Volatile Organic Compounds (VOC	Cs)			
Total VOCs (mg/m ³)	0.078±0.01			





The included subjects were all males. There was no significant difference between the shoe makers and their controls according to their age $(35.4\pm12.0 \text{ and } 37.8\pm9.23 \text{ years respectively})$ and according to their smoking index $(8.83\pm3.02 \text{ and } 3.58\pm1.39 \text{ package/year respectively})$. The duration of exposure of all the examine shoe makers were above 10 years (mean = 20.8 ± 14.7 years).

Neurological examination revealed that 61% of the shoe makers had neurological disorders (Figure 1). While, all the control subjects were normal.

Figure (2) shows that nearly half the shoe makers with neurological abnormality had combined cranial and spinal neurological disorders (47.4%). The details of neurological abnormalities were illustrated in Figures 3, 4, and 5.





Figure 2. Percent of shoe makers according to the neurological examination



Figure (3) Percent of shoe makers with abnormalities in the cranial nerves



Figure 4. Percent of shoe makers with abnormal movement



Figure 5. Percent of shoe makers with sensory abnormalities and disturbance in micturation

There were no significant effects of the age on the occurrence of neurological abnormalities among the examined shoe makers. Duration of exposure in shoe makers with neurological abnormalities both combined cranial and spinal and spinal alone were significantly longer than that in normal shoe makers and those with cranial problems (Table 2).

	A	ge	Duration o	f exposure			
	Mean	SD	Mean	SD			
Normal (24)	31.8	13.40	17.1	15.24			
Cranial problem (10)	33.6	8.40	15.8	10.01			
Spinal problem (10)	42.4	9.18	31.8	11.02			
Both cranial & spinal (18)	37.1	11.50	22.3	15.01			
ANOVA	1.0	19	10.715*				
P-value	N	S	P< 0.01				

Table (2): Role of age	, SI and duration of ex	posure on the neurological healt	problems in the workers
	·		1

*Kruskal Wallis Test

On comparing the levels of the trace elements between the shoe makers and their controls, Se was significantly lower in the shoe makers than in the control subjects, while levels of Cu and Zn levels were not significantly different between the two groups (Table 3).

Table (?	3) Com	narison	hetween	the level	s of Cu	Se	and Zn	in the	shoe	makers	and their	r controls
	$\gamma comp$	Jarison	Detween		s or Cu	, oc,	anu zn .	m uic	SHOC	marcis	and unon	controls

	Worke	rs (62)	Contro	ols (72)	Independent t-test						
	Mean	SD	Mean	SD	t-test	P-value					
Cu (ppm)	4.19	0.69	2.88	0.15	0.559	NS					
Se (ppm)	0.038	0.003	0.403	0.26	4.991	P< 0.0005					
Zn (ppm)	0.130	0.01	0.147	0.08	0.201	NS					

Table (4) showed that there was no significant difference between the levels of Cu, Se, and Zn in the

shoe makers with normal and abnormal neurological signs and symptoms detected through examination.

Table (4) Comparisons between the levels of Cu, Se, and Zn in the shoe makers with normal and abnormal neurological signs

	Cu (pr	om)	Se (pp	m)	Zn (ppm)				
	Mean	SD	Mean	SD	Mean	SD			
Normal (24)	3.24	0.12	0.04	0.01	0.11	0.04			
Cranial problem (10)	3.2	0.16	0.02	0.01	0.17	0.05			
Spinal problem (10)	3.24	0.15	0.04	0.02	0.08	0.02			
Both cranial & spinal problems (18)	6.59	2.37	0.04	0.02	0.08	0.02			
ANOVA	43.03	32	1.94	8	0.653				
P-value	NS		NS		NS				

Discussion

Employees in shoe manufacture are routinely exposed to a mixture of organic solvents used in cleaning and as diluents in glues, primers, and degreasers (Emara et al., 1996). Metals such as chromium can be present in the work environment from the tanned leather used in shoe manufacture. (Saad and Beshir, 2007; Saad et al., 2008). Lead, copper and nickel from dyes and varnishes used in painting and polishing of the shoes (Rom, 2007).

Exposure to organic solvents and other chemicals in shoe factories has been reported to increase the risks for acute and chronic health problems in shoe-makers (Alpay, 2004). Environmental monitoring of indoor workplace pollutants is routinely carried out in many studies in order to correlate levels of pollutants with health effects. Levels of many types of pollutants are measured in shoemaking and shoe repair workshops in previous studies.

In the current study, the concentrations of total VOCs and metals (Cu, Pb, Ni and Cr) as well as respirable particulate matter concentrations were measured in air of the selected workshops. Environmental concentration of total VOCs seemed to be not so high in the air of the selected shoemaking workshops, but there is no Egyptian Standard to

compare with. According to US Occupational Safety and Health Administration (OSHA, 2004), the concentration of VOCs in the selected workshops (0.078 ± 0.01) was within the safe limits, as OSHA has set 3.19 mg/m³ as workplace time weighted average regulation limit for a normal 8-h work day.

According to law 4 for 1994 of Egyptian standards, that modified in the year 2005 it has set a maximum limit of 50 μ g/m³ for Pb, 1000 μ g/m³ for Cu. In case of chromium, it is 500 μ g/m³ for Cr metal and trivalent Cr compounds, and 50 μ g/m³ for hexavalent compounds. Lastly it is 1500 μ g/m³ for Ni. OSHA has set a permissible exposure limits (PEL) of 50 μ g/m³ averaged over 8 hours for Pb. For Cu copper dust and mists 1000 μ g/m³, and 1000 μ g cr/m³ for chromium metal and insoluble compounds. Divalent and trivalent chromium compounds has PEL 500 μ g/m³, while it is reduced to 5 micrograms for carcinogenic hexavalent Cr compounds. While for Ni, it is 1500 µg/m^3 for the inhalation fraction of metal. (OSHA2006). American Conference of Governmental Industrial Hygienists (ACGIH) and NIOSH (2005) have set similar values for these metals as TLVs and PELs. So, it is obvious that indoor metal levels measured in the selected workshops in the present study were below the PEL for all of them (ATSDR, 2010)

In agreement with the present study, the study of Sanni et al. (2002), which detected very low concentrations of insoluble and hexavalent Cr in shoe repair workshops in Finland ($0.10 - 0.32 \ \mu g/m^3$ for insoluble Cr and $0.01 - 0.08 \ \mu g/m^3$ for hexavalent Cr). Levels of airborne particles sampled in front of finishing machines ranged from 70 – 1010 $\ \mu g/m^3$, which is comparable to our results. However, there was high concentrations of a mixture of organic solvent vapors (1.1-13.2) times the occupational exposure limit value.

The present study revealed increased incidence of neurological problems among shoemakers compared to the controls. Both cranial and spinal problems occurred. Affection was more evident in cranial nerves I, VII, VIII, while spinal problems were in the form of muscular weakness, increased sensory abnormalities and micturition disturbances. This was in accordance with many studies which examined neurological or neuropsychatric abnormalities in shoemakers or shoe repair workers. For example, in the study of Elci et al. (2007), they found that muscular leg cramps, imbalance in walking and hand tremors were the most prevalent neurological symptoms among shoemakers in Turkey. Gobba (2006) found in his study occupationally-related olfactory impairment in workers chronically exposed to industrial chemicals such as some metals as cadmium, chromium, manganese, arsenic, mercury, and organic lead, and to other chemicals as acrylates, styrene, and solvent mixtures. This was in agreement with the present study which found that the most affected cranial nerve among our workers was the olfactory nerve (figure 3).

Previously, outbreaks of a disease known as shoemakers paralysis have appeared in a number of footwear factories, presenting with a clinical picture of sever form of flaccid paralysis, localized to the limbs and gives rise to osteotendinous atrophy with areflexia. The condition was explained by functional inhibition or injury of lower motor neurons of the pyramidal tract (Parmeggiani, 1989).

And, in the study of Langauer et al. (1983) on women workers exposed to glue solvents in a shoe factory, Cortical organic changes and subclinical neuropathy were significantly more frequent in workers exposed to high concentrations of extraction naphtha and toluene.

Another group of workers exposed to solvents such as dockyard painters reported significantly higher neurological problems than controls. They were in the form of problems in buttoning and unbuttoning, hands trembling and feeling weak or unsteady in the arms or legs (Chen, 1999).

However, in contrast to these results, Sanni et al. (2002) found in their study that self reported neurological disorders of shoe repair workers in Finland were low (1%) compared to other work related diseases like musculoskeletal disorders, dermatitis, rhinitis and asthma.

The present study revealed no significant effect for age on the incidence of neurological disorders. But, the duration of exposure was significantly increased in the shoe makers with spinal neurological abnormalities compared to the normal workers and those with cranial abnormalities. Similarly, Elci et al. (1) found that the rate of peripheral neuropathy was increased by duration of work, but this was insignificant statistically. Additionally, Hassan et al. (2001) and Emara et al., (1996) proved that the incidence and the severity of neurological and psychiatric symptoms were related to the duration of exposure to neurotoxic solvents in shoemaking and other industries.

The present study revealed no increase in air concentrations of heavy metals (according to the Egyptian standards) in the shoemakers' workshops despite the presence of neurological abnormalities. Some previous studies correlated exposure to chromium, lead, aluminum, manganese and other metals in various industries with neuropsychatric abnormalities in their workers. Examples are the study of Sjogren et al (1990) which was among welders exposed to metal fumes, and the study of Gobba (2006) which detected olfactory dysfunction due to affection of neuroepithelium in workers chronically exposed to industrial metals. So, our suggestion is to revise the present standards, concerning heavy metals, trying to lower their levels to a safer values for CNS functioning.

Analysis of trace elements in the biological fluids became nowadays an important clinical diagnostic test in human diseases (Hussein et al, 2008). Among them are Zn and Se which have an antioxidant effect, that is protective against cellular injury in general and neurological diseases caused by exposure to solvents (Viegas et al., 2000)

In the present study there was no statistically significant difference between the shoe makers and their controls regarding the levels of Cu and Zn. In agreement with the present results, Andrzejak et al. (1992) and El-Gazzar et al. (1997) found no significant variations in levels of Cu and Zn between workers exposed to benzene and toluene and their control subjects. While, Dundaroz et al. (2002) revealed a decrease in plasma levels of Zn due to chronic inhalation of volatile organic solvents.

While, Se of the shoe makers in the current study was significantly lower than the controls. Hussein et al. (2008) attributed the significant decrease in the Se of shoe makers compared to their controls to the exhaustion of the Se as an antioxidant against the oxidative effect of chronic exposure to organic solvents. Contradictory, Georgieva et al. (2002) found that Se was not significantly changed in workers exposed to a mixture of hydrocarbons.

Currently, in the present study, on comparing the levels of Cu, Se and Zn in normal shoe makers with the neurologically affected workers revealed no statistically significant difference between them. Moreover, no difference was found between the groups of workers with cranial, spinal or combined problems.

This findings could be explained the fact that exposure to organic solvents affected the levels of Se in all the exposed workers, but due to individual variation and / or genetic susceptibility, some workers were affected by neurological abnormality while others still are not. Those normal workers may be affected later on with aging or increasing dose and duration of exposure to solvents as both factors have deleterious effects on central nervous system.

Previously, Niels et al. (1981) found that in patients with parkinson's disease and amyotrophic lateral sclerosis the levels of Se were normal. Kapaki et al., 1997 in their study on amyotrophic lateral sclerosis patients, they detected that the serum Zn were unchanged, while, CSF and serum Cu levels were decrease.

Conclusion

Occupational exposure to organic solvents and other chemicals in shoemaking industry was found to have hazardous effects on nervous system both cranial and spinal. The protective role of trace elements has been suggested. Levels of Se were decreased in shoe workers, while, the exact role of Zn and Cu are not clarified in the development of neurological abnormalities and needs further study.

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Ultrastructural Studies On The Effect Of Electromagnetic Field On The Liver Of Albino Rats (*Rattus* Norvegicus)

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Abstract: The aim of the present study was to study the effect of long-term exposure of rats (1 hour per day, 3 days weekly for 4 weeks) to either static or time varying magnetic fields of induced flux densities 2 mT (millitesla) =20G (Gauss), on the ultrastructure of liver tissue. One hundred and ten male rats were divided into three main groups. Animals of the first group (10 rats) were not exposed to the magnetic field and represented the control group. The second group (50 rats) was exposed to static magnetic field (direct current) at strength of 2 mT. The third group (50 rats) was exposed to alternating magnetic field (alternating current) at strength 2 mT. The results revealed structural irregularity in hepatocyte nuclei as the most prominent ultrastructural change in the liver of treated groups. This was manifested as irregularity of nuclear membranes, widening of the nuclear pores and heterogeneous distribution of the chromatin material. Furthermore, swelling and clumping and deformation of mitochondria were observed in the groups exposed to the magnetic field. In addition, the rough endoplasmic reticulum appeared with marked dilation and the lysosomes appeared distorted.

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1. Introduction

In recent years, there has been a great deal of publicity concerning the possible health effects of magnetic fields; however, there is still very little understanding of the interaction mechanisms between magnetic fields and living matter. Nowadays, a new discipline, which can be called "magneto biology" is taking shape (Genva & WHO, 1987; 1989a; 1989b).

The concern about the possible harmful effects of electro-magnetic fields (EMFs) on human health has led to a growing interest in the influence of EMFs on life processes and their interaction with cells, organs , and embryonic development (**Brent**, **1999**).

The most common frequencies in the environment are 50 and 60 Hz, since these frequencies are used in the transmission of electricity (**Coleman** *et al.*, **1988; Bernhardt, 1991**). Therefore the question arises as to whether fields of similar intensity environmental radiation, e.g. by high power electric lines may influence animals and humans.

It was reported that 60 Hz affect normal development of chicken, hormone levels and circadian rhythmicity in rats, reproduction and development of rats and mice nervous system and decreased resistance of mice against cancer cells (Marino, 1990). Exposure to 60HZ magnetic fields was also found to affect cellular processes related to proliferation and tumor promotion (Frazier *et al.*, 1990; Hori *et al.*, 2005). The reported effect of EMF

on cellular activity was reported as due to structural and functional activities of cell membranes leading to intracellular mobilization of intracellular calcium ions (**Tonini** *et al.* **2001**).

Changes in cell structure and function was also suggested as due to liberation of free radicals in cells and tissues exposed to EMF (**Zsont** *et al.*, **2004**). Liberation of free radicals was reported to cause changes in cell surfaces micromorphology in a variety of cells following radiation. These include changes number and size of microvilli, development of surface blebs, membrane ruffling and retraction of pseudopods (**Somosy 2000**). Free radicals also increased the number and fraction volume of lysosomes in the cells and elevated lysosomal enzyme activity (**Hamberg, 1983; Piao** *et al.*, **1983**).

Free radicals and reactive oxygen species result in alterations in the structure and function of mitochondria (Kergonou *et al.*, 1981; Erickson & Koppenol, 1987). Elongation and branching of the mitochondria, and a marked increase of their size and the development of giant forms were the most frequently reported changes (Maeda, 1982; Betzold *et al.*, 1992). Vacuolization of mitochondria and disruption of their outer and inner membranes were also frequently observed (Rene' & Evans, 1970; Yago *et al.*, 1972; Shen *et al.*, 1989; Kim and Shin, 1994).

Excessive liberation of free radicals caused dilatation, vesicularization, and fragmentation of the endoplasmic reticulum cisternae and degranulation of RER membranes in a wide varity of cells (**Skog** *et al.*,

1983; Rosen *et al.*, 1989; Mukerjee & Goldfeder, 1974; Skog *et al.*, 1983).

Liver is a very important organ for the healthy and lasting life of mammals. EMF induced liberation of free radicals and reactive oxygen species can induce liver disease (**Muriel, 2009**).

The aim of the present study, the effect of two electromagnetic fields on the rat liver mainly at the ultrastructural level.

2. Material and Methods

One hundred and ten male albino rats (*Rattus norvegicus*) were used in the present study. These animals aged approximately $2\frac{1}{2}$ - 3 months with an average weight 140 -180 gm. The rats were kept in well ventilated cages at room temperature; and fed on a balanced diet, while water was allowed *adlibitum*.

The animals were divided into three main groups according to the type of the magnetic field induced:

Group : Control group (10 rats)

Animals of this group were not exposed to magnetic fields.

Group : Direct current magnetic field-treated group (50 rats)

The animals were exposed to static magnetic field (direct current DC), at strength 2 mT (milli tesla) = 20G (Gauss), 50 Hz (Frequency) at 2V (Volt), and 0.6A (Ampere); for one month (Exposure period), three days a week, day after day for one hour in each exposure. The animals of this group were subdivided into 5 subgroups each included 10 animals:

Subgroup One (GR1/DC): Animals of this group were sacrificed after 1 day following the end of exposure period.

Subgroup Two (GR2/DC): Animals of this group were sacrificed after one week following the end of exposure period.

Subgroup Three (GR3/DC): Animals of this group were sacrificed after two weeks following the end of exposure period.

Subgroup Four (GR4/DC): Animals of this group were sacrificed after one month following the end of exposure period.

Subgroup Five (GR5/DC): Animals of this group were sacrificed after two months following the end of exposure period.

meter. voltage and current were monitored with a voltmeter.

Group : Alternating current magnetic field-treated group (50rats)

Rats were exposed to alternating magnetic field (alternating current AC), at strength 2 mT (millitesla) = 20G (Gauss), 50Hz (Frequency) at 43V (Volt), and 1.28A (Ampere) for one month (Exposure period), three days a week, day after day, one hour for each exposure. Animals of this group were also subdivided into 5 subgroups each includes 10 animals:

Subgroup One (GR1/AC): Animals of this group were sacrificed after 1 day following the end of exposure period.

Subgroup Two (GR2/AC): Animals of this group were sacrificed after one week following the end of exposure period.

Subgroup Three (GR3/AC): Animals of this group were sacrificed after two weeks following the end of exposure period.

Subgroup Four (GR4/AC): Animals of this group were sacrificed after one month following the end of exposure period.

Subgroup Five (GR5/AC): Animals of this group were sacrificed after two months following the end of exposure period.

Effects and exposure technique to different magnetic fields

Static Magnetic Field

An artificial electromagnet apparatus that generates electromagnetic field (EMF) constructed in the Department of Physics, Faculty of Science, Benha University, was applied. The animals were kept in a perforated glass box (5 rats per time), placed in between two poles, each pole was attached to coil which had 1000 turns of the apparatus, connected with DC unit (stabilizer). A horizontal magnetic induction was applied to the whole body of the animal.

Alternating Magnetic Field

The Electromagnetic field (EMF) applied in this study was generated by an artificial Hilm-Holtiz Coil constructed in the Department of Zoology, Faculty of Science, Benha University. The animals were kept in a perforated glass box, (5 rat per time), placed in between two coils, each coil contained 348 turns of the apparatus and connected with AC unit (varic). Then, a horizontal magnetic induction was applied to the whole body of the animal. The field strength in both cases was monitored with a gauss **Electron microscopy**

For electron microscopy examination, small pieces of the liver (1mm) were cut and immediately fixed in 2-4 % cold phosphate buffered

glutaraldehyde (**Dawson** *et al.*, **1969**) or in cacodylate buffered 2.5% glutaraldehyde solution at pH 7.4 for 2-4 hours (**Sobatini** *et al.*, **1963**). Following fixation, the specimens were washed thoroughly at 4°C in three changes of 0.1 N of the buffer solution and then post-fixed in 1% osmium tetra oxide in phosphate buffer for 2 hours (**Millonig**, **1961**), dehydrated and embedded in epoxy resin (Epikote 812). Ultra-thin sections were stained with alcoholic uranyl acetate followed by lead citrate and then examined using a transmission electron microscope (JOEL).

3. Results

Control group

Figure (1) represents an electron micrograph of a section in liver of control group. Some hepatocytes are binucleated. The nucleus contains one or two nucleoli. The cytoplasm is completely filled with cell organoids. The mitochondria are numerous and distributed all over the cytoplasm. Rough endoplasmic reticulum, Golgi bodies and some lysosomes are evident. Blood sinusoids including red blood cells and surrounded by endothelial cells, Kupffer cells and fat storing hepatic stellate cells can also be observed.



Figure 1. Electron micrograph of a control rat liver showing hepatocytes (H), plasma membrane (PM), double intact nuclei (N), the nucleoli (Nu), scattered mitochondria (M), rough endoplasmic reticulum (RER), numerous lysosomes (L). A blood sinusoid(BS), red blood cells (RBC). (X2700).

One day post-exposure group

Cell injury prevails with different degrees in this group (Figure 2). While cellular changes in the direct current group were in the form of dilatation in RER cisternae (Figure 2A), injury was more rigorous in alternating current group (Figur 2B). In the latter group, the nucleus was indented. Several vacuoles appeared in a swelled cytoplasm. Cell organoids formed cytoplasmic aggregations.

One week post exposure groups

After one week of direct current exposure, some cells appear undergoing apoptosis. Other cells, however appear normal (Figure 3A). The apoptotic cells have foamy cytoplasm and separate apoptotic bodies. Lysosomes appear in both apoptotic cells and apoptotic bodies. In alternating current group (Figure 3B), some nuclei appear enlarged with pale chromatin, showing karyolysis as a sign of necrosis. More signs of cell injury such as fragmentation of rough endoplasmic reticulum are also evident.



Figure 2. EM of liver after one day following the end of (A) direct current magnetic field exposure, and (B) alternating current magnetic field exposure. (A)showing hepatocyte with smooth contoured nucleus (N) with some clumped heterochromatin, one nucleolus (Nu), vacuoles (V) and many lysosomes (L). (X 4000) The insert at the upper left corner demonstrates a higher magnification (X800) of the RER. Notice the dilated cisternae (*). (B) showing a hepatocyte with indented (arrow) nucleus (N) with one nucleolus (Nu), vacuoles (V) and many lysosomes (L) in an area of aggregated organelles. (X 4000).



Figur

hepatocytes (H), plasma membranes (PM), indented (arrow) nucleus (N) with clumped large nucleolus (Nu), mitochondria (M), vacuole (V)& numerous lysosomes (arrows) in apoptotic bodies (**), hepatocytes with foamy cytoplasm (f) blood sinusoid (BS) with red blood cells (RBCs) (X 2000). (B) alternating current magnetic field exposure showing hepatocytes (H) with plasma membranes (PM), a nucleus (N) with an eccentric large nucleolus (Nu), ballooned pale nucleus (***), mitochondria (M) & fragmented rough endoplasmic reticulum (RER) and endothelial cell (E). (X 2700).

Two weeks post exposure groups

After two weeks of exposure to direct current magnetic field, some cells show signs of swelling of the cytosol and clumping of the organelles (Figure 4A). On the other hand, signs of hydropic degeneration appear in hepatocytes of the alternating current magnetic fields exposed group (Figure 4B). Signs of chromatin fragmentation and nucleolar margination suggest the onset of necrosis. The cytoplasmic organelles are relatively few with signs of cytoplasmic swelling.

One month post exposure groups

After one month, signs of hepatocyte recovery appear in the direct current exposed group (Figure 5A). Mitochondria are numerous and evenly distributed in the cytoplasm. The rough endoplasmic reticulum is well developed and sequesters mitochondria. On the other hand, hepatocytes from alternating current exposed rat liver, showed several signs of cell injury (Figure 5B). Swelling of the cytoplasm, loss of cell organoids and karyolysis are demonstrated in cell of this group. There is increase appearance of fat storing (hepatic stellate) cells.

Two months post exposure groups

After two months of exposure, the direct current group showed normal hepatocyte structure (Figure 6A). Signs of recovery appear in the hepatocytes of alternating current exposed rat hepatocytes (Figure 6B). The distribution and number of mitochondria is similar to control. The nuclear chromatin and nucleoli suggest biologically active state. There are hepatic stellate cells. However, fibrosis was evident as collagen fibers were detected in sections (see insert of figure 6B).

4. Discussion

The increasing use of electric power for domestic and industrial appliances has resulted in the exposure of many millions of daily users, in homes and work places, to a complex mix of artificially elevated electromagnetic fields (EMF) that span a wide frequency range. The recent concern about the possible harmful effects of magnetic fields (MFs) on human health calls for continuous research on the influence of MFs on life processes and their interaction with organ tissues and cells.

Liver was used in this study because it is an organ with high iron content (**Ngelucci, 2010**). This makes the liver more susceptible to the effects of magnetic fields (**Yoshikawa, 2000**). Its susceptibility is due to increased liberation of free radicals by electromagnetic fields (EMF); because iron is closely involved in free radical formation from hydrogen

peroxide, when exposed to EMF, via the Fenton reaction, in cells (Meneghini, 1997).

Hepatocytes from the liver of animals exposed to direct or alternating current magnetic fields for one hour every day, 3 days a week for 1 month were examined by electron microscopy after 1day, 1 week, 2 weeks, 1 month and 2 months.

After one day of last exposure, the signs of cell injury were milder in direct current exposed animal hepatocytes than those exposed to alternating current. Such a difference has been previously suggested in several reports (**Tabrah, 1978; Adey, 1979**).

In the direct current group, the injury of the cells was manifested as dilatation of the cisternae of rough endoplasmic reticulum. Although such a change was reported as an early sign of cell death (**Kazunobu, 2004**), it could result from the effect of abnormal osmotic force (McGee 1992). Such osmotic changes may be due to the effect of EMF on membrane permeability (**Ghadially, 1982**).

In hepatocytes from animals exposed to alternating current EMF, the nucleus was indented. Several vacuoles appeared in a swelled cytoplasm. Cell organoids formed cytoplasmic aggregations. Such features were reported in necrotic hepatocytes (**Reynolds & Moslen, 1984**).

After one week of exposure to direct current EMF, apoptotic cells with foamy cytoplasm and separate apoptotic bodies were observed in the electron micrographs. Lysosomes appeared in both apoptotic cells and apoptotic bodies. The ballooning of hepatocytes is one of the earliest, most frequent, and most conspicuous changes seen in liver injured by CCl4 administration. It is also observed in such conditions as viral infection, alcoholic hepatitis, biliary obstruction, starvation, choline deficiency, hypoxia, scurvy, yellow fever, and radiation injury (Phillips, 1987). Although the relationship between the ballooning changes and necrosis and the fate of the ballooned hepatocytes have long been debated, the conventional explanation of cell ballooning is that it is a forerunner of necrosis (Alison, 1994; Shi, 1998). However, evidence was reported based on electron microscopy and immune-histochemistry that ballooning and foamy cytoplasm is a feature of hepatocyte apoptosis (Shi, 1998). The presence of apoptotic bodies in the electron micrographs of the present study demonstrates the apoptotic cell death in direct current exposed animal hepatocytes.

In hepatocytes from animals after one week of exposure to alternating current, some nuclei appeared enlarged with pale chromatin, showing karyolysis. More signs of cell injury such as fragmentation of rough endoplasmic reticulum are also evident.



Figure 4. EM of rat liver dissected after two week following the end of (A) direct current magnetic field exposure showing hepatocyte with sighns of swelling, has a nucleus (N) with somewhat clumped heterochromatin, mitochondria (M), packed rough endoplasmic reticulum (RER) & vacuole (V) clumped at one pole of the nucleus. (X 6700). (B) alternating current magnetic field exposure showing a hepatocyte with an eccentric large nucleolus (Nu) in the nucleus (N), few scattered mitochondria (M), rough endoplasmic reticulum (RER), vacuoles (V), and numerous lysosomes(L).Signs of hydropic degeneration are also observed. (X 4000).



Figure 5. EM of treated rat liver after one month following the end of (A) direct current magnetic field exposure showing hepatocytes (H) with large nucleus (N) containing two nucleoli (Nu), numerous mitochondria (M) and rough endoplasmic reticulum (RER). (X 2700). The insert at the upper right corner is enlarged view of the RER in the rectangle. (X14000). (B) alternating current magnetic field exposure showing a hepatocytes (H) with swelling cytosol, a marginated karylytic nucleus (N), few scattered mitochondria (M), dispersed rough endoplasmic reticulum (RER), vacuoles (V) and a sinusoid Larger view (BS). In addition, a hepatic stellate (fat storing cell FS) enclosed in between hepatocyte, which contains one irregular nucleus (N) and fat droplets (F). (X 2700).



Figure 6: EM of treated rat liver after two month following the end of (A) Direct current magnetic field exposure showing enlarged nucleus (N), mitochondria (M) and rough endoplasmic reticulum (RER). (X 8000). (B) alternating current magnetic field exposure showing hepatocyte (H) a nucleus (N), with three nucleoli, mitochondria (M), rough endoplasmic reticulum (RER) & vacuoles (V). A fat storing cell (hepatic stellate cell) (FS), is adjacent to the hepatocyte (X 2700). The insert at the upper right corner illustrates a magnified view of collagen fibers found in sections of this group. (X8000).

The observed features were described for hepatocytes undergoing necrosis (**Francavilla**, 1989). Induction of hepatocyte necrosis may be through the increased production of tumor necrosis factor alpha induces by alternating current EMF (**Yamaguchi**, 2006) which results in cell necrosis (**Yu**, 1996).

After two weeks of exposure to direct current magnetic field, some cells showed signs of swelling of the cytosol and clumping of the organelles. Such a change could be due to disturbance of the cytoskeleton especially microtubular organization (**Stockem, 1992**). Such disturbance is probably due to mobilization of intracellular calcium by EMF (**Tonini, 2001**) which is essential for microtubular organization (**Masini, 2006**).

signs of hydropic degeneration appear in hepatocytes of the alternating current magnetic fiels exposed group. Signs of chromatin fragmentation and nucleolar margination suggest the onset of necrosis. The cytoplasmic organelles are relatively few with signs of cytoplasmic swelling.

Signs of hydropic degeneration appear in hepatocytes of the alternating current magnetic field exposed group. Signs of chromatin fragmentation and nucleolar margination suggest the onset of necrosis. The cytoplasmic organelles are relatively few with signs of cytoplasmic swelling. All these signs indicate the onset of cell necrosis (**Schaff, 1990**).

After one month, signs of hepatocyte recovery appear in the direct current exposed group. On the other hand, hepatocytes from alternating current exposed rat liver, showed several signs of cell injury. Swelling of the cytoplasm, loss of cell organoids and karyolysis are demonstrated in cell of this group. There is increase appearance of hepatic stellate cells. This indicates that alternating current EMF at 2mT, 50Hz results in a more lasting injury in the liver of treated animals. The appearance of activated hepatic stellate cells indicate the onset of liver fibrosis (**Gressner, 1998**). This is evident in the present study from the appearance of collagen fibers in sections of liver after 2 months of exposure to alternating current EMF.

Finally, the high risk exposed personnel should be regularly examined for liver function tests . Furthermore, for the safe medical use of Magnetic Resonance equipment, monitoring of cardiac and circulatory functions of the patients during examination, should be recommended especially in patients with impairment of excitation stimulation or impairment of conduction of the excitation as they are more susceptible to the imposed cardiovascular stress.

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Seed Morphology and Seed Coat Sculpturing of 32 Taxa of Family Brassicaceae

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Abstract: Seed exomorphic characters of 32 taxa of Brassicaceae were investigated by LM and SEM. The diagnostic characterts at the generic and specific level are, seed shape, dimensions, colour, epidermal cells, and seed coat surface and aspect of anticlinal and periclinal walls. Seed shape among the studied taxa showed wide range of variations. LM revealed most of the studied seeds vary from globose to oblong-ellipsoid or elongate. Most of the seeds have no wings except *Farsetia aegyptia*. SEM investigation at higher magnifications revealed main six types of seed surface sculpture; reticulate, ocellate, foveate, papillate, stellate and domate. The seed exomorphic criteria obtained from LM and SEM were analyzed by the STATISCA program package using the UPGMA clustering method. Produced data facilitate the construction of a dendrogam between the studied taxa. Two groups are represented from the first group included the taxa of Tribe Arabideae, Lepidieae, Matthioleae, Sisymbrieae, Alysseae, Chamireae, Schizopetaleae, Stenopetaleae, Drabeae, Euclidieae, Lunarieae, and Streptantheae. The second group included the most commonly known genera of the tribe Brassiceae.

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1. Introduction

The Brassicaceae, which currently includes 3709 species and 338 genera (Warwick et al. 2006), is one of the ten most economically important plant families (Rich, 1991). In Brassicaceae, much attention was paid to the general anatomy of the seed coat and its taxonomic use particularly in species of economic value (Berggren, 1962). Discrimination between Brassicaceae seeds is very difficult with the naked eye or lens. On the other hand, Heywood, 1993 stated that the cruciferae is classified into 13 tribes, Arabideae, Hesperideae, Lepidieae, Matthioleae, Sisymbrieae, Alysseae, Brassiceae, Chamireae, Schizopetaleae, Stenopetaleae, Helphiteae, Cremolobeae, Drabeae, Euclidieae, Lunarieae, and Streptantheae only two tribes, the Brassiceae and Lepidleae can be regarded as natural. It is the reason why micromorphological structures have been observed on the surface of the seeds (Bernard, 2000). Most systematists agree that data concerning the macro-and microstructure of seeds are very significant for the classification of Angiosperm taxa. In Brassica species, relatively numerous reports concerning the seed coat structure have been published (Mulligan and Bailey, 1976; Buth and Roshan, 1981; Setia and Richa, 1989 and Ren and Bewley, 1998). In Egypt, Täckholm (1974) reported 61 genera and 106 species and El-Hadidi & Fayed (1995) reported 55 genera and 108 species. Several recent studies of different seed coat characters have employed scanning electron microscopy (SEM). For example,

Vaughan and Whitehouse (1971) studied the macro and micromorphological characters of approximately 90 genera and 200 species of Brassicaceae and paid special attention to the relationships between structure and existing taxonomy. The exo-and endomorphic characters of Brassicaceae seeds have been studied by Musil (1948), Murley (1951), Berggren (1962), Corner (1976), Jonsell (1986), El-Naggar (1987, 1996), Fayed and El-Naggar (1988, 1996), El-Naggar and El-Hadidi (1998). Koul et al. (2000) gave detailed descriptions of seed morphology in 44 species of the subtribes Brassicinae, Raphaninae and Moricandiinae, and elucidated the phylogenetic relationships between taxa. More recently, Tantawy, et al. (2004) studied the macro and micromorphological characters of approximately 22 genera, 30 species of Brassicaceae. The present work concern with the exomorphic studies of certain Brassicaceae seeds by using LM and SEM to emphasize seed surface structure considered as a distinguished taxonomic character.

2. Materials and Methods

Seeds of 32 taxa representing 18 genera, 30 species, 1 ssp. and 2 varieties of Brassicaceae were obtained from the Royal Botanic Gardens at Kew, London, UK, and from different localities in Egypt. Studied local taxa were identified according to Täckholm (1974) and Boulos (1999). The studied taxa are given in Table 1.

No.	Characters	Serial Number	Location	Collection Date
	Таха			
1	Brassica oleracea S.I.L	0070498	England	1988
2	B. oler. var. capitata L.		Egypt	2009
3	B. oler. var. botrytis L.		Egypt	2009
4	B. rapa L	0020747	Switzerland	1974
5	B.nigra (L.) koch	0070395	England	1988
6	B. tournefotii Gouan	0184696	Egypt	2002
7	Cardamine flexuosa	1071	England	1988
8	Cardamine hirsuta	387109	Brukina-Faso	2007
9	Capsella bursa-pastoris DC.		Egypt	2009
10	Coronopus didymus (L.) Sm.	0076065	England	1989
11	Descurainia sophia (L.) Webb	0497413	Jordan	2008
12	Diplotaxis erucoides(L.)DC	0113946	Jordan	1996
13	D.harra (forssk) Boiss.		Egypt	2009
14	D. tenufolia(L.)DC	064824	England	1986
15	Eruca Sativa Miller		Egypt	2010
16	Erysimum cheiri (L.) Crantz	0026716	France	
17	Farsetia aegyptia Turra		Egypt	2010
18	Hirschfeldia incana (L.) Lagr.	0115249	Lebanon	1996
	Fossat			
19	Lepidium sativum L.	0071831	Oman	1987
20	Raphanus sativus L.		Egypt	2010
21	R. raphanistrum L.	0071853	Oman	1989
22	Moricandia sinaica (Boiss.)		Egypt	2010
	Boiss.			
23	Matthiola longipetala (Vent)		Egypt	2010
	DC. spp. incana (L.) R.Br			
24	Sinapis alba L.	200095	Jordan	2003
25	S. arvensis L.	0115272	Lebanon	1996
26	<i>S. allionii</i> Jacq.		Egypt	2010
27	Sisymbrium orientale L.	0158808	Jordan	2001
28	S. altissimum L.	0175935	Belgium	1989
29	S. irio L.		Egypt	2009
30	S. officinale (L.) Scop	0053257	England	1984
31	Schouwia thebaica Webb.		Egypt	2010
32	Thlaspi perfoliatum L	0113876	Jordan	1996

Table	1. I	Localities	of	the	studied	taxa	of	the	Brassicaceae.

The dry mature seeds were cleaned, and examined by light microscope to show the different exomorphic parameters viz. shape, dimensions, colour and seed surface texture. Five to ten seeds for each taxon were taken to cover the range of variations. For SEM investigation, the seeds were dried and fixed to specimen stubs with an adhesive and placed on the revolving discs of Joel fine coat ion sputter (Joel, JFC 1100). Each seed was uniformly coated with gold. Specimens stubs were then fixed to the specimen holder of Scanning Electron Microscope (Joel JSM 350) maintained at accelerating potential voltage of 15 Kv. and

photomicrographs were taken at different magnifications in Unit of at the Scanning Electron Microscopy Unit in Alexandria University (Egypt). This study is dependent upon the application of a total of 28 comparative seed exomorphic characters and their states as a binary character (0 &1), on each taxa. The characters and states have been subjected to numerical analysis under an program using similarity and dissimilarity assessment percentage method (Rohlf, 1993). The method applied is based on cluster analysis by using an UPGMA (unweighted pair-group method with arithmetic means).

3. Results and Discussion

Brassica oleraceae: Globose seeds, brownish-black with globrous surface. Seed size 1.5×2.0 mm, seed wing absent. Seed coat ornamentation revealed the anticlinal walls are identical and showed ferrucate pattern, in addition flakes of wax-like structure of various size are seen over the seed surface. Crakes are found on their surface (Fig.1).

B. oler. var. capitata: Globose seeds and black, globrous surface. Seed size 1.0×1.3 mm, seed wing absent. SEM revealed the anticlinal walls are identical and showed regulate ornamentation, randomly reticulate surface pattern with weakly outlined polygonal cells found (Fig. 2).

B. oler. var. botyrtis : Globose seeds, black with globrous surface. Seed size 1.0×1.5 mm, seed wing absent. The seed coat showed; seed surface pattern is identical and regulate in addition irregular reticulate

sculpture with peripheral particles (colliculate) of various size and number (Fig.3).

B. rapa: Globose seeds are found, black in color and globrous in texture. Seed size 1.0×1.5 mm, seed wing absent. Seed coat which examined by SEM revealed seed surface pattern is identical and showed regulate ornamentation, polygonal pits of outer periclinal cell wall (Fig.4).

B. *nigra*: Seeds are globose black in color with globrous texture. Seed size 1.0×1.5 mm, seed wing absent. SEM revealed seed surface pattern is identical. Polygonal pits of outer periclinal cell wall are found on their surface (Fig.5).

B. tournefotii: Globose seeds and dark brown with globrous texture. Seed size 1.0×1.2 mm, seed wing absent. The examinations of seed coat revealed papillate structure; anticlinal walls are more or less broad and smooth and the periclinal walls are deeply depressed smooth (Fig.6).



4- B. rapa



6- B. tournefotii

Cardamine flexuosa: Oblong-elliposid seeds and reddish brown with globrous texture. Seed size 1.0×0.5 mm, seed wing absent. SEM revealed presence of undulate pattern in which unregulated structures are present (Fig.7).

Cardamine hirsute: kidney shape seeds and shiny brown with globrous texture. Seed size 0.8×1.0 mm, seed wing absent. SEM revealed microreticulate structure. The periclinal cell wall with sunken central portion found (Fig. 8).

Capsella bursa-pastoris: Oblong-elliposid seeds, and reddish brown with globrous texture. Seed size is

small 1.0×0.5 mm, seed wing absent. SEM showed reticualte-papillate patterns in which irregular periclinal cell wall has been noticed (Fig.9).

Coronopus didymus: Seeds are small in size, kidney shaped, and shiny brown with globrous texture. Seed size 0.8×1.0 mm, seed wing absent. SEM revealed revealed the smooth surface and stellate-like structure has been noticed. Epidermal cell shape irregular polygonal cells. Anticlinal boundaries raised-channelled, straight to sinuous; smooth to fine folds pericinal cell wall slightly concave smooth to medium folds (Fig.10).



8- Cardamine hirsute



10- Coronopus didymus

Descurainia Sophia: Kidney in shape, and shiny brown in color with globrous texture. Seed size 0.8 $\times 1.0$ mm, seed wing absent. SEM revealed bright dull spherical particles are regularly cover all seed surface or completely sunken in the epidermal cells. (Fig.11). **Diplotaxis erucoides:** Seeds kidney, and yellowish with globrous texture. Seed size 1.0 $\times 0.5$ mm, seed wing absent. SEM revealed outer periclinal cell wall with irregularly formed pits. In addition compactly wax-like materials are present on their surface (Fig. 12).

D.harra: Seeds are oblong, subglobose in shape, brown in color with globrous in texture. The seed size 0.8×1.2 mm, seed wing absent. SEM revealed rugose-striate sculpture on their seed surface (Fig.13). **D.** tenufolia: Seeds are oblong in shape brown in color with globrous in texture. Seed size 0.2×1.0 mm, seed wing absent. SEM revealed reticulate patterns, absence of particles on their surface (Fig.14).

Eruca Sativa: Subglobose seeds, brown and globrous texture. The seed size 1.7×1.4 mm, seed wing absent. SEM revealed epidermal cell shape isodiametric, 5-6 gonal, elongate in one direction Anticlinal boundaries raised, straight to slightly sinuous; smooth to fine folds. In addition periclinal cell wall concave with medium striate structure (Fig.15).

Erysimum cheiri: Oblong ovate seeds, orange-brown in color with globrous texture. The seed size 3.0×1.3 mm, seed wings present. SEM revealed ocellate structure in which flat central portion; periclinal isodimetric and polygonal cell wall smooth to folded are present (Fig.16).

Farsetia aegyptia: Seeds small in size, kidney shaped, brown -orange with globrous texture. Seed size 3.6×5.0 mm, small seed wings present. Seed exomorphic by SEM revealed reticulate ornamentation, epidermal cells patterns show tetra, pentagonal and nearly isodimetric forming a network form. A very small particles are scattered on their surface (Fig.17).

Hirschfeldia incana: Seeds are kidney, and shiny brown with globrous texture. Seed size 0.8×1.0 mm, seed wing absent. SEM revealed reticulate patterns, small particles present. Anticlinal boundaries cell are highly raised in one direction; straight to sinuous; percilinal cell wall flat to concave structure (Fig.18).

Lepidium sativum : Obliquely ovate seeds, brown with globrous texture. Seed size 1.0×2.5 mm, seed wing absent. SEM revealed epidermal cell shape isodiametric, elongate in one direction, 4-5-6 gonal. Anticlinal boundaries highly raised, straight with smooth form (Fig.19).



16- Erysimum cheiri



19-Lepidium sativum

Raphanus sativus: Seed are kidney and shiny brown with globrous texture. Seed size 0.8×1.0 mm, seed wing absent. SEM revealed reticulate ornamentation, epidermal cells patterns show tetra, pentagonal and nearly isodimetric. A small particles are scattered on their surface (Fig.20).

R. raphanistrum : kidney shaped seeds, and shiny brown with globrous texture. Seed size 0.8×1.0 mm, seed wing absent. SEM revealed reticulate seed coat pattern showing isodiametric polygonal cells (Fig.21).

Moricandia sinaica : Seeds are oblong-elliposid in shape, and orange with globrous texture. Seed size 0.2×0.5 mm, seed wing absent. SEM revealed reticulate with spherical particles are completely sunken in the epidermal cells and interwoven appearance were recorded on their surface (Fig.22). *Matthiola longipetala (Vent) DC. spp. incana:*

Globose seeds, and yellowish in color with globrous texture. The seed size 1.5×0.2 mm, seed wing absent. SEM revealed flat to slightly concave; fine to coarse folds is present (Fig.23).



20-Raphanus sativus



23-Mathiola longipetala spp. incana

Sinapis alba: Globose in shape, and brown in color with globrous in texture. The seed size 2.0×3.0 mm, seed wing absent. SEM revealed irregular reticulate patterns (Fig.24).

S. *arvensis*: Globose seeds, and dark brown with globrous texture. The seed size 1.0×0.5 mm, seed wing absent. SEM revealed rugae show folds on their walls, giving the appearance of stellate structures. Thin long cracks radiating from the pleurogram are seen on the seed surface (Fig.25).

S. *allionii* : Globose seeds, orange brown with globrous texture. The seed size 0.8×1.0 mm, seed wing absent. SEM revealed reticulate seed coat pattern. testa cells with highly raised anticlinal cell boundaries (Fig.26).

Sisymbrium orientale: Oblong seeds, yellowish with globrous texture. Seed size 1.0×0.5 mm, seed wing absent. SEM revealed smooth to folded ocellate epidermal cell shape present; domate cell wall with globular central papillae (Fig.27).



25- S. arvensis



27- Sisymbrium orientale

S. altissimum: Seeds are oblong-elliposid in shape, and reddish brown in color with globrous in texture. The seed size 1.0×0.5 mm, seed wing absent. SEM revealed ocellate pattern; periclinal cell wall with sunken central papilla present (Fig.28).

S. irio: Small in size, oblong in shape, and yellowish with globrous in texture. Seed size 1.0×0.5 mm in width, seed wing absent. SEM revealed epidermal cell shape isodiametric, polygonal, anticlinal channeled straight to sinuous, periclinal cell wall appear flat or concave with central position radiate-striate (Fig.29).

S. officinale: Seeds are oblong in shape, and dark brown in color with globrous in texture. The seed size

 1.0×1.5 mm, seed wing absent. SEM showed that periclinal cell wall with little sunken central papilla (Fig.30).

Schowia thebaica: Seeds are circular in shape, brown in color with globrous in texture. The seed size 1.5×2.5 mm, seed wing absent. SEM revealed ocellate pattern with several sunken central papilla on their structure (Fig.31).

Thlaspi perfoliatum: Seeds are globose in shape, yellow in color with globrous in texture. Seed size 1.0×0.5 mm, seed wing absent. SEM revealed sinuous cell wall; periclinal cell wall convex and micropapillate (Fig.32).





32- Thlaspi perfoliatum

The dendrogram produced from the cluster analysis between the 32 taxa based on the seed exomorphic characters is represented in Fig 33. From the dendrogram; the taxa are separated at taxonomic level of 100.0%. Two groups are represented; the first group included the studied taxa of Tribe Arabideae, Lepidieae, Matthioleae, Sisymbrieae, Alysseae, Chamireae, Schizopetaleae, Stenopetaleae, Drabeae, Euclidieae, Lunarieae, and Streptantheae. The second group included the most commonly known genera of the tribe Brassiceae such as the six species of genus Brassica, Diplotaxis, Raphanus and Hirschfeldia) this is in accordance with work of Al-Shehbaz, et al. 2006. Within the second group, the three species of Sinapis are separated at taxonomic level of 17.0%. On the other hand, Schouwia thebaica is separated at single taxonomic level of 78.0%.

The seed shape among the taxa showed wide range of variations. Most of the seeds vary from globose to oblong-ellipsoid or elongate. However, they are globose in the six species of genus *Brassica*, three species of *Sinapis* and *Thlaspi perfoliatum*. Kidney shape in *Coronopus didymus*, *Descurainia* and Cardamine hirsuta and Diplotaxis erucoides, circular in Farsitea aegyptiaca Obliquely ovate in *Raphanus sativus*, oblong in the three species of *Sisymbrium* (orientale, irio & officinale). The seed shape as observed in the present study seems to be diagnostic at the generic level. Most of the

investigated seeds have no wings, but in Farsetia aegyptia wing is present and may be apical (small) or marginal (largely expanded). Presence or absence of wing to be of diagnostic value in distinguishing between the studied taxa. Our data showed marked similarities with earlier studies of Kapil et al. (1980) and Abdel Khalik et al. (2002). Seed size vary greatly between the present taxa. However, the largest seed size of 3-4×1.3-1.5 Farsetia aegyptia and Erysimum cheiri, relatively large (range from 1.2-2.0 mm) in 5 taxa, (Brassica oleraceae, Mathiola longipetalae, sinapis alba, Schowia thebaica and Eruca sativa). The smaller size was found in the studied taxa of Diplotaxis tenifolia and Moricandia sinaica. Several studied taxa are shared in size of 0.8×1.0 ; these taxa Cardimine hirsute, Coronopus didymus, are Hirschfeldia incana, the two species of Raphanus and Sisymbrium allionii.

In similar, the seed size of 1.0×0.5 were noticed in several taxa such as the four taxa of *Sisymbrium, Capsella bursa-pastoris, Cardimine flexus* and *Diplotaxis erucoides* and *Sinapis arvensis.* The seed size as a variable criterion is considered diagnostic for some extent. This is in accordance with the work of Aniszewski *et al.* (2001) and El-Tantawy *et al.*, 2004. On the other hand, seed surface in all taxa, is glabrous and to be unreliable criterion to use at the generic and specific level.

N o.	Characters Taxa	L×W	Shape	Texture	Color	Anticlinal cell wall	Periclinal cell wall	Seed Surface
1	Brassica oleracea	1.5×2.0	Globose	glabrous	brown	raised	concave	wax like materials
2	B. oler. var. capitata	1.0 ×1.3	Globose	glabrous	black	straight	concave	randomly reticulate
3	B. oler. var. botrytis	1.0 ×1.5	Globose	glabrous	brown	raised	concave	Reticulate- foveate
4	B. rapa	1.0 ×1.5	Globose	glabrous	brown	raised	depressed	Foveate pattern
5	B.nigra	1.0 ×1.5	Globose	glabrous	black	raised	concave	microreticulate
6	B. tournefotii	1.0 ×1.2	Globose	glabrous	dark -brown	Highly-raised	domate	papillate
7	Cardamine flexuosa	1.0×0.5	oblong- elliposid	glabrous	reddish brown	sunken	concave	undulate
8	Cardamine hirsuta	0.8 ×1.0	kidney	glabrous	Pale-brown	Slightly raised	concave	microreticulate
9	Capsella bursa- pastoris	1.0×0.5	oblong- elliposid	glabrous	orange brown	raised	sunken	ocellate
10	Coronopus didymus	0.8 ×1.0	kidney	glabrous	shiny - brown	Raised- cannelled	folded	stellate-like
11	Descurainia sophia	0.8 ×1.0	kidney	glabrous	Shiny- brown	raised	sunken	dull spherical
12	Diplotaxis erucoides	1.0 ×0.5	kidney	glabrous	yellow	broad raised	deeply depressed	reticulate
13	D.harra	0.8 ×1.2	subglobose	glabrous	brown	broad raised	deeply depressed	rugose-striate
14	D. tenufolia	0.2×1.0	subglobose	glabrous	Brown	raised	concave	reticulate
15	Eruca Sativa	1.7×1.4	oblong	glabrous	Brown	Raised	Flat to convex	reticulate
16	Erysimum cheiri	3.0×1.3	Oblong	glabrous	orange- brown	raised	Flat to convex	ocellate
17	Farsetia aegyptia	3.6×5.0	circular	glabrous	Brown	slightly raised	slightly concave	reticulate
18	Hirschfeldia incana	0.8×1.0	kidney	glabrous	Brown	raised	pitted	reticulate patterns
19	Lepidium sativum	1.0×2.5	kidney	glabrous	brown	raised	pitted	microreticulate
20	Raphanus sativus	0.8×1.0	Obliquely ovate	glabrous	Shiny- brown	slightly raised	flatted	reticulate
21	R. raphanistrum	0.8×1.0	kidney	glabrous	shiny - brown	raised	pitted	reticulate
22	Moricandia sinaica	0.2×0.5	oblong- elliposid	glabrous	orange	raised	pitted	reticulate
23	Matthiola longipetala spp. incana	1.5×0.2	globose	glabrous	yellow	raised	clumped	domate
24	Sinapis alba	2.0×3.0	globose	glabrous	brown	raised	depressed	reticulate
25	S. arvensis	1.0× 0.5	globose	glabrous	dark brown	raised	depressed	Stellate
26	S. allionii	0.8 ×1.0	globose	glabrous	orange- brown	Raised	Convex	reticulate
27	Sisymbrium orientale	1.0×0.5	oblong	glabrous	yellow	Raised	papillate	ocellate
28	S. altissimum	1.0×0.5	oblong- elliposid	glabrous	orange- brown	Raised	papillate	ocellate
29	S. irio	1.0×0.5	oblong	glabrous	yellow	sinuous	domate	domate
30	S. officinale	1.0×1.5	oblong	glabrous	Dark- brown	domate	sinuous	papillate
31	Schouwia thebaica	1.5×2.5	Circular	glabrous	brown	raised	depressed	ocellate
32	Thlaspi perfoliatum	1.0×0.5	Globose	glabrous	vellow	raised	depressed	micro-papillate

Table 2. T	The seed morphological	characters as seen by LM and SEM	between taxa of the family.
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Taxa Stat Recomized	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Seed colour 1-Black 1/ Yellow 0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2- Brown 1, reddish brown 0	1	0	1	1	0	1	0	0	0	1	1	0	1	1	1	0	1	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0
3- orange 1/ orange brown 0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	1	0	0	1
Seeds Surface 4-globrous 1/ rough 0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L/W 5- 1.0 ×1.5 1/ 1.0 ×1.5 0	0	1	1	1	1	1	1	1	0	1	1	1	1	0	0	0	1	0	1	1	1	0	0	1	1	1	1	1	1	1	0	0
Seed Shape 6- globose 1/ sub globose 0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	1
7- kidney 1/ heart 0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0
8- ovate 1/ obliquely ovate 0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1
9- shiny 1/ shiny brown 0	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1
10- oblong 1/ oblong-elliposid 0	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
Anticlinal cell wall 11- raised, 1/ sunken, 0	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1
12- slightly raised 1/ high raised 0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
13- sinuous 1 /domate 0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
preiclinal cell wall 14- depressed 1 /deeply depressed 0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	1
15- concave 1/ convex 0	1	1	1	0	1	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16- flatted 1 /sinous 0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Seed coat pattern 17-Reticulate	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	1	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0
18-microreticulat	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
19- Reticulate- foveate	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19-undulate	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20-ocellate	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0
21-toveate	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22-domate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
23-Reticulate- foveolate	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24-wax-like materials	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
23-papinate 27-micropapillate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
28-rugose-striate	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
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Table 3. The resulted 29 binary characters of the studied spp. (Characters & States are symbolized for numerical



31 32 28 19 30 27 21 29 16 26 24 25 11 23 17 15 7 8 9 22 10 20 13 14 12 3 2 18 1 6 4 5

Seed colour varies from brown, dark brown, pale brown black, orange-brown to shiny brown (Table 2). Seed colour is diagnostic at the generic and specific level for some extent. The data of seed colour is compatible with that mentioned before by Dahlgren and Clifford (1982) and Barthlott (1984).

SEM investigation revealed main six types of seed sculpture; reticulate, ocellate, rugose, papillate, stellate and domate. The reticulate seed surface pattern is the basic type in the majority of the studied taxa (15 taxa). The separation between them based on the aspects of anticlinal and periclinal walls. Ocellate type was recorded in *Capsella bursa-pastoris, Erysimum cheiri, Sisymbrium orientalen, S. altissimum* and *Schouwia thebaica.* Papillate form was noticed in *B.tournefotii, Sisymbrium officinale* and *Thlaspi perfoliatum.* Stellate structure was found in *Coronopus didymus* and *S. arvensis* and domate structure was

Dissimilarity

80

60

40

20

0

found in only two species *Mathiola longapetalae* and *Sisymbrum irio*. From the SEM data, it was noticed that; the seed surface sculpture, aspects of the anticlinal and periclinal walls can serve as good diagnostic parameters at the generic and specific level in the studied Brassicaceae. This is in accordance with the work of Barthlott (1981, 1984), Fayed and El-Naggar (1996) and Abdel Khalik and Maesen (2002) and Tantawey *et al.*, 2004.

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Evaluation of the Reaction of Major Weeds and Some Rice Cultivars to Colletotrichum graminicola

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Abstract: Alisma plantago-aquatica, Sagitaria trifolia and Echinochloa spp. are among the most important damaging weeds of rice paddies. In this research, Colletotrichum graminicola was isolated from these weeds and studied as a biological agent for controlling weeds. To do so, at first, reactions of five rice cultivars including three indigenous cultivars such as Hashemi, Ali Kazemi and Binam and two bred ones, i.e. Sepidroud and Khazar to Colletotrichum graminicola were evaluated. Thus, a complete random design with three replications and five treatments was used at a greenhouse. Then, Colletotrichum graminicola was inoculated on these weeds. The experimental design was a randomized completed with three replications. Inoculation was done at the 3-4-leaf stage

using a spore suspension consisting of 10^{6} conidia/mL distilled water to which Tween-20 1% was added. Results showed that *Colletotrichum graminicola* caused high disease ratings in *Alisma plantago-aquatica, Sagitaria trifolia, E. crus-galli* and *E. oryzicola,* respectively. In addition, the studied rice cultivars showed a significant reaction in terms of the disease rating among which bred cultivars were less tolerant. Moreover, the fungus reduced fresh weight, dry weight and height in the studied weeds and rice cultivars. Therefore, *C. graminicola* can be used as a mycoherbicide for the biological control of these weeds only when other cultivars except the above-mentioned rice cultivars are planted. This issue particularly requires producing new tolerant cultivars with a combination of the desired traits.

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Keywords: weeds, *Colletotrichum graminicola*, rice, biological control.

1. Introduction

Weeds are the most important biological barriers in rice production in a way that a noticeable part of the production costs are allocated to them and are among the most important inhibiting factors with regards to increasing rice production (Mudge, 2004). Researchers have reported the reduced crop yield to be 10%, which is due to competition with weeds (Moody, 1991), while this rate in rice is 20%, that is more than other crops (Lindquist and Kropff, 1998). Today, herbicides are considered among the most important factors in cropping systems of the developed countries and a noticeable part of the increase in crop yields in these countries is indebted to the use of these chemicals (Zand et al., 2007). Despite the universal acceptance of herbicides in recent years, willingness to lower their consumption rate has had an increasing trend because more consumption of herbicides increases production costs, damages crops and causes weeds to become resistant (Blackshaw et al., 2006). Furthermore, in the last few years, concerns for unknown side effects of herbicides, their environmental effects and the role they have in threatening mankind's health have increased (Blackshaw et al., 2006).

Accordingly, controlling weeds by living microorganisms is of great significance for maintaining natural ecosystems and preventing environmental hazards, especially pollution of ground waters and the emergence of a new pest related to producing crops throughout the world (Marol and Baroet, 2004).

Fungi are some of the most important factors which might be used for the biological control of weeds in rice paddies (Safari Motlagh, 2010). Colletotrichum gloeosporioides f. sp. aeschynomene as the anthracnose-causing fungus is used in the state of Arkansas in the US as a microbial herbicide called Collego for controlling the main weed of soybean fields and rice paddies, which is Aeschynomene virginica (Kouchaki et al., 2001). Studies conducted by Watson and Winder showed that Colletotrichum dematium has strong bioherbicidal characteristics and could control fireweed (Epilobium spp.) quite well (Watson and Winder, 1993).. In the state of Florida, it was found that fungi such as Colletotrichum truncata, Ralstonia solanacearum and Fusarium oxysporum isolated from Sesbania exaltata, Solanum viarum and Striga hermonthica could be effective against these weeds and eradicated them after five days (Charudattan, showed that 2001). Conducted studies some
Colletotrichum species are extensively used for the biological control of weeds in the US fields (Goodwin, 1995). Poa weed as one of the main weeds in the United States reduces the yield of most crops and chemical control does not have much effect on limiting weeds competition (Goodwin, 1995). Also, using Colletotrichum graminicola along with applying chemical and mechanical methods controlled weeds (Goodwin, 1995). This fungus was effective in some corn bred cultivars; however, productive cultivars were sensitive to it (Goodwin, 1995). In another study, for the biological control of Echinochloa crus-galli and Echinochloa sp. in paddy fields in Vietnam, Australia and South Korea, Exserohilum fusiforme and Colletotrichum graminicola were used as two pathogens and inhibited the growth of these two weed species at early stages (Johanson et al., 2003).

Ipomoea locunosa is known as a serious and extensively spreading weed in soybean, cotton and many other crop fields of the southern parts of the United States (Cartwright and Tempelton, 1994). *Colletotrichum capsici* was used for its biological control and proved effective in reducing damages (Cartwright and Tempelton, 1994).

Sesbania exaltata is a problematic weed in soybean cultivation (Boyette et al., 2006). In order to biologically control this weed, microsclerotia of *Colletotrichum truncatum* are mixed with some surfactants and turn into granules called 'Pesta' which effectively control this weed (Boyette et al., 2006).

Abutilon theophrasti is a weed with a wide spreading range which damages many crops (Meir et al., 2009). Therefore, a specific pathovar of *Colletotrichum coccodes* has been considered as a specific bioherbicide for selective controlling of this weed (Meir et al., 2009).

While introducing a microorganism as a biological control agent, it is very important to make sure that it won't damage crops (Watson, 1985). In the present study, *Colletotrichum graminicola* was isolated from three main weeds of rice paddies in Guilan province in Iran, i.e. *Echinochloa* spp., *Alisma plantago-aquatica* and *Sagitaria trifolia* and thus, it was investigated as a probable mycoherbicide for the biological control of the said weeds. Also, to examine the effects of the fungus on rice, the reaction of some rice cultivars to *C. graminicola* was studied.

2. Materials and methods

2.1. Collection and culture of fungal isolates

Leaves with symptoms of the disease weeds (*Alisma plantago-aquatica, Sagitaria trifolia, E. crus-galli* and *E. oryzicola*) were collected in Guilan province of Iran, cut to appropriate sizes and transferred to the laboratory. Samples were surface sterilized with 0.5% sodium hypochlorite solution,

washed by sterile distilled water and placed on potato dextrose agar (PDA) in Petri dishes. Then, Petri dishes were incubated at 28^oC in darkness or light on a 12 hours light/dark photoperiod for 6-15 days. Conidia were single-sporulated and then, monoconidial isolates of the recovered fungi were maintained on halfstrength PDA slants in test tubes as stock cultures (Zhang et al., 1996) or colonial of fungal placed onto sterilized filter paper, then cuts of these filters were incubated in sterilized vials at freezer on -20°C (Safari Motlagh, 2010).

2. 2. Study and identification of fungi

Fungi which had grown were isolated and koch's postulates were completed for most sample after each collection. Cultures of these fungi were submitted to the Research Plant Pathology Institute of Iran for the confirmation of identification.

2.3. Pathogenicity test

Weeds

This reaction occurred as complete random design (CRD) with one treatment and 3 replications. Weeds were planted in plastic pots 2.5 cm in diameter containing farm soil. For each treatment, one control was assigned (Zhang et al., 1996). Pots were placed at 25-30°C, 12 D: 12 L photoperiod and a relative humidity of more than 90%. Inoculation of weeds was performed at its 3-4 leaf stage in greenhouse. To do so, a spore suspension including 10^6 Colletotrichum graminicola spore/mL distilled water was used. In order to increase adsorption, 1% Tween-20 was used. This suspension was sprayed on the leaves using a sprayer. It should be mentioned that before inoculation, all pots were sprayed with distilled water. To create a relative humidity higher than 90%, treated plants were immediately covered with plastic bags for 48 hours (Ghorbani et al., 2000). Evaluation was done 7 days after inoculation based on lesion type and size in reaction to inoculation: 0= lesions absent, 1= small, unexpanded lesions, 2= slightly to moderately expanded lesions, 3= large lesions (Zhang et al., 1996). Therefore, standard evaluation system and Horsfall-Barratt system were applied for Echinochloa spp. system were applied for Echinochloa spp. (Zhang et al., 1996; Bertrand and Gottwald, 1997).

Disease rating =
$$\frac{(N_1 \times 1) + (N_2 \times 2) + \dots + (Nt \times t)}{(N_1 + N_2 + \dots + N_t)}$$

Where N is number of leaves in each of rate, t is number of treatments.

Rice

This reaction occurred as complete random design (CRD) with five treatment and 3 replications. The five rice cultivars including 3 indigenous (Hashemi, Ali Kazemi and Binam) and 2 bred cultivars (Khazar and Sepidroud) were evaluated against inoculation with Colletotrichum graminicola. In order to do so, first, rice seeds germinated and after being transferred to the greenhouse inside pots, 2.5 cm in diameter without any drain, they were planted in the farm soil. When the plants reached their 3-4 leaf stage, thinning was performed. Finally, there were 4 shrubs in each pot. Then, 2 g urea fertilizer was added to the pots. At this stage, inoculation was done by a spore suspension of Colletotrichum graminicola containing 10⁶ spore/ mL of distilled water with 1% Tween-20. Other environmental conditions were similar to those of the weed. Evaluation was done 7 days after inoculation based on Horsfall-Barrat system. Then, disease ratings were calculated (Bertrand and Gottwald, 1997). It is noteworthy that in both experiments, one control was considered for each replication.

2.4. Measuring plant fresh weight, dry weight and height

In order to measure these traits, inoculated weeds and rice cultivars along with controls were transferred from greenhouse to the laboratory. Then, shrubs were cut on the soil surface and weighed by an electric scale. This weight was recorded as their fresh weight. After separately measuring their height, each shrub was placed inside a paper bag and they incubated in an oven at $80-90^{\circ}$ C for 48 hours. When the bags were taken out of the oven, each shrub was weighed, which was considered as its dry weight (Ghorbani et al., 2000).

2.5. Data Analysis

Data analysis was done using SPSS and MSTAT-C softwares. In order to compare average values, Duncan test was used, while for comparing the reaction of rice cultivars and weeds, the difference between the average value of each fungus-treated rice cultivars and the controls was used.

3. Results

According to the variance analysis table for the evaluation of the disease rating, it was found that the studied rice cultivars showed significant reactions to *Colletotrichum graminicola* (Table 1). Also, based on the comparison of the mean traits in the study of disease rating, the greatest effect of the fungus was seen on Sepidroud, i.e. this cultivar was less tolerant compared with others (Figureure 1). Among indigenous cultivars, Hashemi showed less tolerance (Figureure 1). There was no significant difference between Ali Kazemi, Khazar and Binam with only

Binam being more tolerant to this fungus in terms of the number and sizes of the spots created (based on Horsfall-Barratt system) (Figure 1).

On the other hand, based on the variance analysis table for the evaluation of traits including height, fresh weight and dry weight, the studied rice cultivars showed significant reactions (Table 1). According to the comparison of the above-mentioned traits among the cultivars, it was found that for height, there was no significant difference between Hashemi, Sepidroud and Binam cultivars. Also, no significant difference was observed between Ali Kazemi and Khazar. No significant difference was found between the dry and fresh weights of Khazar and Binam as well (Table 2). However, a significant difference was observed in terms of these two traits between Hashemi, Ali Kazemi and Sepidroud (Table 2). Moreover, compared with other rice cultivars, Khazar showed more reductions of the said three traits (Table 2).

In the investigation of the reactions of the studied rice cultivars regarding their heights, fresh weights and dry weights compared with the controls, results showed that for height, Ali Kazemi, Sepidroud and Khazar were more affected by the fungus than the controls (in comparison with the controls, they revealed a decrease in height which compared with that of other cultivars was greater.); however, when compared with each other, they had no significant differences in terms of this trait (Table 3). In the second group, there were Hashemi and Binam for height decrease, yet with no significant difference between each other. But when compared with Ali Kazemi, Sepidroud and Khazar, they were less affected by the fungus (Table 3).

In terms of fresh weight, this fungus was effective on Sepidroud (as a bred cultivar) and indigenous Hashemi and Ali Kazemi compared with the controls, but they showed no significant differences between themselves. *Colletotrichum graminicola* had no effects on the fresh weights of Khazar and Binam. Furthermore, these cultivars did not show any significant differences between themselves (Table 3). In comparison with the effect of the fungus on the fresh weight in bred cultivars, Sepidroud had more fresh weight decrease than Khazar.

Concerning dry weight, it was found that the fungus caused this trait to decrease in the studied cultivars compared with controls and that there was no significant difference between the cultivars.

And in terms of the effect of this fungus on all the three studied traits, it was revealed that the fungus was more effective on height and fresh weight than on the dry weight. Moreover, it was found that bred cultivars were more sensitive to the fungus (Table 3).

Based on the variance analysis table for evaluating the disease rating, the effect of the *Colletotrichum* graminicola on Alisma plantago-aquatica, Sagitaria *trifolia* and *Echinochloa* spp., was significant (Table 4). The greatest effect was seen in *Alisma plantago-aquatica* and then in *Sagitaria trifolia, E. crus-galli* and *E. oryzicola*, respectively (Figure. 2).

According to the variance analysis table for evaluating weeds' height, dry weight and fresh weight, it was found that the weeds showed significant reactions in terms of all three studied traits (Table 4). With consideration of the comparison of the traits' mean values in weeds results revealed that in terms of height, there was no significant difference between two Echinochloa species; however, Alisma plantagoaquatica and Sagitaria trifolia showed a significant difference (Table 5). Moreover, conditions for both fresh and dry weights were the same. Two Echinochloa species showed no significant difference in terms of these traits, while Alisma plantago-aquatica did have a significant difference regarding their fresh and dry weights (Table 5). The examination of the reactions of the studied weeds in terms of the three traits compared with those of the controls revealed that for height, all weeds had reduced heights compared with controls, but the differences were not significant among themselves (Table 6). In terms of fresh weight, Sagitaria trifolia and Alisma plantago-aquatica had more reductions, but did not show any significant difference compared with each other. The fungus reduced the fresh weight in both Echinochloa species as well.

Comparison of the studied weeds' dry weights with those of their controls indicated a reduction in this trait among treatments, but these weeds did not show any significant difference. Therefore, in terms of dry weight, *Sagitaria trifolia* was more affected compared with *Alisma plantago-aquatica* and two *Echinochloa* species and showed more reduction of the trait (Table 6).

4. Discussion

The present study revealed that not only the disease rating caused by *Colletotrichum graminicola* in *A.plantago-aquatica, Sagitaria trifolia, Echinochloa crus-galli* and *E. oryzicola* was high, but also it caused high disease rating in the studied rice cultivars as well.

In this research, *C. graminicola* was effective in both bred and indigenous rice cultivars. This finding was consistent with that of Johanson *et al.* According to these studies, *Colletotrichum* species such as *C. graminicola* and *C. truncata* are considered as effective fungi with strong antagonistic characteristics for the biological control of *Echinochloa* and nightshade, but extensive use of this fungus depends on producing tolerant rice and wheat cultivars (Johanson et al., 2003)

The present research showed that bred rice cultivars were more affected compared with indigenous cultivars, which might be related to these cultivars becoming more adapted to indigenous fungi. While in the study of the reaction of bred and indigenous corn cultivars to *Colletotrichum* spp., isolated from annual mercury in corn fields in the US, bred cultivars were more tolerant and the disease rating caused by the fungus in them was lower (Norris, 1992). However, compared with indigenous cultivars, it was more effective in terms of their dry weight (Norris, 1992). Molecular studies revealed that bred cultivars had more resistant genes, but in indigenous cultivars, fresh and dry weight-controlling genes were more in numbers (Norris, 1992).

In another study, it was found that *C. graminicola* was effective in controlling *Sorghum halepense*, but since the fungus caused higher disease rating in alfalfa, it was not introduced as a biological control agent (Tempelton and Henry, 1990).

It has been found that the competence of a plant is directly related to its genetic composition that is, the less the number of resistant genes in a plant, the lower its competence degree and it would be considered a more suitable host for fungi (Blum, 1998).

In conclusion, with the results of this research taken into account, it can be said that *Colletotrichum graminicola* can be introduced as a biological agent for controlling *Alisma plantago-aquatica*, *Echinochloa* spp. and *Sagitaria trifolia* provided that rice cultivars other than those studied here are planted. This would be especially possible when new bred cultivars have a combination of the useful traits of the existing indigenous and bred rice cultivars all together.

Table 1. Variance analysis of disease rating and the studied traits in rice cultivars affected by C. graminicola.

SOV	DF		Squares Mean							
		Disease rating	Height	Fresh weight	Dry weight					
Treatment	4	0.778 ***	171.278 **	13.814**	0.834 **					
Error	10	0.136	8.761	0.049	0.015					
C.V.	-	15.62	4.21	4.49	14.19					

^{*} Significance at the probability level of 1%.

SOV: sources of variations

DF: degree of freedom

Table 2. Com	parison of the	reactions of rice	cultivars affecte	d by <i>C</i> .	graminicola.
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Cultivars	Height	Fresh weight	Dry weight
Hashemi	$68.206 \pm 0.586b$	$6.09 \pm 0.0832b$	$1.013 \pm 0.0166b$
Ali Kazemi	$82.833 \pm 1.083a$	$7.956 \pm 0.0633a$	$1.673 \pm 0.155a$
Sepidroud	$69.206 \pm 1.149b$	$4.683 \pm 0.0392c$	0.825 ± 0.0173 b
Khazar	$62.33 \pm 2.385c$	$3.157 \pm 0.0179 d$	0.407 ± 0.0121 c
Binam	68.820 ± 2.454 b	2.760 ± 0.261 d	$0.392 \pm 0.0416c$

Treatments having at least one similar letter do not show a significant difference at the probability level of 5%.

Table 3. Comparison of the reactions of rice cultivars affected by *C. graminicola* with those of the controls.

Cultivars	Change of Height	Change of Fresh	Change of Dry
		weight	weight
Hashemi	-0.823 ± 0.151 b	-0.036±0.149a	$-0.133 \pm 0.33a$
Ali Kazemi	-1.96±0.571a	$-0.046 \pm 0.129a$	$-0.24 \pm 0.14a$
Sepidroud	-1.816±0.183a	-0.063 ± 0.044 a	$-0.132 \pm 0.048a$
Khazar	$-1.19 \pm 0.052a$	$0.39 \pm 0.084 b$	$-0.121 \pm 0.05a$
Binam	-0.783 ± 0.859 b	$0.196 \pm 0.066b$	-0.118±0.046a

Treatments having at least one similar letter do not show a significant difference at the probability level of 5%.

SOV	DF		Squares Mean						
		Disease rating	Height(cm)	Fresh	Dry				
				Weight (g)	Weight(g)				
Treatment	3	3.652**	432.828**	97.676**	4.710**				
Error	8	0.489	1.218	0.989	0.192				
C.V.	-	27.74	2.04	8.94	25.16				

**:Significance at the probability level of 1%

SOV: sources of variations

DF: degree of freedom

Table 5. Comparison of means of the studied traits affected by C. graminicola in weeds.

Weeds	Height(cm)	Fresh weight(g)	Dry weight(g)
E. oryzicola	$62.330 \pm 0.0378a$	$7.179 \pm 0.0257c$	$0.998 \pm 0.0569c$
E. crus-galli	$62.123 \pm 0.313a$	$5.475 \pm 0.141c$	$0.630 \pm 0.188c$
Sagitaria trifolia	$54.900 \pm 0.907b$	$17.346 \pm 1.128a$	$3.466 \pm 0.447a$
A. plantago-aquatica	$36.776 \pm 0.837c$	$14.513 \pm 0.891b$	$1.870 \pm 0.227b$

Treatments having at least one similar letter do not show a significant difference at the probability level of 5%.

	Table 6	5. (Comparison	of the	reactions	of wee	eds affec	ted by	С.	graminicola	with	those	of the	controls
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Weeds	Change of Height(cm)	Change of Fresh	Change of Dry
		weight(g)	weight(g)
E. oryzicola	-0.916±0.562a	-0.231 ± 0.093 b	-0.198±0.097b
E. crus-galli Sagitaria trifolia A. plantago-aquatica	-1.12±0.403a -0.933±0.466a -0.483±0.483a	-0.351±0.163b -4.926±1.526a -3.173±0.164a	-0.322±0.031b -1.23±0.309a -0.036±0.433b

Treatments having at least one similar letter do not show a significant difference at the probability level of 5%.



Figure 1. Diagram of the comparison of Colletotrichum graminicola mean disease rating in rice cultivars.



Figure 2. Diagram of the comparison of Colletotrichum graminicola mean disease rating in weeds.

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The Theoretical and Conceptual Framework and Application of Community Empowerment and Participation in Processes of Community Development in Malaysia

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Abstract: This study defines and discusses the concept of participation in the context of social development in general, and in terms of community development and community work specifically. Community development could not be achieved without community participation. The principles and techniques underlying the empowering process are also elaborated. The study describes the application of the concepts defined in some of the community work and community development activities in Malaysia

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Introduction

Community participation is the main factor which can effect on processes of community development. Without community participation and empowerment community development could not be achieved. It is important to note that since this study focuses on people's participation process in community development activities, therefore it is beyond the scope of the study to generate, enrich or to improve the meaning and definition of 'community' deduced from the study. However, since the word 'community' is prefixed to other words, such as 'development', 'participation' and 'empowerment' to become community development, community participation and community empowerment, therefore it is worth examining some of the 'common' definitions used, which are particularly relevant to this study. Community participation and empowerment are considered necessary to get community support for community development projects (Cole, 2007). Community participation refers to peoples' engagement in activities within the community. It plays an essential and long-standing role in promoting quality of life (Putnam, 2000).

Community

The term 'community' has been used in different ways and at different levels (Stacey, 1974). In other words, 'community' as a sociological concept is a contested term, which can be deduced, discussed and argued from and within its own literature or body of knowledge based on community studies (Bell and Newby, 1974; Willmott, 1989; Nisbet; 1967). In reviewing ninety-four definitions of community, Hillery (1955) pointed out that the common components of community are area, common ties and social interaction. This is relevant to Willmot's (1989) concept: territorial community, interest community and community of attachment, though the first two, according to him, are not mutually exclusive because non-local communities. i.e non-territorial communities can also contain interest communities, but are geographically dispersed. Obviously the territorial communities who share and live in a common residential or geographical area contain localized interest communities, at least in terms of sharing some of their common problems or expectations. Working together to pursue shared needs or to overcome common problems implies that the sense of community of attachment could also enhance their existing spirit of community and feelings of identity, the social bonds that tie them together. The close and personal relationships between community members or group members within a small geographical area also resemble the characteristic of gemeinschaft (Tonnies, 1955).

Some portrayed community as an area which contains all or most of the elements of a common life; the politic, economic, social and religious life of the inhabitants - a kind of small scale social system (Ogburn and Nimkoff 1953), (MacIver 1924) and (Worsley 1977). Nevertheless, it is not necessarily to characterize community with a 'particular dominant occupational function'. Improvement in the transportation system can influence the behavior and occupational patterns of the inhabitants, whereby they could seek other jobs in nearby towns or industrial areas. In rural areas for example, farming is no longer the only form of occupation, although initially the establishment of the community was built upon on the agriculture-based economy. As the community grows, and partly due to the scarcity of land for cultivation, younger generations start to find other job opportunities and engage in a new economic sector. Other external economic forces. such as industrialization in urban fringe areas, also play their part in contributing to the diversification of employment patterns among rural people.

In general, people living in one defined territory share the same social and political life. They share the same leader(s), either selected by them or appointed by external bodies. It is within the locality that social life and political life are integrated together. At the local level, people can manage their own lives through local leadership institutions. Nevertheless, to what extent the local community possesses political autonomy in terms of making or influencing decisions on matters related to their social life depends on the degree of relationship between the community and the external bodies at the higher level, such as the state or other main voluntary bodies. Some of these relationships are created through the national policy and its administration structure, and this is then strengthened by the local patronizing political culture. This is especially true if the establishment of the locality is initiated by the state through its development program, such as the planned village settlement, and resettlement scheme. It is within this framework that the local community's political and social life is intertwined with the national aspiration and politico-administration, and within such relationships people maneuver and manage their socio-political life. This social system approach to view community also comes from MacIver, who defines community thus; 'Community is a social unity whose members recognize as common a sufficiency of interests to allow of the interactivities of common life... out of which associations arise... [and]... is the whole incalculable system of relations". (MacIver, 1924:109-129)

From various definitions as reviewed earlier, community can be referred to as a social unit where the locality in which they reside is an integral part; within which members interact together to do things and to achieve what they want. By participating in various groups and collective action, communities are able to act together regarding the common concerns of their lives. This definition of community, which is derived from community studies literature, is particularly relevant to this study on the four basic components surrounding the concept, i.e. people, area, interaction and interest. In this study the http://www.americanscience.org definition of community is used as a convenient term to refer to individuals and groups who live together in a defined geographical area which has its own social, economic and political entity, i.e the planned village settlement, interacting and working together to achieve their common interests in the process of developing their community life.

It can be concluded that, by referring to community as an entity, or in Stacey's term the 'local social system' (Stacey, 1974), which embraces the social, economic and political life of the people within a particular locality, the community life, its associations or groups and activities can be grasped in detail. In relation to the perspective that views community as an object of change, this understanding of people's lives and their activities facilitates us to examine the micro process of people's participation in developing themselves and effecting change in their community life such as: (1) the process of how they stir up members' concerns about problems, needs and interests; (2) how they organize to pursue their interests and meet their needs/objectives, prioritizing, influencing, deciding and working on these; (3) the mode of working practice to achieve group goals; (4) the degree and type of involvement of community members in the respective groups and activities; and (5) the degree to which the community gains control over life through their collective action in the participation process of bringing change and community development. In short, by conceptualizing community as a social unit living in one defined territory with its own social, economic and political entity which consists of individuals as actors and in their interaction with others, they are able to bring change to their community life through participating in activities, and this integrates both the ideas of perceiving community as a subject and as an object of change. This brings us to view some of the definitions on community participation.

Community Participation

The concept of community participation has become one of the most important subject matters discussed in various disciplines that have and need human input in the development process, such as in social policy (Richardson, 1983; Croft and Beresford, 1992), health (Oakley, 1989; Rifkin, 1985), community planning (Moser, 1989; Wandersman, 1979), psychology (Chavis and Wandersman, 1990; Zimmerman and Rappaport, 1988), tourism (Aref, and development 2009) community development and community work (Abbott, 1995; Lackery and Dershem, 1992; Goulet, 1989; Oakley and Marsden; 1984; Gilbert and Ward, 1984; Smith, 1981; Galjart, 1981a and 1981b; Wandersman, 1981). In general, the concept of participation in this literature has been used to describe many kinds of activities and processes carried out, directive or nondirective by the authorities responsible, or initiated by people themselves to bring social development and improvement for the betterment of community members. On reviewing this literature, which is based on observations of the practices as to how activities, program or projects were carried out, there are various models or typologies of community participation put forward by some of the authors. A close examination of these models or typologies shows that the fundamental aspect underlying their discussions is the extent of people's involvement in the decision making process, and the degree of people's control in the activities or projects for them as recipients of the development programs. Oakley and Marsden (1984) in their attempt to define this concept have outlined a continuum of definition based on different interpretations ranging from defining participation merely as a means at one end of the continuum, towards describing it as a process with some element of peoples' control at the other end. As a means, participation is considered as; "... a voluntary contribution by the people to one or another of the public programs supposed to contribute to national development but the people are not expected to take part in shaping the program or criticizing its content" (Oakley and Marsden, 1984:19). On the other hand participation can be defined as a process in itself where people have some control over the whole development process (Oakley and Marsden, 1984).

These definitions of participation bring us to discuss the issue of participation as a means and/or end. As a means, participation is perceived as a vehicle to achieve the pre-determined objectives or goals which may or may not be congruent with the needs of the participants. In this situation the aim to achieve the pre-set objectives, either as determined by the government, its extension agencies or other external bodies, is more important than the act of participation itself. Therefore, the act of participation in such situations can be considered as "an input into a development program" (Oakley and Marsden, 1984: 27) since people are not given the opportunity to decide or influence the decision that has an effect on them later. The mobilization of people in this form of participation is to get things done based on a fixed quantifiable development goal (Moser, 1989: 84) which can be state-directed or externally-directed activities, the 'top-bottom' approach to community development. In such phenomena participation turns into passive and static events which can then be induced or even coerced participation (United Nations, 1981) or a compulsory participation (Oakley, 1989), or manipulative participation (Midgley, 1986)

by the government or other external bodies. It is for this reason that Koneya asserts that 'citizen participation' is not 'community development', as in the former it is the government which decides to include citizens in government-centered programs, whereas the latter, i.e. community development, 'is a citizen-originated activity that organizes and uses citizen power to reach upward toward government' (Koneya, 1978:25). On the other hand, participation as an end in itself focuses on participation as a process in which people are directly involved in shaping, deciding and taking part in the development process from the 'bottom-top' perspective. Here, the development goal is of secondary importance but the process whose outcome is an increasingly 'meaningful' participation in the development process' (Moser, 1989:84) is much more valued. This is because in such a process the authentic form of participation (Midgley, 1986), direct participation (Richardson, 1983) or active participation (Gilbert and Ward, 1984) from people emerges where their confidence and competence are built up. In this situation, participation becomes a process "of achieving greater individual fulfillment, personal development, self-awareness and some immediate satisfaction" (Richardson, 1983: 57). It is an active and permanent form of participation in which the direct involvement of the people does not only help to sustain the life of a project or a group but extends a persons' involvement in creating or establishing other new projects or community groups. The distinctive features in defining participation as a process is that people are given the chance to 'formulate' their own development, to influence or to 'have a say' in the decision making process regarding the programs or projects initiated for them. In this respect, viewing participation as a process can help to develop people's capacities or abilities, recognize and improve their inherent potential, and provides them with opportunities to influence and share power, i.e. power to decide and to gain some control over their lives.

In reality, however, participation as a means is not a dichotomous entity but rather is a continuum based on the degree of people's involvement in deciding or influencing the decision making process concerning the development program or in its implementation. It is along this continuum that the models or typologies of participation are constructed by the writers mentioned above, as shown in Table 1. Although the types of participation are differ between the authors, the main aspects which differentiate between the stages within the typologies are the same. They share common ideas regarding the extent or the degree to which community members have the chance or are given the chance to decide for themselves.

			Table 1: 7	Fypologies or Mod	lels of Particip	ation - A conti	nuum		
Level o	f participation			ТҮРО	LOGIES OF PA	RTICIPATION			
	Brager & Specht (1965)	Arnstein (1969)	Hollnsteiner (1977)	Koneya (1978)	Wandersman (1979-1981)	Johnston (1982)	Oakley (1986-1989)	U.Nation (1981)	Moser (1983)
High	Community control	Citizen control	Community control	Citizens define the problem and decide the action	Self-planning	Participating through creativity	Spontaneous participation (authentic participation)	Spontaneous participation	Participa tion as a end
C O	Community has delegated authority	Delegated power	People's representation on decision making board	Presents problems & boundaries, but citizen decide		Participating by taking initiative			C O N
N T I N U	Plans jointly	Partnership	Consultation starting with plan formulation	Presents problems, ask for ideas, then decide	choice	Participating by giving suggestions and making criticisms	Induced participation (co-optive & manipulative of an activity	Induced participation	T I N U
U M	advises Community is consulted	Placation consultatio n	Community's choice of final plan from among pre determined option	Presents tentative decision, consult citizen, then decide		aimed at improvement of an activity			M
low	Community receive information	Informing	Appointment of local leaders to position in government bureaucracy	Announces decision, permits questions	Feedback	Voluntary participation prompted by awareness			
		Therapy	Unofficial representation by 'solid citizen' group which endorses	"sells" decision to citizen		Voluntary participation stimulated by a reward			
	Non participation	Manipulati on	outside- planned program	Decide, announces decision thro bulletin	No participation	Participation in response to an order or to force	Compulsory participation	Coerced participation	Participa tion as a means

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The degree of intensity of participation is denoted by the stages along the continuum starting from the lowest, which some authors consider as 'no participation' since people do not participate in the decision making process that eventually defines participation as a 'means in response to an order' (Johnston, 1982:203). At its lowest degree or level, people's participation can take the form of being coerced (United Nations, 1981) or compulsory (Oakley, 1989) which can be manipulated (Arnstein, 1969) by the state or other external agencies that practice the anti-participatory mode of social development (Midgley, 1986). There is no 'people's control' at this lowest stage. Even if the state or the government practices the consultative approach to community development in a partnership program by giving people the choice within the predetermined projects, people's participation is induced. This sort of participation can be found in the government or any agency that practices a top-bottom approach in community development (Midgley, 1986), and in this incremental mode to encourage people's involvement, the participation in itself performs dual roles, i.e. instrumental and development roles (Richardson, 1983). In the former, participation is seen as a means, used by the state or the ruling party to achieve some pre-set goals, while the latter relates more to the development of human capacities. Although there is an encouragement or promotion given at this middle portion of the continuum as denoted by 'placation', 'choice', 'induced' or 'partnership', however as White reminds us, participation at this stage is not mainly concerned with the process of mobilizing the people, who should be regarded as the recipient or beneficiaries of the development introduced, but of uppermost importance is 'the participation of the organized community as such' (White, 1982: 19). It is through participating by organizing themselves to define their own problems together with the responsible authority to influence the decision making process, or by taking the creative effort among themselves to plan, decide and initiate their own groups, activities and projects, that authentic community participation is said to be established. At this point people have some control over the development process, the upper most level or degree of participation, and the decisional power taken by the non-elite, i.e. the ordinary community members, enables them to be free from manipulation and cooptation where authentic participation can be promoted (Goulet, 1989).

Whether participation is a means or an end is a matter of debate. But since both lie in a continuum, therefore the most important issue is how participation as a means can be 'upgraded' and has the capability to develop into participation as an end. In http://www.americanscience.org

other words, this polemic can be resolved when the induced participation at the beginning of the topbottom community development program has the ability to develop the people (who were forced or induced to participate at the initial stage) so that they have the potential to resolve problems and to take care of themselves later. This is parallel to the philosophy and definition of community development as a process by which peoples' efforts are united with those of governmental authorities to improve the living conditions of the community, and to enable them to contribute fully to national progress. This also takes into account the ability of the community to participate in local initiative activities without external support to maintain their self-sustaining groups. For this to materialize it depends on both sides, i.e. the openness of government (Abbott, 1995) and its development agencies - the extension worker including the VDSC as the case in Malaysia, and the community itself. As 'participation is not just an end in itself, but it is more than a means' (Cohen and Uphoff, 1980:227), therefore the cooperation and power sharing between the people and the development agencies is essential in promoting participation as a means towards an end so as to increase the level of people's participation towards the top of the continuum where the 'empowerment' is situated (Arnstein, 1969; Oakley and Marsden, 1984; Moser, 1989).

Community empowerment

Theoretically and pragmatically, in exploring the empowerment process with the Malaysian context, there are limitations at the macro level because the control that the state has over the citizen through, firstly, the politico-bureaucratic mechanism in-built within the community development process. and secondly, at the same time mutually supported by the culture of patronage. Structurally, examined it at the micro level, the process of bringing change through community development activities is closely related to government policy, facilitated by and under the auspices of the respective state's agencies. This politico-bureaucratic nature of promoting community change is further strengthened at the micro level through the District Office within the state rural development administration policy. In fact, a close relationship between the District Office, other extension agencies personnel and related departments, and the local people through their representatives, is encouraged by the state (Chee, 1974 & 1975; Siedentopf, 1987). In this politico-bureaucratic structure and patronizing culture, the appointment of voluntary community development personnel, such as the VDSC and the Mosque Committee members, for example, is based on certain procedures introduced by

the state, which intensify and extend the state's controlling power over the people. Interference by the local 'political man', the Member of Parliament (MP) or the State Assemblymen in community life, help to extend the patronizing culture macro-micro relationship. At the local level, this patron-client phenomenon can sometimes become more complex when there are some forms of individual-based patronage between the politician and his supporters, and thus this makes the state's control through its MP's and State Assemblymen over the masses more effective. The impact of these phenomena is much more significant when the patronized individual(s) is/are the community activist or community developer (unpaid). By upholding and promoting the community's traditional working practices; the selfhelp and mutual-help spirit to develop people's selfreliance and self-determination propagated by the state through the local community activists to meet the local needs, reinforces the patronizing framework and network

Generally in the Malaysian context, and specifically in exploring the participation process in community development, it can be said that to a certain degree, empowerment is limited by the social structure. However, this does not mean that empowerment does not take place within the given structure in which people live. This is because, within this structure, there are some real spaces for empowerment to take place. As discussed earlier, although people are socially structured, they are also creating reality. The same experiences that they face in their environment are shared together as subjective meanings, which are then translated into an action process to initiate something to fulfill their collective needs. In the process of participation people are empowered and can be empowered, and the central themes of people's empowerment is the ability that they have to make changes based on their own needs after realizing the problems they face. Hence, within these prescribed circumstances, individuals interact and influence each other, mobilize and organize themselves to decide, perform and take the action collectively to solve common problem(s) and to achieve their goals.

Facilitated by local activists, individual members exercise their abilities - the 'power to' act through collective action, mobilizing themselves in initiating and establishing various community groups, conducting group activities, organizing self-help and other communal projects, based on their interests in an effort to solve and alleviate common problems they experience. In other words, these problems and needs, which are shared by individuals, are objectified and manifested in the establishment of the groups and activities. These are also the tangible http://www.americanscience.org products of empowerment. Such activities or groups cannot materialize if they are not empowered to do so. It can be argued that it is through the relationship with others within the environment and the structure in which they live, that they are able to translate the problems (and needs), their subjective meanings, into concrete action by participating to acting upon it. It is through this process that individuals are able to exercise and experience their power with others. The process whereby individuals exercise their ability and capacity effectively to achieve certain goals, and to further develop those capabilities, is referred to as individual empowerment. By exercising those capabilities through participation in establishing, organizing, implementing and managing self-initiated groups or activities, individuals can gain more control over their lives, while at the same time strengthening their existing personal ability. Participating in such actions allows individuals to practice their potentialities and experience the actual empowering process.

The process of achieving goals or objectives, and to bring about changes however, cannot be attained successfully individually, moreover the problems needing to be addressed are shared between the individuals as group members. Support and commitment is needed from other community or groups members to form the group or collective empowerment. Individual empowerment actually can contribute to group empowerment (Staples, 1990; Kieffer, 1984). This takes place in the participation process itself when the empowered individuals, who realize their personal responsibility for bringing some changes to their social environment, help to enhance the functioning of the group and community members by informing, inviting, encouraging and organizing them to participate in identifying the problem, prioritizing the needs, deciding (making decisions) and taking part in conducting the group activity, project or even action. By mobilizing, integrating, utilizing and coordinating local resources into a selfhelp effort for community change (Kahn and Bender, as one collective action. 1985) collective empowerment is said to be generated. The ability of individual members to influence their friends and other community members to participate together in pursuing the action process is their interpersonal empowerment. Once these interrelationships are established collective empowerment is thus tightened and stabilized, which eventually not only sustains but further encourages individual empowerment to take place (see also Parsons, 1991: Kieffer, 1984: Longres and McLeod, 1980). In other words, when the group(s) are established and the members are able to define the boundaries of its actions and activities to meet the shared (felt) needs or to solve the common problems which correspond to the changing environment or situation, group members can become active participants in implementing the decisions and the process which affects them. At the point where they are responsible for implementing their own choices and decisions, and are accountable for actions taken, that collective empowerment is exercised. Indeed, empowerment as a process of developing and exercising power - the ability to make decisions and to take the initiative on matters related to their lives is an ongoing process of developing the 'power to' act to achieve their objectives.

An increase in individual empowerment has the ability to promote or build up and generate collective or group empowerment, which can effect change; to improve their living or community life conditions, providing care or help for the community, setting up community education centers and so forth. At the community level, the effort to bring these changes is related to the objectives of the groups or activities initiated by the empowered individual members. Empowerment in itself is a reflexive activity in which the process can be initiated and sustained by individuals, as the agents who seek to determine their own destiny or lives. In this reflexive process, which takes place within an action is empowerment; individuals gain and develop skills, competence and confidence. Furthermore, this individual empowerment is reinforced by continued involvement with, and support from, the group (Evans, 1992). Developing and gaining skills, competence and confidence in such a process is, in general, knowledge gathering. Here, the dictum 'knowledge is power' according to Foucault, could advance people's action collectively as a group, when they reflect on past actions and experiences, and know where their capabilities are in relation to the social arrangement in which they live. With regard to this, collective action can promote and bring collective empowerment nearer to the 'power over' situation.But it is important to note that empowerment through people's participation within the enclosed situation, shaped by the structure, is not equivalent to a change in the power structure, or a change in the distribution of power because power is held somewhere else by the state, submerged within the politico-bureaucratic framework they themselves promote, which is then strengthened by the culture of patronizing. Nevertheless, within this structure reinforced by the culture, the participation process takes place. People involved in setting up community groups and organizing their activities learn and gain knowledge. This is a real discourse experienced by them. Through this, empowered people know where the structure is and to what extent they can infiltrate the 'membrane' that surrounds the structure, while http://www.americanscience.org

participating in exercising the ability to develop and initiate some changes in community life. Consequently, they could also act collectively to take action to secure and improve their position, as the subject in the process of developing themselves through influencing, negotiating, demanding, and even, in some instances, using threatening and confronting strategies in the process of interacting with others to achieve their group goals.

Although, one can argue that people are gaining some power when they successfully influence other party, this does not mean that power is being taken or seized by them. Even if they succeed in exercising their power over the state's representative body in implementing development programs by controlling them, this does not mean that power has been taken or transferred directly from the power holder (the state) to the people - the status quo remains. The reason for this is that the action taken, and the interaction process between both parties, takes place within the structures and frameworks which are regulated and approved by the state. But it is justified to say that people have exercised their power over the state representative by making some adjustments to the power relationship, which disadvantages them in the prior place. Similarly, the state agency's power is not reduced if it complies with the demands made by the people through their collective action in campaigning activities. Their power still exists. In short, the action taken by the people is that of 'negotiation', and the effort put forward by them within the permissible surrounding structure. Since empowerment is not directly power which has been given or taken by the people from the power holder (because they do not have the power to execute this), empowered people have the ability to see the boundary of flexibility within the social structure, and to take this opportunity to try their best to maneuver within those real spaces available to meet their own needs at the group or local level. In other words, empowered people are in the process of checking the limits of the membrane surrounding the structure - its elasticity - and to what extent it can be tolerated, while participating in taking their collective action. Therefore, empowered people are both reproducing, and at some point exerting a kind of 'challenge' to the structure. Surrounded by, and living in, a paternalistic society or environment where the relation still dominates patronizing human interrelationships, the notion of empowerment is not synonymous with a process whereby people gain, seize or take power, and later develop an absolute control over the structure.

Pragmatically, in the Malaysian context, it is within the given structure that people participate and empower themselves by exercising their inherent editor@americanscience.org

ability to develop and initiate change at the micro level, to fulfill their immediate and future needs without changing the power structure, and thus the superstructure. Also, at the macro level, it is within this structure that the process of empowering the people, propagated by the state through promoting and encouraging the joint effort between them with the aim of improving the economic, social and cultural condition of the community, which eventually could enable them to contribute to the national progress (see the United Nation's definition of community development adopted by Malaysia) is inculcated. Under this 'guided' environment, local people, together with the state-sponsored leadership institution such as the VDSC and other patronized local indigenous leaders, take the opportunity to develop themselves by stirring up local issues through group meetings (dialogue), raising members' awareness about the problems faced, and further, engaging into an action process to achieve the decided goal. Through learning by doing, facilitated by relatively more 'literate' individuals, the community activists, community members collectively learned to develop themselves and to achieve their objectives. In fact, the whole idea of the self-help campaigns and joint-venture or partnershipliked activities promoted by the state since the early 60's can be referred to as the empowering strategy used by the top authorities. Since the people's empowerment, participation and its processes take place within the existing structure, thus this 'symbolic empowerment' is used by the state as a strategy in promoting and providing the right for the people to participate in government programs and to enable them to initiate their own community development projects/activities under the auspices of the local rural development administration personnel, the District Officials, VDSC members and other state appointed personnel. This relates to the issue of product and process of people's participation process.

Conclusion

In this study, the people's participation in community development activities is viewed as a process by which individuals are involved in initiating, deciding, planning, implementing and managing the group and its activities. It is also a process of social development in which people, as subjects in their own environment, seek out ways to meet their collective needs and expectations and to overcome their common problems. In pursuing this collective action, the self-help and mutual-help spirit that underlies the Asian traditional community spirit of working, helped to hasten the achievement of these shared interests through group-based-activities. Thus, by understanding this collective action in which http://www.americanscience.org

members participate, it is possible to comprehend the dynamic aspect of the group process within which participation took place. Participation is a dynamic process. Hence, it is difficult to predict or even to quantify using a standard 'measurement'. Participation is rather moulded by, and originates from, individuals' experiences in participating. As such, the qualitativeethnographic approach employed in this study was able to assist in understanding the process of people's participation in community development activities. This approach has also helped to deepen the knowledge about participation itself. This was not achieved merely by putting 'participation' into a measurable variable that can be operationalized into four quantifiable aspects, i.e. decision making, implementing, benefit sharing and evaluation (Cohen and Uphoff, 1977, 1980), but more importantly it involves understanding of how people organize themselves to meet their needs. It was through indepth interviews, follow-up interviews and group discussions with community members that the participation process was grasped. It was through intermingling the inductive and deductive processes, incorporated within and between these three main techniques of data collection on different groups of respondents that people's participation process in community activities was scrutinized and better understood. Although the main source of information in this study was based on individuals' participation experiences in the process of establishing groups and implementing group activity, it was also supported and validated by direct observation on the actual process they engaged in, watching and studying video clips, and other recorded materials kept by the group. Therefore, these two interchangeable processes, inductive and deductive, supported by triangulating the methods within and between different sources of evidence helped to complement, integrate and simultaneously verify the information gathered within which people's experiences can be comprehended. From the sequential analysis carried out during the fieldwork and detailed post-fieldwork analysis, the people's action process in participation were analytically constructed.

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Level set segmentation method in cancer's cells images

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Abstract: For early detection in cancer, it is necessary that cells be monitored on time. One of the first steps in the monitoring process is segmenting the cancer's cells. In this paper, we focused on the level set method and compared with snake active contour that use in image segmentation. Level set method is a fast and accurate approach that can be used in segmentation and reduce human interaction as possible. A set of cancer cells images is selected to serve as the representative test set. The selections are different sizes and resolutions.

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Keywords: Level set; GVF snake; cancer's cell; Image segmentation;

Introduction:

Medical imaging allows scientist and physicians to decide about life saving information regard to the human physiological activities. It plays an important role in the diagnosis, therapy and treatment of various organs, tumors and other abnormalities. Image segmentation is typically used to locate objects and boundaries in images and should stop when the object of interest in an application have been isolated .It is used to calculate the geometric shape and size of tumors and abnormal growth of any tissue. There are many techniques available for auto-segmentation of images like Active contours [13, 14], Fuzzy based classifiers [16], Gradient Vector Field theory, Tensor based segmentation, Level set theory etc. But many of them are suffering from problems like optimization, initialization and insufficient results in noisy images. Most widely used segmentation is level set segmentation in biomedical medical images [15] such as X-ray, CT and MRI. The level set technique or active contour is a powerful numerical technique for image processing. A predecessor to the level set methods: Level set method was first introduced by Osher and Sethian [1] that it is an implicit (nonparametric) technique and introduced in the medical vision community by malladi et al.[2], the active contour snakes [3] used parameterized representation of curves to segment images[3]. The geodesic active contours [17] had an added advantage of handling changing topology of the evolving curve implicitly,

but could only use boundary information, such as image gradients. The region competition algorithm [18] introduced a way of incorporating statistical region based information to evolve curves. But the Gaussian distribution specifically assumed for each region could prove restrictive in many cases. The active contours without edges method [19] provides a robust way of taking into account region information, including textures. However, the method is expected to perform unsatisfactorily whenever intensity distributions of the regions have the same first order moment but different higher order moments. The geodesic active contour method [4] unifies boundary and region based information along lines similar to those separately proposed in [17, 18] and so on. Since level set introduction, by Osher and Sethian [1], it has become a popular numerical method for the purpose of capturing the evolution of moving interfaces. In its simplest form, the method is very elegant, and offers some significant advantages over other interface tracking/capturing methods. Probably the most appealing feature of the method is its ability to handle changes in topology without complicated mesh generation, surface reconstruction, or collision detection. Particularly when considering applications in three dimensions or higher, this becomes a significant advantage: some other methods do not generalize so easily to higher dimensions. Another appealing feature of the level set method, which is often over looked, is its ties to numerical methods derived for hyperbolic conservation laws. This

connection allows the method to capture corners and cusps in the interface properly without non-physical loops and oscillations. Level set has been applied in many fields like physics, chemistry, fluid mechanics, image processing, computer Vision and a collection of other areas .its applications cover most fields in image processing ,such as noise removal , image in painting, image segmentation and reconstruction that one of the critical problems in computer vision and image analysis is segmentation. Image segmentation plays a central role in numerous useful applications such as satellite image analysis, biomedical image processing, scene interpretation, video image analysis, content-based image database retrieval, and many others. For early detection in cancer it is necessary that cells be monitored on time.one of the first steps in the monitoring process is segmenting the cancer's cells .While this can be done manually, the process can be time consuming. Level set method be a fast and accurate approach that can be used in segmentation and reduce human interaction as possible.

Gradient Vector Flow (GVF)

This approach is external force model for active contours and deformable surfaces, which we called the gradient vector flow (GVF) field [5]. The field is calculated as a diffusion of the gradient vectors of a gray-level or binary edge map. It allows for flexible initialization of the snake or deformable surface and encourages convergence to boundary concavities .we define the gradient vector flow field to be the vector field V(x, y) = [u(x, y), v(x, y)] that minimizes the energy functional:

 $E = \int \mu \left(u_x^2 + u_y^2 + v_x^2 + v_y^2 \right) + |\nabla f|^2 |V - \nabla f|^2 dx dy$ (1)

This variation formulation follows a standard principle that of making the result smooth when there is no data. In particular, we see that when $|\nabla f|$ is small; the energy is dominated by sum of the squares of the partial derivatives of the vector field, yielding as lowly varying field. On the other hand, when $|\nabla f|$ is large, the second term dominates the integrand, and is minimized by setting $V = \nabla f$. This produces the desired effect of keeping V nearly equal to the gradient of the edge map when it is large , but forcing the field to be slowly varying in homogeneous regions. The parameter μ is a regularization parameter governing the tradeoff between the first term and the second term in the integrand. This parameter should be set according to the amount of

noise present in the image (more noise, increase μ).We note that the smoothing term the first term with in the integrand of (1) is the same term used by Horn and Schunck in their classical formulation of optical flow [9]. It has recently been shown that this term corresponds to an equal penalty on the divergence and curl of the vector field [9]. Therefore, the vector field resulting from this minimization can be expected to be neither entirely irrational nor entirely solenoidal. Using the calculus of variations [10], it can be shown that the GVF field can be found by solving the following Euler equations.

$$\mu \nabla^2 u - (u - f_x) (f_x^2 + f_y^2) = 0$$
(2a)

$$\mu \nabla^2 v - (v - f_y) (f_x^2 + f_y^2) = 0$$
(2b)

Where ∇^2 is the Laplacian operator. These equations provide further intuition behind the GVF formulation. We note that in a homogeneous region [where I (x,y) is constant], the second term in each equation is zero because the gradient of f(x, y) is zero .Therefore, within such a region, u and v are each determined by Laplace's equation, and the resulting GVF field is interpolated from the region's boundary, reflecting a kind of competition among the boundary vectors. This explains why GVF yields vectors that point into boundary concavities. Equations (2a) and (2b) can be solved by treating u and v as functions of time and solving

$$u_{t}(x, y, t) = \mu \nabla^{2} u(x, y, t) - [u(x, y, t) - f_{x}(x, y)] [f_{x}(x, y)^{2} + f_{y}(x, y)^{2}]$$
(3a)

$$v_t(x, y, t) = \mu \nabla^2 v(x, y, t) - [v(x, y, t) - f_y(x, y)] [f_x(x, y)^2 + f_y(x, y)^2]$$
(3b)

The steady-state solution of these linear parabolic equations is the desired solution of the Euler equations (2a) and (2b). Note that these equations are decoupled, and therefore can be solved as separate scalar partial differential equations in u and v. The equations in (3) are known as generalized diffusion equations, and are known to arise in such diverse fields as heat conduction, reactor physics, and fluid flow [11]. Here, they have appeared from our description of desirable properties of snake external force fields as represented in the energy functional of (1). For convenience, were write (3) as follows:

$$u_t(x, y, t) = \mu \nabla^2 u(x, y, t) - b(x, y)u(x, y, t) + c^1(x, y)$$

$$\begin{split} v_t(x,y,t) &= \mu \nabla^2 v(x,y,t) - b(x,y) v(x,y,t) + \\ c^2(x,y) \end{split}$$

Where

$$b(x, y) = f_x(x, y)^2 + f_y(x, y)^2$$

$$c^1(x, y) = b(x, y)f_x(x, y)$$

$$c^2(x, y) = b(x, y)f_y(x, y)$$

Any digital image gradient operator [7] can be used to calculate f_x and f_y . To setup the iterative solution, let the indices I, j, and n correspond to x, y, and t, respectively, and let the spacing between pixels be Δx and Δy and the time step for each iteration be Δt . Then the required partial derivatives can be approximated as

$$\begin{split} u_t &= \frac{1}{\Delta t} (u_{i,j}^{n+1} - u_{i,j}^n) \\ v_t &= \frac{1}{\Delta t} (v_{i,j}^{n+1} - v_{i,j}^n) \\ \nabla^2 u &= \frac{1}{\Delta x \Delta y} (u_{i+1,j} + u_{i,j+1} + u_{i-1,j} + u_{i,j-1} - 4u_{i,j}) \\ \nabla^2 v &= \frac{1}{\Delta x \Delta y} (v_{i+1,j} + v_{i,j+1} + v_{i-1,j} + v_{i,j-1} - 4v_{i,j}) \end{split}$$

Substituting these approximations into (4) gives our iterative solution to GVF as follows:

$$u_{i,j}^{n+1} = (1 - b_{i,j}\Delta t)u_{i,j}^n + r(u_{i+1,j}^n + u_{i,j+1}^n + u_{i,j+1}^n + u_{i,j-1}^n - 4u_{i,j}^n) + c_{i,j}^1\Delta t$$

$$v_{i,j}^{n+1} = (1 - b_{i,j}\Delta t)v_{i,j}^n + r(v_{i+1,j}^n + v_{i,j+1}^n + v_{i,$$

Where

(4a)

(4b)

$$r = \frac{\mu \Delta t}{\Delta x \Delta y}$$
(6)

Convergence of the above iterative process is guaranteed by a standard result in the theory of numerical methods. Provided that, c^1 , c^2 and are bounded, (5) is stable whenever the Courant – Friedrichs-Lewy step-size restriction $r \leq \frac{1}{4}$ is maintained. Since normally Δx , Δy , and μ are fixed, using the definition of r in (6), we find that the following restriction on the time-step Δt must be maintained in order to guarantee convergence of GVF:

$$\Delta t \le \frac{\Delta x \Delta y}{4\mu} \tag{7}$$

The intuition behind this condition is revealing. First, convergence can be made to be faster on coarser images—i.e., when Δx and Δy are larger. Second, when μ is large and the GVF is expected to be a smoother field, the convergence rate will be slower (since Δt must be kept small).

An example of contrast between traditional snake and GVF snake is shown in Fig. 1. Fig.1 (a) shows an image $(100 \times 100 \text{ pixels})$ having a concave region. Fig.1 (b) shows the initial border (=0.5, =0.0). Fig.1 (c) and (d) shows the border output of traditional snake and GVF snake. Fig.1 (e) and (f) shows the potential force field of two snakes. Because the initial border is far from the true boundary, the active contours cannot converge to the true boundary. Clearly, the capture range of traditional snake is very small and GVF snake has a much larger capture range than traditional snake.

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Figure 1. (a) An image having a concave region (b) initial border (c) convergence of traditional snake (d) convergence of GVF snake (e) force field of traditional snake (f) force field of GVF Snake

Level set:

In idea of level set method [6,12], a contour C is represented by the zero level set which is called a

level set function that of a higher dimensional

function (3Dsurface) with the motion of the curve embedded in the motion of the higher dimensional surface. The motion of the front is matched with the zero level set of a signed distance function H .In the level set method, the curve is represented implicitly as a level set of a 2D scalar function referred to as the level set function which is usually defined on the same domain as the image. The level set is defined as the set of points that have the same function value. It is worth noting that the level set function is different from the level sets of images, which are sometimes used for image enhancement. The sole purpose of the level set function is to provide an implicit representation of the evolving curve. Level set

function take positive and negative values

outside and inside the contour C. the energy functional e defined by:

$$e(\phi, f_{1}(x), f_{2}(x)) = \sum_{i=1}^{2} \lambda_{i} \underbrace{\int k_{\sigma}(x-y) |I(y) - f_{i}(x)|^{2} N_{i}(\phi(y)) dy}_{M}$$
(8)

Where
$$N_1$$
 () = H () and N_2 () = 1 – H (),

k is a Gaussian kernel. The f1(x) and f2(x) are two values that approximate image intensities in outside and inside of C, respectively. I is input image. Energy functional for contour C that converts to level set formulation written by

$$(\phi, f_1, f_2) = \sum_{i=1}^{2} \lambda_i \int M \, dx + v \int |\nabla H(\phi(x))| dx$$

(9)

where the last term | H ((x)) |dx computes

the length of the zero level contour of **that can** be

equivalently expressed as the integral $() \mid dx$

with the Dirac delta function $\ ,$ which has often been used in variation level set methods. In practice, the

Heaviside function H in the above energy functional

is approximated by a smooth function H defined by

$$H_{\epsilon}(x) = \frac{1}{2} \left[1 + \frac{2}{\pi} \arctan(\frac{x}{\epsilon}) \right]$$
(10)

The derivative of H is

$$\delta_{\epsilon}(x) = H'_{\epsilon}(x) = \frac{1}{\pi} \frac{\epsilon}{\epsilon^{2} + x^{2}}$$
(11)

By replacing H in (9) with H_{ϵ} , the energy

functional e in (8) is then approximated by

$$\begin{aligned} (\phi, f_1, f_2)_{\epsilon} &= \sum_{i=1}^2 \lambda_i \int k_{\sigma}(x - y) |I(y) - f_i(x)|^2 N_i^{\epsilon}(\phi(y)) dy + \nu \int |\nabla H_{\epsilon}(\phi(x))| dx \end{aligned}$$
(12)

As proposed in [14], we introduce a level set regularization term that is necessary for accurate computation and stable level set evolution.

$$\rho(\phi) = \int \frac{1}{2} (|\nabla \phi(x)| - 1)^2 dx$$
 (13)

Which characterize the deviation of the function

from a signed distance function. Therefore, we

propose to minimize the energy functional

$$\mathcal{F}(\phi, f_1, f_2) = (\phi, f_1, f_2)_{\epsilon} + \mu \rho(\phi)$$

Where μ is a positive constant. To minimize this energy functional, its gradient flow is used as the level set evolution equation in this method.by keeping f_1 and f_2 fixed, the energy functional

(, f1, f2) with respect to using the standard

gradient descent method by solving the gradient flow equation as follows:

$$\frac{\partial \phi}{\partial t} = -\delta(\phi)(\lambda_1 v_1 - \lambda_2 v_2) + v \delta_e(\phi) div \left(\frac{\nabla \phi}{|\nabla \phi|}\right) + \mu(\nabla^2 \phi - div \left(\frac{\nabla \phi}{|\nabla \phi|}\right))$$

Where n_1 and n_2 are the functions

$$r_i(x) = \int k_\sigma(y-x) |I(x) - f_i(y)|^2 dy, \quad i = 1,2$$

The term $-\delta(\phi)(\lambda_1 r_1 - \lambda_2 r_2)$ is derived from the data fitting energy, and, therefore, is referred to as the data fitting term. This term plays a key role in this model, since it is responsible for driving the active contour toward object boundaries. The second term

() div (/| |) has a length shortening or

smoothing effect on the zero level contours, which is necessary to maintain the regularity of the contour. This term is called the arc length term. The third

termµ (
$$\nabla^2 \phi$$
 – div (/| |)) is called a level set

regularization term, since it serves to maintain the regularity of the level set function.

Image test set

A set of four 3D images is selected to serve as the representative test set as shown in Figure 2 cells is selected from different cancers. In 2007, the American Cancer Society estimated that 559,650 people will die in the United States of cancer in that year alone. This amounts to more than 1,500 people per day [20]. On a positive note, studies show that the five year relative survival rate for all cancers diagnosed has increased to 66% (1996-2000) from 51% (1975-1977). This increase in survival rates is attributed in part to the progress made in detecting certain cancers at an earlier stage .for early detection it is necessary that cells are segmented .Each test image was one of several similar images or image slices in our database and the experiments were comprehensive within The database .The typical

di culties of image processing presented in the test

set include : blur or weak edge , strong edge near the missing edge, profile contour in overlapping objects, complex contour shape with accentuated protrusions and concavities, in homogeneous interior intensity distribution. These are some of the typical challenges that would fail any simple segmentation schemes.









GVF snake has been proposed to deal with the traditional snake's problems of short capture range and inability to track at boundary concavity. But GVF cannot capture object contours in some complicated images. In order to compare the segmentation performance between level set and GVF snake, we run matlab code and show results in this section. Usually, the GVF snake method only works when the boundary is clear and complete because of the boundary detector term and the initial contour that is located close to object, this problem is shown in figure3, tumor is not segmented when initial contour is far away from object. GVF model cannot work in complicated images too (see figure4 and figure5). In figure4 is shown initial contour and segmented cell.



(a)

(b)





Figure 2. (a) Breast cancer cell. (b) Pancreatic cancer. (c) Cancer cell. (d) Breast cancer cell SME (e) breast cancer tumor

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(e)

Figure 3. (a) Far away initial contour (b) segmented tumor (c) closed initial contour (d) segmented tumor



Figure4. (a) Initial contour (b) segmented breast cancer's cell



Figure 5 .segmented cells with GVF snake

Level set method has been tested with images too. Unless otherwise specified, we use the following parameters in this paper: =3, 1=1, 2=2, time step t=0.1, $\mu=1$, and =0.02*255*255. We use relatively small scale parameter for the experiments in this section. In general, method with a smaller scale can produce more accurate location of the object boundaries, while it is more independent of the location of the initial contour when a larger is used .figure 6 shows the segmentation results by level set method.





(a) Breast cancer cell



(b) Pancreatic cancer



(e) Breast cancer tumor



(c) Cancer cell



(d) Breast cancer cell, SME,

Figure6. Segmented cells with level set method

Conclusion:

GVF is used in image segmentation, but when the image does not have clear boundary or divided to multipart, GVF cannot segment the image, whenever, Level set is a method that can change the topology and segment complicated image. In this paper we demonstrated superior performance of level set in image segmentation compared with GVF.

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Determinants of urban Land Price in Freetown, Sierra Leone

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Abstract: Internal migration has over the last two decades increased demand for residential land in Freetown. Unfortunately, access to land via the government functionary has not kept pace with demands. The majority of the population therefore depends on the unofficial land market. This study addressed two objectives. The first attempted to identify the most important variables that determine urban land price. The second compared the east, and west sections of the city, in terms of which factors are significant in determining land price in each area. Taking eight settlements, four on either side of the city centre, 160 residents were interviewed for objective one. A model was developed using 10 variables, and a regression equation, based on land maximization theory was ran. The obtained parameters $r^2=0.81$, P=0.000, =0.05, indicated a strong overall positive correlation between the dependent and explanatory variables, and the regression model highly significant. Positive correlation coefficients were shown by income, population, infrastructure, social status, environmental concerns and neighbourhood. These indeed explained the factors that influenced residential land use in Freetown, especially in the western half of the city. For objective 2, a total of 40 land traders and 40 land buyers were interviewed, and the results presented in percentages. These showed that the two sides agreed only on income, and population as significant in determining land price. We concluded that this had to do with class division, as the west houses the three arms of government, diplomats, and weathy, as against the middle and low class in the east.

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1. Introduction

Developing countries in the last three decades have recorded enormous transformations in their social, economic and demographic status. Increase in population, and urbanization have particularly been singled out. The attraction by the cities of the population places sustained pressure on urban services and facilities such as hospitals, educational institutions, housing, transport, telecommunication systems and energy supply (Sivam, 2002). This is because the growth of the urban population out paces services provided, thus creating a wide margin between demand and supply of urban infrastructural facilities and services (Olayiwola et al, 2005).

The demand for land has particularly been of an increase in cities all over the world, as world population swells. With this, has been singled out the demand for residential land use. Residential use is the largest consumer of land in urban areas. All things being equal, population increase goes with a proportionate increase in demand for urban land for residential purposes. Access to residential land has been the concern of researchers, in terms of the socioeconomic and environmental implications.

In urban areas, access to land is through the land market. A land market is defined as, "a framework through which seekers of land for various uses can acquire it for its development into the required uses" (Dowall, 1991; cited in Owei, 2007). Like most economic transactions, economic forces of demand and supply are expected to determine price of urban land. In general, the relative scarcity of land allocated to different land uses influence the price greatly. If urban land supply is very responsive to demand, land prices will tend to reflect the productive value of land. On the other hand, if urban land markets are constrained and cannot effectively respond to demand pressure, land prices will tend to be much higher, exceeding their productive value.

However, Bryant (1972) has shown that the laws of supply and demand are not adequately observed in

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land markets. There are a number of reasons for this. In the first place there is an inherent monopoly in terms of supply. What this means is that land is different from goods, services and other commodities because its supply is relatively fixed. In addition, the heterogeneity of land plots in terms of location and quality makes the urban land market imperfect in nature (Zhu, 2002). Land price is generally nonstationary when its temporal dimension is considered (Hannonen, 2008).

David Ricardo and Johann Heinrich von Thunen's 19th century literatures provided the first, though opposing theories on land use and land valuation, and on which the present-day theories of land use and land value are based. Whilst Ricardo's economic theory was based on relative productivity of agricultural land, von Thunen's geographical theory considered the locational attributes of land in terms of its values and use.

Using soil fertility as a dependent variable, Ricardo pointed out that the most fertile lands on an agricultural landscape are the first to use during cultivation, followed by the less favored land, which is put to use only when the demand for agricultural products increases. The rent on the most productive land is a function of its advantage over the least productive, with competing farmers insuring that the full advantage goes to the landlords in the form of rent (Alonso, 1964). To Ricardo, land value depends on absolute and relative productivity of land. A notable shortcoming of Ricardian land rent theory is that it does not account for location. This was addressed by von Thunen, who focused on absolute and relative spatial locations of human activities on a landscape. To him, the economic forces of demand and supply determine the value of land. That is, the decision on establishing a given value (price) for land is made when land owners and buyers interact. The goal of the landowner is to maximize returns, whilst the buyer pays the price that could give good returns on the land. Land users bid against one another for the right to use land. His market model for land value is mathematically expressed as:-:

Land rent = (total revenue) — (total cost) — (total transportation cost) (1)

where, total revenue is money received by the land user from the sale of product at the central market; total cost is all agricultural inputs as well as *opportunity cost* of the land user; and transportation cost is total expenditure of getting goods to market.

Following Ricardo and von Thunen, Weber (1909) analyzed the distribution of Industrial

locations around a central city, taking into consideration the volume of materials to be shipped, the distance that the goods had to be shipped, and the unit distance cost of shipping; consequently. In essence, industries become located in particular concentric zones a round a central city. Burgess (1925) did a similar model by analyzing the distribution of urban land uses in Chicago using a concentric zone model of both industrial and residential uses around a city centre. This was followed by Christaller's (1933) Central Place Theory on a Germany city. His theory focused on the number, size and location of human settlements in an urban system. To Christaller, each settlement functions as a central place that provides services to neighbouring areas.

Thence, several scholars (Muth, 1969; Alonso, 1964; Barlowe, 1978; Mills, 1981; Baross and Linden, 1990) determined the optimal pattern of land prices in zones, located at different distances from the center of a city. They proposed distance decay models for assessing the impact of distance on the land values. They have argued that under efficient market conditions, land values tend to go down slowly but systematically with increase in distance from city core [otherwise known as the Central Business District (CBD)] and suburbs centre. Hurd (1903) had earlier built upon Ricardo's idea to present the case of a city or urban land. His theory is summarized as:-

"Since value depends on economic rent, and rent on location, and location on convenience, and convenience on nearness, we may eliminate the intermediate steps and say that value depends on nearness".

Such nearness could be nearness to city center, social amenities, market areas, sea port or other such factors that buyers and sellers consider important in influencing land prices on the urban land market. It should be hastened to say here that the theories and models described above are more applicable in the industrial north, and not necessarily to developing countries in determining the value of land situated at varying distances around the city.

Urban land markets play a critical role in shaping urban development outcomes. They determine the location, density, form and price of residential, commercial and industrial development (Serra et al 2004). In general, the demand for urban land is influenced significantly, but not limited to population growth, income, and level of economic activity. The supply on the other hand is determined by locationspecific characteristics of the land itself, such as topography and physical conditions; amenities such as availability of infrastructure: roads, water, electricity; patterns of land ownership and government policy.

In Sierra Leone, like, most African countries, the concept of land markets has not received sufficient attention in the analysis of processes of access to land. Whilst there is no denial that it does exist, it operates outside the realm of official cycles. Access to land is either through the official government channel, or the unofficial land market. In the official government scheme, land is leased to individuals (upon application), or granted, but not sold. Owners of such lands are not permitted to trade or exchange it in cash or kind. In the unofficial system, access to land is by way of a number of channels including purchase (Kironde, 2000). Baross and Van der Linden (1990) (cited in Sivam, 2002) observed that the informal market is growing and becoming more diverse and increasingly commercialized in Africa countries, This is because the official means of access to land has not been able to adequately address the demands for land. What's more, it has also been proved to be corrupt and bureaucratic.

The dramatic increase in population during and after the war has exacerbated the scarcity of land further. And expectantly, this has raised the price of land in the city. In addition, there is now marked difference in land price between the two halves of the city divide. Land price/value is higher in the west of the city than in the east for the same parcel size. As mentioned earlier, the land market in Sierra Leone is unofficial and this may have led to the shortage of literature on its study. This paper is therefore a ground breaking study. It addresses two main objectives i) to analyze the factors that determine land price in Freetown ; and ii) to analyze the factors which buyers consider important in paying for land in the east and/or the west of Freetown. The results would provide information to town planners, social service providers, and other policy makers in identifying areas attracted to different category of people in the city. It would help city planners identify trends in urban land use across the city, and make valuable adjustment to existing town planning maps such as housing structures, and access roads. It would also help in the location and distribution of social services and facilities, commercial and business houses across the city. Areas prone to environmental disasters could be identified. And finally, it would also serve as reference for further research.

Modeling urban land

In addition to the models mentioned above, several empirical and theoretical models (example,

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see, Briassoulis, ;Lambin and Geist, 2006; Koomen et al, 2007) have been produced on urban and rural lands, and their uses. In urban studies however, Alonso's (1964) urban land market theory (borrowing from von Thunen's analysis) and model is regarded as providing the platform on which a series of urban econometric models have developed to explain various land study issues. These models carry common characteristics: the description and explanation of urban spatial structures based on land rent and transportation costs, and the assumption of utility maximizing individuals.

Land rent maximization is a theoretical basis of econometric models in land use studies (Barlowe, 1978; Alig et al, 2004). In Barlowe's (1978) view, land rent is total revenue or residential economic surplus realized on land, less the total cost. From the perspectives of Ricardo and von Thunen, the competing uses to which land is put is determined by the value or rent of that land. This theoretical approach forms the thrust of this paper.

In recent times, hedonic methods and models are among several popular econometric approaches in land studies. Examples include the Lancastrian analysis (Lancaster, 1991); multidimensional scaling (Cooper, 1983); and random utility models. Marko (2008) for example compared four different hedonic approaches (ordinary least squares estimation, robust MM-estimation, structural time series estimation and robust local regression) in a model to predict urban land prices in a local market of Espoo, Finland. He found out that four independent variables: permitted building volume, house price index, northing and easting had more influence on land price in the study area, than parcel size variable and different indicator variables, which had weaker influence.

Earlier, Shimizu and Nishimura (2007) estimated commercial and residential land prices in Tokyo for a 25-year period (from 1975 to 1999), and investigated possible structural changes in the price equations, using ordinary least squares hedonic price equations,. Their result showed significant differences in price in different locations, which was owed to supplier pricing and end-user preferences. In addition, they identified significant structural changes in the underlying price structure, identifying pre-bubble, bubble and post-bubble periods.

Another approach by Colwell and Munneke (2003) used piecewise parabolic regression to examine urban land prices within a nonparametric framework, with specific interest in the land price gradient with respect to distance from the inner city.

The concluded that the approach can indeed be used to examine very complex land value evaluations.

Clapp et al. (2001) using the ordinary least squares method estimated a hedonic price index equation to determine the value of residential structures in Fairfax county, Virginia, yearly from 1975 to 1992. Three simultaneous equations were used to explain changes in population density and percentage of workers at home. One of their findings was that land value changed dramatically over time.

The principle behind the Hedonic method considers a variable as a bundle of characteristics. Shown mathematically, the hedonic function describes the relationship between price of a product (P) and its attribute vector (x):

P = f(x)(1)These implicit characteristic prices can be seen as parameters that relate the dependent variable (P)and the independent variables of the hedonic model (*x*). This approach is used in this study. This research used the price of urban land as dependent variable, and locational attributes of land, such as amenities, and socio-economic characteristics of respondents as independent variables. This dependent variable-land price is ran against independent variables in regression analysis based on land rent maximization theory to obtain results for objective1. Regression analysis derives a statistical equation for making quantitative predictions of one variable from the values of other variables. For Objective 2, descriptive statistics (in the form of percentages) was used to compare the most important factors residents in the west and the east consider important in buying land for residential purpose.

2. Materials and methods

2.1 Study area

Freetown is the capital city of the West African state of Sierra Leone. It is part of the western area of the country (Figure 1). Sandwiched between the Atlantic Ocean on the North West, west and south west and the Freetown peninsula mountains on the north, east, and south east, this city was founded by the British for freed slaves in the 18th century. The land referred to as "crown land", was bought from local chiefs and made available to freed slaves from Nova Scotia and the Americas. The whole of the western area, including Freetown was subjected to Acts directly, based on the principle of "eminent domain" through ordinances of Crown Lands, Public lands and Unoccupied Lands (National Lands Policy, 2005). Access to land by the public is made possible as a result of several ordinances: Ordinance 1 of 1872; Ordinance cap 117 of 1906; Ordinance 8 of 1912; No.19 ordinance of 1960 among others. For a thorough explanation of these ordinances, the reader is referred to Turay, 2006; Unruh and Turay, 2004; and Asiama, 2003. But it is important to note that freehold tenure is allowed under these ordinances. Transferability of land is allowed, either through purchase (private land) or lease, with the possibility of becoming permanent (from government).

As a result of migration from the rest of the country, Freetown has grown into the most populated city in the country. During and after the civil war (1991-2001), its population swelled to between eight to nine times its original size of two hundred and fifty thousand (SSL, 2004). Pressure on the land has been mounting as urbanization takes its toll. Access to land through official means has unfortunately not alleviated the land problem in the city. The price of land along the urban-rural gradient has not only deluded the conventional models of urban land use, but is non-linear. A number of factors have been identified to be responsible. This is the main thrust of this paper. It is therefore a good laboratory to study urban land market scenarios.

2.2 Method

2.2.1 Data source

Data collected were both primary and secondary. Primary data collected were through questionnaires administered to landlords/house owners, and land dealers. Eight zones (referred to here as settlements) situated at varying distances from the reference point were identified in the city as the study population (Fig.1). Four of these (Kissy, Calaba Town, Hastings and Grafton) are found on the east of Paterson Zochonis (PZ), the point considered as the city centre or Central Business District, and the other four (Hill Station, Regent, Wilberforce and Goderich) run westwards. The selection of four settlements on both sides of the city centre is borne out of the fact that social services and/or infrastructure development vary and unevenly distributed on either side, and we believe these differences could affect urban land prices greatly.

For each settlement, a random selection of 20 households was made, giving a total of 160 respondents. Random selection was the chosen statistical option because the unplanned nature of some of these settlements makes it difficult to use any other sampling method. The 160 respondents formed the study population for objective one.

To obtain the study population for the second objective, the snowball sampling technique was used to select a total of 20 land sellers (on each side of the city centre) involved in land sale, and 20 land buyers

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who were in the process of building residential structures. This method was used because it was difficult to identify land traders. Once one was identified, we were led to another land trader, until the total of 40 respondents (20 land traders, and 20 land buyers) was obtained in the east and also in the west. The questionnaire prepared sought information from respondents on the most significant factors they believe determine land price in Freetown.

Fig.1: Map of Sierra Leone showing the Western area and study Area



2.2.2 The model

This model was developed for the first objective. Like other land use models cited earlier in this work, we made a number of assumptions in this study. These include:

- 1. the city is monocentric;
- 2. land market operates as a free market;

3. all residents purchase land (away from the city centre) for residential use only;

4. all residents have work places in the city centre;

5. income is not the same for all residents;

6. residential lands are of the same size per lot;

7. agricultural lands at the urban fringe could be converted to residential use.

Given these assumptions, and following a preliminary testing of the questionnaire, we were able to develop a general model/equation (for the total sampling population); factoring those variables which we believe explain the land price in the study area. A total of 18 variables were tested, but 10 proved to be most important and best for the model. Race n ethnicity for example affect land value but it is not included in the model because land areas are not demarcated by government but private land owners sell their land to those that pay the asking price. Bank loan as variable is also not included because the link between loan and purchase of land in Sierra Leone has not come onto the forefront of existing literature. As a matter of fact, such loans exist where real estates are developed, a situation that has just been introduced in Sierra Leone. Transportation is not included but is subsumed in distance because it is often connotated in Sierra Leone in not only physical and monetary terms but convenience. People tend to consider the difficulty involved in getting away from their localities (as determined by distance) to other parts of the city than the means involved. Also, such a situation is applicable only to those without personal vehicles; in which case distance is more applicable because it affects all. In fact multicollinearity exists between these two The influence of stock market on land price, as observed in industrial countries is not included in this model because the stock market is yet in its rudimentary state in Sierra Leone.

The regression equation of the model is:-Urban land price (ULP) (in Leone) =

+ $_{7}$ (population) + $_{8}$ (distance) + $_{9}$ (neighborhood) + $_{10}$ (income) (2)

where ULP is dependent variable; 0 an intercept term; and $_{1}$ through $_{10}$ are independent variables.

Though it is simplistic in nature, this methodology captures the main variables that play in the hands of buyers and sellers of land outside of governmental cycles. This model has policy as well as environmental considerations. The dependent variable is price of urban land in Leone (\$1=3800Le as at 29th.March, 2010), the official currency of Sierra Leone. The model seeks to identify the factors that determine the land price per "town lot" (16.40 metres by 24.60 metres) across the city: from the east, across the city, passing through the centre, to the west.

The inclusion of social status as an independent variable owes to the fact that people are often conscious of their social status during economic transactions. They pay amounts for parcels of land as long as the location befit their social standings. For infrastructure, literature has it that people cloud around areas that have good infrastructural development, viz a viz, good houses in the neighborhood, and the presence of amenities. Roback (1982) analyzed the effects of amenities on land rent and showed it to be significant. On environmental concerns, areas with poor drainage network, foul smell, noise and prone to environmental disaster are expected to attract a small number of people. For topography, hilly areas attract less residential areas than flat areas, and hence could be sold at reduced price. But is this the case in the west, where vacant lands are mainly on hilly slopes? Perception is one of the variables that has been identified as tool used by land owners to postpone sale of land. The sale of land is mostly done amidst expectations that, conditions (demand and supply) could change at any time. Season is included in the model because it was discovered that the price of land in the study areas varies with the two main seasons in the country: the dry and the wet seasons. Population density and population growth are represented by population. Population density is perceived in this study as number of people per family. It is assumed here that households consider their number when undertaking economic activities. Maisel (1964) and Witte (1975)^a analyzed population density and population growth in their empirical work on interurban land price variation. As a result of the 11-year civil war, the population of Freetown swelled to more than six

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times its original size. This scenario has been discovered to push the demands for residential land. The inclusion of population as a variable in the model is therefore understandable. Income is in the model because it is a very significant variable in economic transactions, not least the purchase of land in a specific location (Witte, 1975)^b. Households with similar incomes tend to agglomerate, and in this case, and this model would establish whether income does indeed play a significant role in land value determination. Distance is included because all the settlements in the study area are situated at varying distances from the city centre, and if land prices are to vary, it is important to know how significant it is in the equation. The influence of neighborhood also plays a role in attracting residents to a point. We want to know whether land buyers consider persons with the same or similar status in their purchase of residential land in certain places. How significant it is in our model, is the reason for its inclusion. For seasons, Sierra Leone has two distinct seasons: wet and dry seasons. It is generally believed that land owners become desperate to sell land during the rainy season when many people need money to run their homes. At the same time, land buyers become reluctant to buy land because there are no immediate plans to start housing construction. The inclusion of this variable would therefore explain an interesting situation for the land market.

For Objective 2, responses from respondents (in the east and west of the city) were computer using percentages. The rationale is to see how geographical location on both sides of the city affect urban land price. These are presented in Tables 2.

3.0 Results

3.1 Regression analysis based on land rent maximization theory

Results of the regression models are presented in Eq. 3.

```
Urban land price = 4367.32
+21.274(social status)
+ 16.665(infrastructure)
+ 2.387(environmental concerns)
- 11.765(topography)
-25.176 (expectations)
- 4.398 (seasons)
+ 18.246(population)
- 4.197(distance)
+27.529 (neighborhood)
+32.156 (income) (3)
```

As expected, nearly all of the explanatory variables are positively correlated with urban land

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price. The obtained parameters-r²=0.81, P=0.000, =0.05, indicated a strong overall positive correlation between the dependent and explanatory variables, and the regression model highly significant. The positive coefficient shown by income, suggests that income plays a significant role in determining the price of urban land. The positive show of neighborhood confirms the assertion that neighbor effect has a determining factor on the residential location of individuals in specific places. The positive coefficient returned for population also confirms that population increase, which most times means increased demands pushes buyers and sellers to enter into economic transactions. Infrastructure, environmental concerns, and social status, as expected, all showed strong positively correlation with the price of urban land because they all carry strong locational attributes that influence the decision of buyers and sellers of residential land. Unfortunately, distance, and topography were expected to return positive correlations. However, together with seasons and expectations, they all showed negative correlations.

3.2 Differences in response to land price from residents in east and west of Freetown

Table 2 presents result of responds from those interviewed in the east and west of Freetown on the factors they consider important in determining the price of land in Freetown. These are also represented by histogram (Fig. 2).

End to the determinants of urban land price	Tabl	2: Percentage response	e of East	t End	and	Wes	t
1	End	o the determinants of url	oan land	price			

	%	%
Variable	East	West
Distance	75	10
Environmental Concerns	45	90
	30	60
Perception/Expectation		
Infrastructures	30	75
Neighborhood	20	90
Population	62.5	90
Season	80	30
Social Status	30	70
Topography	80	10
Income	100	90

Source: Author, 2010

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Fig.2. Percent Response- east and west

Although results showed that more than half of respondents on either side of the city identify income and population as important determinants of land price in Freetown, there was significant disparity however in the remaining eight variables. For example, whilst topography of the land was important for people in the east, it was not the case for those in the west. This could be attributed to the fact that people in the east are considered to be generally not as rich as those in the west. Because of this, lands in the east that are steep are sold for quite reduced prices compared with similar land in the west. In addition, the west of the city is generally a hilly area, unlike in the east where the topography is a bit gentle.

4.0 Discussion4.1 The model equation

The positive correlation coefficients shown for population, infrastructure, neighborhood, income, environmental concerns, and social status do not only agree with much of existing literature, but present the status quo in the study area. Population growth in recent years increased the demand for land for residential use in Freetown. The aftermath of the civil war saw a surge in population in the capital as due to internal migration. With infrastructure and/or services in most parts of the country destroyed or absent, many citizens made their stay permanent in the capital city. Unfortunately, land allocated by the central government for residential use has not met demands. This increase in demand for residential land, pushed prices (of parcels of land) up. Buyers are sellers of land enter into frequent land transactions as a result of population factor. As observed by Kironde (2000); and Sivam (2002), in their studies in Tanzania and India respectively, the people turn to either the unofficial land market for purchase, or in the case of the poor, construct illegal

and indiscriminate structures on sometimes "badlyshaped" lands and hills slopes. A good number of residential areas on hill slopes and valleys in the east end of the city belong to the low-class populace. These unplanned settlements are constructed outside the legal cycles, such as construction work without permit, and not taking into consideration any environmental effects. In fact some of these lands are government owned and have not been leased to citizens. In recent years demolition exercises have been on-going. That has however not stopped "land grabbing" in the city.

The positive coefficient showed by income agrees with earlier works (Cruz, 2001; Olaviwola1 et al; 2005; Owei, 2007) that the value of land increases with individual income. With increase in an individual's earning, the need to have a decent housing arises. In Freetown, much of residential development has taken place mostly in the west end of the city. As cited above, the "high class" populace is found in that part of the city. The cost of a "town lot" in the west goes for two to three times the cost in the east. High quality houses are therefore seen in these neighbourhoods, as against those in the east, where, the built-up area is characterized by dilapidated structures, congestion, environmental related problems and poor urban image, shortage of and low quality infrastructure, basic services and inefficiencies in land utilization

Social status and neighborhood also showed positive correlation coefficients in the model. The political elite, diplomats, foreign nationals, the wealthy and/or educate are mostly found in the west of the city. They consider their social status, in addition to security, amenities, environmental concerns, and neighborhood in locating their residents. This is in agreement with Haurin et al, (2003 in Grimes and Liang, 2007), who indicated that neighborhood effect allow people to bid more highly for land located near wealthier and/or higher status individuals, than go for cheaper ones amongst the poor. In the west of Freetown are found the official residents of the Exective, Legislative and Judiciary Arms of Government, including those of The President and Ministers; top civil servants, law enforcement personnel and judges, lawyers; bank governors, chancellors of the universities, members of the diplomatic and Consular corps and their Missions; the British International Military Training unit; two military barracks of The Arm Forces Of Sierra Leone; private hospitals; top educational institutions; Five Star Hotels and the beautiful beaches in their vicinity, and quiet neighborhoods. Thus in addition to income, social status, neighborhood effects and infrastructure have been

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contributed significantly to the model in determining the price of land in Freetown. Such a point was also made by Sazak (2004) on the role of land owners in the transformation of land affected by the metropolitan city. Among other things, he stated that rather than singling out one variable such as distance in determining the value of land in a location, other site characteristics should also be considered.

The negative correlations shown by topography, and distance in the model, point to the fact that vacant lands are now available only outside of the city, on sometimes relatively high grounds. This means buyers make do with what is available, instead of going without. Similarly, seasons and expectations/perceptions are not important because people need land throughout the year, and also do not consider future price rises or demands probably because of the absence of real estate markets in the country.

4.2 Comparing east and west

In Freetown, distance has not played a major role in residential location of respondents in the west (10 percent) of the city, but other social factors (Table 2) are much more important to them. This though contradicts the classical models of land rent discussed earlier. Residents in the west are ready to pay" high rent" for parcels of land away from the city centre, irrespective of the distance. This happens because the actors (respondents) consider a multiple of factors order than distance. For example, Regent in the west end of the city is outside the Freetown municipality, but as a result of its proximity to The British International Military Training Team (IMATT), The American Embassy, a military barracks, dignitaries, including Presidents, past and present, this rural area, far from the city centre is attracting more residents than such areas as Calaba Town and Kissy in the east end that are within the municipality. Again distance is not seen to be of significance. Goderich in the west also benefits from its strategic location. Both Goderich and Regent offer good examples of the extent to which the city is sprawling. Many residents now prefer to move away from the city centre into low-density development suburban areas and urban fringes. The Tiebout-based "flight from blight" hypothesis argues that perceived urban ills (e.g., higher crime rates and lower school quality) push households to live in lower density, suburban communities that offer a preferred bundle of public goods and services. The evidence of high crime rate and other social ills are greater in the east end than in the west. It also reveals that, while commercial and industrial developments have become more clustered over time, the pattern of

residential development has become increasingly fragmented and dispersed. This urban spillover is feasibly seen in areas such as Hill Station, Goderich, Grafton and Hastings.

Although topography of the land shows an overall positive relationship with the dependent variable, it is not significant in the west (10 percent) as in the east (80 percent).The explanation for this could be that because the west end is perceived by many as the "best" place to live, many wouldn't mind building on hill slopes. But in the east, land prices inevitable fall with gradient.

Environmental concerns also showed a high percentage in the west (90 percent). The east end of the city is plagued with environmental problems. This is attributed to poverty, overcrowding, poor drainage, illiteracy, poor sanitation and infrastructure, lack of good roads and poor planning, and generally poor quality of life. The west on the other hand has good road network, tap borne water, drainage, and sanitation. Security against criminals and arm robbers is also better in the west than in the east. And as Sivam (2002) noted, adequate housing means privacy, useful per capita space, physical accessibility, physical security, structural stability and durability, and adequate basic physical and social infrastructure and transportation within healthy local and citywide environments. Unlike the east end of the city, the west end carries most of these features. Noise and other forms of pollution are very evident in the east of the city.

6. Conclusion

The price of land in Freetown is not determined by the Laws approved in parliaments, but by a number of factors. These range from the perception of the populace, to population size, income, infrastructure and service, neighborhood, social status, environmental considerations and a host of others that could be site specific. And as seen in this work, the distance decay models of earlier scholars have not provided strong evidence that distance determines the price of land in Freetown. In addition, this work has also added to existing body of knowledge that there is a difference in land price between the west and east ends of the city. This has got a lot to do with class division. Most of the residents in the west belong to the elite and decision making class, although some in the east also belong to this influential and high class group.

It could be concluded that residents in the west of Freetown consider many social factors (infrastructure, security, neighborhood, income) in locating residential areas than those in the east. Telling by their social status, the "elite" or "high

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class" people have a number of considerations in locating their residents than "low class".

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Determination of the Appropriate Time of Relaying Cassava into Pepper in Intercropping System in Nigeria

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ABSTRACT: Relay intercropping of cassava (*Manihot esculenta* Crantz) into pepper (*Capsicum annum* L.) is a common practice among farmers in Nigeria. However, there is high variation in the time of the relay thus leading to variability in yields of cassava and pepper. Field experiments were conducted to determine the appropriate time to introduce cassava into pepper in a relay intercrop. The experiment was a randomized complete block design replicated three times. Five different planting dates of relaying cassava into pepper were evaluated . Pepper and two cassava varieties were the test crops. Sole pepper was included for comparison. Delayed relay planting of cassava into pepper beyond 1 MAT (Months after transplanting) adversely affected the yield of both cassava varieties while simultaneous planting of pepper and cassava significantly (P 0.05) delayed flowering and maturity of pepper with a resultant effect of significant (P 0.05) reduction in fruit yield of pepper. The most appropriate time to introduce cassava into pepper in a relay intercrop was 1 MAT.

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Key Words: Capsicum annuum, cropping pattern, Manihot esculenta, relay intercrop, sole crop

INTRODUCTION

Pepper (*Capsicum annuum L.*) is the second most important crop after tomato (*Lycopersicon esculentum*) among the family *Solanaceae*. Pepper is usually grown in various combinations with other food crops like maize (*Zea mais*), yam (*Dioscorea spp.*) and cassava (*Manihot esculenta* Crantz) in traditional cropping systems.

Cassava is the main staple food for 40 % of the population in West and Central Africa (FAO 1998).The plant is highly adapted to various edaphoclimatic conditions and serves as a major source of starch to the people in tropical Africa. It is an important crop commonly grown in mixtures with other crops on the field by farmers in Nigeria.

Few studies reported on the performance of pepper in intercropping. Manu-Aduening and Boaamposem (1998) reported that maize and pepper components showed differences in plant height, and the extent of leaf conductance was higher than in sole crops in three spatial arrangements of yam-maizepepper intercrop. In Nigeria, some subsistence farmers plant pepper as sole crop, while others relay cassava into pepper when the harvesting of pepper fruits is nearing completion. The delay in the introduction of cassava usually leads to its poor establishment and exposure to attack by pests and diseases at the onset of the dry season. The compatibility and the appropriate time to interplant these two crops are yet to receive adequate research attention. This study was designed to determine the appropriate time to introduce cassava into pepper crops.

Materials and Methods

Field experiments were conducted at the Research Station of Oyo State Agricultural Development Programme (OYSADEP) in Ogbomoso during 2001 – 2003 cropping seasons. Ogbomoso lies on latitude 8° 01' N, longitude 4° 06' E, about 310 m above sea level in the derived savannah belt of southwestern Nigeria.

The mean annual rainfall of the experimental station during the period of the experiment was 1,062 mm with high intensity over a period of seven months (April to October). The land used for the experiments had been previously cropped to staple food crops such as cassava, maize, yam and guinea corn. Composite samples of the topsoil (0 - 15 cm depth) were taken from the site and analyzed for their physical and chemical properties (Table 1) before the commencement of the experiment in each year.

The experiment was a randomized complete block design replicated four times. Five different planting dates of relaying cassava into pepper were evaluated. Pepper and two cassava varieties were the test crops. Sole pepper was included for comparison. Each plot measured 5 m x 4 m with 1 m gaps between plots and 2 m margin between blocks to facilitate field operations. Pepper cultivar NHVI-B, released by the National Horticultural Research Institute (NIHORT) was used. Pepper seedlings were raised in nursery beds measuring $1.2 \times 10 \text{ m}^2$. Sowing was done in drills of about 2 cm deep at 10 cm apart. Six-week old seedlings were transplanted into the crest of the ridges and cassava stem cuttings of about 20 - 25 cm length were planted at the side of the ridges. . Two cassava varieties namely TMS30572 (improved) and Oko iyawo (a local variety) were used in the study. The pepper crops were planted in mid June in each year and cassava was relayed into the pepper crops at the five planting dates namely; OMAT (mid-June, simultaneous planted with pepper), 1MAT (mid-July), 2MAT (mid-August), 3MAT (mid-September), and 4MAT (mid-October). The usual planting dates of cassava in the study area ranges between mid May to late July in relation to rainy seasons. Cassava was planted at 1 m x 1 m and pepper at 1 m x 0.5 m spacing. Plant population density was thus 10,000 per hectare for cassava and 20,000 plants per hectare for pepper. Hoe weeding was done each time cassava was introduced into pepper plots with an average of four weedings carried out before total harvesting of both crops.

Ten plants of the cassava and 15 plants of pepper were randomly sampled from the net plot to record observations adopting the partial replacement procedure by Gomez and Gomez (1984). Days to 50 % flowering were recorded. Numbers of days to first time of harvest were recorded from sowing date to the time of picking the first fully ripe fruits of pepper. Ripe fruits of pepper were harvested at weekly intervals. Number of fruits per plant was obtained from randomly selected plants/plot while the cumulative fresh weight of the total fruits picked from the net plot (12 m^2) was recorded. Fruit length was obtained from 30 randomly picked fruits using transparent ruler. Residual harvest of pepper was carried in the following season. Total root yields of cassava from the net plot $(12m^2)$ were recorded at 12 MAP Data were analyzed using ANOVA.

RESULTS

Fresh fruit yield of pepper

Fresh fruit yields of pepper recorded in the treatments where the cassava varieties were planted simultaneously with pepper were significantly (P 0.05) lower than those recorded for other treatments. Sole pepper produced the highest fruit yield of 4.6 t/ha, about 309 % more than the fruit yield of 0.9 t/ha recorded in pepper plots where TMS30572 was introduced at 0 months after transplanting (MAT) in 2001/2002 cropping season. Similar trend was observed in 2002/2003 cropping season (Figs. 1 &2).

Flowering and days to first time of harvest

Pepper plots in which cassava was introduced at 0 MAT required 6 - 7 more days to attain 50 % flowering, as compared with other treatments in both years. Similarly, pepper took an average of 151 days to reach first time of harvest when both cassava varieties were relayed into pepper at 0 MAT this was significantly (P 0.05) higher than the average of 143 days required by other treatments (Table 2). Sole pepper gave the highest number of 27 fruits per plant which was about 375 % higher than number of fruits per plant recorded under TMS30572 relaved into pepper at 0 MAT. However, introduction of both cassava varieties at 1, 2, 3 and 4 MAT had no significant (P > 0.05) effect on fruit length of pepper in both years (Table 3). Residual harvest of pepper fruits recorded in the following season showed significant differences across the treatments with the highest yield recorded where TMS30572 was introduced at 2 MAT in the two successive cropping seasons (Figs. 3 & 4).

Cassava root yield

Fresh root yield of cassava decreased significantly (P < 0.05) with progressive delay in the time of relaying cassava into pepper in both years of the experiment. The highest fresh cassava root weight of 20.30 t/ha was recorded from the treatment with introduction of *Oko Iyawo* at 0 MAT followed by treatment with introduction of *Oko Iyawo* at 1 MAT with a fresh tuber yield of 19.4 t/ha but they were not significantly different (P > 0.05) from each other (Figs. 5 & 6).

DISCUSSION

Introduction of cassava at the late-vegetative or early reproductive stage of pepper did not adversely affect fresh fruit yield of pepper. Conversely, the low fresh fruit yield recorded for pepper when simultaneous planted with cassava may be attributed to competitive interaction between both crops at earlier stages of crop growth. Lowe et al., (1982) also reported that root bulking in cassava was visible at 6 weeks after planting; a stage at which the cassava could had probably started competing with pepper. Intercrop competition, according to Palaniappan (1985), occurs essentially in a response of one species to the environment as modified by the presence of another species. Crop competition could be minimised by spatial arrangement and choosing crops best able to exploit soil nutrients, and with different times of maturity, thereby separating periods of maximum demand of growth resources above and below ground.

Soil Properties	2001	2002
pH (H ₂ 0)	6.70	6.70
Organic Carbon (g kg ⁻¹)	0.39	0.51
Total N g kg ⁻¹	0.040	0.051
Available P (mg kg ⁻¹)	8.05	7.35
Exchangeable Bases		
cmol kg ⁻¹ soil		
Ca	2.33	2.03
Κ	0.13	0.12
Na	0.60	0.61
Exchangeable Acidity	0.14	0.28
C. E. C.	2.84	2.56
Physical Properties		
Sand (g/kg)	781	798
Clay (g/kg)	65	58
Silt (g/kg)	154	144

Table 1. Selected pre-planting physico-chemica	characteristics of soil of the	e experimental sites (0 -	15 cm depth)
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Table 2. Effects of different times of relaying two cassava varieties into pepper on 50% flowering of pepper and first time of harvest

Cropping system Days to 50% flowering Days to matu			Days to maturity	urity		
	2001/2002	2002/2003	2001/2002	2002/2003		
P Sole						
0 MAT	87c	87c	143c	143c		
P + C1						
0 MAT	95a	95a	150a	152a		
P + C1						
1 MAT	88b	89b	143c	144bc		
P + C1						
2MAT	86c	87c	142d	145b		
P + C1						
3 MAT	88b	89b	143c	143c		
P + C1						
4 MAT	88b	88bc	143c	143c		
P + C2						
0 MAT	92a	94a	151a	151a		
P + C2						
1 MAT	88b	88bc	143c	143c		
P + C2						
2 MAT	89b	88bc	142d	142d		
P + C2						
3 MAT	88b	87c	144b	144b		
P + C2						
4 MAT	87c	87c	144b	143c		

Means in the same column followed by the same letters are not significantly different at p<5% according to Duncan's multiple range test.

P = Pepper; C1 = Oko Iyawo; C2 = TMS30572, 0, 1, 2, 3, and 4 are

MAT: Months after transplanting

Cropping system	Fruit length (cm) 2001/2002	2002/2003	No. fruits/Plant 2001/2002	2002/2003
P Sole				
0 MAT	9.9a	9.7a	27a	27a
P + C1				
0 MAT	7.6b	7.4b	7c	6c
P + C1				
1 MAT	9.3a	9.3a	25b	26ab
P + C1				
2MAT	9.7a	9.4a	26ab	26ab
P + C1				
3 MAT	9.3a	9.2a	25b	25b
P + C1				
4 MAT	9.4a	9.3a	27a	26ab
P + C2				
0 MAT	7.5b	7.4b	6с	6c
P + C2				
1 MAT	9.6a	9.2a	25b	26ab
P + C2	- -			
2 MAT	9.5a	9.6a	26ab	25b
P + C2		- -	A F 1	
3 MAT	9.6a	9.7a	25b	26ab
P + C2				
4 MAT	9.5a	9.6a	26ab	27a

Table 3. Effect of different times of relaying two cassava varieties into pepper on number and fruit length of pepper

Means in the same column followed by the same letters are not significantly different at p<5% according to Duncan's multiple range tests.

P = Pepper; C1 = Oko Iyawo; C2 = TMS30572, 0, 1, 2, 3, and 4

MAT: Month After Transplanting



Time of relaying cassava into pepper (MAT)



In this study competition was reduced by introducing cassava into pepper at 1 MAT of pepper which probably did not coincide with period of critical demand for growth resources, especially light, nutrient and water Effect of shading on the growth and yield of pepper had been reported (AVRDC 1997). Okoli et al. (1996) also observed that the longer the delay in introducing cowpea into cassava, the greater was the shading effect of the well established cassava genotype on cowpea.

Mutsaers *et al.* (1993) opined that crop combinations where cassava yield was least affected by competition were the most efficient ones. In this study, the yield decline recorded in cassava planted at later dates was probably due to negative effects of water deficiency in relation to rainy season and shift in planting dates of cassava experienced during vegetative and root development stages. Earlier report by El Sharkaway et al. (1998) indicated that early and mid-season water stresses significantly reduced top and root biomass than late or terminal stress that may occur at the maturity stage in cassava. The findings of this study also agree with that of Agbaje and Akinlosotu (2004) that variation in time of planting, which resulted in yield difference, was due to severe stress suffered by late planted cassava at both the vegetative stage and root initiation. The practical significance of productivity in intercropping could only be fully assessed when related to monetary returns (Willey, 1979). In this study, introducing cassava into pepper at 1 MAT had lowest cost benefit ratio compared to other stages of relaying cassava into pepper at 1 MAT that lowest cost benefit ratio compared to pepper at 1 MAT under the circumstances prevailing at the study site.



Time of releving cases we into nenner (MAT)

Fig. 3 .Residual harvest of pepper in the second season as affected by different times of relaying cassava into pepper (2001/2002) cropping season.

Bars labelled with the same letter are not significantly different by DMRT at 5 % probability level.. C1 = Oko Iyawo; C2 = TMS30572; 0, 1, 2, 3, and 4 are MAT = Months after transplanting



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Time of relaying pepper to pepper (MAT)

9/19/2010

Enhancement the teaching and learning methods of some zoological courses (invertebrate, parasitology and animal physiology) in Taif University, KSA

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Abstract: The main objective of this study is to outline specific manners by which learning and teaching processes can be enhanced in the university campuses for the sake of superior learning capabilities and experiences. This paper describes the advantages of various approaches of improving the teaching of some biological courses (invertebrate, parasitology and animal physiology), including the disciplinary, the problem-oriented and combined approaches. In the disciplinary approach, the previous courses are taught in the classical manner as a coherent subject, covering classification of invertebrate, biology of invertebrate and parasites, molecular biology, pathology and immunology, as well as clinical manifestations, diagnosis, therapy, control and prevention of parasitic diseases. Animal physiological course included the description of different systems in the human body and compared with the other animals. Problem-oriented teaching approaches the subject, starting from diseases in animal species or from organ systems; it also tackles training of skills for problem solving and self-learning. Combined approaches include elements of the disciplinary approach and those of other methods. A list of the developed courses and the way of presentation has been discussed. The course syllabus put in student guide to be given to the student in the beginning of the semester. Improving the practical courses has been done by recording them in video. The strategy in the lectures has been changed with the students to minimize the distance with them. Moreover, three workshops have been hold during improving the courses to enhance the efficacy of the technician in the using the computer and modern equipments. Based on the observations and experiences regarding teaching biological courses (branch of zoology), some suggestions have been made which may be helpful in the development of curriculum of zoology in Taif university, KSA to approach to online biological programs (E-learning) at leading research universities. [Ismail Shalaby, Nahla S. El-Shenawy, Mahi Ghobashy and Amaal Mohammadein. Enhancement the teaching and learning methods of some zoological courses (invertebrate, parasitology and animal physiology) in Taif University,

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Keywords: Zoology; teaching, disciplinary, problem-oriented, combined and Region-specific approaches; E-learning, preparation of syllabus, evaluation of courses.

1. Introduction

During the past few decades, the zoological profession has been exposed to numerous new developments science. agriculture, in animal husbandry, disease control, public health and other relevant fields. Zoological education has to respond to new challenges, particularly in KSA, where E-learning comprises all forms of electronically supported teaching. learning and The Information and communication systems, whether networked or not, serve as specific media to implement the learning process (Tavangarian et al., 2004). Saudi Arabia spent some \$15bn on educational development in 2007, mainly to fund existing and open new universities as part of a priority programme to develop higher education (Zahrani, 2010). The aspects of higher education; Saudi students studying abroad, foreign students studying in Saudi Arabia, female education, the role of the Ministry of Higher Education and the

financing of higher education have been examined (Saleh, 1986; Sohail and Shaikh, 2003).

Quality in education is becoming a requirement and a demand by the market. Employers are becoming more and more selective in their choices of new graduates. Educators are challenged by the strong and growing global economic forces, rapid developments in technology and its impact on products and processes, and conflicting values. Students are more engaged with their learning and perform better if they know what is or will be expected of them (Huba and Freed, 2000).

E-learning is essentially the computer and network-enabled transfer of skills and knowledge. Elearning applications and processes include Web-based learning, computer-based learning, virtual classroom opportunities and digital collaboration. Content is delivered via the internet, intranet/extranet, audio or video tape, satellite TV, and CD-ROM. It can be selfpaced or instructor-led and includes media in the form of text, image, animation, streaming video and audio (Allen and Seaman, 2003). The worldwide E-learning industry is estimated to be worth over \$48 billion US according to conservative estimates (EC, 2000). Developments in internet and multimedia technologies are the basic enabler of E-learning, with consulting, content, technologies, services and support being identified as the five key sectors of the E-learning industry (Nagy, 2005). By 2006, 3.5 million students were participating in on-line learning at institutions of higher education in the United States (Allen and Seaman, 2008). There has been an increase of around 12-14 percent per year on average in enrollments for fully online learning over the five years 2004-2009 in the US post-secondary system, compared with an average of approximately 2 per cent increase per year in enrollments overall. Allen and Seamen (2008) claim that almost a quarter of all students in post-secondary education were taking fully online courses in 2008, and suggests that in 2009, 44 per cent of post-secondary students in the USA were taking some or all of their courses online, and projected that this figure would rise to 81 per cent by 2014. Thus it can be seen that Elearning is moving rapidly from the margins to being a predominant form of post-secondary education, at least in the USA.

Many higher educations, for-profit institutions, now offer on-line classes. By contrast, only about half of private, non-profit schools offer them. Private institutions may become more involved with on-line presentations as the cost of instituting such a system decreases. Properly trained staff must also be hired to work with students on-line. These staff members need to understand the content area, and also be highly trained in the use of the computer and Internet. Online education is rapidly increasing, and online doctoral programs have even developed at leading research universities (Hebert, 2007).

However, it should be noted that many early online courses, such as those developed by Murray Turoff and Starr Roxanne Hiltz in the 1970s and 80s at the New Jersey Institute of Technology (Hiltz, 2005) and the online distance courses at the University of British Columbia (where Web CT, now incorporated into Blackboard Inc. was first developed) (Bates, 2005) have always made heavy use of online discussion between students.

Biology is a fascinating subject to study and can lead on o a variety of rewarding career paths. Biology is the 'science of life' and as such involves the study of areas such as animals, people and plan life. The subject is therefore essential for those who wish o pursue a career in medicine or in veterinary professions. The topics studied within the broad title of biology can include; environmental issues, genetics and zoology etc. Currently, there is a broad agreement that (a) future zoology education should produce competent graduates, who are able to act both professionally and effectively, and flexible enough to respond to changes in the zoology profession (Zahrani, 2010); (b) all students should graduate from their courses with the same general certificate that will be a qualification to practice in all fields of zoological science; and (c) zoological specialization should occur after the primary qualification has been obtained. This means that future undergraduate education in zoology has to provide the basis for obtaining a universal certificate which confers the right to practice, as in the past, but should offer more flexibility and improved options for postgraduate specialization and continuing education.

There are eleven standards that have been identified for self evaluation scales provided in different documents for institutional evaluations and for assessments of the quality of programs (Web 1 and 2):

Mission and Objectives

Governance and Administration

Management of Quality Assurance Processes

Learning and Teaching

Student Administration and Support Services

Learning Resources

Facilities and Equipment

Financial Planning and Management

Faculty and Staff Employment Processes

Research

Institutional Relationships with the Community

Learning and teaching one of the most important points for institutional evaluations and for assessments of the quality of programs. Therefore, the learning, teaching subjects and methods have to be reconsidered and more emphasis has to be laid on flexibility, understanding and solving problems, and on self-learning of students in order to impart skills to cope with new challenges in their professional lives. Keeping in view these challenges, many biological institutions around the world are restructuring their undergraduate teaching programmes, and some have modified the curriculum using or evaluating various teaching approaches, including new methods, such as computer and internet-assisted learning (Maki, 2002).

2. Objectives

In this paper, various approaches used to teach invertebrate, parasitology and animal physiology have been discussed. The second aim of this publication is to stimulate discussions on teaching approaches and methodologies for some zoological courses and to provide information about appropriate educational strategies for teaching zoology. We would like to make most effective use of the internet to improve education about zoology, and help to ensure the accuracy of zoological information on the internet. This information would be helpful to give considerable advice in the complicated process of changing and improving curricula for education in some zoological courses. The developed courses will be the first courses on line in Taif University for E-learning programs in biology department. This can be used as a guideline/model for the development of revised zoological curriculum in KSA. The third aim concern about the change from what has been a traditional emphasis on rote learning and shifting to creative thinking and problem solving, and the development of personal attributes of personal and group responsibility, leadership.

3. Analysis, Discussion and Conclusion

3.1 Teaching Approaches

There are several choices of teaching invertebrate, parasitology and animal physiology which have been discussed in detail earlier. These include the disciplinary approach, problem-oriented approaches focused on diseases of animal species, organ systems or other subjects, and combined approaches thereof.

3.1.1 Disciplinary Approach

In this method, invertebrate, parasitology and animal physiology are conventionally taught as a coherent subject, covering classification of invertebrate, invertebrate and parasite morphology, biology, molecular biology, epidemiology, pathology and immunology, together with clinical manifestations, diagnosis, therapy, prevention of parasitic diseases as well as covering the structure of different system in the human body and compared with the animal system. The same method has been applied in other project concerning the development the zoological courses (Adel Nabi et al., 2007). Using invertebrate and parasite taxonomy as a primary pattern, the disciplinary approach provides an effective and easy access for the students to gain an overview on invertebrate, parasitology and parasitic diseases and, most importantly, to understand the complex networks of epidemiological key factors, the parasite-host relationships and their clinical significance. In this regard, it has to be emphasized that many of the invertebrate and parasitic infections involve several host species and several organ systems or in most cases the whole host organism. Experience gained during the last two decades has shown that the disciplinary approach can provide an adequate basis of knowledge and skills of invertebrate and parasitology for professional life if it is well structured, concise, focused to relevant parasitic diseases and well balanced regarding the depth and breadth of knowledge. This approach has certain disadvantages like insufficient integration between invertebrate, parasitology and other disciplines, overlapping between disciplines, lack of training of the students for self-directed learning and for applying their knowledge to practical problems.

3.1.2 Problem-Oriented Approaches

The problem-oriented approaches have been applied during teaching the developed courses. Curricula based on problem-oriented teaching were first introduced in medical schools, e.g. at Harvard Medical School in 1985 and at Bowman Gray School of Medicine (USA) in 1987/1988 (Philip and Camp, 1990). At the latter school, 25 students were accommodated per year, six to eight students per group, each with a basic scientist and clinician as a supervisor. The students had confronted with clinical cases and patients already in the first year. The main objectives of the curriculum included training of independent learning, critical thinking, learning of problem solving skills, enhancement of understanding of disease mechanisms, training of team-work and lifelong learning habits. Several veterinary schools or faculties have also introduced curricula using problembased teaching, e.g. Cornell University, Ithaca, USA in 1983 and the Faculty of Veterinary Medicine, University of Utrecht, Netherlands in 1995 (Eysker, 2002). Such curricula have been focused on diseases affecting various animal species, on organ-oriented diseases or both. For many years, schools in Europe practiced some elements of problem-oriented teaching, especially in the clinical part of the curriculum, with relatively small groups of students having access to clinical patients under supervision of academic staff members. Similarly, in Pakistan, one credit hour course for parasitology clinic has been started in revised curriculum in 1998.

1. We believe that problem-based learning (PBL) provides a forum in which these essential skills will be developed. The basic principle supporting the concept of PBL is older than formal education itself; namely, learning is initiated by a posed problem, query, or puzzle that the learner wants to solve (Boud and Feletti, 1998). In the problemapproach, real-world based complex, problems are used to motivate students to identify and research the concepts and principles they need to know to work through those problems. Students work in small learning teams, bringing together collective skills at acquiring, communication, and integrating information. Problem-based instruction addresses directly many of the recommended and desirable outcomes of an undergraduate education: specifically, the ability to do the following:

- 2. Think critically and be able to analyze and solve complex, real-world problems.
- 3. Find, evaluate, and use appropriate learning resources.
- 4. Work cooperatively in teams and small groups.
- 5. Use content knowledge and intellectual skills acquired at the university to become continual learners.

3.1.3 Combined Approaches

Another choice of teaching some zoological courses is a combined disciplinary and problemoriented approach (Eckert, 2000). In this approach, various disciplines should initially present concise overviews on invertebrate or parasitic or physiological function and other infectious diseases, including all aspects from etiology to control. Enough time has to be reserved for practical training and self-learning of the students, who should have access to computer-based and interactive learning facilities. General principles of diagnostic techniques can be taught in an interdisciplinary approach together with structure various systems and pathology. In this period, some modest options for specialization may be offered as electives, e.g. tropical parasitology or parasitic diseases of wild and zoo animals. In the clinical part of the curriculum, cases of infectious diseases should be approach presented in а problem-oriented predominantly focused on animal species. Ideally, these presentations should be interdisciplinary endeavors with participation of clinicians, infectiologists, pathologists, physiology and other specialists.

3.1.4 Region-Specific Approaches

There are certain parasitic diseases of animals which are specific for a particular area e.g. fascioliasis is a pertinent parasitic disease of marshy areas. Similarly, the diagnosis and control practices used by the local farmers are also equally important and helpful in field conditions. Other examples are concern about the present of certain invertebrate in certain place of world and some anemia are related to certain region. The knowledge on these area specific parasites and control measures should be gathered and be included after scientific validation. This approach will help the students to easily tackle with region-specific parasitic or physiological problems.

3.2 Computer-Based Learning in Classrooms Approaches

The use of computers in classrooms has not only enhanced the level of interest among students but has also opened new avenues for teachers to explore novel methods of student-centered teaching. Research confirms that computers aid collaborative learning environments in the classroom, enabling students to use audio-visual aids to understand core concepts (importance of computers in the classroom). Most importantly, computers have made it possible for learners to further their education through virtual or online learning courses, without having to sacrifice their jobs (Veletsianos and Kleanthous, 2009). Computers help students share important knowledge and help them build positive relationships which enhance their learning processes.

3.3 Preparation of Components of Comprehensive Course Syllabus

We prepared course syllabus for each developed course that contained the following items; basic information, course description and materials. Basic information include name of university, semester, year, course title, number, unit value, course meeting times and location, instructor and how to contact him. Course description included four points that are prerequisites, overview of course, student learning objectives and methods of instruction (lecture, discussion, group work, etc.). Materials concerned about primary or required books//readings for the course, supplemental or optional books//readings, websites and links, other materials (lab equipment, software, etc). The course syllabus put in student guide to be given to the student in the beginning of the semester.

3.4 Practical Courses

Some practical courses have been prepared in power point presentation also video for some practical courses has been made. The dissection of birds to prepared fixed slides for parasite, dissection of shrimp, some experiments for the hematological (hemoglobin determination, counting red blood cells, counting white blood cells and measuring the packed cell volume) have been recorded in videos. Kaltura (2010) and Loutchko et al. (2002) mentioned that the multimedia course for internet should depend on virtual education.

3.5 Minimizing the Distance Between the Students

and the Teacher

During the developing the way of teaching the developed courses, we change our strategy in the lecture with the students to minimize the distance with them. The strategies include we arrive early and chat with students, show them a sample of exam, offer early assistance to students having difficulty, acknowledge students who are doing well in the course, mule topics for office hours, listen attentively to all questions and answer them directly (Gleason, 1986).

3.6 Recommendations for Administering and

Analyzing Student Course Evaluations

Student course evaluations (or student ratings) are one source of data about teaching. Although departments may use any type of evaluation form to obtain student opinion, since 1975 it has been required that students numerically rate each instructor on the following question: considering both the limitations and possibilities of the subject matter and course, how would you rate the overall teaching effectiveness of the instructor?. During the project we make procedures for administering student evaluation forms as following: A set number of blank evaluation forms are distributed to each faculty member for each class. Sufficient class time is designated for students to fill out questionnaires (evaluations are best not distributed at the final exam, when students have other things on their minds, but rather during the last two weeks of the term). Students are informed about the purpose of the evaluation. The instructor designates a student from the class (or a staff person) to supervise the evaluation. Students complete the questionnaires while the faculty member is absent from the room. The designated student (or departmental staff person) collects the evaluation forms and places them in an envelope, noting on the outside the instructor's name, the course number, the total number of students present, the total number of forms collected and the date. The student the signs the envelope and files it with the department. Summary information (including statistical data and syntheses of open ended responses) should become a permanent part of the teacher's file (Johnson-Eilola, 2005).

3.7 Enhancement the Efficacy of the Administrators and Technician

During the project, three workshops have been organized; one of them to encourage the administrators to improve the practical class and they should encourage the students for the non curriculum activities. The second workshop for the technician concerned about using the computer and how to work on the word program. The last one was concerned on training the technician on all the equipments and instruments in the department.

3.8 E-learning Services Approaches

The main objective from during this study was to approach to the E-learning service in Taif University. E-learning services have evolved since computers were first used in education. There is a trend to move towards blended learning services, where computer-based activities are integrated with practical or classroom-based situations. Bates and Poole (2003) and the OECD (2005) suggest that different types or forms of E-learning can be considered as a continuum, from no e-learning, i.e. no use of computers and/or the Internet for teaching and learning, through classroom aids, such as making classroom lecture Power point slides available to students through a course web site or learning management system, to laptop programs, where students are required to bring laptops to class and use them as part of a face-to-face class, to hybrid learning, where classroom time is reduced but not eliminated, with more time devoted to online learning, through to fully online learning, which is a form of distance education. This classification is somewhat similar to that of the Sloan Commission reports on the status of E-learning, which refer to web enhanced, web supplemented and web dependent to reflect increasing intensity of technology use. In the Bates and Poole continuum, 'blended learning' can cover classroom aids, laptops and hybrid learning, while 'distributed learning' can incorporate either hybrid or fully online learning (Bates and Poole, 2003). It can be seen then that E-learning can describe a wide range of applications, and it is often by no means clear even in peer reviewed research publications which form of Elearning is being discussed (Lowenthal et al., 2009). However, Bates and Poole argue that when instructors say they are using E-learning, this most often refers to the use of technology as classroom aids, although over time, there has been a gradual increase in fully online learning (Bates and Poole, 2003).

Technology enhanced learning (TEL) has the goal to provide socio-technical innovations (also improving efficiency and cost effectiveness) for Elearning practices, regarding individuals and organizations, independent of time, place and pace. The field of TEL therefore applies to the support of any learning activity through technology. The recent trend in the E-Learning sector is screen casting. There are many screen casting tools available but the latest buzz is all about the web based screen casting tools which allow the users to create it directly from their browser and make the video available online so that the viewers can stream the video directly. The advantage of such tools is that it gives the presenter the ability to show his ideas and flow of thoughts rather than simply explain them, which may be more confusing when delivered via simple text instructions. With the combination of video and audio, the expert can mimic the one on one experience of the classroom and deliver clear, complete instructions. From the learner's point of view this provides the ability to pause and rewind and gives the learner the advantage of moving at their own pace, something a classroom cannot always offer. The Elearning system not only provides learning objectives, but also evaluates the progress of the student and credit can be earned toward higher learning institutions. This reuse is an excellent example of knowledge retention and the cyclical process of knowledge transfer and use of data and records.

In conclusion improving the contents, curricula, methods, structures of courses and improving the teaching were made. During the development the course everyone was thinking about how to make the teaching more effective. Each course has content, clarified objectives, establishes the expectations, lecture summary, quizzes and the new point that will be discussed next lecture. A lecture has been planned to cover less than the entire period. Briefly, we summarize the previous lecture; introduce the topic(s) for the day; present the material; summarize briefly; preview any homework and the next lecture. However it is a more substantial and difficult task to spread this commitment to a majority of teaching staff and to ensure they are able and willing to use appropriate teaching strategies to develop these abilities. Some resistance is to be resistance from faculty members who are not convinced of the need for these changes, or who lack the skills in different forms of teaching. Institutions are beginning to introduce training in teaching and a lot of attention will need to be given in institutions to management of change strategies.

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Water use efficiency in rice hybrid under different water intervals and nitrogen levels

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Abstract: Shortage of water in rice cultivation area of Iran is going to be a major problem in near future therefore water use in rice production systems has to be reduced and water use efficiency increased. Experiment was conducted in a split plot based on completely randomized block design with 3 replications during 2007-2008. Four levels of nitrogen (N1=0, N2= 90, N3= 120 and N4= 150 kg/ha) were splited on 4 different irrigation managements (I1=continuous submergence, I2= 5, I3= 8 and I4= 11 days interval). Grain yield was 7342, 7079, 7159 and 5168 kg/ha in I1 to I4 and 5303, 6628, 7398 and 7418 kg/ha in N1 to N4 respectively. Water use efficiency was 1.41, 1.53, 1.68 and 1.31kg m3 in I1 to I4 and 1.16, 1.48, 1.67 and 1.63 kg/m3in N1 to N4 respectively. Water used was 5190, 4630, 4270 and 3950 m3 in I1 to I4 and 4590, 4470, 4440 and 4540 m3 in N1 to N4 respectively. The results clearly showed that continuous submergence irrigation is not essential for rice production and we can use irrigation interval 8 days. The application of 120 and 150 kg/ha nitrogen produce same grain yield, therefore consumption of 120 kg/ha nitrogen for rice hybrid advised.

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Keywords: irrigation, nitrogen, water use efficiency, rice, Iran

1. Introduction

Rice is the most important staple in Asia, providing on average 32% of total calorie uptake (MacLean et al., 2002). Iran with near 165 million hectare of land area and amount of rainfall equal to 300mm in 67% of its territorial land is considered as a semi-dry region in the world. About 75% of the global rice volume is produced in the irrigated lowlands (MacLean et al., 2002). 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).Although in recent years the growth of consumption rate of fertilizers in Iran has increased sharply and a large amount of fertilizer in addition to domestic productions has been imported from abroad nevertheless unfortunately during this period not only yield of crops has not increased in accordance with the consumption growth rate of fertilizers but also yield in hectare of crops has declined to many reasons such as water shortage, different irrigation methods, lack of scientific knowledge by farmers and method of fertilizer usage. 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).

Biomass and yield did not significantly differ between ASNS and CS, but water productivity was significantly higher under ASNS than under CS.grain yields were 4.1-5.0 t ha⁻¹ with 0 kg N ha⁻¹ and 6.8– 9.2 t ha⁻¹ with 180 kg N ha⁻¹ (Belder et al., 2004). 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).

Belder et al (2004) investing compared continuous submerge method and interval irrigation methods in China and the Philippines through different nitrogen levels, water saving in interval irrigation methods was 18-15 % higher than submerge method, they also found the water productivity amount in the Philippines and China, 0.73- 1.48 and 0.5 – 1.3 kg yield for $1m^3$ of input water. Tabbal et al (2002) in a research in the Philippines compared the conditions of submerge and interval irrigation in direct and transplanting plantation and concluded that the water productivity in saturated situation for direct and transplanting plantation is less than other treatments. Bouman and Tuong (2001) for watered farms of India and Philippine reported the water productivity as 0.2-1.1.

The purposes of this experiment determine the best appropriate level for usage of nitrogen fertilizer and irrigation management.

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. 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 6m² harvesting of every plot. 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

Water interval had significant effect on grain yield, biomass, harvest index, water used and water use efficiency (table 1). Nitrogen level had significant effect on grain yield, biomass and water use efficiency (table 1). Grain yield was 7342, 7079, 7159 and 5168 kg/ha in I1 to I4 (fig1a) and 5303, 6628, 7398 and 7418 kg/ha in N1 to N4 (fig2a) respectively.I1N3, I3N4, I1N4, I2N3, I3N3 and I4N1produced maximum and minimum grain yield (table 2).

Continuous submergence, irrigation interval 5 and 8 days had no differences on grain yield and produced same grain yield. Irrigation interval 11 days decreased grain yield to 27%. The consumption of 120 and 150 kg/ha nitrogen fertilizer produced same grain yield. Minimum grain yield obtained in 0 kgN/ha. Reported that application of N fertilizer increased grain yield of rainfed lowland rice even when the rice crop was exposed to water deficit (Castillo et al, 1992). Also, reported that grain yield and dry matter increased as the applied N rate was increased (Zhong and Huang, 2002).

Biomass was 13369, 13653, 13111 and 12046 kg/ha in I1 to I4 (fig1b) and 10328, 12635, 14139 and 15077 kg/ha in N1 to N4 (fig2b)

respectively.I1N4, I2N4, I3N3 and I4N1produced maximum and minimum biomass (table 2).

Harvest index was 50, 50, 51 and 46 percentage in I1 to I4 (fig1c) respectively and nitrogen level was not effective on harvest index (fig2c).

Water used was 5190, 4630, 4270 and 3950 m3 in I1 to I4 (fig1d) and 4590, 4470, 4440 and 4540 m3 in N1 to N4 (fig2d) respectively. Amount of irrigation in 8 days interval compare to continuous submergence decreased 18% (4270 and 5190 m3 respectively).

I1N4 and I4N3, had maximum and minimum water used respectively (table 2). Water use efficiency was 1.41, 1.53, 1.68 kg/m3 and 1.31 in I1 to I4 (fig1e) and 1.16, 1.48, 1.67 and 1.63 kg/m3 in N1 to N4 (fig2e) respectively. I3N4, I3N3, I1N3 and I4N1 had the highest and lowest WUE respectively (table2).

4. Discussions

Shortage of water in rice cultivation area of Iran is going to be a major problem in near future therefore water use in rice production systems has to be reduced. The results clearly showed that continuous submergence irrigation is not essential for rice production and we can use irrigation interval 8 days in paddy fields of north of Iran. The application of 120 and 150 kg/ha nitrogen produce same grain yield, therefore consumption of 120 kg/ha nitrogen for rice hybrid advised.

Water saving irrigation can lead to reduce water consumption in paddy fields and conservation of natural water resources of which is important goal of achieving sustainable development in agriculture.

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S. O. V	df	Yield	biomass	Harvest Index	amount of irrigation	Water use efficiency
Ι	3	12452331**	5364477.1*	333.7**	33888.8**	0.375*
Е	6	1077622	885869.2	21.1	1735.3	0.09
Ν	3	11838173**	44436179.4**	33.6 ns	580.5 ns	0.669**
I*N	9	1211276*	4554914.2*	43.1 ns	4127.3**	0.125**
Е	24	501150	1882779.7	22.6	795.1	0.43
CV (%)		10.59	10.93	9.48	6.25	10

Table 1. Analysis	of variance	in some	parameters	of rice
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Table 2. Effects of water interval and nitrogen levels on some parameters of rice

water interval	Nitrogen level	Yield (kg/ha)	biomass (kg/ha)	Harvest index (%)	Water used (m3)	Water use efficiency(kg/m3)
	0	5495d	10340f	49.3abcd	5440a	1.01cd
T1	90	7117bc	13370bcde	53.3abc	4697f	1.52bc
11	120	8912a	15400bc	58.0a	5327b	1.67ab
	150	8247ab	18000a	45.7cde	5480a	1.50bc
	0	5971cd	10370f	57.3a	5160c	1.16c
10	90	7074bc	14530bcd	49.0abcd	4950d	1.43bc
12	120	7730ab	13960bcde	55.3ab	5000d	1.54bc
	150	7543b	15760ab	47.7bcde	4717f	1.60b
	0	5344d	10390f	51.3abcd	4410g	1.21c
10	90	7328b	13770bcde	53.3abc	5063d	1.45bc
13	120	7679ab	15680ab	49.0abcd	4587f	1.67ab
	150	8284ab	14990bc	55.3ab	4597f	1.80a
	0	4004e	10300f	43.0de	4057i	0.98d
T.4	90	4994d	12890cdef	39.0e	4840e	1.03cd
14	120	5274d	12240def	43.3de	4033j	1.31c
	150	5600d	12970cde	44.0de	4173j	1.34c
					5	

а







7159 a

13

5168 b

14

water interval



Fig 1- effect of nitrogen levels on grain yield (a), biomass (b) harvest index (c), water used (d) and WUE (e).



Fig 2- effect of nitrogen levels on grain yield (a), biomass (b) harvest index (c), water used (d) and WUE (e).

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Pathological Evaluation of Probiotic, Bacillus Subtilis, against Flavobacterium columnare in Tilapia Nilotica (Oreochromis Niloticus) Fish in Sharkia Governorate, Egypt

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Abstract: Fifteen out-of eighty-five of collected Tilapia nilotica fish (17.64%) showing skin lesions, were positive for Flavobacterium columnare with cultural, morphological and biochemical characteristics. These skin lesions were large erosions with loss of scales and red-grayish patches, particularly at the frontal head region and abdomen. All of the positive isolates (Flavobacterium columnare) were molecularly tested by means of PCR. With consistent with F. columnare standard ATCC 49512 strain, these isolates produced a 675 bp band. One hundred apparently healthy Tilapia nilotica fingerlings (30±5 gm) were used to evaluate the effectiveness of probiotic, Bacillus subtilis, in water or diet against the intramuscular challenge with Flavobacterium columnare infection. They were equally divided into 10 groups (10 fish for each group). Five groups were experimental control {placebo (gp 1), intramuscularly infected with 0.2 x10⁸ F, columnare CFU (gp 2), received 0.1 gm/L probiotic in water (gp 3), 0.2 gm /L probiotic in fish diet (gp 4), or 1 gm/L oxytetracycline (gp 5)}; two were prophylactic experiment {received 0.1 (gp 6) or 0.2 (gp 7) gm of probiotic in water and diet, respectively 2 months before bacterial infection and continued for a week later}; and three were treated experiment {intramuscularly infected with 0.2 x10⁸ F. columnare CFU and then received the probiotic in water (gp 8), diet (gp 9) or 1 gm/L oxytetracycline for a week (gp 10)}. Specimens from the skin, gills, liver, kidney and intestine were collected, fixed in 10% buffered neutral formalin solution and were routinely processed for pathological examination. Exposure of the fish to F. columnare infection produced focal coagulative necrosis, ulcerations besides severe hydropic and spongiosis in the epidermis, particularly at the necrotic areas of the fins and heavily infiltrated with granulocytes and few lymphocytes. The dermis was infiltrated with neutrophils and the underlying muscles were necrotic. The gills showed coagulative necrosis in the gill-filaments with neutrophils infiltration and few extravasated erythrocytes. Focal proliferation of the respiratory epithelium was noticed particularly those covering the secondary lamellae which frequently sloughed. The probiotic (prophylactic experiment) in water or diet was alleviated the lesions of the Flavobacterium columnare infected fish with an increase of water quality, while such changes were still similar to those described with infected fish in probiotic treated experiment. The oxytetracycline-treated group showed significant reduction of these lesions and the treated fish appeared normal. Collectively, it could be concluded that the probiotic, B. subtilis, in water or diet (as prophylaxis) are effective in amelioration the lesions of F. columnare infections that have wide spread among Egyptian freshwater fish. Oxytetracycline is the drug of choice to treat such disease and minimize the lesions of F. columnare.

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Keywords: Flavobacterium columnare, Probiotic B. subtilis, Tilapia nilotica, PCR and Oxytetracycline

1. Introduction:

Columnaris disease previously referred to as myxobacterial infection and reported by Davis in 1922. It remains one of the most frequently encountered and devastating bacterial diseases of freshwater fishes. This disease is also known as saddleback disease, cotton-wool disease and fin rot. These names reflect the gross lesions of affecting fish. Columnaris disease is caused by the Gramnegative bacterium (*Flavobacterium columnare*). Such bacteria can infect fishes of any age, under a variety of water condition and during any season of the year (Griffin 1992). Acute disease is characterized by an incubation period of less than 24 hrs and the resulting mortalities are seen two to three days post exposure (Holt et al 1975).

Columnaris is a contagious disease that can be transmitted horizontally through direct contact and skin wounds as well as through orofecal route (Bullock et al 1986, Welker et al 2005 and Austin and Austin 2007). Due to the ubiquitous nature of the *F. columnare* in freshwater, an injury to the skin or gills of fish with elevation of water temperature may quickly initiate the Columnaris infection. The disease can be clinically diagnosed through its characteristic clinical picture in the affected fish. In scaly fishes,

the infection initially appears as milky veiled erosions on the dorsal aspect of fish body which may progress to extensive ulcers as dull whitish or yellow necrotic areas (Sakr 1996). The infected skin loses its natural sheen and a gray, white or yellowish margin surrounds the focal lesions. The mouth and inner walls of the oral cavity may be covered with a vellowish mucoid material (Kumar et al 1986). In scaly-less fishes, the lesions start with simple ulcers which predominately end with extensive saddlebacklike ulcers exposing the underlying musculatures (Morrison et al 1981). Fin and gill rot is another lesion of the progressive infection in both scaly and scaly-less fishes (Bullock et al 1986 and Latremouille 2003). The histopathology may provide useful information concerning the severity of infection. The gills showed branchial epithelial cells and goblet cells hyperplasia. These lesions rapidly progress to severe neutrophilic inflammation and gill necrosis. Erosions and ulceration of the epidermis besides necrosis and edema in the underlying muscles were recorded in the affected skin (Eissa et al 2006 and Rasha 2008).

Nowadays, we have the ability to diagnose Columnaris disease by modern molecular methods: such as polymerase chain reaction (PCR) based techniques employing species-specific primers (Wakabayashi and Riyanto 1999, Darwish et al 2004, Bader et al 2003 and Eissa et al 2010) and by DNA sequence analysis (Tiirola et al 2002, Thomas-Jinu and Goodwin 2004 and Zhang and Arias 2009).

Currently, common control measures of the diseases focused on the use of antibiotics and chemical agents. The potassium permanganate at concentration of 2 ppm for 8-10 hours (Francis-Floyd 1998) and copper sulfate 1mg/L (Thomas-Jinu and Goodwin 2004) can be treated Columnaris disease. Moreover, Columnaris disease is most effectively treated mainly with tetracycline (Hawke and Thune 1992 and Smith et al 1994). Soltani et al (1996) reported the minimal inhibitory concentration of amoxicillin (0.06µg/ml), oxytetracycline (0.06- $0.12 \mu g/ml$), oxolinic acid $(0.06-0.12\mu g/ml),$ norfloxacin $(0.12 \mu g/ml)$ and trimethoprim (>64µg/ml). However, the use of antibiotics as treatment has been problematic by acquisition of genes that result in antibiotic resistant strains of bacteria and residues of antibiotics can diffuse into the aquatic environment (Ellis 1991 and Smith et al 1994). The use of some antibiotics, disinfectants and other chemical agents to control fish diseases in pond have been prohibited because resistance can develop in environmental bacteria, negative effect on human health and restriction for export commodities (WHO 2006).

An alternative method to prevent and cure fish disease is by using probiotic. Probiotic is defined as microbial cell preparation or components of microbial cells, which have a beneficial effect on the health and well being of the host (Vivas et al 2004, Bagheri et al 2008, Zhou et al 2009 and Son et al 2009). Other benefits of probiotic in aquaculture are competitor for nutrient, source of nutrients and enzymatic contribution to digestion and improve water quality (Abraham et al 2008 and Dalmo and Bogwald 2008).

probiotic Common product used in aquaculture such as Bacillus species can improve water quality by reducing the number of bacteria pathogens in farm (Wang et al 2008). Furthermore, several researchers have been proved the effectiveness of probiotic in fish and shrimp to resist to pathogen (Liu et al 2010), Aeromonas salmonicida (Irianto Austin 2002) and enhance immunity (Randelli et al 2008 and Nayak 2010). Moriarty (1998) and Balcazar and Rojas-Luna (2007) reported that probiotic Bacillus species can reduce number of Vibrio species in penaeid cultured pond. He was observed after fed with feed containing probiotic Bacillus subtilis for 14 days. Significant increase in respiratory burst activity, differential leukocyte count (neutrophil and monocyte) and serum bactericidal activity are seen in comparing with the control (Aly et al 2008, Kumar et al 2008 and Marzouk et al 2008). Nayak et al (2007) reported that after Indian carp were fed with probiotic B. subtilis for 60 days, the total serum protein and globulin content were significantly higher in probiotic fed group than the control.

Probiotic containing in fish food can build up the beneficial bacterial flora in skin and intestine while they grow competitively over certain bacteria (Ziaei-Nejad et al 2006 and Abd El-Rhman et al 2009). *Flavobacterium columnare* grows by stacking adhesion layer on fish skin and the consequence of colonization caused skin damage lesion and thus, including fish mortality. However, manipulation of the composition of the bacterial community on fish skin may be used in prevention to Columnaris disease. This studyencourages work on more ecological and natural method to prevent *F. columnare* infection in Nile tilapia by using commercial probiotic product *B. subtilis*.

The objective of this work was to survey the prevalence of natural infection with *F. columnare* and to evaluate the effectiveness of commercial probiotic product, *Bacillus subtilis* as competitor in prevention against experimental infection on *Tilapia nilotica*, employing the pathology besides the PCR for confirmative diagnosis in naturally infected fish.

2. Materials and methods

Fish:

A total of 85 tilapia species, showing skinlesions (erosions, ulceration and fin or tail rot), were collected from Abbassa Fish Farm in Sharkia governorate. The clinical signs were recorded and fish were transferred alive in plastic bags to the laboratory and subjected to full microbiological and Real time PCR-isolation of *Flavobacterium columnaris*.

Bacteriological Isolation and Identification:

Sterile bacteriological swabs from skin lesions were cultured into tryptic soy broth (TSB) and onto Hsu-Shotts agar (Bullock et al 1986) or Cytophaga agar plates (Farmer 2004). The inoculated broth and agar plates were inoculated at 25°c for 48-72 hours. Bacterial isolates were presumptively identified using broth cultural characteristics and conventional biochemical tests recommended by Griffin (1992).

Molecular Identification:

Chromosomal DNA was extracted from 100 µl of bacterial suspension (a single colony of each of the isolated bacteria suspended in 100 ul of sterile saline) using DNeasy tissue extraction kit (QIAGEN, Valencia CA) according to the manufacture's instructions. The extracted DNA was amplified using specific primer set specifically for F. columnare (Darwish et al 2004). Two specific primers were efficiently used {Forward (598-CAGTGGTGAAATCTGGT-614) and Reverse (1260-GCTCCTACTTGCGTAGT-1276)}. The negative controls consisted of a PCR mixture with molecular grade water (negative QC control) and another with Pseudomonas florescence DNA template (negative QA control). Positive control (FC+) consisted of a PCR mixture with DNA extracted from known F. columnare (ATCC 49512). Thermal cycling and amplification procedures were done according to the method described by Darwish et al (2004).

Experimental Design:

One hundred apparently healthy *Tilapia nilotica* fingerlings (30 ± 5 gm) were equally divided into 10 groups (10 fish for each group). Five groups were experimental control {placebo (gp 1), intramuscularly infected with 0.2 x10⁸ *F. columnare* CFU (gp 2), received 0.1 gm/L probiotic in water (gp 3), 0.2 gm /L probiotic in fish diet (gp 4), or 1 gm/L oxytetracycline (gp 5)}; two were prophylactic experiment {received 0.1 (gp 6) or 0.2 (gp 7) gm probiotic in water and diet, respectively 2 months before bacterial infection and continued for a week later}; and three were treated experiment {the fish

firstly infected with 0.2×10^8 *F. columnare* CFU and then received the probiotic in water (gp 8), diet (gp 9) or 1 gm/L oxytetracycline for a week (gp 10)} as shown in table (1). All fish were daily observed for the clinical signs and mortalities and then they were sacrificed at the end of each experiment.

Probiotic and its Safety:

The probiotic, *Bacillus subtilis* (ATCC 6633), was obtained as lyophilized cells from Sigma. This probiotic was prepared according to previous studies (Liu et al 2009 and Tseng et al 2009). The fish were daily immersed in the probiotic at 3 constant times (bath exposure) or thoroughly mixed with the diet (*ad libitum*). The safety of such probiotic was evaluated by absence of the clinical sign, lesions and mortalities (Aly et al 2008).

Water quality assessment:

Water temperature and pH were measured using a waterproof digital combo pH meter and thermometer {HI98127 (pHep 4)-Hanna instruments Inc., RI, USA}. Dissolved oxygen (DO) concentration were measured using a digital dissolved oxygen meter (HI 9142-Hanna instruments Inc., RI, USA). Total ammonia nitrogen (TAN mg/L) was determined following the method described by Chattopadhyay (1998).

Histopathological Examination:

Specimens from the examined organs (skin, gills, liver, kidney and intestine) were collected after necropsy and fixed in 10% buffered neutral formalin solution. Five-micron thick paraffin sections were prepared, stained by hematoxylin and eosin HE and then examined microscopically for histopathology (Bancroft and Stevens 1996).

3. Results

Prevalence and Microbiological Findings:

Fifteen out-of eighty-five of collected *Tilapia nilotica* fish (17.64%) showing skin lesions, were positive for *Flavobacterium columnare* with cultural, morphological and biochemical characteristics (table, 2). All of the positive isolates (*Flavobacterium columnare*) were molecularly tested by means of PCR. Consistent with *F. columnare* standard ATCC 49512 strain, these isolates produced a 675 bp band (Fig 1).

Water Quality Parameters:

Water quality parameters of the affected aquaria including pH, temperature, DO and TAN, were determined in different groups and compared to the control one (table, 3).

Gps		Treatments					
	Classifications	Flexibacter Infection 0.2 x 10 ⁸ CFU	Prob. in water 0.1 gm/ L	Prob. in ration 0.2 gm/ Kg	Oxytetracycline 1 gm/ 10 L	Withdrawal 2 weeks later	
1	al	-	-	-	-	-	
2	ol	+	-	-	-	-	
3	antro	-	+	-	-	-	
4	cc	-	-	+	-	-	
5	Щ	-	-	-	+	-	
6		+	+ *	-	-	+	
7	Prophylactic	+	-	+ *	-	+	
8		+	Simultaneously	-	-	-	
9	m	+	-	Simultaneously	-	-	
10	Treatment	+	-	-	Simultaneously	-	

Table (1): Groups and treatments of experimental study.

2 months before infection and a week after (simultaneously) then 2 weeks withdrawal.

Pathological Findings:

Groups (1, 3 and 4): control or received probiotic, *Bacillus subtilis*, in the water (0.1gm/L) and diet (0.1gm/L).

Neither gross nor microscopic abnormalities were seen in the skin, gills, liver and kidney. Groups (2, 8 and 9): *Tilapia nilotica* infected with 0.2

 $x = 10^8$ CFU of *Flavobacterium columnare* or they simultaneously received the probiotic in the water and ration as treatment.

Clinical Signs and Postmortem Examination:

The infected fish showed loss of appetite and respiratory distress. They swam near the water surface, gasping and engulfing the atmospheric air. The fish showed rapid movement of the opercula with nervous manifestation and 70% mortalities. The postmortem lesions were similar to natural infected fish. The skin lesions were presented as large erosions with loss of scales and red-grayish patches (plaques), particularly at the frontal head region and abdomen. Some frontal ulcers were deeply penetrated the skull to form hole-in-the head-like lesion (head cysts). Hemorrhagic small ulcers surrounded by red eroded zone were visualized on the pelvic and anal areas. Sometimes, the ulcers appeared yellow or orange in color. Hemorrhage was noticed at the base of the pectoral fins besides fragmented or frayed caudal fin with edematous, gravish discolored margin. Necrosis of the membranous portion of the caudal fin (fin-rot) was also detected. The gill filaments were congested, swollen and covered with profuse mucus. The visceral organs (liver, spleen and kidney) were congested. Sometimes, corneal opacity was recorded (Fig 2).

Histopathological Findings:

The significant lesions were restricted to the skin and gills besides septicemic lesions in the other organs. The skin showed focal coagulative necrosis and severe spongiosis in the epidermis, particularly at the necrotic areas of the fins and heavily infiltrated with granulocytes, EGC and few lymphocytes (Fig 3). Sometimes, the epidermal necrosis and pustules (Fig 4) was serious in the affected areas, sloughing off to induce erosions or ulcerations (Figs 5 and 6). These ulcers showed masses of basophilic bacterial colonies. Spongiosis and hydropic degeneration were observed with mucous cell proliferation and mucous cysts formation, which showed eosinophilic mucus (Fig 7). The ulcerations spread by radial expansion and may penetrate into deeper tissues, producing necrotic dermatitis, myositis and perimyosititis (Fig 8). The affected dermis was infiltrated with neutrophils and the underlying muscles were necrotic (Fig 9). Excessive edema and hemorrhage were seen among the necrotic skeletal muscle fibers (Fig 10). The latter revealed central myolysis and gradually disappeared and replaced by cavitations that empty or filled with fine eosinophilic granular material. The edematous fluid was usually entrapped leukocytes. The dermal capillaries were congested, especially in the areas of ulcerations. Some ulcers were undergoing healing by granulation tissue formation which was infiltrated by leukocytes and a great number of melanin-carrying cells and extended to the underlying necrotic muscles. Inflammatory cell aggregations together with numerous EGC were noticed, particularly near the bony elements. The gill infections are less common but more serious. Columnaris begins at the tips of the lamellae and causes a progressive necrosis that extends to the base of the gill arch. The affected gills showed coagulative necrosis in the gill-filaments with neutrophils infiltration and few extravasated erythrocytes (Fig 11). Focal proliferation of the respiratory epithelium was noticed particularly those covering the secondary lamellae which frequently sloughed (Fig 12). Telangiectasia of the branchial blood capillaries and hemorrhages besides congestion of the lamellar blood capillaries and focal aneurysms were detected (Fig 13). The interlamellar spaces were obliterated by epithelial and mucous cells hyperplasia as well as neutrophils infiltrations (Fig 14). Large basophilic bacterial clumps were tightly adhered to the primary lamellae, within the mucous cells and on the necrotic areas. Sometimes, lamellar epithelial degeneration, exfoliation, fusion and clubbing were mildly visualized besides few inflammatory cells infiltrations that leading to lamellar thickening. The most severe lesions were seen in the gill arches. These lesions were represented by multifocal ballooning degeneration, spongiosis, and hydropic degenerations and necrosis (Fig 15). Hypertrophy and hyperplasia of the mucous cells were noted. Edema, congestion, hemorrhages and leukocytic infiltrations mainly with neutrophils and lymphocytes were These cells were extended to the detected. submucosa and lamina propria. The basement membrane of the superficial epithelium of the gillarch was hyalinized. Sometimes, the gill rakers showed necrosis and complete sloughing of the lining epithelia. The liver revealed coagulative necrosis and neutrophils infiltrations (Fig 16). Focal vacuolations and hydropic degeneration were seen besides congestion of the hepatic blood vessels. The pancreatic acini were atrophied and showed focal coagulative necrosis (Fig 17). The blood sinusoids and the central veins were highly congested. Sometimes the epithelial lining of the bile ducts showed vacuolar degeneration and others revealed hyperplasia in the lining epithelia. Focal replacements of the hepatic parenchyma with fibrous connective tissue infiltrated with numerous macrophages, lymphocytes and polymorphnuclear cells were detected. The kidney revealed multifocal areas of coagulative necrosis and hemorrhages (Fig 18). Moderate hydropic degenerations in the lining epithelium of the renal tubules and shrunken glomeruli were seen. Sometimes, the glomeruli were contracted with dilated Bowman's capsule with eosinophilic material. Focal congestion, hemorrhages and depletion of the hemopoietic elements were uncommon besides activation of the melanomacrophages centers. Focal area of fibrosis and mononuclears were seen replacing the renal tissue. Some renal tubules showed hyaline droplet

degeneration and few interstitial leukocytes infiltrations.

Group (5): received 1 gm / 10 L of oxytetracycline.

Clinical Signs and Postmortem Examination:

Loss of balance, loss of appetite and sluggish movement were the most common clinical signs. No mortalities were recorded.

Histopathological Findings:

The liver of treated fishes showed diffuse vacuolations (Fig 19) and hydropic degeneration besides necrotic changes in the pancreatic acini. Multiple areas of interstitial aggregations of mononuclear cells were observed throughout the hepatic parenchyma (Fig 20). The hepatic blood vessels and sinusoids were congested. The kidney revealed focal hydropic degeneration and individual-cell necrosis in the affected renal tubular epithelium (Fig 21). The hemopoietic cells were focally necrotic with activation of the melanomacrophage centers. The gills showed severe congestion of lamellar capillaries (Fig 22). The skin was normal.

Groups (6 and 7): received the probiotic in the water and diet then infected with 0.2×10^8 CFU of *Flavobacterium columnare* (prophylactic experiment).

Clinical Signs and Postmortem Examination:

The affected fish were mostly normal. Sometimes, they showed dullness and darker-colored skin with slight frayed tail (Fig 23). Focal petechial hemorrhages were rarely encountered besides easily detached scales. The liver and kidneys were slight congested with congested gills. No mortalities were recorded.

Histopathological Findings:

The probiotic in water or diet was alleviated the lesions of the challenged *Flavobacterium columnare*. where it activated the skin immune system. The latter was represented by thickening of the epidermis with acanthosis and mild spongiosis with no evidence of ulcerations (Fig 24). Sometimes, the apical layers of the epidermis were sloughed and numerous melanomacrophages were detected at the junction with the underlying dermis (Fig 25). Proliferation of mucus secreting cells was focally noticed in the epidermis of some cases. The dermis revealed few lymphocytes and polymorphnuclear cells infiltrations among normal or mild degenerated muscle fibers (Fig 26). The gills showed mild proliferative changes in the respiratory epithelium with focal fusion of the secondary lamellae (Fig 27). Congestion, telangiectasis and hemorrhage were also frequent in gill filaments, rakers and arches. The liver showed

moderate vacuolations of the hepatocytes and focal necrosis in the pancreatic acini (Fig 28) in some cases. Severe congestion of some hepatic blood vessels and hemorrhage were also visualized. The liver of most fishes was nearly normal. The kidney was normal except for hydropic degeneration in some renal tubular epithelia (Fig 29).

Group (10): infected with *F. columnare* and treated with oxytetracycline.

Clinical Signs and Postmortem Examination:

The experimental fish were emaciated and showed no other clinical signs. The mortality rate was 10% (1 out-of 10 fish).

Histopathological Findings:

Almost all columnaris-lesions were reduced to become normal. The skin showed mild Zenker's

degeneration and edema in the dermal skeletal muscles. Few round cells aggregations were also noticed. The gills were severely congested or hemorrhagic with hyperplasia in the lining epithelium and lymphocytes infiltrations (Fig 30). The liver showed mild hydropic degeneration and glycogen depletion. Mild congestion of the hepatic blood vessels was noticed. Few round cells infiltrations were detected in the portal areas and interstitial tissue. The pancreatic acini were normal. The kidney revealed few polymorphnuclear cells and lymphocytes among the renal tubules. The latter showed mild hydropic degeneration. The hemopoietic elements were normal besides activation of the melanomacrophage centers particularly in the anterior kidnev.

No	Morphological and biochemical characteristics	Results		
1	Gram stain	Gram –ve bacilli (rod-like)		
2	Gliding Motility	+ ve		
3	Colony on Hsu-Shotts agar	Round, yellow and strongly adherent to the agar.		
4	Chromo-shift (adding 3% KOH)	The yellow pigmented colonies change to pink		
5	Glucose fermented	+ ve		
6	Raffinose	- ve		
7	Citric utilization	+ ve		
8	Flexirrubin-type pigment	+ ve		
9	Congo Red test	+ ve		
10	Catalase test	+ ve		
11	Gelatin hydrolysis	+ ve		
12	Urea hydrolysis	+ ve		
13	Indole production	- ve		
14	Casein hydrolysis	+ ve		
15	Starch hydrolysis	- ve		
16	Nitrate reduction	+ ve		
17	H2S production	+ ve		
18	Growth on Trypticase Soy broth	- ve		
19	Growth with 0.5% NaCl	+ ve		
20	Growth with 1.0 % NaCl	- ve		
21	Growth in the presence of Neomycin sulfate	+ ve		

 Table (2): Morphological and biochemical characteristics of isolated F. columnare.

Table (3): Water quality parameters in different groups. (probiotic in water or diet was similar).

Water quality parameters	Control	Infected	Probiotic	Prob + Inf	Inf + Prob	Inf + Oxyt
Water pH	6.6	8.7	6.5	6.6	8.0	7.2
Water temperature	24° C	30° C	26° C	25° C	28° C	27° C
Water dissolved oxygen / ppm	6.5	3.1	6.6	6.0	4.5	5.0
Total ammonia nitrogen / ppm	0.4	3.2	0.65	0.9	2.1	1.6



Figs (1-6): Groups (2, 8 and 9) are similar.

1-PCR-results. 2-Tilapia showing skin erosions, ulcers and grayish hemorrhagic patches. 3-Skin showing focal coagulative necrosis in the epidermis. 4-Skin showing pustule. 5-Skin showing hydropic degeneration and spongiosis with dermal edema. 6-Skin showing ulceration of the epidermis. (HE: Bar = $100 \mu m$).



Figs (7-12): Groups (2, 8 and 9) are similar.

7-Skin showing proliferation of mucus cells with cyst formation. 8-Skin showing deep ulcer with necrotic dermatitis. 9-Skin showing neutrophils infiltrations among necrotic muscles. 10-Skin showing excessive edema and hemorrhage among necrotic muscles. 11-Gill showing coagulative necrosis in the gill-filaments with neutrophils infiltration and few extravasated erythrocytes. 12-Gill showing sloughing of the epithelial lining of the secondary lamellae. (HE: Bar = $100 \mu m$).



Figs (13-18): Groups (2, 8 and 9) are similar.

13-Gill showing congestion of the lamellar blood capillaries and focal aneurysms. 14-Gill showing interlamellar spaces obliterated by epithelial and mucous cells hyperplasia and neutrophils infiltrations. 15-Gill-arch showing multifocal ballooning degeneration, spongiosis, and hydropic degenerations and necrosis. 16-Liver showing coagulative necrosis and neutrophils infiltrations. 17-pancreatic acini showing atrophy and focal coagulative necrosis. 18-Kidney showing coagulative necrosis in tubular epithelia. (HE: Bar = 100 μ m).



Figs (19-24): Groups (5, 6 and 7).

19-Liver (5) showing severe vacuolations in the hepatocytes. 20-Liver (5) showing interstitial aggregation of mononuclears. 21-Kidney (5) showing focal hydropic degeneration and individual-cell necrosis in the affected renal tubular epithelium. 22-Gill (5) showing severe congestion of lamellar capillaries. 23-Tilapia (6) was darker in color with slight frayed tail. 24-Skin (6) showing thickening of the epidermis by acanthosis and mild spongiosis with no evidence of ulcerations. (HE: Bar = $100 \mu m$).



Figs (25-30): Groups (6, 7 and 10).

25-Skin (6) showing sloughed apical layers of the epidermis and numerous melanomacrophages at the junction with the underlying dermis. 26-Skin (7) showing few lymphocytes and polymorphnuclear cells among mild degenerated muscle fibers. 27-Gill (7) showing mild proliferative changes in the respiratory epithelium. 28-Liver (6) showing moderate vacuolations and focal necrosis in the pancreatic acini. 29-Kidney (7) showing hydropic degeneration in some renal tubular epithelia. 30-Gill (10) showing severe congestion and hemorrhage. (HE: Bar = 100 μ m)

4. Discussion:

It is evident that the columnaris is primarily an epithelial disease of freshwater fish. It causes erosive / necrotic skin and gill lesions that may become systemic. It often presents as large erosions with loss of scales and red-grayish patches that may have a red margin on the head, back (saddleback lesion), and / or fins (fin rot) especially the caudal fin. Fragments of the fin rays may remain after the epithelium has sloughed, leaving a ragged appearance. Ulcerations with yellow or orange color were deeply seen in underlying tissue due to masses of pigmented bacteria growth (Sakr 1996 and Latremouille 2003). The gill infections are less common but more serious. Columnaris begins at the tips of the lamellae and causes a progressive necrosis that extends to the base of the gill arch (Bullock et al 1986). The gill filaments were congested, swollen and covered by profuse mucus. Such lesions were the main cause of large mortalities among infected fish. The previous findings could be attributed to the adherence and irritation of the bacteria with gill structure (Eissa et al 2010).

When the probiotic, Bacillus subtilis, included in the diet and in water of aquaria for 2 months before the challenge with Flavobacterium columnare, the fish showed no clinical signs with rare focal petechial hemorrhage and easily detached scales. No mortalities were recorded. The addition of probiotic to the water or diet of fish could improve the water quality (Eissa et al 2010) and the immune system (Randelli et al 2008 and Nayak 2010). Balcazar et al (2006) reported that the use of probiotics, which control pathogens through a variety of mechanisms, is increasingly viewed as an alternative to antibiotic treatment. Nevertheless, some possible benefits linked to the administering of probiotics have already been suggested as: competitive exclusion of pathogenic bacteria (Balcazar 2003 and Balcazar et al 2004), source of nutrients and enzymatic contribution to digestion (Sakata 1990 and Prieur et al 1990) direct uptake of dissolved organic material mediated by the bacteria (Moriarty 1997) enhancement of the immune response against pathogenic microorganisms (Irianto and Austin 2002) and antiviral effects (Girones et al 1989 and Direkbusarakom et al 1998).

The histopathological findings observed with the *F. columnare* were similar to those described by Roberts (1989) and Sakr (1996). The skin and gills, primary target organs, showed necrosis with intense neutrophilic response. Erosions and ulcerations were seen as sloughing the necrotic tissue. Such reaction was deeply penetrated the underlying tissue as dermis and muscle (myositis and perimyositis) in skin and gill-arch. The dermal lesions besides those of the epidermis are probably the reasons for easily

detached scales. The aforementioned lesions were milder or completely absent with probiotic treated fish with numerous melanomacrophages infiltrations in these organs. The melanin pigment inside these cells is supposed to share in the defense mechanism as it is a response to probiotics. Melanin pigments are also found in the melanomacrophages within lysosomes; such pigments are considered to have a bactericidal effect through the production of free radicals (Ellis 1981). The treated groups with simultaneously received the probiotic during infection were similar to those the control (gp 2), where the probiotic has no time to enhance the immune system or improve the water quality (Eissa et al 2010). Meanwhile the use of oxytetracycline antibiotic was ameliorated the lesions of F. *columnare*. The previous findings could indicate that the oxytetracycline is sensitive and a drug of choice in treatment of columnaris disease in fishes (Hawke and Thune 1992 and Smith et al 1994).

Collectively, it could be concluded that the probiotic, *B. subtilis*, in water or diet (as prophylaxis) are effective in amelioration the lesions of *F. columnare* infections that have wide spread among Egyptian freshwater fish. Oxytetracycline is the drug of choice to treat such disease and minimize the lesions of *F. columnare*.

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Metabolic Effects of Estrogen and / or Insulin in Ovariectomized Experimentally Diabetic Rats

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Abstract: Postmenopausal adverse metabolic changes increase cardiovascular risk and impair quality of life. This study was planned to evaluate the benefits gained by estradiol treatment alone and insulin treatment alone versus combination of these two hormonal therapies on the metabolic derangements accompanying estrogen deficiency with diabetes. Rats were divided into five groups: control sham-operated group, ovariectomized streptozotocin diabetic group (OVX-STZ), estradiol-treated OVX-STZ diabetic group that received daily subcutaneous injection of estradiol (50µg/kg) for 4 weeks, insulin-treated OVX-STZ diabetic group that received daily subcutaneous injection of insulin (10 or 20 IU/kg) for 2 weeks and combined estradiol-treated, insulin-treated OVX-STZ diabetic group. Rats in all groups were subjected to determination of body weight, body mass index (BMI), blood glucose, plasma levels of total cholesterol, triglycerides, HDL-c, insulin, estradiol, leptin and malondialdehyde (MDA). In addition, in vitro glucose uptake by the diaphragm and glucose output by both kidneys were measured. Insulin treatment alone increased peripheral glucose uptake, reduced renal gluconeogenesis, normalized blood glucose and plasma total cholesterol, decreased triglycerides, LDL-c and atherogenic index and increased HDL-c. Plasma MDA was reduced however, still higher than controls. Estrogen therapy alone lowered blood glucose although not fully normalized, increased peripheral glucose uptake and decreased renal gluconeogenesis, reduced plasma triglycerides, total cholesterol, LDL-c and MDA and elevated HDL-c as compared to untreated groups, yet, not completely normalized. Combined estradiol and insulin therapy returned all measured parameters towards control values with complete normalization of peripheral glucose uptake and blood glucose levels as well as plasma triglycerides, HDLc, atherogenic index and MDA. While BMI, gluconeogenesis, total cholesterol and LDL-c approached control values although still not fully normalized. It is concluded that either insulin or estrogen therapy provided only partial improvement of the metabolic error of estrogen deficiency with diabetes while the best cure was found with combined estradiol and insulin therapy which achieved successful optimization of weight gain, reduced adiposity, tight glycemic control, alleviated dyslipidemia and normal oxidative state. Thus, insulin therapy together with hormonal replacement therapy as a coadjuvant might be the most advisable line of treatment in post menopausal diabetic women.

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Keywords: Metabolic Effect; Estrogen; Insulin; Ovariectomize; Diabetic; Rat

1. Introduction:

Many postmenopausal women live with diabetes mellitus; however, little information is available about how the changes that occur around the time of menopause might uniquely affect management of diabetes mellitus ⁽¹⁾.

As noticed in the literature, postmenopausal diabetic patients encountered the reality of increased atherogenic lipid profile ⁽²⁾, as well as redox imbalance ⁽³⁾, and thereby increased cardiovascular risk factors. However, the role of hormonal replacement therapy in reversing such threats remains a subject of debate.

There are conflicting data on the effect of hormonal replacement therapy in postmenopausal diabetic women. On one hand, Borissova *et al.* ⁽⁴⁾ recommended the use of hormonal replacement therapy in diabetic postmenopausal women because of its favorable effect. On the other hand, Feher and

Isaacs ⁽⁵⁾ denied the potential benefits of hormonal replacement therapy. In addition, some evidence suggests that estrogen therapy may improve insulin sensitivity ^(6,7).

It was therefore, worthwhile to investigate the effect of estrogen treatment alone and insulin treatment alone versus combination of these two hormonal therapies on modulating the metabolic error presented by altered glycemic and lipid metabolism in estrogen deficient experimentally diabetic rats.

2. Materials and Methods Experimental animals:

The present study was performed on 92 female Wistar rats. The rats were purchased from Research Institute of Ophthalmology (Giza) and were maintained in Physiology Department Animal House, under standard conditions of boarding and given regular diet consisting of bread, vegetables and milk with free access to water.

Experimental protocol: Rats were divided into 5 groups:

- Group 1: Sham-operated control rats (Sham) (n =16). Rats in this group were subjected to all surgical procedures of ovariectomy except for removal of ovaries. 2 weeks later, they received a single i.p. injection of 0.05 M citrate buffer (1 ml/kg) and were studied 2 weeks later.
- Group 2: Ovariectomized streptozotocin diabetic rats (OVX–STZ) (n =32). Rats in this group were subjected to bilateral ovariectomy. 2 weeks after the operation, they received a single i.p. injection of STZ and were studied after Two weeks.
- Group 3: Estradiol-treated ovariectomized STZ diabetic rats (E_2 +OVX–STZ) (n =16). Rats in this group were subjected to bilateral ovariectomy and on the next day, they received subcutaneous injection of estradiol (50µg/kg), daily 6 days/week for 4 weeks⁽⁸⁾. Two weeks later, they received a single i.p. injection of STZ then studied 2 weeks later.
- Group 4: Insulin-treated ovariectomized STZ diabetic rats (Insulin+OVX–STZ) (n =13). Rats in this group were subjected to bilateral ovariectomy. 2 weeks later, they were rendered diabetic then received daily subcutaneous injection of insulin in a dose of 10 or 20 IU/kg/day, 6 days/week for 2 weeks according to their blood glucose level ⁽⁹⁾.
- Group 5: Estradiol-treated, Insulin-treated ovariectomized STZ diabetic rats (E_2 + Insulin + OVX – STZ) (n =15). Rats in this group were subjected to bilateral ovariectomy followed on the next day by subcutaneous injection of estradiol for 4 weeks. Two weeks after the operation, rats were rendered diabetic then treated with daily subcutaneous injection of insulin for 2 weeks.

Experimental Procedure

On the day of experiment, overnight fasted rats were tested for re-estimation of blood glucose level via rat tail sampling using blood glucose test strips. Then, rats were weighed and anaesthetized i.p with thiopental sodium 40 mg/kg (Sandoz, Austria). The length of the anaesthetized rat was measured from tip of the nose to the anus to calculate body mass index (BMI) according to the following equation: DMI = Body weight (kg) (length (m2))

BMI = Body weight (kg) / length (m²).

A midline abdominal incision was made and blood samples from the abdominal aorta were collected into two plastic tubes. One tube containing sodium fluoride / potassium oxalate, for immediate determination of blood glucose concentration. The other tube containing EDTA, for preparation of plasma which was stored at -20 °C for later determination of plasma leptin, estradiol, insulin, malondialdehyde (MDA) and lipid profile (total cholesterol, triglyceride and high density lipoproteincholesterol (HDL-c).

Immediately after blood collection, both kidneys were exposed and excised from the renal pedicle and placed separately in ice cold Krebs Ringer solution for 10 minutes after which cortical kidney slices were prepared for *in vitro* estimation of glucose output by both kidneys. Then, the diaphragm was exposed, quickly and carefully excised then immediately placed in ice cold Krebs' solution for *in vitro* estimation of glucose uptake by diaphragm.

Methods:

I. Biochemical studies

Blood glucose was determined by glucose oxidase enzymatic colorimetric technique, according to the method described by Trinder ⁽¹⁰⁾, using kits supplied by Stanbio, USA. Plasma lipids (triglycerides, total cholesterol and HDL-cholesterol) were measured by quantitative enzymatic colorimetric methods ^(11, 12) using kits supplied by Stanbio-laboratory, Texas, U.S.A. Plasma LDL-cholesterol and atherogenic index (AI) were calculated according to Friderwald et al. ⁽¹¹⁾ and Grundy et al. ⁽¹³⁾ respectively as follows:

LDL-c = Total cholesterol - (triglyceride/5 + HDL-c)

Atherogenic index = <u>Total cholesterol</u> HDL-c

Malondialdehyde (MDA) was assayed in plasma, according to the method of Esterbauer and Cheeseman ⁽¹⁴⁾, as thiobarbituric acid reactive substance.

Plasma estradiol was estimated by radioimmunoassay using RIA estradiol kit, supplied by Immunotech, France. Plasma insulin was measured quantitatively by immunoradiometric assay using Insulin (e) IRMA kit supplied by Immunotech, Czech Republic. The measurement of plasma estradiol and insulin levels were performed in Middle Eastern Regional Radioisotope Center for Arab Countries, Cairo.

Plasma leptin was determined quantitatively by Elisa technique using leptin (sandwich) ELISA kit supplied by DRG, Germany. The measurement was performed in Oncology Diagnostic Unit, Biochemistry Department, Faculty of Medicine, Ain Shams University.

II. *In vitro* determination of glucose uptake by the diaphragm was performed according to the method described by Mohamed *et al.* ⁽¹⁵⁾.

III. *In vitro* determination of glucose output by both kidneys
Cortical kidney slices from both right and left kidneys were separately used to measure glucose output by the kidney according to the method described by Randall ⁽¹⁶⁾ with few modifications.

Statistical Analysis:

Results were statistically analyzed by one-way ANOVA for differences between means of different groups. Further analysis was made by least significant difference (LSD) to find inter-groupal significance. For differences within the same group, analysis was determined by Student's 't' test for paired data. Correlations and lines of regression were calculated by linear regression analysis using the Least Square Method. All data were analyzed using SPSS statistical package (SPSS Inc.) version 8.0.1. A probability of P<0.05 was considered statistically significant.

3. Results:

Body weight and body mass index (BMI) changes

In table (1), treatment of OVX-STZ diabetic rats with estrogen alone, insulin alone as well as with combined estradiol and insulin resulted in significant increase in final body weights as compared to their initial values (P<0.001).

The final body weight was significantly increased in insulin-treated OVX-STZ rats as compared to OVX-STZ, estradiol-treated and sham-operated groups (P<0.001, P<0.001 & P<0.01 respectively). Estradiol-treated OVX-STZ diabetic group demonstrated non significant difference as compared to OVX-STZ group. Combined treatment with estradiol and insulin showed non significant difference as compared to OVX-STZ and estradioltreated groups but significant decrease as compared to insulin-treated group (P<0.001).

BMI showed significant increase in insulintreated OVX-STZ diabetic rats as compared to OVX-STZ (P<0.001) and estradiol –treated (P<0.001) groups. Combined treatment with estradiol and insulin showed significant decrease in BMI as compared to insulin-treated OVX-STZ rats (P<0.001) while non significant difference as compared to both OVX-STZ and E_2 +OVX-STZ groups.

Glycemic parameters

As shown in table (2), OVX-STZ diabetic rats demonstrated significant increase in blood glucose (P<0.001) as well as in glucose output by both right and left kidneys (P<0.001) but significant decrease in glucose uptake by the diaphragm (P<0.001) as compared to sham control rats.

Treatment with either estradiol alone or insulin alone as well as combined therapy with estradiol and insulin resulted in significant decrease in blood glucose (P<0.001) and glucose output by right and left kidneys (P<0.001) and significant increase in glucose uptake by the diaphragm (P<0.001) as compared to OVX-STZ rats. Compared to estradiol treatment, insulin treatment caused significant decrease in blood glucose (P<0.001) and in right and left kidneys glucose output (P<0.05 & P<0.001 respectively) but a similar effect on diaphragmatic glucose uptake. Rats receiving combined treatment with estradiol and insulin demonstrated significantly lower blood glucose (P<0.001) and glucose output of both kidneys (P<0.001) together with significantly higher glucose uptake (P<0.001) than rats receiving estradiol treatment alone as well as significantly higher glucose uptake (P<0.001) than rats receiving insulin treatment alone.

Lipid profile

OVX–STZ group showed significant increase in plasma triglycerides (P<0.001), total cholesterol (P<0.001), LDL-c (P<0.001) and atherogenic index (P<0.001) but significant decrease in HDL-c (P<0.001) as compared to sham-operated group.

In estradiol-treated OVX–STZ diabetic rats, plasma triglycerides, total cholesterol and LDL-c showed significant decrease as compared to OVX-STZ group (P<0.001) but significant increase as compared to sham control group (P<0.05, P<0.001 and P<0.001, respectively), the atherogenic index was decreased as compared to OVX-STZ group (P<0.001) but was insignificant from sham control group whereas plasma HDL-c was increased as compared to OVX-STZ group (P<0.001) but decreased as compared to sham control rats (P<0.05).

In insulin-treated OVX-STZ diabetic rats, plasma triglycerides was lower than in OVX-STZ group (P<0.001) but higher than in estradiol-treated rats (P<0.01) and sham control rats (P<0.001), total cholesterol was lower than in both OVX-STZ (P<0.001) and E_2 -OVX-STZ (P<0.01) rats, plasma HDL-c was higher than in OVX-STZ group (P<0.001) but lower than in sham-control (P<0.001) and estradiol-treated (P<0.001) groups and atherogenic index was lower than in OVX-STZ group (P<0.001) but was higher than in sham-control group (P<0.001).

In combined estradiol and insulin treated ovariectomized diabetic group, plasma triglycerides showed significant decrease when compared to non-treated OVX-STZ (P<0.001) and insulin-treated groups (P<0.001), total cholesterol was decreased as compared to OVX-STZ rats (P<0.001) but was increased as compared to sham-operated control (P<0.01) and insulin-treated (P<0.05) groups, HDL-c was increased when compared to OVX-STZ (P<0.001) and insulin-treated ovariectomized diabetic (P<0.001) and insulin-treated ovariectomized diabetic (P<0.001) groups, LDL-c was decreased as compared to OVX-STZ (P<0.001) groups, LDL-c was decreased as compared to OVX-STZ (P<0.001) groups, LDL-c was decreased as compared to OVX-

STZ group (P<0.001) but was insignificant from either estradiol treatment or insulin treatment alone and the atherogenic index was significantly decreased as compared to OVX-STZ (P<0.001) and insulintreated (P<0.05) groups but was insignificant from estradiol-treated and sham control groups (table 3).

Plasma levels of malondialdehyde, estradiol, insulin and leptin are shown in figure 1.

Correlation studies among the experimental groups:

Correlations of plasma estradiol levels and plasma insulin levels versus other parameters in untreated ovariectomized STZ-diabetic (OVX-STZ), estradiol-treated ovariectomized diabetic (E_2 +OVX–STZ), insulin-treated ovariectomized diabetic (Insulin+OVX–STZ) and estradiol-treated, insulin-treated ovariectomized diabetic (E_2 + Insulin + OVX – STZ) groups are displayed in tables 4 & 5; figures 2 &3.

		Initial BW	Final BW	BMI
		(g)	(g)	(Kg/m²)
Sham	(16)	181 <u>+</u> 11.5	214 <u>+</u> 13.3 *	5.6 <u>+</u> 0.2
OVX -STZ	(32)	195 <u>+</u> 5.0	195 <u>+</u> 4.8	5.1 <u>+</u> 0.09 ^a
$E_2 + OVX - STZ$	(16)	174 <u>+</u> 5.7	$188 \pm 6.6 * a$	5.2 <u>+</u> 0.12
Insulin+ OVX – STZ	(13)	212 <u>+</u> 4.6	258 <u>+</u> 5.9 * ^{a b c}	6.3 <u>+</u> 0.13 ^{a b c}
E ₂ +Insulin +OVX – ST	TZ (15)	164 <u>+</u> 5.1	198 <u>+</u> 5.2 * ^d	5.1 ± 0.06^{ad}

Number in parenthesis is the number of rats in each group. Results are expressed as means \pm SEM. *: Significance by student's "t" test at p<0.05 from respective baseline value for paired data.

a: significance from sham group by LSD. b: significance from OVX-STZ group by LSD. c:significance from E_2 + OVX-STZ group by LSD. d: significance from insulin + OVX-STZ group by LSD.

output by the left kinney in the studied groups.								
	Blood	Glucose uptake	Glucose output	Glucose output				
	glucose	by diaphragm	by right kidney	by left kidney				
	(mg/dl)	(mg/g/90min)	(mg/g/hr)	(mg/g/hr)				
Sham	78 <u>+</u> 2.6	6.7 <u>+</u> 0.2	3.4 <u>+</u> 0.15	3 <u>+</u> 0.2				
	(16)	(16)	(15)	(16)				
OVX – STZ	480 <u>+</u> 15.4 ^a	1.3 ± 0.04^{a}	9.1 <u>+</u> 0.32 ^a	9.1 <u>+</u> 0.23 ^a				
	(32)	(32)	(32)	(32)				
$E_2 + OVX - STZ$	213 <u>+</u> 4.2 ^{a b}	3.9 <u>+</u> 0.18 ^{a b}	6.1 <u>+</u> 0.22 ^{a b}	6.5 <u>+</u> 0.25 ^{a b}				
	(16)	(16)	(16)	(16)				
Insulin + OVX –	97 <u>+</u> 4.5 ^{b c}	3.7 <u>+</u> 0.23 ^{a b}	4.9 <u>+</u> 0.19 ^{a b c}	4.8 <u>+</u> 0.32 ^{a b c}				
STZ	(13)	(13)	(13)	(13)				
E ₂ + Insulin +	94 <u>+</u> 2.7 ^{b c}	$6.4 \pm 0.23^{b c d}$	4.3 <u>+</u> 0.11 ^{b c}	4.4 ± 0.18^{abc}				
OVX-STZ	(15)	(15)	(15)	(15)				

Table (2): Blood glucose, glucose uptake by the diaphragm, glucose output by the right kidney and glucose output by the left kidney in the studied groups.

Number in parenthesis is the number of rats in each group. Results are expressed as means \pm SEM. a: significance from sham group by LSD. b: significance from OVX-STZ group by LSD. c:significance from E₂ + OVX-STZ group by LSD. d: significance from insulin + OVX-STZ group by LSD.

Table	(3): Plasma	triglycerides	(TG),]	plasma	total	cholesterol	(TC),	plasma	high	density	lipoprotein
	cholestero	ol (HDL-c), pla	isma low	density	/ lipop	rotein chole	sterol (LDL-c) a	and at	herogeni	c index (AI)
	in the stud	lied groups.									

	0					
		TG (mg/dl)	TC (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	AI
Sham	(16)	46 ± 1.40	85 ± 1.1	52 ± 0.93	24 ± 1.19	1.6 ± 0.05
OVX –STZ	(32)	107 ± 1.9^{a}	129 ± 1.1^{a}	26 ± 0.57 ^a	82 ± 1.33 ^a	5.1 ± 0.12^{a}
E ₂ + OVX–STZ	(16)	56 ± 1.14 ^{ab}	98 ± 2.4 ^{ab}	48 ± 2.1^{ab}	39 ± 1.03 ^{ab}	2.1 ± 0.05 ^b
Insulin + OVX–STZ	(13)	$69 \pm 3.89^{\text{abc}}$	88 ± 1.4 ^{bc}	$37 \pm 1.19^{\text{ abc}}$	37 ± 1.28 ^{ab}	2.4 ± 0.06 ^{ab}
E ₂ +Insulin + OVX–STZ	(15)	52 ± 1.11 ^{bd}	94 ± 1.5 ^{abd}	49 ± 0.98 ^{bd}	34 ± 1.37 ^{ab}	1.9 ± 0.03 ^{bd}

Number in parenthesis is the number of rats in each group. Results are expressed as means \pm SEM. a: significance from sham group by LSD. b: significance from OVX-STZ group by LSD. c:significance from E₂ + OVX-STZ group by LSD. d: significance from insulin + OVX-STZ group by LSD.



Figure (1): Mean values of plasma levels of malondialdehyde (MDA), estradiol, insulin and leptin in the different groups studied.

¶: P<0.05, #: P<0.01, *: P<0.001, a: significance from sham group by LSD. b: significance from OVX-STZ group by LSD. c: significance from E_2 + OVX-STZ group by LSD. d: significance from insulin + OVX-STZ group by LSD.

	01	X - STZ	01	$E_2 + \sqrt{X - STZ}$		nsulin + VX –STZ	$E_2 - O$	+Insulin + VX_STZ
Parameters		Sham	-	+ Sham	-	- Sham	-	+ Sham
	r	Р	r	Р	r	Р	r	Р
Blood glucose	-0.88	< 0.001	-0.86	< 0.001	-0.57	< 0.01	-0.63	< 0.01
	(28)		(24)		(22)		(23)	
BMI	0.22	NS	0.13	NS	-0.63	< 0.01	0.36	NS
	(28)		(24)		(22)		(23)	
Plasma	-0.91	< 0.001	-0.62	< 0.01	-0.54	< 0.05	-0.46	< 0.05
TG	(28)		(24)		(22)		(23)	
Plasma	-0.85	< 0.001	-0.42	< 0.05	-0.20	NS	-0.72	< 0.001
TC	(28)		(24)		(22)		(23)	
Plasma HDL-c	0.88	< 0.001	0.49	< 0.05	0.82	< 0.001	0.30	NS
	(28)		(24)		(22)		(23)	
Plasma LDL-c	-0.86	< 0.001	-0.76	< 0.001	-0.81	< 0.001	-0.72	< 0.001
	(28)		(24)		(22)		(23)	
Atherogenic	-0.87	< 0.001	-0.75	< 0.001	-0.82	< 0.001	-0.61	< 0.01
index	(28)		(24)		(22)		(23)	
Plasma Insulin	0.86	< 0.001	0.80	< 0.001	0.59	< 0.05	0.70	< 0.01
	(19)		(19)		(17)		(18)	
Plasma Leptin	0.76	< 0.001	0.28	NS	0.50	NS	0.06	NS
	(18)		(15)		(15)		(17)	
Plasma MDA	-0.83	< 0.001	-0.68	< 0.001	-0.87	< 0.001	-0.34	NS
	(28)		(24)		(22)		(23)	

Table (4): Correlation co	oefficients (r) between	plasma estradiol	levels and other	parameters in the groups of
rats studied.		-		

In parenthesis is the number of observations.

NS: not significant

Table (5): Correlation coefficients (r) between plasma insulin levels and other parameters in the groups of rats studied.

	0V +	'X – STZ - Sham	01	$E_2 + VX - STZ$	II VO	nsulin + /X –STZ	$E_2 + OV$	Insulin + /X–STZ
Parameters			-	+ Sham	+	- Sham	+	Sham
	r	Р	r	Р	r	Р	r	Р
Blood	-0.91	< 0.001	-0.90	< 0.001	-0.49	< 0.05	-0.69	< 0.001
glucose	(28)		(24)		(22)		(22)	
Glucose uptake	0.97	< 0.001	0.89	< 0.001	0.79	< 0.001	0.47	< 0.05
by diaphragm	(28)		(24)		(22)		(22)	
Glucose output	-0.86	< 0.001	-0.74	< 0.001	-0.48	< 0.05	-0.47	< 0.05
by right kidney	(28)		(24)		(22)		(22)	
Glucose output	-0.94	< 0.001	-0.83	< 0.001	-0.61	< 0.01	-0.65	< 0.01
by left kidney	(28)		(24)		(22)		(22)	
BMI	0.19	NS	-0.09	NS	- 0.62	< 0.01	-0.03	NS
	(28)		(24)		(22)		(22)	
Plasma TGs	-0.91	< 0.001	-0.66	< 0.001	-0.59	< 0.01	-0.40	NS
	(28)		(24)		(22)		(22)	
Plasma TC	-0.93	< 0.001	-0.56	< 0.01	-0.23	NS	-0.48	< 0.05
	(28)		(24)		(22)		(22)	
Plasma HDL-c	0.94	< 0.001	0.31	NS	0.62	< 0.01	0.29	NS
	(28)		(24)		(22)		(22)	
Plasma HDL-c	-0.94	< 0.001	-0.71	< 0.001	-0.54	< 0.05	-0.49	< 0.05
Athono gonio indov	(28)	<0.001	(24)	<0.001	(22)	<0.01	(22)	<0.05
Atherogenic index	-0.93	<0.001	(24)	<0.001	(22)	<0.01	(22)	<0.05
Plasma Lentin	0.88	< 0.001	0.61	< 0.01	0.75	< 0.001	0.55	<0.05
r monia Depun	(27)		(20)		(20)		(20)	
Plasma MDA	-0.87	< 0.001	-0.71	< 0.001	-0.65	< 0.01	-0.21	NS
	(28)		(24)		(22)		(22)	

In parenthesis is the number of observations.

NS: not significant



Figure (2): Graphs showing relationships between plasma estradiol and blood glucose in (A) Untreated OVX-STZ diabetic group + Sham-operated controls (r = -0.88, P<0.001, n = 28). (B) Estradiol-treated OVX-STZ diabetic group + Sham-operated controls (r = -0.86, P<0.001, n =24). (C) Insulin-treated OVX-STZ diabetic group + Sham-operated controls (r = -0.57, P<0.01, n =22). (D) Combined estradiol and insulin-treated OVX-STZ diabetic group + Sham-operated controls (r = -0.63, P<0.01, n =23).



Figure (3): Graphs showing relationships between plasma estradiol and plasma MDA in (A) Untreated OVX-STZ diabetic group + Sham-operated controls (r = -0.83, P<0.001, n=28). (B) Estradiol-treated OVX-STZ diabetic group + Sham-operated controls (r = -0.68, P< 0.001, n = 24). (C) Insulin-treated OVX-STZ diabetic group + Sham-operated controls (r = -0.87, P<0.001, n=22). (D) Combined estradiol and insulin –treated OVX-STZ diabetic group + Sham-operated controls (r = -0.34, NS, n=23).

4. Discussion:

The present work studied the extent by which either estradiol treatment or insulin treatment can improve the metabolic derangements in estrogen deficiency with diabetes and finally evaluated the gain achieved by combination of these two hormonal therapies.

Estradiol treatment was found to exert an obvious effect in improving adiposity, dyslipidemia, oxidative stress and hyperglycemia, yet still diabetic; however, with the exception of adiposity, none of the metabolic parameters were back to control values. Insulin therapy successfully normalized blood glucose, reduced dyslipidemia and oxidative stress; however, estradiol therapy was more effective in reducing oxidative stress and adiposity compared to insulin therapy. Combination of both hormonal therapies in estradiol-treated, insulin-treated ovariectomized diabetic group offered the most beneficial effect as it successfully resulted in optimization of weight gain, reduced adiposity, good glycemic control, reduced dyslipidemia and normal oxidative state. This denotes that insulin therapy together with hormonal replacement therapy as a coadjuvant might be the most advisable line of treatment in post menopausal diabetic women.

In insulin-treated group, blood glucose was normalized, diaphragmatic glucose uptake was increased as compared to untreated group but did not differ from estradiol-treated group and was still significantly lower than the control values. Renal glucose output was decreased as compared to untreated and estradiol treated groups but still significantly higher than control values. It seems that insulin therapy exerted better euglycemia compared to estrogen therapy and this effect is probably via decreased gluconeogenesis rather than complete normalization of peripheral glucose uptake which could be due to insufficient dose or duration.

More favourable lipid profile was seen following insulin control of hyperglycemia, where it had prominent effect in lowering total cholesterol towards normal as well as improving triglycerides, HDL-c, LDL-c and AI as compared to untreated ovariectomized diabetic group but not back to control values. When compared to estradiol-treated group significant increase in triglycerides and decrease in total cholesterol as well as HDL-c was recorded together with non significant difference in LDL-c and AI. Insulin is the major hormone to inhibit hydrolysis of triglycerides (TGs) in adipose cells into glycerol and FFAs. Together with glucose, insulin may also play a role in the reesterification of FFAs in adipose cells, promoting TG storage (17). By these mechanisms, insulin lowers plasma FFA levels which are the major substrates for TG synthesis in the liver, and they stimulate apoB secretion from the liver ⁽¹⁸⁾. Thus, the ability of insulin to suppress plasma FFA concentrations plays a major role in hepatic VLDL TG synthesis and secretion, apoB secretion ⁽¹⁸⁾, and plasma VLDL cholesterol and apoB concentrations ⁽¹⁹⁾.

Following insulin treatment body weight and BMI were significantly high, this could be attributed to insulin role in promoting lipogenesis as well as its inhibitory role on protein catabolism (positive nitrogen balance) at the same time, the persisted estradiol deficiency promoted central fat deposition, so it is most likely that the increase in weight is mostly due to fat deposition as confirmed by increased BMI. The associated increase in plasma leptin level seems to be insulin-induced as insulin was reported to stimulate leptin mRNA and protein expression, due to increased activation of the leptin gene promoter⁽²⁰⁾.

Suppression of oxidative stress was one of the goals achieved by insulin-mediated metabolic control. Hyperglycemia is the major causative factor of raised oxidative stress in diabetes, but in this group of rats, the insulin treated ovariectomized diabetic, participates; estrogen deficiency also SO administration of insulin alone reversed hyperglycemia with no obvious effect on estradiol values which led to partial improvement of oxidative stress, yet not back to normal values. It is worthmentioning here that, estradiol treatment showed more effective role in reducing oxidative stress than do insulin treatment although none of them succeeded to get it back to normal control values.

Unexpectedly, insulin treatment did not enhance aromatase activity and thereby estradiol production, instead estradiol values were very close to untreated ovariectomized diabetic rats. This could be attributed to the duration of insulin therapy or less than needed dose further investigations are needed to clarify this point.

Estradiol replacement was another line of treatment in the present study, the overall judgment about how much estradiol treatment was able to improve the metabolic state is that, although most of the measured parameters were significantly improved when compared to untreated ovariectomized diabetic rats, yet they were still significantly different from control values, a result that make it possible to say that hormonal replacement therapy per se is not sufficient to maintain good glycemic and metabolic control in postmenopausal women who developed diabetes. The significant decrease in weight gain following estradiol treatment seems to be a combined effect of estradiol and diabetes, where besides the diabetic effect on lipid and protein metabolism, estradiol goes through different pathways to achieve an obesity reducing property, where it is known that estrogen decreases central adiposity ⁽²¹⁾. This represents a major health problem because abdominal visceral fat show greater lipolytic sensitivity than femoral and gluteal subcutaneous fat due to fewer inhibitory alpha adrenergic receptors in abdominal regions and greater alpha adrenergic receptors in gluteal and femoral regions ⁽²²⁾.

Estradiol treatment was associated with significant rise in plasma leptin, this could be another mechanism decreasing food intake and increasing energy expenditure and thereby decreasing body weight. Our results are in agreement with Shimizu et al. (23) who experimentally proved that estradiol supplementation reversed the inhibitory effect of ovariectomy on ob gene expression and circulating leptin levels and that serum leptin concentration was higher in premenopausal women than in men and postmenopausal women; this allowed them to declare that estrogen increased in vivo leptin production in rats and human subjects. However studies evaluating the effect of estrogen replacement therapy on leptin levels were contradictory, with some authors supporting a stimulatory effect of estrogen whereas others suggested that estrogens do not have a stimulatory action on leptin in humans ^(24, 25).

Estradiol relation to ghrelin hormone provides another mechanism explaining the decrease in weight gain in this group, where, estradiol was found to attenuate the orexigenic action of ghrelin ⁽²⁶⁾ and the drop of estrogen levels following ovariectomy was associated with an increase in plasma ghrelin that was associated with increased food intake, body weight, and hypothalamic neuropeptide Y ⁽²⁶⁾. From the above mentioned estradiol hormonal interactions, it could be suggested that estrogen, indeed provides protection against weight gain.

Better glycemic control was clearly demonstrated after estradiol treatment where renal gluconeogenesis was significantly decreased and skeletal glucose uptake was significantly improved and thereby blood glucose level was significantly reduced compared to untreated group, yet, their improvement was not to such an extent that their values were back to normal. This finding is consistent with results obtained from ovariectomized diabetic group that showed tendency to hyperglycemia. All these positive changes towards better glycemic control could be attributed to the roles played by estradiol both at -cells of the pancreas as well as peripheral insulin-sensitive tissues. It was found that 17 -estradiol at

physiological concentrations protects pancreatic cells against lipotoxicity, oxidative stress, and apoptosis ⁽²⁷⁾. Estrogens and their receptors (ER) have direct effects on islet biology. The estrogen receptor ER, ER, and the G-protein coupled ER are present -cells and enhance islet survival. They, also, in improve islet lipid homeostasis and insulin biosynthesis ⁽²⁷⁾. In vivo, estradiol treatment rescued streptozotocin-induced -cell apoptosis, helped sustain insulin production, and prevented diabetes. In vitro, in mouse pancreatic islets and -cells exposed to oxidative stress, estradiol prevented apoptosis and protected insulin secretion. Estradiol protection was through activation of ER as it was partially lost in cells and islets treated with an ER antagonist ⁽²⁸⁾.

At the peripheral insulin-sensitive tissues, estradiol is known to modulate insulin sensitivity and, consequently, glucose homeostasis. Estradiol was found to counteract the effects of hyperglycemiainduced downstream of the insulin receptors, as well modulating insulin receptors tyrosine as phosphorylation⁽²⁹⁾. Some data, also, revealed a surprising role for estradiol in regulating energy metabolism and opened new insights into the role of the two estrogen receptors. ER and ER, in this context. New findings on gene modulation by ER and ER of insulin-sensitive tissues indicate that estradiol participates in glucose homeostasis by modulating the expression of genes that are involved in insulin sensitivity and glucose uptake ⁽³⁰⁾. Therefore, drugs that can selectively modulate the activity of either ER or ER in their interactions with target genes represent a promising frontier in diabetes mellitus coadjuvant therapy.

Skeletal muscle glucose uptake is maintained by one of the isoforms of the glucose transporter family, GLUT4 ⁽³¹⁾. The rate of glucose transport into muscle cells is limited by the concentration of GLUT4 at the cell surface. The enhancement of diaphragmatic glucose uptake following estradiol administration shown in this study could partly be attributed to the elevated glucose transporter–4 protein expression. It was discovered that estradiol acts on ER and not ER to enhance glucose transporter-4 expression ⁽³²⁾.

The antiatherogenic cardiovascular protective properties of estrogen emerge from its ability to direct the lipoprotein metabolism towards higher HDL-c and lower LDL-C. It is obvious from our results that treatment with estradiol gave the ovariectomized diabetic females the opportunity of lowering their plasma lipids, and this effect was prominent by the significant improvement of all the measured lipid parameters as compared to non treated group of rats.

The mechanisms of such effects are mediated through the ability of estradiol to stimulate the

expression of LDL- receptor gene and increasing the number of LDL receptors. This effect was confirmed by Parini *et al.* ⁽³³⁾ who found that treatment of rats with ethyl estradiol for 7 days increased the hepatic LDL receptor protein and mRNA level from 3 to 4 folds. Also, Distefano et al. ⁽³⁴⁾ reported that the expression of LDL-receptor gene is stimulated by estrogen in vivo. Also, our results came in accordance with Granfone et al. ⁽³⁵⁾ and Walsh et al. ⁽³⁶⁾ who reported that estrogen replacement is effective in decreasing LDL-c and apo B concentrations and increasing HDL-c and apo A concentrations in dyslipidemic postmenopausal women. LDL-c internalizes into the cells through the process of LDL-receptor mediated endocytosis accelerating LDL catabolism. The expression of LDL-receptor on the cell surface is a function of various hormone regulated transcription of the receptor gene; -estradiol is considered the prime hormonal regulator of LDL-receptor expression ⁽³⁷⁾.

Another protective mechanism offered by estradiol in lowering LDL-c and increasing HDL-c is through depression of hepatic lipase enzyme activity ⁽³⁸⁾, thereby decreasing HDL-c catabolism. The elevated levels of HDL-c following estradiol treatment seen in our study is in agreement with Walsh et al. ⁽³⁹⁾ who demonstrated that HDL elevation following oral estradiol treatment in postmenopausal women is dose dependent. Estradiol fatty acyl esters incorporate into HDL and enhance the atheroprotective properties of HDL by mediating the initial steps of reverse cholesterol transport ⁽⁴⁰⁾.

Another benefit offered by estrogen replacement therapy in ovariectomized diabetic rats was the significant decrease in plasma MDA, adding extra evidences that estrogen is more than a sex hormone and that its loss after menopause requires therapeutic intervention. It was found that lipid peroxidation is most often induced by reactive oxygen species, O_2^{-1} and H_2O_2 , and this damage is inhibited by superoxide dismutase and catalase. The remaining amount of damage appears to be caused by peroxyl radicals. It was documented that estradiol alone collectively blocks 70% of such damage ⁽⁴¹⁾.

This indicates that estradiol is acting as a chainbreaking antioxidant, inhibiting the effect of H_2O_2 , $\cdot O_2^{-}$ and hydroperoxyl radicals. E_2 action in inhibiting DNA damage supports this view. E_2 prevented DNA strand breaks in a manner similar to the free radical scavengers; catalase and superoxide dismutase. E_2 might be preventing oxidative DNA damage to some extent by inhibiting the formation of superoxides ⁽⁴¹⁾. Our results came in accordance with Kii et al. ⁽⁴²⁾ who demonstrated that acute treatment with 17betaestradiol showed a protective effect against ischemiareperfusion injury through its antioxidant effects. Also, Hernández et al. ⁽⁴³⁾ showed that the lower plasma total antioxidant status, reduced thiol groups and the increase in plasma lipoperoxides presented in ovariectomized animals were reestablished with the estrogen treatment. It is to be noted here that estradiol treatment although successfully lowered plasma MDA yet it was still higher than control values this is attributes to that these rats are diabetic which represents other cause of oxidative stress.

Combination of estradiol and insulin therapy was the last line of treatment investigated in ovariectomized diabetic rats. Rats received combined treatment of estradiol and insulin showed normal pattern of gaining weight with lower BMI; this proves that normal hormonal state is essential to direct metabolism towards optimal balance between opposite metabolic pathways as lipolysis versus lipogenesis, glycolysis versus gluconeogenesis and positive versus negative nitrogen balance. It seems that the lowered adiposity seen in these rats with normal weight gain reflects the anabolic action of insulin on protein metabolism. The additive action of estradiol and insulin supplementation on leptin hormone was manifested by higher levels of this hormone in combined hormone-treated than in estradiol-treated alone or insulin-treated alone ovariectomized diabetic rats. Estradiol enhanced leptin gene expression ⁽²³⁾ and also insulin stimulated leptin mRNA and protein expression ⁽²⁰⁾. The resulted increase in leptin values ultimately contributes in optimization of body weight.

Combined estradiol and insulin treatment successfully normalized blood glucose through optimization of skeletal muscle uptake of glucose as well as renal gluconeogenesis. It is believed that estradiol not only increases insulin secretion from -cells but also enhanced insulin pancreatic sensitivity in target organs ⁽⁴⁾, an effect that is most obviously seen in our study through increasing diaphragmatic glucose uptake. Also, the significant positive correlation between plasma estradiol and insulin hormones seen in untreated ovariectomized diabetic rats showed less positivity following combined estradiol and insulin treatment implying an effect of estradiol on insulin hormone and thereby glycemic control.

Insulin and estradiol teamed up to shift lipid profile towards more protective healthy picture, actually triglycerides, HDL-c and atherogenic index values were completely normalized. In addition, significant negative correlations existed between both estradiol and insulin and each of total cholesterol, LDL-c and atherogenic index, whereas, the significant positive correlation between both levels of plasma estradiol and insulin with HDL-C were abolished. These results, therefore, encourages postmenopausal diabetic women not only to control their blood glucose but also to start hormonal replacement therapy.

Moreover, marked reduction of oxidative stress was recorded, following estradiol and insulin therapy, as the lipid peroxide marker, MDA, was significantly reduced to control values. Also, the significant negative correlations between plasma levels of each of estradiol and insulin and levels of MDA, found in the ovariectomized STZ-diabetic group were abolished by combined treatment.

In explanation of these results, it could be suggested that estradiol by its antioxidant effect reduces reactive oxygen species and insulin by its hypoglycemic effect reduces blood glucose and thereby glycosylation and autooxidation of glycation products⁽⁴⁴⁾.

In view of the aforementioned data, insulin therapy alone which induced euglycemia, reduced dyslipidemia and oxidative stress yet their values were still higher than controls; while estrogen therapy in ovariectomized diabetic rats succeeded to some extent in reducing hyperglycemia, dyslipidemia and oxidative stress yet not completely normalized. Thus, from the above discussion it is clear that postmenopausal diabetic women suffer the consequences of both estradiol and insulin deficiency, and trials to reverse any of them although to some extent improved the condition yet they were not optimally successful. Combination of both estradiol and insulin therapies in ovariectomized diabetic rats showed synergistic effects and was superior in terms of optimization of blood glucose and plasma malondialdehyde together with alleviation of dyslipidemia.

Therefore, we may advocate the use of estrogen replacement therapy side by side with insulin in postmenopausal diabetic women to achieve better glycemic control and thereby improving the general metabolic state.

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Evaluation of Lumbo-Pelvic Stabilizing Exercises in the Treatment of Backache after Normal Labour.

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Abstract: This study was designed to evaluate the effect of Lumbo-pelvic stabilizing exercises in the treatment of post partum backache. Twenty volunteers women diagnosed with post partum backache, their ages ranged from 25-35years, they were delivered normally and received stabilizing exercises for lumbo-pelvic muscles (24 sessions), 3 sessions per week for 8 week. All women were evaluated before and after the end of the treatment programme using visual analogue scale for pain assessment and Oswestry disability questionnaire for the assessment of functional disability The obtained results showed highly statistically significant decrease in pain intensity.(p<0.001), and improve the functional disability (p<0.01) at the end of the study Accordingly, it could be concluded that lumbo-pelvic stabilizing exercises appears to be effective in the management of post partum backache.

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Key words: post partum – backache – lumbo-pelvic stabilizing exercise -visual analogue scale – Oswestery disability questionnaire

1. Introduction:

More than 50% of women complain of some degree of low back pain during pregnancy, and many describe pubic, pelvic, hip, knee and various other joint discomforts. Backache often persists after delivery and may last up to one year .While the etiology of low back pain during pregnancy remains theoretical, three mechanisms regularly are described: biomechanical, musculoskeletal, hormonal and vascular (Darryl et al., 2007).

The classical hypothesis of low back pain postulates that weight gain experienced during pregnancy results in postural changes that produce pain. Due to the anterior displacement of the center of gravity of the trunk and abdomen, women may unconsciously shift their head and upper body posteriorly over their pelvis, inducing hyperlordosis of the lumbar spine. This shift generates stress on intervertebral disks, facet joints and ligaments, promoting joint inflammation. Inflammation and distension of the joint capsule create pain and increase sensitivity to movement (Macevilly and Buggy, 1996).

On the 917 women, who entered the study, 817(89%) responded completely, the presence of back pain at the time of delivery was reported by 67% of these. At follow up, 63% stated that they had no back pain, whereas 37% still had some. Although 26% were in the "much better" group, 4% were in the "somewhat improved" group, as compared to immediately after delivery,7% of the women said they had serious back pain 18 months after delivery. The pain was most often in the posterior pelvic and lumbar areas and was, on average, 3.2 on the visual analogue scale, which ranged from 0= no pain to 10=severe and intolerable pain (Ostgaard and Andersson, 1992).

Back pain after the birth can come from several sources. Muscle Strain during the actual birth can occur. The lower back muscles are used, along with the pelvic muscles, during a vaginal birth. Sometimes this pushing can strain the muscles or ligaments in the lumbar region of the back. Coccyx Pain is sometimes the result of a vaginal birth. The coccyx is flexible during labor and is supposed to move out of the way of the birth passage, allowing an easier delivery. Sometimes, the coccyx is more in the way than out of it and can be injured. These injuries occur mostly from the baby's head, as the baby descends the birth canal. Coccyx injuries can be very painful. Psychological back pain can begin or carry over as a continuation of pregnancy back pain. The subconscious mind might take the opportunity to use the end of pregnancy as a chance to start a psychologically induced pain syndrome (include depression, fatigue, listlessness, pain, malaise and anger) (Ostgaard and Andersson, 1992).

Recent research has focused on the importance of activation of muscles for motor control and stability of the lumbo-pelvic region (Vleeming et al., 1997).

Specific stabilizing exercise program in women with post partum pelvic pain improved functional status and reduced pain (Stuge et al., 2004).

The stabilizing sequence includes strengthening of the segmental muscles, neutral spine stabilization and finally strengthening the prime movers. The inter segmental muscles act as tonic or postural stabilizers of the spine, tending to be fatigued and atrophied after spinal injury. Therefore initial stabilization exercises are directed toward these muscles, which can control individual segmental mobility. The next phase is the stability training; it involves direct and indirect strengthening of muscle groups in neutral spine posture. Training begins with exercises designed to locate the neutral spine in a variety of body positions as prone lying, sitting and jumping which increase the awareness of lumbar and pelvic motion .This is followed by exercises of extremities while maintaining neutral spine and later with addition of resistance to the extremities, either manually or with weights. These exercises are performed slowly with the emphasis on precise pelvic control. This will facilitate neuromuscular coordination, enhance endurance, strength and also emphasize the smaller postural stabilizer (Weinstein et al., 1998).

Finally, strengthening the prime movers. Strengthening of the large and more superficial muscles of the trunk. These muscles are not only involved in moving the spine, but are also responsible for transferring load directly between the thoracic cage and the pelvis .The main function of the global muscles is to balance the external loads applied to the trunk so that the residual forces transferred to the lumbar spine can be handled by the local muscles (Britt et al., 2004).

The stabilization exercises were created to address the following goals: to focus training on particular muscles that are important for increasing stability, to represent the full range of potential levels of difficulty and to provide clear increase in difficulty based on increasing moment to the muscles stabilizing the lumbar spine. Lumber stabilization exercise should focus on the transverses abdominis and multifidus, because these muscles are the primary stabilizers of the spine (Wohlfart et al., (1993).

Contraction of the transversus abdominis significantly decreases the laxity of the sacroiliac joint in non pregnant women, and it might therefore be better to postpone the exercises until the post partum period (Richardson et al., 1999).

The co-contraction of the transversus abdominus and multifidi muscles occurred prior to any movement of the limbs. This suggests that these muscles anticipate dynamic forces, which may act on the lumbar spine and stabilize the area prior to any movement. They also showed that the timing of coordination of these muscles was very significant, and that back injury patients were unable to recruit their transversus abdominus and multifidi muscles early enough to stabilize the spine prior to movement. Furthermore, the multifidi muscle showed poor recruitment in back injury patients, again showing how the recruitment of these deep trunk muscles is very important (Saal and Saal 1989).

Mike et al. (2009) defined dynamic stabilization as the stability of the patient to be active through the day without increasing symptoms, a corner stone of this approach is to make every attempt to work the patient as vigorously as possible without increasing symptoms. As the patients progress in their ability to control the spinal functional position during various exercises, there should be a corresponding increase in the ability to perform activities of daily living without increasing symptoms.

The stabilizing sequence includes strengthening of the segmental muscles, neutral spine stabilization, and finally strengthening of the prime movers. The inter-segmental muscles act as tonic or postural stabilizers of the spine, tending to fatigue and atrophy after spinal injury. Therefore initial stabilization exercises are directed toward these muscles, which can control individual segmental mobility. The next phase is the stability training involves direct and indirect strengthening of muscle groups in neutral spine posture. Training begins with exercises designed to help locate the neutral spine in a variety of body positions such as prone, lying, standing, sitting and jumping which increases the awareness of lumbar and pelvic motion. This is followed by exercises of the extremities while maintaining neutral spine and later with addition of resistance to the extremities, either manually or with weights. These exercises are performed slowly with the emphasis on precise pelvic control. This will facilitate neuromuscular coordination, enhance endurance and strength, and also emphasize the smaller postural stabilizers (Weinstein et al., 1998).

Because of the higher incidence of post partum backache which may reach to 37% of women after normal labour. It has a profound psychosocial impact not only on women but also, on their families as it has an effect on their performance of the daily living activities and decrease their ability to maintain an independent life style, so it adversely affects all aspects of life. Accordingly, it seems to be important to find out a safe effective exercises program to treat post partum backache.

2. Subjects, Material and Methods Subject's Criteria: -

This study was carried out on 20 patients, their ages ranged from 25-35 years old, their BMI ranged from 30-35 kg/ m2 and the number of parity

not more than three times. All patients were referred from orthopedic after examination at least two months after delivery. None of the patients took any medication or specific treatment for low back pain during the study. Patients were free from any diseases which cause low back pain (disc prolapse, spondylosis,...) confirmed by X- Ray Patients were delivered normally without epidural anesthesia. They were treated by stabilizing exercises, three sessions per week for eight weeks (24sessions), and repetition 10-15 of each exercise. The duration of this study was six months.

Instrumentation:

1) Weight –height scale to estimate the patient body weight and height to calculate the body mass index (BMI) for each patient.

2) Visual Analogue Scale (VAS) for measuring pain intensity in clinical practice.

3) Oswestry disability questionnaire for measuring functional disability

Methods: -

Each patient in this study was instructed about the different evaluation and treatment procedures to gain her confidence and cooperation through the study. Methods for patient evaluation were done before starting and after the end of the treatment (2months) for all patients participated in this study as following: A detailed medical and gynaecological history was taken from each patient including number of deliveries (not more than 3 times), age, weight and history of neurological disorder.

Assessment of low back pain severity: Pain assessed by Visual Analog Scale (VAS).

VAS is a scale that allows continuous data analysis and uses a 10cm line with 0 (no pain) and 10 (worst pain) on the other end. Patients were asked to place a mark along the line to denote the level of pain (Scrimashaw and Maher, 2001).



(Visual analogue scale)

Assessment of functional disability:

Functional disability of each patient was assessed by Oswestery disability questionnaire. It is valid and reliable. It consists of 10 multiple choice questions for back pain; patient select one sentence out of six that best describe her pain, higher scores indicated great pain

The treatment procedure:

Each patient performed stabilizing exercises program every other day for 2 months (24sessions), repetition of each exercise between 10-15 times (10 times in first 12 sessions after that they were 15 times at others 12 sessions) and 3 session per week. Lumbopelvic stabilizing exercises training program consisted of abdominal hollowing ,quadruped abdominal hollowing, ,unilateral abduction ,unilateral knee raise, bilateral knee raise ,unilateral heel slide , bilateral heel hover and bilateral heel slide

Statistical analysis:

The collected data were statistically analyzed by using t-test for comparing before and after treatment programme. Significance level of 0.05 was used throughout all statistical tests within this study; p-value <0.05will indicate a significant result, p-value<0.01 will indicate a highly significant result (Bendate and Piersol, 1991).

3. Results

In this study twenty patients were suffering from post partum backache, they received stabilization exercises. Medical and gyaecological history had been taken at the beginning of the study and after the end (after 2 months) of the treatment programme. All data had been collected and statistically analyzed.

I-Physical characteristics of the patients

The mean values of patients' age, weight, height and BMI were 28.35 ± 1.84 yrs, 80.20 ± 1.88 Kgs, 155.85 ± 3.01 Cms and 32.95 ± 1.50 Kg/m2 respectively.

II- Pain Severity.

As observed in table (1) the mean value of pain severity for patients before starting the study was 7.30 ± 1.08 and it was decreased after the end of the treatment programme to 3.15 ± 1.18 with mean difference of 4.150. The difference is highly statistically significant (p<0.001) with percentage of 56.85% improvement in pain severity at the end of the programme (Fig.1).

	Pre-	Post-				
	treatment	treatment				
Х	$7.30{\pm}1.08$	3.15±1.18				
Mean diff	4.1	50				
% of change	56.8	35%				
T-Value	21.208					
P-Value	0.001					
Significance	High signi	ficance				
-						

 Table (1): Mean value of pain severity at pre and post treatment for patients.

III-Oswestery functional disability.

As observed in table (2); the mean value of oswestery disability for patients before starting the study was 60.25 ± 15.21 and it decreased after the end

	Pre- treatment	Post- treatment			
Х	60.25±15.21	35.65±11.25			
Mean diff	24.600				
% of change	40.85%				
T-Value	9.440				
P-Value	0.001				
Significance	High significance				

Table (2): Mean value of Oswestery functionaldisability test at pre and post treatment forpatients.

4. Discussion:

Post partum lumbo-pelvic pain is a serious problem for the women, as it has an effect on their performance of the daily living activities so that, it has an effect on her family and society (Britt et al .,2004).



Fig. (1):. Mean value of pain severity at pre and post treatment for patient

of the treatment programme to 35.65 ± 11.25 with the mean difference of 24.600. The difference is highly statistically significant (p<.001) increase with percentage of 40.85% improvement in functional disability at the end of treatment programme (Fig.2).



Fig. (2): Mean value of Oswestery disability at pre and post treatment for patients.

More than 50% of women complain of low back pain post partum and backache may persist up to one year (Darryl et al., 2007).

A specific stabilizing exercises program in women with post partum pelvic pain improve the functional status, reduce low back pain and improve the quality of life (Stuge et al.,2004). Lumbar stabilization exercises that are directed at the local muscle system have been advocated by physiotherapists as an effective means of treating chronic low back pain by enhancing the dynamic stability of the lumbar spine (Richardson and Jull, 1995).

In this study twenty patients were suffering from post partum low back pain and received stabilization exercises. Result of this study showed a highly statistically significant (p<0.01) decrease in pain severity , this result agree with Hides et al., (1996) who compared between lumbar stabilization exercises and medical treatment on 39 patients complaining from acute low back pain . They measured pain severity by visual analogue scale and pain diaries they reported significant decrease in pain severity in both the lumbar stabilization exercise program and control group.

Also Taimela et al .,(2000) conducted a study to examine the effect of lumbar stabilization exercises on low back pain patients .They used visual analogue scale to measure pain intensity .They reported decrease in low back pain severity .

Also the results showed significant differences in function disability, this agree with Richardson and Jull (1995) who reported that he specific sub maximal training of lumbar stability muscles of lumbar spine and integration of this training into functional tasks decrease both pain severity and functional disability in patients suffering from low back pain.

The results were also supported by O'Sullivan et al., (1997) who used Oswestry disability questionnaire to assess patient's level of functional disability; they reported decrease in functional disability in stabilizing exercise group. A accordingly, it was found that lumbar stabilizing exercise for lumbo-pelvic muscles were effective in decreasing pain intensity and improving the functional disability.

5. Conclusion:

It can be concluded that lumbar stabilizing exercises for lumbo-pelvic muscles decrease the pain intensity, and improve the function disability postnatal.

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Oswestery disability Questionnaire Section 1: Pain Intensity

I have no pain at the moment The pain is very mild at the moment The pain is moderate at the moment The pain is fairly severe at the moment The pain is very severe at the moment The pain is the worst imaginable at the moment

Section 2: Personal Care (eg. washing, dressing)

I can look after myself normally without causing extra pain

I can look after myself normally but it causes extra pain

It is painful to look after myself and I am slow and careful

I need some help but can manage most of my personal care

I need help every day in most aspects of self-care I do not get dressed, wash with difficulty and stay in bed

Section 3: Lifting

I can lift heavy weights without extra pain

I can lift heavy weights but it gives me extra pain Pain prevents me lifting heavy weights off the floor but I can manage if they are conveniently placed eg. on a table

Pain prevents me lifting heavy weights but I can manage light to medium weights if they are conveniently positioned

I can only lift very light weights

I cannot lift or carry anything

Section 4: Walking

Pain does not prevent me walking any distance Pain prevents me from walking more than 2 kilometres

Pain prevents me from walking more than 1 kilometre

Pain prevents me from walking more than 500 metres I can only walk using a stick or crutches

I am in bed most of the time

Section 5: Sitting

I can sit in any chair as long as I like

I can only sit in my favourite chair as long as I like Pain prevents me sitting more than one hour Pain prevents me from sitting more than 30 minutes Pain prevents me from sitting more than 10 minutes Pain prevents me from sitting at all

Section 6: Standing

I can stand as long as I want without extra pain I can stand as long as I want but it gives me extra pain

Pain prevents me from standing for more than 1 hour Pain prevents me from standing for more than 30 minutes

Pain prevents me from standing for more than 10 minutes

Pain prevents me from standing at all

Section 7: Sleeping

My sleep is never disturbed by pain My sleep is occasionally disturbed by pain Because of pain I have less than 6 hours sleep Because of pain I have less than 4 hours sleep Because of pain I have less than 2 hours sleep Pain prevents me from sleeping at all

Section 8: Sex Life (if applicable)

My sex life is normal and causes no extra pain My sex life is normal but causes some extra pain My sex life is nearly normal but is very painful My sex life is severely restricted by pain My sex life is nearly absent because of pain Pain prevents any sex life at all

Section 9: Social Life

My social life is normal and gives me no extra pain My social life is normal but increases the degree of pain

Pain has no significant effect on my social life apart from limiting my more energetic interests e.g. sport Pain has restricted my social life and I do not go out as often

Pain has restricted my social life to my home I have no social life because of pain

Section 10: Travelling

I can travel anywhere without pain

I can travel anywhere but it gives me extra pain Pain is bad but I manage journeys over two hours Pain restricts me to journeys of less than one hour Pain restricts me to short necessary journeys under 30 minutes Pain prevents me from travelling except to receive treatment

Score: / *x* 100 = %

Scoring: For each section the total possible score is 5: if the first statement is marked the section score = 0, if the last statement is marked it = 5. If all ten sections are completed the score is calculated as follows:

Example: 16 (total scored)

50 (total possible score) $/50 \ge 100 = 32\%$ If one section is missed or not applicable the score is calculated: 16 (total scored) 45 (total possible score) $\ge 100 = 35.5\%$

Minimum detectable change (90% confidence): 10% points (Change of less than this may be attributable to error in the measurement).

- Scores (0-20%) (Minimal disability).
- Scores (20%-40%) (Moderate).
- Scores (40%-60%) (Severe)
- Scores (60%-80%) (Crippled).
- Scores (80%-100%) (Patients are confined to bed). (Fairbank and Pynsent,2000).

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The Association between Lichen Planus and Hepatitis C Virus

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Abstract: Background: Hepatitis C virus, has been associated with a wide variety of extrahepatic manifestations (EHM) in the natural history of the disease. Lichen planus (LP) has also been reported in association with the hepatitis C virus. Objective is to determine the frequency of HCV in patients with LP in Sohag University Hospital. Patients and methods: The study was carried out in Sohag University Hospital in Clinical Pathology Department and Dermatology Venereology Department from October 2009 to April 2010, on 70 patients with LP (34 males, 36 females) their age ranged from 25 to 80 years old, and 20 patients (11 males, 9 females) their age ranged from 22 to 70 years old as a control group with other dermatological manifestations. Patients were recruited from the out patient clinic of dermatology department, full history, clinical examination, biopsy in some cases to confirm the diagnosis of LP and laboratory investigations for anti-HCV detection, liver function tests were done. Results: There was insignificant difference between LP group and the control group regarding the age or the sex (p = 0.176). There was a highly significant increase in HCV infection between LP group (p = 0.000), a significant increase in HCV infection and duration of LP showed a significant correlation (p = 0.022), There was an increase in aspartate aminotransferase (AST) in actenic LP patients and mucous LP patients compared with those of classic type (p = 0.028).Conclusion: A possible relationship between LP and HCV, thus, it seems that the HCV antibody test is necessarily required for LP patients.

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Key words: Lichen Planus- HCV Infection

1. Introduction:

Infections with hepatitis viruses, have been associated with a wide variety of extrahepatic manifestations (EHM)¹. Several (EHM) had been reported in the natural history of HCV infection². This included mixed cryoglobulinemia, , autoimmune thyroiditis, non-Hodgkin's lymphoma, lymphoproliferative disorders and some cutaneous manifestations, such as porphyria cutanea tarda, leukocytoclastic vasculitis, LP (lichen planus), sicca syndrome and others³.

It was found that Egypt reports the highest prevalence of HCV worldwide, ranging from 6 % to more than 40 % with an average of 13.8%. Injection therapy for schistosomiasis, and needles were frequently reused, despite termination of this program and the implementation of measures designed to reduce hospital-related infection, transmission continues, including a cut puncture and cutting of the skin with unsterilized knives were likely transmission modes^{4,5}.

Elevations of aspartate amino transferase (AST) and alanine amino transferase (ALT) level indicated the presence of liver injury. Patients with chronically elevated aminotransferase values should

undergo a workup to exclude the possibility of chronic liver disease¹. Due to the lack of symptoms, the vast majority of chronically infected individuals remain undiagnosed and unaware of the infection for several years⁶. Fibroscan is not currently licensed for use in the United States⁷. Future generations of serologic fibrosis markers may have greater accuracy and may obviate the need for liver biopsy⁸.

Lichen planus (Greek *leichen*, "tree moss"; Latin *planus*, "flat") is a unique, common inflammatory disorder that affects the skin, mucous membranes, nails, and hair. The appearance of lichen planus (LP) and lichen planus-like or lichenoid dermatoses has been likened to the scurfy, finely furrowed, dry excrescences of the symbiotic vegetation known as lichen⁹. Cell-mediated immunity plays the major role in triggering the clinical expression of the disease. Both CD4+ and CD8+ T cells are found in the skin lesion of LP¹⁰.

LP had also been reported in association with the hepatitis C virus $(HCV)^{10}$. While other studies had rejected such an association ^{11,12}.

LP may be the first presentation of HCV infection,^{10,13}. The oral form of LP seems more common¹⁴. The differential diagnosis of LP actinicus

includes polymorphous light eruption, granuloma annulare, sarcoidosis, melasma and erythema dyschromicum perstans¹⁵. The LP-HCV association cannot be explained on the basis of high HCV seroprevalence in the general population only. Possible genetic differences among the populations studied (HLA-DR6 allele in Italy)^{16,17}.

The aim of the present study is to determine the frequency of HCV in patients with LP in Sohag University Hospital.

2. Patients and methods:

This study was carried out in Sohag University Hospital in Clinical Pathology Department and Dermatology Venereology Department from October 2009 to April 2010, on 70 patients with LP (34 males, 36 females) their age ranged from 25 to 80 years old as the study group, and 20 patients (11 males, 9 females) their age ranged from 22 to 70 vears old as a control group with other dermatological manifestations. Patients were recruited from the out patient clinic of dermatology department. A written informed consent was taken from them. The study was approved by the Ethical Committee of Sohag Faculty of Medicine.

Exclusion criteria included cases with lichenoid drug eruption.

Each subject was evaluated for full history, clinical examination to detect the type, extent and severity of the disease. Biopsy was taken from some cases to confirm the diagnosis of LP and laboratory investigations included anti-HCV detection, liver function tests.

Details of blood sampling and laboratory techniques:

Five ml of venous blood was drawn from every patient, added to the appropriate vacutainer for liver function tests; anti-HCV detection.

Liver Function Tests:

Liver function tests were performed on fully automated chemical autoanalyzer Synchron CX-9 (Beckman) system by the use of kits supplied by Beckman Coulter/France, serum aspartate aminotransferase (AST) Cat No. 446265, serum alanine aminotransferase (ALT) Cat No. 442620, serum total bilirubin (TBIL) Cat No. 442745 and serum albumin Cat No. 442765.

Anti-HCV Detection:

Anti-HCV detection was performed by using ARCHITECT Abbott System, MEIA (microparticle enzyme immunoassay) technology; which is a variation of the ELISA principle HCV version 3 (recombinant HCr43, c200, c-100-3, NS5).

Statistical methods:

The data was processed and analyzed using the Statistical Package of Social Sciences (SPSS) version 9. Frequency, percentage, mean and standard deviation were calculated. Chi square test was used to compare the percentage while t-test and ANOVA test were used to compare means, p-value significant at level < 0.05.

3. Results:

The demographic data of the studied groups were illustrated in table 1. There was insignificant difference between LP group and the control group regarding age or sex (p = 0.176).

HCV was found to affect 27 patients (38.6%) of LP group, and only 2 patients (10%) of the control group. There is highly significant increase in HCV infection among LP group compared to the control group (p = 0.000) (table 2).

The relation between age and anti-HCV antibodies in LP group showed that HCV was found to affect 18 patients (50%) out of 36 patients more than 50 years old, and 9 patients (33.3%) out of 22 patients aged 30-50 years old. Patients aged less than 30 years old were all not affected by HCV, they were 12 patients (17.2%). Generally there was a significant increase in HCV infection among older patients (p = 0.008)(table 3).

Relation between anti-HCV antibodies and the duration of LP showed the negative HCV antibody was found in patients less than 1 year duration and the positive HCV antibody was found among those with duration more than 10 years (p = 0.022), but the relation was not significant in those LP patients with duration 1-10 years (table 4 fig 1).

Liver function tests examination in LP subtypes represented an increase in the total bilirubin concentration in actenic LP patients (1.53 mg/dl) compared to those with mucous LP (1.37 mg/dl) and classic type (1.08 mg/dl) however this increase was insignificant (p = 0.095). There was an increase in AST level in actenic LP patients (40.42 IU/L) and mucous LP patients (40.10 IU/L) compared to those of classic type (27.04 IU/L) , this increase was significant (p = 0.028).

Also there was an increase in ALT level in actenic LP patients (45.00 IU/L) and mucous LP patients (44.33 IU/L) compared with classic type (31.87 IU/L), this increase was significant (p = 0.041). The serum albumin concentration was higher in both classic and actenic LP patients than in mucous type (3.58 g/dl, 3.568 g/dl, 3, 44 g/dl respectively), but this increase was insignificant (p = 0.906) (table 5).

Relation between anti-HCV antibodies and pruritis, 20 patients (74.1%) out of 27 patients with LP and HCV positive were suffering from severe pruritis, but generally the relation between pruritis and HCV

infection was insignificant (p = 0.399),(table 6).

Table (1): Dem	ographic data in I	LP group and	the control group

Group / Gender		Mean age (year)	S.D	p - value
Control group (n= 20)	Male (n=11)	42.09	17.28	
	Female (n=9)	42.11	13.76	0.176 NS
LP group (n = 70)	Male (n = 34)	46.09	18.14	
	Female (n = 36)	49.47	15.70	

N S: non significant p > 0.05

Table (2) The LP and control groups regarding the anti HCV antibodies:

Group	Control group (n= 20)		LP group (n= 70)		p –Value		
Anti-HCV	No.	%	No.	%	Value	Comment	
Negative	18	90%	43	61.4%	0.000002	H.S	
Positive	2	10%	27	38.6%	0.000002	H.S	

H.S: Highly Significant p<0.001

Table (3): The relation between age and anti-HCV in LP patients

Anti HCV	Negative HVC (n= 43)		Positi (n=	ve HCV = 27)	Total (n= 70)	
Age group	No.	%	No.	%	No.	%
< 30 years	12	27.9%	0	0.0%	12	17.14%
30-50 years	13	30.2%	9	33.3%	22	31.43%
> 50 years	18	41.9%	18	66.7%	36	51.43%
Total (n= 70)	43	61.4%	27	38.6%	70	100%
p - value	p = 0.008	significant				

Table (4): Relation between anti-HCV antibodies and duration of LP

Anti HCV	Negative (n= 43)		Positive (n= 27)		Total (n= 70)		p - value	
Duration	No.	%	No.	%	No.	%	Value	Comment
< 1 year	29	67.4%	13	48.1%	42	60.0%	0.006	HS
1 -10 years	13	30.2%	8	29.6%	21	30.0%	1.00	NS
> 10 years	1	2.3%	6	22.2%	7	10%	0.0001	HS
p – value	0.022	5						

p - value0.022SS: Significant p < 0.05 HS: Highly Significant p < 0.001</td>NS: Non Significant p > 0.05

LP Subtypes		lucus $a = 21$)		Actenic $(n = 19)$		Classic $(n = 41)$		p – value	
1	Mean	S.D	Mean	S.D	Mean	S.D	Value	comment	
Total	1.3762	1.331	1.532	1.425	1.083	0.6356	0.095	NS	
Direct	0.4429	0.829	0.200	0.0882	0.298	0.6136	0.417	NS	
	40.105	29.85	40.421	31.117	27.049	15.129	0.028	S	
	44.338	30.63	45.00	33.543	31.878	15.302	0.041	S	
	3.443	0.572	3.568	0.656	3.588	0.5528	0.906	NS	
	Total Direct	Subtypes Mucus (n = 21) Mean Mean Total 1.3762 Direct 0.4429 40.105 44.338 3.443 3.443	Mucus (n = 21) Mucus (n = 21) Mean S.D Total 1.3762 1.331 Direct 0.4429 0.829 40.105 29.85 44.338 30.63 3.443 0.572	Mucus (n = 21) Actenic (n = 19) Mean S.D Mean Total 1.3762 1.331 1.532 Direct 0.4429 0.829 0.200 40.105 29.85 40.421 44.338 30.63 45.00 3.443 0.572 3.568	Mucus (n = 21) Actenic (n = 19) Mean S.D Mean S.D Total 1.3762 1.331 1.532 1.425 Direct 0.4429 0.829 0.200 0.0882 40.105 29.85 40.421 31.117 44.338 30.63 45.00 33.543 3.443 0.572 3.568 0.656	SubtypesMucus $(n = 21)$ Actenic $(n = 19)$ Classic $(n = 41)$ MeanS.DMeanS.DMeanTotal1.37621.3311.5321.4251.083Direct0.44290.8290.2000.08820.29840.10529.8540.42131.11727.04944.33830.6345.0033.54331.8783.4430.5723.5680.6563.588	Mucus (n = 21) Actenic (n = 19) Classic (n = 41) Mean S.D Mean S.D Total 1.3762 1.331 1.532 1.425 1.083 0.6356 Direct 0.4429 0.829 0.200 0.0882 0.298 0.6136 40.105 29.85 40.421 31.117 27.049 15.129 44.338 30.63 45.00 33.543 31.878 15.302 3.443 0.572 3.568 0.656 3.588 0.5528	SubtypesMucus $(n = 21)$ Actenic $(n = 19)$ Classic $(n = 41)$ $p - value$ MeanS.DMeanS.DMeanS.DValueTotal1.37621.3311.5321.4251.0830.63560.095Direct0.44290.8290.2000.08820.2980.61360.41740.10529.8540.42131.11727.04915.1290.02844.33830.6345.0033.54331.87815.3020.0413.4430.5723.5680.6563.5880.55280.906	

Table (5): Liver function tests in LP subtypes

NS: Non Significant p > 0.05 S: Significant p < 0.05

Table (6): Relation between anti-HCV antibodies and pruritis

Anti HCV	Negative (n= 43)		Positive (n=27)		Total (n = 70)		p- Value	
Pruritis	No.	%	No.	%	No.	%	Values	comment
No.	8	18.6%	3	11.1%	11	15.7%	0.113	NS
Mild	10	23.3%	4	14.8%	14	20.0%	0.149	NS
Severe	25	58.1%	20	74.1%	45	64.3 %	0.0006	S
p- value	0.399	NS						

S : Significant p<0.05 NS : Non Significant p>0.05



Figure (1): Relation between anti-HCV antibodies and duration of LP

4. Discussion:

LP is a chronic papulos qamous inflammatory disease of unknown etiology that affects the skin, mucous membranes, nails and hair with the prevalence up to 2%. Theories of infectious (e.g HCV, herpes simplex viruses), autoimmune, metabolic or genetic causes have been proposed¹⁸.

HCV is an enveloped, single-stranded positive-sense RNA virus that was isolated in 1989 from a chimpanzee chronically infected by contamination with a human factor VIII concentrate ¹⁹. HCV is the main causative agent of parenterally transmitted non-A, non-B viral hepatitis with 3% of the world population being infected^{20,21}.

Chronic hepatitis C infection is characterized by wide spectrum of liver damage ranging from mild chronic hepatitis to cirrhosis and even hepatocellular carcinoma, as well as the leading indication for liver transplant²².

Extra hepatic manifeststion (EHM) of HCV infection is predominantly of possible autoimmune origin and many of them clinically manifest mucocutaneously. One of the possible mucocutaneous manifestation besides cutaneous vasculitis, prophyria cutanea tarda and prurigo is considered to be LP, thus LP could be overt clinical sign for an occult chronic hepatitis ³.

The association between HCV infection and LP comes from epidemiological studies that have shown that the prevalence of antibodies against HCV antigens is higher in patients with LP than in general population 23 .

In our study we found HCV antibodies in 27 of 70 cases of LP (38.6%) with a highly significant (p = 0.000), compared to the control group (10%). Our results support possible relationship between LP and HCV infection. These observations were in accordance Tonsi and Samdani who found that HCV antibodies were higher in patients suffering from LP (26.3%) than those in the control group (4.6%)¹⁰. On the contrary Stojanovič had found that there was no statistically significant difference between the study and the control group regarding anti-HCV antibody prevalence¹¹.

In our study, total bilirubin was insignificant higher in the study group (LP) than in the control group (p = 0.094), also serum AST (p = 0.298). These observations were in accordance with the findings of the study conducted by Ghodsi who found that AST levels and bilirubin concentrations were higher than the normal range in patients with LP but without significant difference in the liver function tests between HCV infected and non infected groups²⁴.

The estimated mean age of LP in the present study was (46.1 years old) this quite dentinal to the belief of LP being a primarily disease of middle age with mean age of oncet in the fourth to fifth decade²³

In the present study, males and females were nearly affected with LP. This observation was in accordance with study conducted by Mignogna et al , who found that LP most commonly affected middle aged adults of both sex with a slight predominance in women²³.

5. Conclusion:

Our results support a possible relationship between LP and HCV. Thus, from this study, it seems that the HCV antibody test is necessarily required for LP patients with no signs of liver disease, because it has been established that early successful treatment of HCV infection prior to overt end-stage disease leads to dramatic improvement of inflammatory liver damage and fibrosis.

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Protective Effect of some Antioxidants against CCl₄-Induced Toxicity in Liver Cells from BRL3A Cell Line.

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Abstract: An in vitro experiment was conducted to investigate the protective effect of ascorbic acid, mannitol and aminoguanidine at different concentrations against carbon tetrachloride induced toxicity and oxidative stress in hepatocytes cell line (BRL3A) from buffalo rats. Results were compared with those of vitamin E as standard hepatoprotective agent. Treatment of BRL3A with CcL_4 lead to generation of free radicals detected after two hours incubation using ESR technique and produced cell injury demonstrated by increased leakage of LDH, ALT and AST to the media. Exposure to CcL_4 caused apoptosis to cells but did not induce lipid peroxidation as tested by the TBARS technique. Treatment with vitamin E has significant hepatoprotective effect by lowering the leakage of intracellular enzymes, reducing the oxidation of proteins and decrease incidence of apoptosis. Ascorbic acid, mannitol and aminoguanidine were ineffective against CcL_4 toxicity.

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Keywords: Protective Effect; antioxidants; CCl4-Induced Toxicity; Liver; Cell; BRL3A Cell Line

1. Introduction:

The liver occupies a vital role in the main functions of the organism. It is particularly susceptible to chemically induced injury due to its extensive metabolic capacity and cellular heterogeneity. Oxidative stress occurs when there is an imbalance between reactive oxygen species (ROS) formation and scavenging by antioxidants. Excess generation of ROS can cause oxidative damage to biomolecules resulting in lipid peroxidation, mutagenesis and carcinogenesis (Khan and Sultana, 2009).

In vitro liver systems represent a better experimental approach to screen potential hepatotoxic compounds and to investigate the mechanism by which chemicals induce liver lesions (*Kucera et al.,* 2006). Liver cell lines are characterized by unlimited subcultivation and cell availability in large number) Guillouzo, 1998). The BRL3A cell line is an epithelial cell line from buffalo rat liver which is able to divide in the absence of serum.

Carbon tetrachloride (CcL₄) has long been known as a model toxicant and has been the focus of many *in vitro* and *in vivo* toxicological studies (Manibusan et al., 2007). The liver is the major target organ of CcL₄ toxicity owing to its high content of cytochrome P-450 (Södergren et al., 2001). Antioxidants are used to antagonize the deleterious action of free radicals and to protect hepatocytes from damage. Vitamin E (-tocopherol) is considered the most important lipophilic antioxidant in biological tissues (Weber et al., 2003). Ascorbic acid is an important dietary antioxidant. It significantly

decreases the adverse effect of reactive species such that can cause oxidative damage to macro-molecules such as lipids, DNA, and proteins (Alpsoy et al., 2009). Mannitol is a polyol which develops an antioxidant activity by scavenging ROS (Mendoza et al., 2007). A number of physiological functions have been ascribed to mannitol, including serving as a reserve carbon source, as an antioxidant, and to store reducing power (Ruijter et al.. 2003). Aminoguanidine is an irreversible inhibitor of the inducible form of nitric oxide synthase (iNOS), which also inhibits endothelial NOS (eNOS) and neuronal NOS (nNOS) (Mohamad et al., 2009).

In the present study, *in vitro* antioxidant activities of ascorbic acid, mannitol and aminoguanidine were assessed in comparison with vitamin E against hepatotoxicity induced by carbon tetrachloride in liver cell line from rats.

2. Material and methods:

a. Material:-

 CcL_4 (99.5%), vitamin E "-tocopherol acetate" and L (+) ascorbic acid "vitamin C" were purchased from Wako Pure Chemicals Co., Japan. Dmannitol and aminoguanidine hemisulfate were obtained from Sigma-Aldrich

Chemical Co., St. Louis, MO, USA. Ham's F-12K media was used for hepatocytes'cell line culturing (Wako Pure Chemicals Co., Japan). LDH cytotoxicity detection kit (Takara Company, Tokyo, Japan), mitochondria extraction kit (Sigma-Aldrich Chemical Co., St. Louis, MO, USA.), Oxyblot protein oxidation detection kit (Chemicon International Co., USA), Qproteome for detection of Apoptosis and In situ cell death detection kit (Roche Company, Germany).

b. Methods:

1- Cell culture, liver cells injury induction and treatment with different antioxidants:-

Liver cell line BRL3A derived from buffalo rats (Nissley et al., 1977) was used. Approximately 1×10^5 cells/ml (5 ml final volume) were cultured into a 25 ml flask and incubated under standard conditions (37 °C and 5 % CO2) in Ham's F-12K medium with L-Glutamine, phenol red and sodium pyurvate supplemented with 10% Fetal Bovine Serum (FBS) and penicillin and streptomycin as antibiotics. Carbon tetrachloride (7.5 µl) was added in the 25 ml headspace of the flask to a paper attached to the stopper. During incubation, CcL₄ evaporated and equilibrium was reached between the gas phase and the medium (Azri et al., 1990). Media samples were collected at 30 min, 1, 3, 6, 9, 12, 14, 24 and 48 hrs. Different antioxidants (vitamin E, ascorbic acid, mannitol and aminoguanidine) were added separately to the media just before the addition of CCL₄. Each agent was individually tested on the cells using a dose range. For vitamin E this was 100, 50 and 25 µM; for ascorbic acid 500, 250 and 125 µM; for either mannitol or aminoguanidine the dose range was 400, 200 and 100 µM. Media samples were collected at 14, 24 and 48 hrs.

2- Detection of Free radicals by Electron Spin Resonance (ESR) method:-

Electron spin resonance signals were measured by preincubation of the hepatocytes in the presence of 40 mM 5, 5-dimethyl-pyrroline-*N*-oxide (DMPO) (*Matsuki, et al., 1999*). After induction of CcL4 toxicity, media samples were collected after 1, 2, 4 and 6 hrs. A control group was incubated with DMPO only. All ESR spectra were recorded at room temperature. AJEOL Model JES – FA 100 ESR spectrometer (JEOL Co., Tokyo Japan) was used. Spectrometer settings were; magnetic field 335.00 ± 5 mT, sweep time 8.0 min, modulation frequency 100 kHz, modulation amplitude 0.2 mT, receiver gain $8x10^3$, time constant 0.03 S, microwave frequency 9.418GHz and microwave power 12 mW.

3- Leakage of intracellular enzymes as (Lactate dehydrogenase (LDH) and aminotransferase (ALT and AST) leakage in cell culture medium: Leakage of LDH enzyme in the cultured media was measured by a kit while the activities of ALT and AST were determined by using automatic multifunction-biochemical analyzer (DRI-CHEM 5500, Japan).

4- Measurement of Lipid Peroxidation: It was carried out by measuring thiobarbituric acid – reactive species (TBARS) according to *Kikugawa et al.*, (1992).

5- Protein oxidation detection:

Using Qproteome mitochondria extraction kit, mitochondria was extracted from control and treated cells after 24 hours incubation with CcL4 to assess the formation of protein carbonyl groups as an index of protein oxidation. Protein concentration was determined by the Bradford assay (Bio-Rad) according to Bradford (1976). An OxyBlot protein oxidation detection kit (Chemicon International Co., USA.) was used according to the manufacturer's detailed protocol. Subsequently, 5 µL protein sample was added with 5 μ L of 12% SDS and 10 μ L of 1× DNPH solution into a tube. Ten microliters of $1 \times$ neutralization solution instead of the DNPH solution served as the negative control. Tubes were incubated at room temperature for 15 minutes. Neutralization solution (7.5 µL) was added to each tube. The DNPderivatized protein samples were separated by polyacrylamide gel electrophoresis followed by Western blotting. Following electrophoresis and transfer to nitrocellulose membranes, the membranes were blocked in Tris buffered saline containing 0.1% Tween 20 and 1% bovine serum albumin for 1 hour at room temperature. Membranes were incubated overnight at room temperature with the primary antibody stock (1:150) and then incubated with secondary antibodies (1:3000) at room temperature for 1 hour. Blots were developed by an enhanced chemiluminescence detection system. Proteins that underwent oxidative modification (i.e., carbonvl group formation) were identified as a band in the derivatized sample, but not in the negative control. Levels of oxidatively modified proteins were quantified and expressed via measurement of optical density using molecular analyst alias program.

6- Analysis of oxidative DNA strand breaks by TUNEL assay:-

The principle of the test is that cleavage of DNA during apoptosis may yield double stranded as well as single strand breaks "nicks" which can be identified by labeling with modified nucleotides in an enzymatic reaction (TUNEL staining, according to Gavrieli et al., 1992. Cells were grown on a small cover glass which was placed inside the flask. After induction of CcL₄ toxicity, cells were washed in PBS and fixed with 4 % paraformaldehyde buffered saline for 1 h at 15-25 °C. Permeablisation of the cells was carried out by incubation with 0.2% Triton X for 2 min. on ice (2-8 °C). Positive control cells were made by treatment with DNAse I for 10 minutes to break up the DNA.

TUNEL reaction mixture (50 μ l) was added on sample, for the negative control, 50 μ l of label solution was added for 60 min. at 37 °C in a humidified atmosphere in the dark. The samples were analyzed under a fluorescence microscope using an excitation wavelength in the range of 450-500 nm. Converter AP (50 μ l) was then added on sample, and incubated in a humidified chamber for 30 min. at 37 °C. Substrate solution (100 μ l) was added to the slides which were incubated for 10 min. at 15-25 °C in the dark. Cells were washed in PBS between each of these steps. The slides were counterstained with haematoxyline and eosin, examined under light microscope, and the apoptotic cells were counted in 10 random fields.

c. Statistical analyses:-

Data are expressed as mean values \pm SE. Student's t-test was used for observations. One-way analysis of variance (ANOVA) was used to assess significant differences among treated groups followed by Tukey's test. Statistical analyses were performed using GraphPad prism software (GraphPad, Inc., California USA). Differences were considered statistically significant when p < 0.05.

3. Results

I- Toxic effect of CcL4 on BRL3A cell line:-

a- Generation of free radicals

The ESR signals were observed after 2 hours incubation of BRL3A cell line with CcL_4 and DMPO (Fig.1, A) reached its peak at 4 hr and began to decline at 6 hr (Fig.1, D&E).

b. Leakage of intracellular enzymes:-

Activities of LDH, ALT and AST enzymes were increased significantly (P<0.05) at 14 and 48 hrs. (Table, 1)

c- Lipids peroxidation:-

Lipids peroxidation revealed no significant change between control and treated cells with CcL_4 (Table, 1).

d -Oxidation of proteins:-

The amount of protein carbonyls was markedly increased in cells treated with CcL₄ compared with that of normal control group (Fig. 2, B). e -Oxidative DNA strand breaks-:

Examination of control cells by the fluorescent microscope revealed no signals, while CcL4 treated cells showed fluorescent signals. The percent of apoptotic hepatocytes stained with the TUNEL method was significantly greater in the CcL4-treated cells (4.2%) than that in the control group (0.66%) (Fig. 3) (Photo, 1).

II- Effect of different antioxidants against CcL4-induced hepatotoxicity

in BRL3A cell line:-

A-Leakage of intracellular enzymes:-

Comparing with the CcL₄ treated group, All the used doses of vitamin E significantly (p<0.05)reduced leakage of LDH enzyme to the media. Ascorbic acid at a dose of 500 µM was able to reduce LDH enzyme leakage to the media after 24 and 48 hrs. Mannitol failed to show reduction in leakage of LDH except at a dose of 200 µM at 48 hr, while aminoguanidine failed to show reduction in leakage of LDH. As regards to leakage of ALT and AST enzymes to the media, vitamin E treatment (100, 50 and 25 µM) significantly (p<0.05) reduced enzymes' leakage. Ascorbic acid, mannitol and aminoguanidine at all doses were unable to reduce the leakage of ALT and AST enzymes to the culture media compared with CcL₄ treated group throughout the experiment (Tables, 2,3 and 4).

b- Oxidation of proteins:-

Treatment with vitamin E showed marked reduction in the amount of protein carbonyls with all doses. Similar results were observed in Ascorbic acid treated groups. No changes in protein carbonyls were observed in groups treated with different doses of mannitol and aminoguanidine, (Figs. 4A & 4B),

c- Oxidative DNA strand breaks:-

Percentage of apoptotic cells stained with the TUNEL method was markedly decreased in vitamin E (VE) 100 µM treated group. The percentage of positive TUNEL cells was 0.85% in control group, 3.8 % in CcL₄ group, 1.79 % in VE 100 µM group, 2.1 % in VE 50 µM and 2.3 % in VE 25 µM treated group (Fig. 3B). Ascorbic acid (AA) did not reduce the incidence of apoptosis in cells treated with CcL₄. Percentage of positive apoptotic cells were 2.8% in AA 500 µM, 2.5% in AA 250 µM, 2.8% in AA 125 μM and 3.2% in AA 62.5 μM treated group. Treatment with different doses of mannitol (M) did not show protective effect against CcL₄ induced apoptosis in BRL3A cells. The percentages of positive TUNEL cells were 2.8% in M 400µM, 2.6% in M 200 µM and 2.5% in 100 µM (Fig. 3C). Aminoguanidine failed to reduce the incidence of apoptosis occurred in BRL3A cells due to CcL₄ toxicity. The percentage of apoptotic cells were 2.66% in AG 400 µM, 2.42% in AG 200 µM and 2.44% in AG 100 µM group (Fig. 5).

Incuba	ation time	LDH	ALT	AST	TBARS
		(U/ml)	(U/L)	(U/L)	(µmol/L)
30 min	Control	0.026±0.001	1.667±0.333	9.000±1.723	0.006±0.001
	CcL4	0.032±0.002	2.000±0.577	8.000±1.000	0.012±0.002
1 h	Control	0.030±0.003	1.333±0.333	10.000 ± 1.000	0.005±0.003
	CcL4	0.025±0.001	1.678±0.333	10.000 ± 1.000	0.014±0.006
3 hs	Control	0.041±0.001	1.333±0.333	10.330±1.528	0.005 ± 0.001
	CcL4	0.042±0.002	1.770±0.667	13.670±1.528	0.005±0.003
6 hs	Control	0.044 ± 0.000	3.333±0.882	6.670±0.333	0.007 ± 0.002
	CcL4	0.043±0.000	4.330±0.881	8.660±1.667	0.004 ± 0.001
9 hs	Control	0.040 ± 0.001	4.000±0.577	6.790±0.333	0.016±0.001
	CcL4	0.041±0.001	4.333±0.882	8.560±0.577	0.026±0.007
12 hs	Control	0.039±0.001	6.000±0.577	3.667±0.333	0.017±0.001
	CcL4	0.042±0.002	7.333±0.882	4.667±0.067	0.025±0.004
14 hs	Control	0.006±0.002	3.000±0.577	5.333±0.333	0.018±0.002
	CcL4	0.023±0.001**	7.335±0.333 **	.667±0.666*	0.024±0.006
24 hs	Control	0.013±0.003	4.333±0.333	6.300±0.330	0.022±0.003
	CcL4	0.028±0.001*	9.320±0.881**	11.67±0.333**	0.022±0.002
48 hs	Control	0.126±0.002	6.000±0.577	11.33±0.577	0.022 ± 0.000
	CcL4	0.378±0.008**	12.67±1.453*	23.67±2.082**	0.031±0.004

Table (1):- LDH, ALT and AST enzyme activiti	es & TBARS va	alues of BRL3A of	cell line treated with Co	L ₄ at
different incubation times.				

(*) Significantly different from the control group at P < 0.05.

(**) Significantly different from the control group at P < 0.001.

Table (2): Activities of LDH, ALT and AST enzymes of BRL3A cell line treated with different doses of vitamin E, ascorbic acid, mannitol and aminoguanidine for 14 hr against toxicity of CcL₄.

	Groups	LDH	ALT	AST
Antioxidant		(U/mL)	(U/ L)	(U/L)
	Control (0.5%ethanol)	0.005±0.002	4.33±0.333	3.670±0.333
X [*] 4	CcL ₄	0.077 ± 0.003^{a}	9.67±0.333 ^a	11.330±1.202 ^a
	VE 100 µM+ CcL ₄	0.066 ± 0.001^{b}	5.33±0.333 ^b	4.330±1.453 ^b
(VE)	VE 50 μ M + CcL ₄	0.068 ± 0.000^{b}	6.33±0.333 ^b	4.667 ± 1.202^{b}
	VE 25 μM+ CcL ₄	0.062 ± 0.000^{b}	7.00±0.577 ^b	3.000 ± 1.155^{b}
	Control	0.028±0.000	4.00±0.577	3.67±0.333
Accorbio soid	CcL4	0.036±0.001 ^a	9.00 ± 0.577^{a}	8.00 ± 0.577^{a}
ASCOLDIC ACIU	AA 500 μM+ CcL ₄	0.033±0.001	7.67±0.333	6.00±0.577
(AA)	AA 250 μM+ CcL ₄	0.036±0.000	7.33±0.333	6.33±0.881
	AA 125 μM+ CcL ₄	0.034±0.001	7.00±0.577	7.00±0.577
	Control	0.009±0.000	3.33±0.667	4.33±0.333
Mannital	CcL4	0.030 ± 0.002^{a}	7.33±0.333 ^a	9.00±0.577 ^a
(M)	M 400 μM+ CcL ₄	0.049±0.002	6.67±0.667	9.67±0.333
(191)	M 200 µM+ CcL ₄	0.041±0.003	6.00±0.577	9.33±0.333
	M 100 μM+ CcL ₄	0.039±0.000	5.33±0.333	8.33±0.333
	Control	0.009±0.000	3.33±0.667	3.67±0.333
	CcL4	0.022 ± 0.000^{a}	7.33±0.330 ^a	10.67±0.333 ^a
Aminoguanidine	AG 400 μM+ CcL ₄	0.039±0.001	7.34±0.882	10.33±0.333
(AG)	AG 200 μM+ CcL ₄	0.028±0.002	6.33±0.660	9.67±0.333
	AG 100 µM+ CcL ₄	0.027±0.002	5.67±0.670	10.00±0.577

(^a) Significantly different from the control group at P<0.05.

(^b) Significantly different from the CcL4 treated group at P<0.05

	groups	LDH	ALT	AST
Antioxidant	_	(U/ml)	(U/L)	(U/L)
	Control			
		0.004 0.000	8 000 ± 0 577	7 (70+0 222
		0.004 ± 0.000	8.000±0.377	1.070 ± 0.555
	ethanol)	0.045.0.003	15 000 0 000	14.660.0.000
	CcL ₄	0.045 ± 0.002	15.330±0.882	14.660 ± 0.333
Vitamin E		" 		u
(VE)	VE 100	0.007 ± 0.000	6.330±0.881 ^b	8.660±0.333 ^b
	µM+CcL4	b	0.0000_01001	01000_010000
	VE 50 µM+	0.017 ± 0.001	$7.000+1.13^{b}$	9 000+0 577 ^b
	CcL4	D	7.000±1.15	9.000±0.577
	VE 25 µM+	0.020±0.000	10.000±0.577	9 330+0 333 b
	CcL4	b	b	7.550±0.555
	Control	0.029 ± 0.000	5.00±0.577	4.67±0.333
	CcL4	0.041 ± 0.001	10.33±0.333 ^a	10.00±0.577 ^a
		а		
A	AA 500 µM+	0.032 ± 0.001	10.00±0.577	9.00±0.577
Ascorbic acid (AA)	CcL4	b		
	AA 250 µM	0.042 ± 0.000	12.00±0.577	9.00±0.577
	+CcL4			
	AA 125 µM+	0.042±0.001	9.00±0.577	9.33±0.333
	CcL4			
	Control	0.027±0.001	4.00±0.577	5.67±0.333
	CcL4	0.040±0.000	9.00±0.577 ^a	12.33±0.333
		a		a
	M 400 µM	0.073+0.000	7.33+0.33	11.30±0.667
Mannitol	+CcL4			
(M)	М 200 µМ	0.069 ± 0.004	7.33+0.33	11.33±0.333
	+CcL4			
	М 100 µМ	0.047±0.001	9.33±0.33	10.67±0.333
	+CcL4			
	Control	0.027±0.001	4.00±0.577	5.00±0.577
	CcL4	0.04±0.002 ^a	9.00±0.577 ^a	12.33±0.333 ^a
Aminoguanidine		0.052 ± 0.001	8 33+0 577	10.67+0.333
	$\pm CeI 4$	0.052±0.001	0.33±0.377	10.07±0.555
(AG)		0.045+0.000	8 00+1 15	11 33+0 333
(10)		0.045±0.000	0.00 ± 1.13	11.55±0.555
		0.028+0.001	8 00 +0 000	11.67+0.222
		0.038±0.001	0.00±0.000	11.0/±0.333
	+UCL4			

Table (3):- Activities of LDH, ALT and AST enzymes of BRL3A cell line treated with different doses of vitamin E, ascorbic acid, mannitol and aminoguanidine for 24 hr against toxicity of CcL₄.

(^a) Significantly different from the control group at P<0.05.

(^b) Significantly different from the Ccl4 treated group at P<0.05

	Groups	LDH	ALT	AST
Antioxidant		(U/mL)	(U/L)	(U/L)
	Control (0.5%ethanol)	0.004±0.00	6.000±0.577	18.000±0.577
	CcL ₄	0.031±0.001 a	11.000±0.577 a	25.000±0.577 ^a
Vitamin E (VE)	VE 100 µM+ CcL4	0.004±0.000 b	7.000±0.577 ^b	12.000±0.577 b
	VE 50 μM+ CcL4	0.012±0.000	5.000±0.577 ^b	14.000±0.577 ^b
	VE 25 μM+ CcL4	0.014±0.000	7.000±0.577 ^b	13.000±0.577
	Control	0.047 ± 0.001	4.00 ± 0.577	10.33±0.333
	CcL4	0.068±0.002 a	8.00±0.577 ^a	22.67±0.333 ^a
Ascorbic acid (AA)	AA 500 µM+ CcL4	0.058±0.002 b	8.00±0.577	20.00±0.577
	AA 250 µM +CcL4	0.070±0.001	7.00±0.577	21.33±0.333
	AA 125 μM+ CcL4	0.068 ± 0.002	6.00 ± 0.577	21.33±0.882
	Control	0.018±0.003	5.00±0.577	12.00±0.577
Mannitol	CcL4	0.031±0.004 a	8.00 ± 0.577^{a}	23.00±0.577 ^a
(M)	M 400 µM +CcL4	0.041 ± 0.001	7.33±0.330	21.00±0.577
	M 200 µM +CcL4	0.045 ± 0.001	5.00±0.577 ^b	21.00±0.577
	M 100 µM +CcL4	0.048 ± 0.001	6.00±0.577	20.67±0.333
	Control	0.017±0.003	5.00±0.577	9.00±0.577
	CcL4	0.030±0.004 a	8.00±0.577 ^a	22.00±0.577 ^a
Aminoguanidine	AG 400 µM +CcL4	0.046±0.002	6.67±0.882	20.33±0.333
(AG)	AG 200 µM +CcL4	0.034±0.001	6.00±0.577	20.30±0.333
	AG 100 µM +CcL4	0.027±0.000	5.33±0.33	19.67±0.333

Table (4):- Activities of LDH, ALT and AST enzymes of BRL3A cell line treated withdifferent doses of vitamin E, ascorbic acid, mannitol and aminoguanidine for 48 hr against toxicity of CCL₄.

(^a) Significantly different from the control group at P<0.05.

^(b) Significantly different from the CcL4 treated group at P<0.05



Fig. (1):- ESR spectra of supernatants of BRL3A cell line treated with CCL4 at different periods of incubation.

(Spectrum A) ESR spectra of the 40 mM DMPO adduct detected in BRL3A cell line. (Spectrum B) Same as in spectrum A, but cells were incubated with Ccl4 for one hour. The spectrum shown in C is from cells incubated 2 hours with CcL4, while the spectrum in D after 4 hours incubation with CcL4. Spectrum shown in E after 6 hour incubation period with CcL4. Instrumental settings of a JEOL Model JES-FA 100 ESR spectrometer: magnetic field 335.000 ± 5 mT, sweep time 8.0 min, modulation frequency 100 kHz, modulation amplitude 0.2 mT, receiver gain 8×103 , time constant 0.03 S, microwave frequency 9.418GHz and microwave power 12 mW.





Fig. (2,B):-Densitometeric measurement of the oxyblot lanes of fig. (2,A)



Fig. (3):- Quantitative evaluation of TUNEL positive cells in BRL3A cells treated with CcL₄.



Photo (1):- Cell apoptosis of BRL3A cell line after exposure to Ccl4 as detected with TUNEL assay under fluorescent microscope.







Fig. (4,B):- Densitometeric measurement of the oxyblot lanes of Fig. (3,A)



Fig (5):- Quantitative evaluation of TUNEL positive cells in BRL3A cells treated with ccl4 and different doses of vitamin E (VE), ascorbic acid (AA), Mannitol (M) and aminoguanidine (AG).

4. Discussion

Various pharmacological or chemical substances are known to cause hepatic injury. It is believed that the occurrence of drug/chemicalsinduced liver injury is especially associated with oxidative stress and a cellular imbalance between the production and elimination of free radicals (*Castro and Freeman, 2001*). Overproduction of free radicals could directly injure hepatocellular membrane by lipid peroxidation, or other means, followed by a series of cascades of cellular events such as massive release of inflammatory mediators or cytokines, which eventually lead to liver injury (*Higuchi and Gores, 2003*). Therefore, it is valuable to identify natural drugs or compounds that can antagonize the deleterious action of free radicals and act as an antioxidant to protect hepatocytes from damage.

In the present experiments, hepatocytes cultured cells were utilized. In an *in vitro* system, compounds affect the cells directly and continuously until the removal of compound -containing medium. In addition, when cells are cultured using single type of cells, there are no interactions from other interstitial cells (*Kikkawa et al., 2006*).

Cytotoxicity of CcL_4 has been selected as a model for induction of oxidative damage. Carbon tetrachloride is one of the most extensively studied hepatotoxicants (*Bhattacharjee and Sil*, 2007). It is now generally believed that CcL_4 hepatotoxicity depends on its reductive dehalogenation catalyzed by cytochrome P-450 enzyme in the endoplasmic reticulum of hepatic cells leading to the generation of an unstable complex trichloromethyl radical. The superoxide anion, H₂O₂, and the hydroxyl radical (OH) are reactive oxygen species (ROS) mainly produced in mitochondria (*Wang et al.*, 2007).

The possible protective effect of some antioxidants (ascorbic acid, mannitol and aminoguanidine) has been attempted on hepatocytes cultured cells treated with CcL_4 . Trials were compared to vitamin E known to be important lipophilic antioxidant in biological tissues. The activity of an antioxidant may depend on its reactivity towards particular radicals, its ability to concentrate near the critical target in the cell or its inhibitory action on radical formation (*Anderson and Phillips, 1999*).

Electron spin resonance (ESR) was employed to measure directly reactive oxygen species (ROS) production in the liver of CcL_4 -treated hepatocytes cell line. Results indicated that ROS production was highly elevated in the cultured media of the cell line at 4 h after CcL_4 treatment, thus further confirming that free radicals and oxidative damage certainly play a vital role in the pathogenesis of acute liver injury (*Wu et al.*, 2007).

Cell viability depends directly on the structure of the membrane, and damage to the cellular membrane can be detected by enzyme leakage. It has been generally accepted that leakage of the cytosolic enzyme LDH correlates well with cellular viability. thus being a useful indicator of plasmatic membrane damage (Grajeda-Cota et al., 2004). In the present study, observations revealed that CcL₄ induced significant increase in the leakage of LDH, ALT and AST enzymes into the medium after 14 hr. The obtained results are in harmony with those reported by Chandan et al. (2007). It is interesting to note that there was difference in the degree of leakage of ALT and AST enzymes, where AST enzyme leakage was higher than ALT enzyme leakage. McQueen and Williams (1982) reported differences in the extent of leakage of enzymes from the cytosolic fraction of hepatocytes. These differences could be due to differences in the tightness of binding of cytosolic enzymes to cellular organelles and in the size of the enzyme molecule (Nakamura et al., 1985). Moreover, AST is present in two isozymes, one located in the cytoplasm and the other in the mitochondria (Latner,

1975). The presence of enzymes outside the cell represents damage to the hepatic cell.

Involvement of lipid peroxidation in the mechanism of carbon tetrachloride-induced hepatotoxicity has been a point of controversy. The present results denoted that CcL₄ did not induce lipid peroxidation as there was no significant difference in the level of thiobarbituric acid-reactive substances (TBARS) between CcL₄ treated cells and control cells. These data are comparable to that of Ikeda et al. (1998) who reported that TBARS, which are widely used index of lipid peroxidation, don't increased significantly in the liver of rats treated with CcL₄. Previous investigators reported absence of lipid peroxidative degradation products in mice after exposure to carbon tetrachloride and have used this evidence against the hypothesis that lipid peroxidation is an integral part of the events that cause tissue damage (Lee et al., 1982). On the contrary, Krithika et al. (2009) recently proved the involvement of lipid peroxidation on exposure to CcL₄.

As regards to oxidation of proteins, the present study was able to detect that there was a significant increase in the formation of carbonvl proteins in the CcL₄ treated cells comparing to control cells. Free radical-mediated oxidation of proteins results in the formation of carbonvl groups in quantities that reflect the intensity of the oxidative stress (Robinson et al.; 1999). Protein carbonyl content is widely used as both a marker for oxidative stress and a measure of oxidative damage (Luo and Wehr, 2009). Carbonyl (CO) groups (aldehydes and ketones) are produced on protein side chains when they are oxidized. These moieties are chemically stable, which is useful for both their detection and storage (Dalle-Donnea et al., 2003). Protein oxidation may play a role in the pathogenesis of CcL₄ induced liver injury and that the accumulation of oxidized proteins may be an early indication of CcL₄ induced liver damage(Sundari et al. 1997). The usage of protein (CO) groups as a marker may have some advantages in comparison with lipid peroxidation products because the formation of protein bound (CO) groups seems to be a common phenomenon of protein oxidation and because of the relatively early formation and stability of oxidized proteins (Dalle-Donnea et al., 2003).

Apoptosis is characterized by morphological changes such as membrane "blebbing", nuclear condensation and fragmentation (Ramage, et al., 2006). These morphological changes are a result of a cascade of biochemical changes occurring within the cell, resulting in activation of caspases and DNA fragmentation (Hengartner, 2000). The present data indicate that along with oxidative damage, apoptosis plays a crucial role in CcL_4 induced hepatotoxicity which was indicated by marked increase in DNA fragmentation (increase in the percent of TUNEL positive cells) in the CcL_4 treated cells compared to control one. Apoptosis represents one of the mechanisms of cell death after CcL_4 induced liver injury (Shi et al, 1998).

Effects of antioxidants on oxidative damage in vitro systems are variable. In most cases, endogenous antioxidant enzymes, particularly catalase, are very effective in preventing damage. Exogenous antioxidant chemicals, on the other hand, can have a pro-oxidant effect at high doses. The chemical composition of culture media and the doses of antioxidants that can be applied in culture systems are major factors in confounding interpretation of in vitro results (*Anderson and Phillips, 1999*).

Vitamin E antioxidant functions mainly in and around the membrane/lipid bilayers acting as the chain breaking antioxidants (Janero and Burghardt, 1989). Results of the present experiments showed that incubation of hepatocytes with vitamin E at concentrations of 100, 50 and 25 µM induced hepatoprotective effect against CcL₄ induced toxicity. This was manifested by an increase in the stability of the cell membrane and decreased leakage of cytoplasmic enzymes (LDH, ALT and AST). Although we cannot rule out a direct scavenging action of vitamin E on CcL3 radical, inhibition of lipid peroxidation is not a likely explanation for vitamin E effects in the present study. Although the ability of CcL₄ to initiate lipid peroxidation in various biological systems has been equivocal, an important observation of the present study was that there was no evidence of CcL_4 -initiated lipid peroxidation.

Ascorbic acid, in the present study, was ineffective against the elevation of enzymes leakage (LDH, ALT and AST). Also it was not able at any concentration to reduce the oxidation of protein. Ascorbic acid did not show any protective effect against DNA damage caused by CcL₄. Similar results were obtained by Winter et al. (2005) who reported that ascorbic acid was ineffective at reducing oxidative DNA produced damage by camphorquinone which generates reactive oxygen species and causes oxidative DNA damage in vitro. On the contrary Qin et al. (2006) found that ascorbic acid reduced myocyte apoptosis and ameliorated myocardial damage after acute myocardial infarction. Park and Lee (2008) reported that high ascorbic acid concentrations might act as a pro-oxidant due to its auto-oxidizing properties.

Mannitol is generally accepted as being a hydroxyl radical scavenger (Van Zandwijk, 1995). Previous studies have used mannitol to determine whether hydroxyl radicals are the predominant

species responsible for Copper mediated DNA damage (Li et al., 1995). However, these studies have found that mannitol is generally ineffective at preventing Cu- mediated oxidative damage in the presence of an ROS-generating system and at mannitol concentrations less than 100mM. The present results showed that mannitol did not effectively reduce CCL₄ induced hepatotoxicity. It showed no reduction in the leakage of cytoplasmic enzymes, protein oxidation or DNA damage. Winter et al. (2005) reported that mannitol treatments of 10.0, 5.0, 2.5, 1.0, and 0.5 mM did not influence the extent of DNA damage generated by camphorquinone. In explanation, it is possible that some ROS generated from CcL₄ are not effectively scavenged by mannitol. Additionally, mannitol may not be a very potent hydroxyl radical scavenger or mannitol may not have adequate access to the damaging ROS (Winter et al., 2005). Shinar et al. (1983) demonstrated that mannitol effectively scavenged hydroxyl radicals at concentrations greater than 1M.

In the present experiments, aminoguanidine (AG) treatment against CCL_4 induced hepatoxicity was ineffective in reducing the leakage of enzymes, the oxidation of protein, and the DNA damage. A notable finding was that AG at a dose of 400 μ M significantly increased leakage of LDH into the media comparing with CcL_4 group. Our results confirmed reports of other investigators (*Ou and Wolff, 1993 and Skamarauskas et al., 1996*) that AG has pro-oxidant activity where it generates H₂O₂ at a low rate in vitro.

In conclusion, the present study indicates that CcL_4 has a potential cytotoxic effect in BRL3A cell culture, and apoptosis represents one of the mechanisms of cell death after CcL_4 - induced liver injury. There was no evidence of CcL_4 -initiated lipid peroxidation. Vitamin E seems effective and provides complete protection against CcL_4 induced hepatotoxicity in culture cells. Ascorbic acid, mannitol and aminoguanidine were ineffective against CcL_4 toxicity. Aminoguanidine, in addition, generates H_2O_2 in vitro, so it has pro-oxidant activity.

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Recognition of Geographical diffusion Esophagus Cancer in Southwestern of Caspian Sea

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Abstract: Esophagus Cancer is an important disease in Iran and has second rank of death after heart disease, in north of Iran this disease has maximum diffusion and has arrangement of Iran in Asian belt of this disease. Environmental and climatically conditions in each area could be helpful toward diffusion and out breaking diseases, like as Esophagus Cancer. Rate of appearing this disease in recent years in Iran and especially in Gilan province (Southwestern of Caspian Sea) has increased that this problem could be very importance by cost ill and its problems. The aim of this paper is recognition of geographically diffusion of Esophagus Cancer in Gilan and presents it by map. The research method of this paper has used from medical documents diseases from hospital, library document studies (Soil, Geology and climate) and field work from 2001-2005 years. Results of this paper has showed that Esophagus Cancer (most rate) rather than on mans ,and it has more diffusions in central area in province of Gilan(Talesh, Lahijan, Someh sara and Rasht) and environmental factors such as, soil factor (Lithosel and Brown forest, climatically factors (season and cold course) and has relationship effectives on Esophagus Cancer diffusions and its presenting by map. This paper has written in framework at Geography of Health (Medical geography) that Geographical scattering an important of this paper, than this essay must be complete by other specialists (Environmental and medicines).

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Keywords: Esophagus Cancer, Geography of health, Caspian Sea

1. Introduction

Esophagus Cancer is the ninth prevalent cancer in the world and one of those diseases that has become prevalent among the people of the world in the recent years, and after cardiovascular diseases is in the second degree of importance (Hoda, 2003, P. 84). Between each nine death in the world, one of them is because of cancer (Rezvani, 1995, P. 1). About 70000 cases of a new cancer happen in Iran, annually, that 450 cases are head and neck cancer, and in the world, 500,000 persons catch this cancer that about 300,000 persons of them die (Harirchi, 2007. P. 6). Mechanism of this disease (Esophagus Cancer) has not been known up to now (Mirhosseini, 1998, P. 100). Cancer of digestion system diseases that Esophagus Cancer is in the first rank, is in the first rank in northern side of Iran and Iran is located at the Asian Belt of this disease (Porahmad & Yavar, 2001, P. 14). Geographical distribution of this disease is surrounded between western coasts of Caspian Sea and China in the eastern side. Some regions in Iran, regions of central part of Asia, former Soviet Union, Afghanistan, Siberia and Mongolia are those regions where are consisted by this disease. In China, the first reason of death is Esophagus Cancer among all different types of cancers. This belt passes across the northern side of Iran and south side of Caspian Sea,

and in the highest level in Torkaman Sahra. Outbreak of this cancer is 130 persons per each 100 thousands persons in China and 114 persons per each 100 thousands persons in Coastal Parts of Caspian Sea (Rabinz, 1990, P. 17, 18).

Rampancy of this disease has been known related to environmental factors and type of nutrition. Introduction to geographical spreading out of this disease and its relation with human environment is one of interesting specialist cases for geographers that is classified in Medical Geography Branch or Geography, such a way that with Health consideration to the dependence of human to the environment and its gradual effect on human body physic, dependence of health and sickness in human life, is one of environmental important roles. Therefore, a physician may treat accurately through knowing the geographical environment and its related factors for diagnosis of a disease. As Boghrat said, "Each person who is eager in Medicine Science, must pay attention to the effects of seasons." (Shokouhi, 1975, P. 345, Houshvar, 2002, P. 10). Effect of climate conditions on diseases is called by researchers of Medicine Science as Meteorpathology, Climo-pathology and Bio-climatology Medicine (Houshvar, 1998, P. 6). Duty of the Medical Geographer is discovery of the center of the disease, determination of geographical dispersion of the disease, determination of those geographical reasons and factors that cause to appearance and distribution of the disease, and also the natural dams (such as sea, mountain, plain, desert, wave, coldness and so on) against each epidemic disease (Houshvar, 2002, P. 6-28). Regarding to this matter, Mac Gelashen has discussed about the environmental facts and elements in its all dimensions, human and disease and health, and in a two-way relation for human health and disease societies, in Medical Geography Book, and more than the other facts, climate and weather factors has been paid attention (Jafarpour, 1996. P. 153), and for drawing up of the relation between human and nature, Topo-clima factors and preparation of the related maps for diagnosis of disease have been known very important (Meloinhou, 1970 and Janioso, 2003 and Graham, 2004). In Ghanoun, Medical encyclopedia, Ebn-e-Sina, geographical distributions of a lot of contagious and non-contagious diseases, and climatic conditions on health and sickness, even type of disease and its outbreak have been explained (Khatami, 2004, P. 755). Different studies related to types of cancers indicate the importance of the effect of environmental and genetic facts on sickness, whereas, identification of the genetic facts is so hard, it seems that through changing of environmental conditions, prevention of cancer diseases may be possible in a wide range (Mirhosseini, 1994, P. 101) such a way that the environmental facts have been considered up to 80-90% for all types of human cancers. Mirhosseini during study on health and standard of disease thinks that a high ratio of cancers is related to the environment facts . Primary study of this disease indicates that it is related to five factors: Atmosphere, soil, natural geography, living creatures and time, such a way that shortage of vitamin and Tanon in foodstuffs. Mo. (Molvbdenum) in soil, increase of Ammoniac and Nitrate in the air and water may cause to increase of Nitrosamine (Mirhosseini, 1994, P. 103).

The most important environmental facts that are known related to Esophagus Cancer consist of:

- 1. Cigarette, Alcohol and Opium
- 2. Ray radiation and usage of alkaline
- 3. Social-economical status (shortage of some vitamins such as A and B2)
- 4. Different kinds of pickles, hot drinks and foodstuffs (hot tea)
- 5. Pollution of foodstuffs with Silica (Si) crystals and polluted fugues in corns.

Food shortage of Molybdenum, Zinc and Vitamin A cause to increase of this disease. As Molybdenum would be in low amount in soil (less than 0.2 micro-gram, availability of Molybdenum is measured by soil PH. Soils with PH more than 6-6.5, rarely need to Mo., soils with PH less than 6, because of surface absorption with hydrous oxides, are low and critical amount in alkaline soils, is 0.95 microgram in the lieu of one gram soil, too (Tandon, 2004, P. 73), change of Nitrate Amine increases in the plant (Rabinz, 1969, P. 18). Accumulation of Nitro Amine in stomach of people will cause to increase the probability of cancer and through increasing of Molybdenum to the soil it change to the form of Molybdate Ammonium that will decrease the danger of cancer (Malakouti & Kahrati, 2005, P. 170). Increase of Nitrosamine in foodstuffs may cause to be increased in cheese, mutton, yoghurt, baked bread and paste indicate its high outbreak. With consideration to this matter that Esophagus Cancer is the ninth prevalent cancer in the world, and in developing countries is in the fifth rank (Rezaei, 2001, P. 113) and in most European countries such as United Kingdom, Fenland, Island, Australia, Eastern South part of Africa, Brazil, Kenya, Malawi, France, Kourachao (in western north side of Venezuela) it may be seen and is in the high degree of importance in the view of introduction to sickness, relation between sickness and environmental facts and its prevalent (Paoliko, 2004, P. 420).

> 6- Environmental pollution (Azote and Nitrate fertilizers): Azote fertilizers and usage of them in agriculture may cause to increase Nitrate in soil, botanic, human and animal organisms. Experiments indicate that accumulation of Nitrate in potato is very high with consideration to usage of Azote fertilizers.

> 7- Radiation of radioactivity of isotopes in mother stones, specially, acidic stones is very high, those soils with heavy and clayey structure that are radiator are of light soils, in gravelly soils, amount of Molybdenum is low, and in soils made of clayey and Ganity minerals, amount of Molybdenum is high. In new volcanic soils and those soils that have high organic materials, amount of Molybdenum is high (Shakouri, 2004, P. 134-140).

Target in the present research is introduction to geographical distribution of Esophagus Cancer outbreak and its relation with geographical environmental facts in Guilan Province.

2. Material and Methods

Study method of the research is usage of field observations, analyzing and description. At first, through drawing up of questionnaires and related tables, direct observance of medical files in hospitals, repeated reference to medical-treatment centers, and interview with specialists, statistic of patients during statistical period between (2001-2005) has been gathered that totally 351 cases of this sickness, confirmed by specialists (physician) has been selected (Hanife, 2006, pp 45). Then, with consideration to the responds to the related questioners and requirement to the present research, the responds have been classified and geographical variance of Esophagus Cancer sickness in Guilan province, on the basis of the place of residence, gender, monthly and seasonal references, the related maps has been prepared using the software of data systems. Then, with consideration to the weather and meteorology statistics and using the soil map (required elements of this disease (Molybdenum)), also using the type of effective soil, the related map has been prepared and by gathering the maps, the probable centers for its prevalent have been known and also the relation between the location of sickness (region) and environmental conditions in the maps has been determined.

3. Results

Registered statistical society (population) has been the numbers of suffering persons to Esophagus Cancer in Guilan Province, as from year: 2001 to 2005, who were 351 cases and has an notable increasing process that between these persons, about 77 cases, their places of birth and residence are not clear. The most rampancy of this disease is related to the year: 2005 (35%) and the less statistic is related to the year: 201 (6%). The form based on the age and gender, out of total cases of affliction to Esophagus Cancer during the statistical period is 210 men (60%) and 141 women (40%). Esophagus Cancer in the ages between 61-80 years are more prevalent, and in age group of 51-60, affliction rampancy in two genders (female and male) has a small difference. But in higher ages, affliction of the men is more than the women (Table 1,2).

Table 1. Affliction rampancy to Esophagus Cancer inGuilan Province (/2001-2005)

Total	Number	Year
%		
6	23	2001
7	24	2002
24	83	2003
28	98	2004
35	123	2005
100	351	Total

	Num	bers of	observed	cases		
Age	Tot	Tot	Wom	Wom	Men	Men
Groups	al	al	en	en	%	
		%				
0-10	-	-	-	-	-	-
11-20	-	-	-	-	-	-
21-30	2	0.7	1	0.3	0.3	1
31-40	14	4	10	2.7	1.2	4
41-50	39	11	13	3.7	7.4	26
51-60	56	16	26	7.4	8.6	30
61-70	110	31	44	12.5	18.8	66
71-80	114	32.6	38	10.8	21.7	76
81-90	13	3.7	8	2.3	1.4	5
91-100	3	1	1	0.3	0.6	2
101-	-	1	-	-	-	-
110						
	351	100	141	40%	60%	210
		%				

Table 2. Number and percentage of rampancy of the patients suffering from Esophagus Cancer, divided to the gender and age (2001-2005)

Out of the entire statistical society (population), 274 cases, their place of birth ware clear. Number of 17 cases (6%), place of their birth were out of the province, and the most rampancy of the place of birth of the patients suffering from Esophagus Cancer are in Rasht, Talesh, Lahijan, Fouman, Somehsara and Roudbar.

Out of the entire statistical society (population), 300 cases, their residence place were clear. The most rampancy of the place of residence has been in Rasht, Talesh, Lahijan, Somehsara and Fouman. It seems that Esophagus Cancer has had more outbreak among those persons that was born in their residence places, and on the other hand, genetic transfer of this disease indicates that must be studied by medicine specialists (Figure 1).

In the view of the season of suffering patients from Esophagus Cancer, the most reference has been in autumn season (26.49%) and the less reference has been in winter season (23.36%) (Table 3) that in another similar research in the eastern side of Mazandaran, the reference season has been as the same as seasonal distribution in Guilan province (Farzanehfar, 206, P. 83).



Figure 1 Geographical rampancy of Esophagus Cancer in Guilan Province on the basis of the (place of birth) residence (2001-2005)

Table 3. Seasonal distribution of the patientssuffering from Esophagus Cancer

Season	Total	Men	Women
	numbers		
Spring	87	53	34
Summer	89	55	34
Autumn	93	56	37
Winter	82	46	36
	351	210	141

In the view of spatial and seasonal rampancy, reference of the patients to the hospital in winter season, in Talesh and Rasht counties, and in autumn season in Roudbar and Roudsar counties, in summer in Astaneh Ashrafieh and Anzali counties, and in spring season in Roudsar and Rasht counties are seen that its geographical distribution, except Rasht city, is in the most level in southern regions of the province (autumn season), western north regions (winter season), northern regions (summer) and eastern regions (spring season), (Table:4).

Table 4. Seasonal percentage of the numbers of thepatients suffering from Esophagus Cancer in thecounties

	Winter	Autumn	Summer	Spring
Talesh	36	20	28	16
Lahijan	40	15	30	15
Anzali	12.5	12.5	50	-
Roudsar	8	31	23	28
Rasht	28	27	20	25
Roudbar	22	33	33	12
Astaneh	9	18	73	-
Ashrafieh				

In order to study of the relation between regional elements and spreading out of the numbers of the patients suffering from Esophagus Cancer, the monthly changes of the regional elements and numbers of the patients have been used. In order to usage of Pierson Correlation Method and introduction to the type of the relation between regional parameters and the sickness, the statistics of those cities were used that had the most or the less numbers of the patients. P-Value method was used for the related test. Whereas, the subject of the research is related to the human life, the least relation (weak correlation) has been important and meaningful for the researcher, such a way that in some cases P-Value would be more than 1%, there is not any sufficient reason in a=1% that correlation is zero (Negahban, 2001, P. 71).

In the view of monthly changes in rainfall and numbers of the male and female patients suffering from Esophagus Cancer in Rasht city, indicate that in those months that the amount of rainfall decreases, references of the male and female patients decrease. Monthly changes of temperature and numbers of the male and female patients indicate that in those months that the temperature is in increasing process, reference of the patients decrease, visa versa, in those months that the humidity increases, references of the patients decrease and visa versa. Such a way that in another research that was about the Topo-clima relation of the cardiovascular patients of Rasht and Manjil has been studied, increase of relative humidity has had a converse relation with cardiovascular patients that this matter needs to more investigation in the medical and genetically view. It must be added that this research has been fulfilled in the geographical-medical (health) view and need to more specialty studies (regional data are in daily aspect) (Ramezani, 2005, P. 26, Table 5).

Table 5 Final relationship between regionalelements and numbers of male and female patientssuffering from Esophagus Cancer in some counties inGuilan Province (1=positive relation), (-1=negativerelation) and (0=without relationship)

	Rainfall	Max. Temp.	Min. Temp.	Max. Humidity	Min. Humidity
Total in	1	-1	-1	0	1
Talesh		•		0	
Male in	1	1	1	-1	-1
Talesh					
Female	1	-1	-1	1	1
in					
Talesh					
Total in	-1	-1	-1	-1	1
Rasht					
Male in	-1	-1	-1	-1	-1
Rasht					
Female	1	-1	-1	-1	1
in Rasht					
Total in	1	-1	-1	1	1
Lahijan					
Male in	1	-1	-1	1	1
Lahijan					
Female	-1	-1	-1	1	1
1n					
Lahijan	1	1	1	1	1
Total in	-1	-1	1	-1	1
Kouddar Molo in	1	1	1	1	1
Roudbor	-1	1	1	-1	-1
Female	1	1	1	1	1
in	-1	-1	-1	1	1
Roudbar					

In the view of relation of the soil with spreading out of those patients suffering from Esophagus Cancer in the Province indicates that the amount of Molybdenum in the soil is one of environmental facts that is effective on development of Esophagus Cancer. Such a way that its low amount in soils will cause to increase the absorption of Nitrosamine and consequently to increase the Esophagus Cancer. In an experiment that has been fulfilled on one gram of soil indicates that when the amount of Mo would be less than 0.2 micro gram, in the lieu of 1 gram of soil, that type of soil is classified in those soils that have shortage. In the case of dispersion of type of soils in different regions in the

province, in the view of the amount of PH and consequently, availability of Mo in them, we can say that in the regions of Astara, Talesh, totally, western side of Guilan Province, Lahijan and Roudsar regions, because of high rainfall and mass botanic covering, and consequently accumulation of organic materials in the surface of the soil, the interaction of the soil is acidic and is variable between 4.5 to 6.7. In eastern side of Guilan Province, (Sepidroud delta, as from Rasht to Astaneh Ashrafieh) interaction of the soils are neutral to a little alkaline and is variable between 7 to 7.8, because the resource of its precipitates originate from aquriferous basin of Sepidroud and out of the province. Consequently, its original materials are limy and because of being new of the precipitates, cleaning of the lime is low and does not continue in high depth, but also it has happened on the surface of the soil. forests

4. Discussions

Study of geographical spreading out of Esophagus Cancer in Guilan Province indicates that in the view of gender, outbreak of this sickness among men is more than women. Difference of affliction of this sickness in men and women, about Stomach Cancer is less. Esophagus Cancer happens in age group of 61-80 years old, more. The most reference of patients suffering from this sickness to treatment centers has been in Oct, Feb of Autumn and Summer seasons, and the least reference is in winter. We can say that this sickness is related to the seasonal outbreak arising the environmental facts that needs to be studied through more specialty studies.

Dispersion of the type of soil and its relation in those regions that Esophagus Cancer is more prevalent in there, a wide area of these regions are covered by soft alluvial soils, humid and semi-humid grassy soils, forestall gray and brown soils, and Lithosel soil consisting of external stones. And in those regions that this sickness is seen less, are covered by Lithosel soils in the regions of forestall brown soils, Lithosel consisting of external stones in the regions of brown soils, limy Lithosel and Sirozem in the region of brown soils. We can say that the soils, in the view of physical and chemical specifications, may affect on geographical spreading out of Esophagus Cancer, such a way that the most outbreak of this sickness can be seen in the around of those regions that are covered by Pouzolick red soils, alluvial and semi-marshy soils, and the regions that the sickness can be seen in the least degree, are those regions that are covered by limy and brown Lithosel soils and forestall Lithosel soils. This matter should be completed by more researches and studies of the related specialists.

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Development of ELISA Method for primary Detection of HCV using core Antigen

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Abstract: Studies show that Hepatitis C Virus (HCV) antigens appear before antibody while the early days of infection. Therefore detecting antigens could lead us to diagnosing the infection on time. The aim of this study was to develop a simple and sensitive enzyme immunoassay for the detection of hepatitis C virus (HCV) core antigen in order to evaluate the role of core antigen as a marker of HCV infection. A total of 280 samples was tested by third generation anti-HCV, and the reverse transcription polymerase chain reaction (RT-PCR) was performed only when the anti-HCV enzyme immunoassay (EIA) was positive. All samples were tested with HCV core antigen using Elisa kits. Among the 280 samples, 95 samples were anti-HCV positive. Among those 95 samples, 75 samples were RT-PCR-positive. The cut-off value was set at 0.15 unit of optical density (equivalent to 2.5 pg/ml of core antigen based on the distribution of healthy subjects (anti-HCV-negative subjects). The difference between the mean optical density values of HCV-ribonucleic acid-positive (HCV-RNA-positive) samples and HCV-RNA-negative samples in the HCV core antigen assay was highly significant (1.4 us 0.08, p < 0.005). The sensitivity and specificity of the core antigen assay were 88% and 96%, respectively. The pretreatment of the anti-HCV-positive samples with a solution that contained 1.5 M glycin buffer (pH = 2) increased the sensitivity of the assay (from 57.3% to 88%). This assay is a simple, sensitive, and useful method for use as a screening strategy for HCV infection in anti-HCV-positive individuals.

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Keywords: Hepatitis C virus (HCV); Core antigen; ELISA; Polymerase Chain Reaction (PCR)

1. Introduction

Hepatitis C virus (HCV) is a single-strand, ribonucleic acid (RNA) virus that contains approximately 9500 nucleotides for coding a polypeptide with a length of roughly 300 amino acids. This virus is the cause of hepatitis C disease, and it has been well established that the initial origin of this infection is the injection of contaminated blood or blood products. At present, the suggested method for recognizing when an individual is infected by the hepatitis C virus is to determine the antibody against HCV. This approach is problematic, because the test produces both false-negative and false-positive results. In addition, this test cannot distinguish between chronic and acute infections. So. Immunoblotting is applied in order to study falsepositive results (1, 2). It is especially important to recognize the infection in the initial steps to prevent secondary infections in blood transfer (3). Therefore,

a method is required that can recognize HCV in samples, especially in the initial phases of infection. Presently, molecular methods are used to determine the presence of viral RNA in samples. These methods use the polymerase chain reaction (PCR) technique, which has advantages and drawbacks, for investigating treatment efficiency. The application of monoclonal or polyclonal antibodies, especially against the viral core antigen, is under investigation (4, 5, and 6). The aims of the present study is to use the Elisa kit approach and demonstrate high sensitivity and specificity for recognition of this antigen and to compare the effectiveness of the approach with other related techniques. Based on the results of this study, the problems with other techniques mentioned earlier can be alleviated.

2. Material and Methods Samples:

280 serum samples were collected from clients of the molecular biology sections of some medical laboratories of Tehran.

Testing the antibody against the hepatitis C virus:

Using the kit from the Pishtaz Teb Zaman Company, all 280 samples were examined according to the procedure provided in the enclosed brochure. Among the 280 samples, 95 samples contained the antibody, while the optical density (OD) values of the remaining 185 samples that were less than the cut-off value specified in the kit, which was an average OD of +02 as the negative control. The kit uses antigens NS3, NS4, NS5, and the Core to identify the antibody, and its use has been approved by the Iranian Reference Laboratory of the Ministry of Health and Care.

RT-PCR for positive samples:

RNA was extracted using the QIAGEN Company's QIAamp Circulating Nucleic Acid Kit Quantitative RT-PCR was accomplished by an Artus 3000 device. The characteristics of the cycle's program of this device are provided in Table 1.

 Table 1. Characteristics of the cycles of RT-PCR device

Cycle information	Quantities						
Cycle quantity	45						
Analysis mode	Quantitative						
Heat turns	First	Second	Third				
Heat degree	95	55	72				
Incubation time(min)	8	20	20				

Testing Core Antigen by the Elisa method:

We utilized monoclonal antibodies from mice, which were provided by the American company Genwaybio. The monoclonal antibodies were available at various concentrations in a carbonate coating buffer with pH = 9.6. Coating was performed at a temperature of 4 o C, after which the specimens were stored at room temperature for 24 hours. Subsequent to washing the Elisa plate with a phosphate buffer solution containing Polysorbate 20 (Tween 20), the wells were blocked for one hour at room temperature with a blocker solution containing 1% bovine serum albumin (BSA) and carbohydrate. The contents of the wells were then removed and dried at room temperature for six hours. These plates were stored in foil, along with a desiccant, at 2 - 8 oC until it was time for the experiment. This antibody identifies a marker of the antigen in the amino acid range of 1 to 80.

Conjugate 1: A polyclonal antibody of goat origin, prepared by the American company Genwaybio, was conjugated by biotin using the following procedure. This antibody identifies an index of the antigen in amino acid range of 80 to 120. An antibody solution with a concentration of 1 mg/ml was prepared with a buffer containing 0.2 M bicarbonate and 0.15 M sodium chloride at pH = 8.8. Then, a biotin solution (Act Biotin LKB) was prepared at a concentration of 4 mg/ml in dimethy formamide (DMF). Subsequently, 200 µl of the biotin solution were added to 10 mg of the 1 mg/ml-antibody solution, and the mixture of the two solutions, at room temperature, was shaken for 15 minutes. After that, 100 µl of 1 M ammonium chloride were added to stop the reaction, and the resulting solution was thoroughly mixed. Then, the solution was incubated for 15 minutes at room temperature while the mixing continued. In order to bring out the free bioptin, dialysis was performed against 0.05 N PBS buffer using a dialysis bag with a cut-off value of 30. Dialysis was repeated after changing PBS buffer solution. Considering the initial volume of antibody and its final volume, the concentration of the antibody conjugated by bioptin was calculated to be about 2.5 mg/ml.

Conjugate 2: In order to identify the antibodyantigen complex, we utilized different concentrations of HRP-conjugated streptavidin prepared by Sigma Company. This conjugate was diluted with a stabilizer solution that is specifically used for maintaining HRP-conjugated streptavidin. The stabilizer solution was obtained from Pishtaz Teb Company.

Test Procedure: A 100-µl serum sample and 50 µl of conjugate 1 diluted in a solution of phosphate buffer containing 0.5% BSA were added to each well. For positive control, the Core recombinant antigen of hepatitis C virus with a concentration of 100 pg/ml in phosphate buffer containing 1% BSA was used. This recombinant antigen was prepared by the Russian RPC Company. The wells were incubated for one hour at a temperature of 37 o C in a water bath. After incubation, the wells were washed out five times by washing solution containing phosphate buffer and Tween 20. Subsequent to completely taking out the washing solution, 100 µl of conjugate 2 were added to each well, followed by incubation for 30 minutes at 37 oC. After incubation, the wells were washed five times with the buffer solution, and 100 µl of the

chromogen substrate solution were added to each well after each well was completely emptied. The chromogen substrate solution contained tetramethyl benzidine and H2O2, prepared by Pishtaz Teb Company. Fifteen minutes later, the color of the solution changed from colorless to blue, indicating that the reaction had taken place. When 100 µl of stopping solution (1 N hydrochloric acid) were added, the reaction stopped, and the color of the solution changed from blue to yellow. The OD value of the wells at a wavelength of 450 nm was measured to be 630 using a reference filter. To determine the cut-off point, we calculated the mean and standard deviation (S.D.) of optical density pertaining to negative samples. Afterward, a cut off was determined for the kit according to the formula (cut off = mean + 3*S.D.).

For excluding the probable interference effect of the presence of the antibody with the Core antigen from the samples, serum samples with positive antibody tests were pretreated by two different solutions, including 1.5 M glycine buffer with pH = 2, 0.5 N hydrochloric acid, and Triton X-100 with a concentration of 0.1%. Then, 100 µl of the sample was mixed with 100 µl of each of the different solutions and kept for 30, 60, 90, and 120 minutes at 37 o C. For acidic solutions, the pH of the sample pH was adjusted to 7 by adding Tris buffer with pH = 10.4. In order to study the probable effect of these solutions in causing background optical density, several negative samples were also investigated in addition to the positive samples. Also, to make sure that the effect of these solutions on the antibodies contained in the serum on the samples that were treated by the Triton X-100 and acidic solutions, the antibody test was repeated once more, and the results were compared to titration of the antibody in serum samples that were not treated by these solutions. With the aim of investigating the diagnostic sensitivity by BBI International panels, seroconversion panel numbers PHV906, PHV914, and PHV920 were selected for study. Evaluation of analytic sensitivity was then studied by serial dilution of recombinant core antigen (amino acids 2 - 119) in the HCV antibody negative sample.

Imprecision Test: To facilitate studying the imprecision of the assay, three samples with different concentrations of antigen were tested ten runs over a five-day period, and each test was performed as duplicate.

3. Results

Among the 280 samples, 95 samples had positive antibody tests, while 75 of these 95 samples had positive PCR results. Among the 75 samples with positive results in both PCR and antibody tests, 43 cases (57) had positive results in the Core antigen test; for the 20 samples with positive antibody tests and negative PCRs, one case was positive in the Core antigen test, whereas five cases were positive in the Core antigen test but had negative results in both the PCR and antibody tests (Table 2).

Table 2. Correlation of antigen test with PCR method

PCR	results	Core Antigen test		
		+	-	
+	75	43	32	
-	205	8	197	

Considering the results, the sensitivity and specificity of the identification test were estimated to be 57% and 96%, respectively. The mean OD value of samples with negative antibody tests and PCRs was calculated to be 0.080 with a standard deviation of 0.023. The cut-off of the OD value for discriminating negative samples from positive samples was determined by adding three times the S.D. to the mean OD value, resulting in 0.15. Therefore, samples that had OD values greater than 0.15 were considered positive. The OD value values for the positive samples ranged from 0.35 to 2.91 with mean value of 1.44

Table 3. The correlation between the antigen test andthe PCR method after pretreatment of samples byglycine buffer

PCR 1	esults	Core An	tigen test
		+	-
+	75	66	9
-	205	8	197

Effect of pretreatment of serum samples to acidic solutions and Triton X-100:

The titration of antibodies present in the serums that were treated by acidic solutions had a considerable decrease compared to serum samples without this treatment. Even so, no such decreases were observed in samples that were treated by Triton X-100. In addition, antibody titration had a substantial decrease after treatment by acidic solutions in 22 samples with negative antigen test and positive PCR test with verified antibody presence (Fig. 1).

This pretreatment led to increases in optical density and positive antigen tests in 23 cases out of 32 samples. Regarding the comparison of optical density of samples with positive PCR tests and negative antigen tests, pretreatment by glycine buffer (pH = 2) for one hour at a temperature of 37 oC resulted in more increase in optical density than treatment by 1 N hydrochloric acid at 37 oC. On the other hand, treatment by Triton X-100 had no effect on increase of optical density. Furthermore, optical density of samples with negative antibody tests, which were used as controls, showed no increase. This indicates that the increase in optical density in samples with positive antibody tests after pretreatment by acidic solutions for testing the antigen is a specific phenomenon caused by dissociation of the antibody from the antigen and subsequent identification of Core antigen using this evaluation technique. (Fig. 1) Comparison of pretreatment effect of serum samples with different solutions on dissociation of antigenantibody complex in HCV Core antigen test:

The correlation between the PCR method and antigen determination after pretreatment of serum by acidic solutions is depicted in Table 3. Considering the results, the sensitivity and specificity of the identification test were estimated to be 88% and 96%, respectively. We investigated the value of different markers of HCV in commercially-available, seroconversion panel samples. These panels include groups of serial samples that have been acquired from an individual at different time periods, and they show the time when the antibody can be identified in the samples. The kit provided by the Pishtaz Teb Company was used to measure data related to antibodies, but the data pertaining to HCV RNA were similar to panel insert.

Considering serial dilution of Core recombinant antigen in a negative serum sample for evaluating the analytical sensitivity of the test and regarding the defined cut off for negative samples, the least amount identifiable through this test was determined to be 2.5 pg/ml, which is equivalent to 20000 IU/ml RNA (Fig. 4).

Imprecision Test: The extrapolated precision results obtained for three samples with different concentrations of antigen are presented in Table 4.

4. Discussions

The ELISA method is practical for identifying Core antigen of hepatitis C virus via specific antibodies that capture the antigen. Although identification of viral RNA has been known as a gold standard in detection of HCV infection, studies reveal that the Core antigen of the virus can be identified in a one-day period after appearance of RNA (3-5). The sensitivity of the test before and after pretreatment of serum with 1.5 M glycine solution was 57% and 88%. respectively, which indicates that its sensitivity and specificity results are favored compared to the results of other studies. In 2005, Gaudy et al. reported sensitivity and specificity values of 96.7% and 100%, respectively, using the kit of Core antigen made by American Ortho Company. Eight out of 2,395 cases with antigen concentrations lower than 8.5 pg/ml were verified using the neutralization technique (2).

In 2005, Fabrizi et al. reported a sensitivity value of 92.7% and a specificity of 97% by using the Ortho kit and comparing it to the PCR method. The population that was studied included 125 people with negative antibody titration and 167 people with positive antibody titration (6). In 2006, Reddy and his colleagues reported a sensitivity of 60% and a specificity of 83% by using the Ortho kit and comparing it to the PCR method. These researchers conducted their studies on a population of 111 patients with chronic renal failure (7).

An increase in test sensitivity subsequent to pretreatment by 1.5 M glycine buffer indicates the elimination of the interference of antibodies against Core antigen present in serum. These antibodies prevent the binding of antigen to antibodies captured in the wells by forming a complex with antigen and masking the antigenic epitopes. pretreatment by specific buffers has also been applied using the Ortho kit. In 1999, Aoyagi et al. verified that pretreatment before the test for identification of antigen is followed by an increase in optical density in the antigen test and a decrease in antibody titration against the virus. These results were produced using a buffer containing Triton X-100 with 0.3% concentration, Chaps with 1.5% concentration, and SDS with 15% concentration. These researchers suggested a incubation period of 30 minutes at 56 oC (1).

All of the nine cases with a negative antigen test, even after pretreatment, had high antibody titrations, and it is possible that in the pretreatment step, all antibodies don't dissociate completely. Another reason for negativeness of these samples may be the existence of incomplete antigenic particles that have not been identified by the antibodies test. Furthermore. used in the concentration of nucleic acid was determined similarly in HBV infection. In the PCR method, there may be no correlation between the concentrations of antigen in blood flow (10).

Heterogeneity of sequence has been reported in the HCV genome. Several studies have specified that there are differences in test sensitivity for determination of different genotypes, both in the PCR technique and in the identification of antigen and antibody (1). In order to minimize the heterogeneity effect in the amino acid sequence for test sensitivity, the antibodies utilized should be capable of identifying the conserved amino acid sequence in constant in core antigen.

Most studies that have used the kit from the Ortho Company have reported an analytical sensitivity of 1.5 μ g/ml, which is equivalent to 1*104 IU/ml RNA (11). Regarding serial antigen dilutions in negative serum, the analytical sensitivity of the test

in current research has been shown to be approximately 2.5 pg/ml, which is equivalent to 2*104 IU/ml RNA. The imprecision index in the designed kit in the present research was based on a variation coefficient of 4 - 12%. In the same way, Havs et al., in 2004, reported the inaccuracy of interpolation and extrapolation as 6% - 25% 1% - 9%, respectively (12).

According to results of the seroconversion serum from the BBI Company used in the present study, the identification of the Core antigen was accompanied by a considerable decrease in the serologic window period. The ELISA method for identification of the Core antigen is a direct technique for detecting HCV infection. It is practical in most laboratories, is affordable, and there is no need for expert and experienced personnel. Crosscontamination problems of PCR do not exist in this test, and maintenance of the sample is simpler due to higher stability of antigen compared to RNA. In conclusion, the method is useful for identifying HCV infection in patients with deficiency of homoral immune systems or those who take immunosuppressive drugs for immune suppression.

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Diversity of Medicinal Plants in the Biospherical Reservation Areas of Iran

(A Case Study of the protected area of Miankaleh)

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Abstract: Awareness of people concerning the side effects of chemical drugs has caused an increasing interest in traditional medicine. This study was carried out to gather and identify medicinal plants, their curative effects and the part of them which is used from the reservation area of Miankaleh. The region under study has an area of 68800 hectares situated 12 kilometers north of the city of Behshahr and northwest of the city of Gorgan. During numerous visits to the area, plants were gathered and, after their identification using specialized references of medicinal plants, the part used and the curative effects of the plants were determined. Results obtained showed that out of a total of 43 families, 125 genera, and 155 species found in the region, 33 families, 52 genera, and 61 species (39% of all the species) belonged to medicinal plants, among which the class Asteraceae with 6 species and the class Chenopodiaceae with 5 species had the most medicinal species. The most used parts of the plants were the leaves with 31%, the whole plants with 19%, and the roots with 15%.

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Key words: Miankaleh, Medicinal plants, Boispherical reservation area, traditional medicine

1. Introduction

Since ancient times, plants have been one of the first and most available resources usable for treating illnesses, and throughout history there has always been a close relationship between man and plants, and the medicinal effects of plants and their uses have been known by everybody (UNESCO, 1996). Today, chemical medicines, because of their harmful and irreversible effects on people, are slowly being replaced by medicines extracted from plants (Banerjee, 2002). More than 422000 species of flowering plants have been reported from all over the world about 5000 species of which are used for medicinal purposes. There are about 8000 plant species out of which 569 genera and 2300 species are medicinal (Mozaffarian, 2005).

There is a considerable and growing interest in herbal medicines in the world since, according to international statistics, the value of trade in herbal medicines enjoys a yearly growth of 12-15% de Silva (1997). It is worth mentioning that in Germany, which is a big center of chemical drugs production, more and more herbal medicines are used by patients and prescribed by doctors (Pande et al., 2004). Medicinal plants are so important that pharmaceutical experts search among plants to find medicines of the 21st century and these experts believe that plants are the solution to medical problems of the future. Use of traditional and medicinal plants in developing countries is widely attracting attention as the main basis for maintaining health (Nazir et al, 2010). For this same reason, identification, preservation, and sustainable management of these valuable resources are necessary (Hamilton 2003). This study was carried out with the purpose of directly accessing herbarium samples, identifying the medicinal plants of the region, finding

out the parts of the plants used, and what illnesses they are used for.

2. Materials and Methods

2.1. The Characteristics of the Region Studied

The protected region of Miankaleh, which consists of two wet and dry ecosystems, has an area of 68800 hectares, 18000 hectares of which belong to the arid part and the rest to the wetland part. The region is 60 kilometers long and its width varies from 5 to 12 kilometers. Miankaleh is 12 kilometers north of Behshahr and northwest of Gorgan, with a longitude of 53 35 54.2 east and a latitude of 36 45 64.55 north and an altitude of 21-22 meters below sea level, at the extreme southeast of the Mazandaran Sea (the Caspian Sea). To the north of Miankaleh lies the Caspian Sea and to the south and to the east, there is the Gulf of Gorgan.

Miankaleh consists of two wet (the Gorgan Gulf and the Miankaleh wetlands) and dry ecosystems, includes a complex of beaches, marshes, pools and lowlands. It is a suitable place for various plant species due to its unique features as a habitat, and is the only remaining one of the wooded coastal and wetland types of the coast of the Caspian Sea.

2.2. Climate

Climate is the result of various elements of weather, is formed after a long time in adaptation to the geographical position of each region, and plays an important role in relation to the renewable resources of the region. By acquiring a complete awareness of the capacities and limitations the climatic factors impose on each region, we can make optimal use of these resources. The weather in this region is affected by the climate of the southern plains and is considered wet temperate, according to climate classification.

2.3. Soil

The soil in the region is alkaline and it has a light (sandy or sandy silt) and deep texture. The available phosphorous is low to medium. The land area in this region is composed of low sand dunes and seaside beaches with a little to medium expanse of rolling lands. In areas near the coast, the topsoil is salty due to the salty sea water which causes the establishment of halophytic plants.

2.4. The Geomorphology of the Region

The coastal provinces of Mazandaran and Gilan were formed during the Quaternary and after the glaciation periods with the substantial decrease in the water level of the Caspian Sea. The formations in this region are limited to the Quaternary and include sediments relating to the Cenozoic era. Sediments in the region are sandy, calcareous, fine-grained, contain a little clayey soil, and are completely different from the sediments in Gorgan, which contain mineral clay soils.

2.5. The Vegetative Cover of the Region

In general, life forms in various plant communities are different from each other, and in fact it is this very difference that forms the basis of the structure of plant communities. In all, 179 species and sub-species were identified in the wildlife protected area of Miankaleh, most of which belong to the classes Asteraceae, Poaceae, and Fabaceae. Many of the classes found in the region have only one genus and one species. The dominant plants in the wildlife protected area of Miankaleh belong to the Iranian-Turani core and type which makes up 26.1 percent of the plants in the region. The European, the Siberian, and the Mediterranean types comprise 7.5, 3.7 and 26.1 percent of the plants in the region, respectively.

3. Methodology

The plant samples were gathered from the region and identified at the herbarium of the Agricultural Sciences and Natural Resources University of Sari. Information such as the Persian names, the parts of the plants used, and usages of the plants was obtained by using references found at the university library. This information is shown in table 1.

4. Discussion and Conclusions

Results of the study showed that, with reference to the floristic list, there are 43 classes, 125 genera and 155 species in the region, out of which 33 classes, 52 genera and 61 species belong to medicinal plants (Zargari, 1985-1991). The classes Asteraceae with 6 species and Chenopodiaceae with 5 species included the most number of medicinal species. The parts of the plants used most were the leaves (in 27 species), the whole plant (16 species), and the roots (13 species). The other parts used in the plants mentioned were seeds, bark, flowers, flower bearing browses, tubers, rhizomes, mental, and tree buds (Prajapati, 2003).

Se. No	Scientific Name	Class	Form	The Part Used
1	Heliotropium europaeum	Boraginaceae	Tr	Leaves, flower bearing
2	Cincium amongo	Compositore	T.	Drowses, seeds
2	Antomiaia annua	Compositeee	11 Tr	Roois A arrial parts
3	Artemisia annua	Compositeae		Aerial parts
4	Anthemis cotula	Compositeae	Не	The whole plant
5	Xanthium spinosum	Compositeae		The whole plant
0	Xanthium strumarium	Compositeae		The whole plant
/	Cicnorium intybus	Compositeae	Не	leaves and the roots
8	Chenopodium botrytus	Chenopodiaceae	Ch	flower bearing browses
9	Chenopodium albom	Chenopodiaceae	Tr	Leaves, seeds
10	Chenopodium murale	Chenopodiaceae	Tr	Leaves
11	Salsola kali	Chenopodiaceae	Tr	The whole plant
12	Salicornia herbacea	Chenopodiaceae	Tr	Sap
13	Capsella Bursa-pastoris	Cruciferae	Tr	The whole plant
14	Convolvulus arevensis	Convolvulaceae	Tr	The whole plant
15	Cyperus rotundus	Cyperaceae	Cr	Roots tubers
16	Stellaria media	Carvophyllaceae	Tr	The whole plant
17	Euphorbia turcomanica	Euphorbiaceae	Tr	Leaves
18	Granium rotundifolium	Geraniaceae	He	Stem
19	Erodium cicutarium	Geraniaceae	Tr	Seeds
20	Cynodon dactylon	Gramineae	Cr	The whole plant
20	Phragmites australis	Gramineae	He	Rhizomes roots
21	Hypericum perforatum	Hyperiaceae	He	Flower hearing browse
23	Linum album	Linaceae	Ch	Seeds
23	Mentha pulegium	Lahiateae	Tr	The whole plant
25	Marruhium vulgae	Labiateae	He	The whole plant
26		Labiaceae	He	Shoots
20	Malva silvestris	Malvaceae	Cr	Leaves Flowers
28	Malva neolecta	Malvaceae	Cr	Flowers
20	Morus alba	Moraceae	He	Leaves Bark Roots
30	Figus carica	Moraceae	Ph	San Stem
31	Oralis corniculata	Oxalidaceae	Tr	The whole plant
32	Anagalis arvensis	Primulaceae		The whole plant
33	Samolus valerandi	Primulaceae		Leaves
34	Rumer acetosella	Polygonaceae	Ch	Leaves
35	Rumex accroseria	Polygonaceae	Ch	Leaves Roots
36	Polygonum hydroniner	Polygonaceae	Cr	The whole plant
37	Portulace oleraceae	Portulação	$\frac{Cr}{Tr}$	Shoots
38	Plantago psyllium	Plantaginaceae	Tr	Lagyas
30	Plantago major	Plantaginaceae	Cr	Leaves Leaves Roots Seed
<u> </u>	Plantago lanceolata	Plantaginaceae	T_r	Leaves Roots Sads
<u>40</u> //1	Punica granatum	Punicaceae	Dh	The whole plant Sap
41	Ranunculus scalaratus	Ranunculaceae	Tr Tr	San
42	Ranunculus muricatus	Ranunculaceae	11 Tr	Sap
43	Paliurus spina abristi	Rhamnaccac	11 Tr	Boots Laguas
44 15	Potentilla rentance	Rosaceae		Rhizomes Doots Laguas
45	Maspilus garmanica	Posacaa		Eruit Laguas
40	Cratagus sp	Rosaceae	TH Dh	Flower Bark
18	Ailanthus altissima	Simaruhaceae	Ph	Bark Roots
+0	manning anissing	Sinarabaceae	111	DUIN, NOOIS

Table 1. The Plant Parts Used in the Species Present in the Protected Area of Miankaleh

49	Salix alba	Salicaceae	Ph	Bark, Branches, Leaves
				mental
50	Datura stramonium	Solanaceae	Tr	Leaves, Seeds
51	Solanum nigrum	Solanaceae	Tr	Leaves, flower bearing
				browses
52	Pimpinella anisum	Umbelliferae	Ch	Fruit
53	Foeniculum vulgare	Umbelliferae	He	Roots, Leaves, Fruit
54	Urtica dioica	Urticaceae	Ch	Leaves, Roots, Sap
55	Urtica urens	Urticaceae	Ch	Shoots, Roots
56	Verbena officinalis	Verbenaceae	He	Shoots
57	Viola odorata	Violaceae	Cr	The whole plant
58	Ulmus minor	Ulmaceae	Ph	Secondary, bark
59	Celtis australis	Ulmaceae	Ph	Leaves, Roots, Leaf buds
60	Peganum harmala	Zygophyllaceae	Tr	Seeds
61	Tribulus terrestris	Zygophyllaceae	Tr	Roots, Fruit, Leaves

Cr: Criptophyte, Ch: Chomophyte, Ph: Phanerophyte, Tr: Trophyt, He: Hemophytee



Figure 1. Parts of plants used from the species present in the protected area of Miankaleh.

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Effective Use Of Teaching Methodologies At Secondary Level In Pakistan

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Abstract: The objective of the study was to investigate the effective use of teaching methodologies at Secondary level in Pakistan. All the participants i.e.100% of Fourth Six week training workshop on "Educational Leadership and Institutional Management" for educational managers at Academy of Educational Planning and Management, Ministry of Education, Government of Pakistan were included in the sample. For the purpose of data collection, a questionnaire was prepared. Data collected through the questionnaire was tabulated, analyzed and interpreted by applying percentage. Major findings of the study reveal that (1) teacher's presents a brief overview of the contents; (2) teacher's uses A.V. aids to enhance the student's comprehension of the concepts; (3) teacher speaks at a rate which allows students time to take notes; (4) teacher evaluates the success of his teaching by asking questions about the topic at the end of the session and; (5) teacher assigns homework and checks it regularly. It was concluded that teachers probe questions answer is incomplete, repeats questions when necessary and also responds students queries politely and carefully; teacher establishes and maintains vigilant contact with the student's body movements do not contradict the speech and takes notes to respond students curiosity and the teachers voice can be heard easily, he raises and lowers his voice for variety and emphasis. It has been recommended that A.V. materials should be used more vigilantly by teachers to make their teaching effective, teacher must pay attention to remove sign of puzzlement to make students learned better and teacher should pay more attention to his own personality and manners and be cooperative with student's words.

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Keywords: Teacher, teaching methods, strategies, tactics, secondary level.

1. Introduction

Teaching is a complex process which requires proper teaching methodologies, in order to inculcate knowledge in the minds of the students and to transfer knowledge to next generation. Secondary education is a turning state therefore; effective teaching methodology is to be adopted according to the needs of the students so that proper guidance can be given.

The progress of country depends upon the quality of its teachers. Ranga (2005, p.5) has commented on teacher education as "the irony of fate, however, is that teaching is the most unattractive profession and teacher no longer occupies an honorable position in the society. Teaching can regain its earlier noble status in case the quality of teacher education in our country is improved."

The term "Teacher" is used for the person, who teachers the students, guides the learners and enables them how to read and write. Encyclopedia of education defines teacher education as, "education and preparation of individuals enabling them become professional teachers." Frank and Wagrall (1987, p.11) have emphasized the need for making teacher education dynamic. They suggested that, "in order to keep pace with- technology changes in society the teacher education programs of all levels in country must be planned in such a way that the teachers produced by these programs, are broadly educated, scientific minded, uncompromising on quality innovative, but sympathetic towards students. Aggarwal (1990, p.26) has concluded that "teacher education is that knowledge, skills and abilities which is relevant to the life of teachers as teacher."

It is also important to provide in-service training to teacher for adopting proper teaching methods. Teacher education is not teaching the teacher how to teach. It is the initiative, to keep it alive, to minimize the evils of the "hit and miss" process: and to save time, energy, money and trouble of the teacher and the taught. The necessity of the teacher to perceive that the course in teacher education would he, help him minimize his trouble, and to appreciate that it would save the children from much of the painful process through which he has himself passed. Teacher education is needed for developing a purpose and for formation of a positive attitude for the profession. "Teacher education refers to the policies and procedures designed to equip teachers with the knowledge, attitudes, behaviors and skills they require to perform their tasks effectively in the school and classroom."(http://www.wikipedia.com).

There are several teaching methods which are used to teach various disciplines at secondary level in Pakistan. However, majority of the teachers use only lecture method to teach even science subjects i.e. Biology, Chemistry, and Physics whereas, these subjects demand practical demonstration. There are many excuses of not adopting modern methods of teaching, the most important is that majority of the teachers have argument that curricula is lengthy and working environment is not provided in public sector.

2. Good Teaching

Teachers are the builders of our new generation. Unless we have the most dedicated hardworking and trained teachers. We cannot educate children for tomorrow. No system of education can rise about the teacher who serves it and its quality depends ultimately on the efforts of the teacher. This depends on the effectiveness with which they have been taught by their own teachers in the classrooms. Swarup and Oberoi (1994, pp.19-21) have described the nature and characteristics of good teaching as discussed in the following paragraphs:

2.1 Provision of Desirable Information

The human knowledge is going on increasing since rise of civilization. He has learnt all this by trial and error, insight and initiation. Desired information should be provided to the students is a well organized form regarding this store of knowledge. It saves time and pupils will not feel any difficulty in achieving knowledge.

2.2 Causing to Learn

A good teaching is not merely to impart information to the students, but it also to arouse the will of self learning in them. The teacher should explore the interests, attitudes, capabilities, competencies and needs of the pupils and guide them accordingly. From this point of view, the teaching should be so much natural and interesting that the pupils get motivated for self learning.

2.3 Efficient Planning

The pupil cannot be taught anything everything all the time. Various stages are meant for achieving the different aspects of knowledge. The pupils have different interests, competencies and needs on the basis of individual differences. There must be a useful planning of teaching.

2.4 Selective

The human is struggling continuously since he came on the earth. As a result of his struggle his knowledge-store is going on enriching day-by-day.

Such an explosion is knowledge cannot be learnt in short-time-span. The students should be taught selected useful things by teaching.

2.5 Provide Opportunity for Activity

The pupil remains active by nature. This activeness is based upon his basic instincts. Hence, each pupil performs desirable and undesirable activities under the influence of his instincts.

2.6 Sympathetic

Psychology has proved that when the pupil gets involved in emotional disturbance while struggling mentally, his all mental powers cease to function smoothly. From the point of view the successful teaching essentially requires emotional stability and security. Therefore, good teaching should be sympathetic.

2.7 Cooperative

Teaching does not mean forceful imposition of knowledge in the pupils. Braia teaching and learning process is an alive and active. Hence desirable results can only be achieved when the teaching is based on the cooperation of the teacher and the pupil.

2.8 Organization of Learning

Mursill (1954) has commented that the organization of learning means the unification of all the components of teaching. Hence, activities of the teacher and pupil should be unified.

2.9 Democratic

Modern age is the age of democracy. We should prepare the pupils for democratically and to achieve the objective, the teaching should be based on democratic ideas.

2.10 Progressive

The real education of the pupils is based upon his personal experience. The good teaching is progressive.

2.11 Needs to Emotional Stability

Every pupil has inborn instincts. These powerful instincts make efforts for their expression. If these instincts are left increased, then the pupil will not behave properly in the society.

2.12 Helping the Child to adjust himself to his Environment

As the pupil develops, he has to face the complicated and complex natural and social environments. In other words the whole life of the pupil is a story of struggle. Good teaching helps the pupil in adjusting in both types of environment.

2.13 Means of Preparation

To prepare the pupil for future life is an important function of education. The teaching is such a process by which the physical, mental, emotional and social development of the pupil occurs. Good teaching is an important means of present and future preparation.

2.14 Successful Teaching

Mursell (1954) describes successful teaching as, "it has revealed a number of specific aspects or emphases in the total pattern of meaningful learning, which make the general orientation more definite.

- i). Learning is essentially purposive. It is meaningful in the sense that it "matters" to the learner.
- ii). The basic process of learning is one of exploration and discovery: not of routine repetition.
- iii). The outcome or result achieved by learning is always the emergence of insight, or understanding or intelligible response.
- iv). The result is not tied to the situation in which it was achieved but, can be used also in other situations."

Ibrahim ((1990, p.12) has also described effective teaching as:

objective of teacher Main education program has always been to prepare effective teachers such teachers should be capable of bringing desired behavioral changes in their students to an optimal level in relation to the infant in terms of human energy and material resources expended in the process. Teachers while teaching in a class room will have to meet their challenged also to pass through various processes, such as communication of the content style of presentation, use of audio visual aids. In brief, teacher effectiveness in an area which concerned is with relationship between the characteristics of teachers. teaching acts and their effects on the educational outcomes of classroom teaching.

It is evident that the teacher has always adopt variety of teaching methods and teaching strategies in teaching their subjects in classroom situation to make teaching more effective and result oriented.

3. Teaching Methods

For effective teaching take place a good method must be adopted by a teacher. A teacher has many options when choosing a style to teach by. The teacher may write lesson plans of their own, borrow plans from other teachers, or search alive or within books for lesson plans. When deciding that what teaching method to use, a teacher will need to consider students background, knowledge, environment, and learning goals. Teachers know that students learn in different ways but almost all children will respond well to praise. Students have different ways of absorbing information and of demonstrating their knowledge. Teachers often use techniques which cater to multiple learning. Styles to help students retain information and strengthen understanding. A variety of strategies and methods are used to ensure that all students have equal opportunities to learn. A lesson plan may be carried out in following ways (http://www. Google.com):

3.1 Questioning

A teaching method that includes questioning is similar to testing. A teacher may ask a series of questions to collect information of what students have learned and what needs to be taught. Testing is another method of testing.

3.2 Explaining

Another teaching method is explanation. This form is similar to lecturing. Lecturing is teaching, giving a speech, by giving a discourse on a specific subject that is open to the public, usually given in the classroom. This can also be associated with demonstrating and modeling.

3.3 Demonstrating

Demonstrations are done to provide an opportunity in learning hew exploration and visual learning tasks from a different perspective. Demonstration can be exercised in several ways.

3.4 Collaborating

Students working in groups are another way a teacher can enforce a lesson plan. Collaborating allows students to talk among each other and listen to all view points of discussion or assignments. It helps students think in unbiased way.

4. Teaching Method, Teaching Strategy and Tactic

It is usually considered that teaching methods, teaching strategies and teaching tactics are same, but there is visible difference in the meaning. Swarup (1994, p.6-8) has explained these terminologies as follows:

4.1 Teaching Method

In the teaching method, the main aspect is the way of presentation and the contents. The teaching method is determined according to the nature of the contents. There can be three methods of the content. There can be three methods of presentation.

- i. **Telling Method:** Such as learning, questioning etc.
- ii. **Doing Method:** Such as project method
- iii. Showing Method: Such as demonstration

observation etc.

4.2 Teaching Strategy

The term strategy refers to pattern of acts that serve to attain certain outcomes and to ground against certain others. The word strategy means the determination of some policy by planning before presenting the contents with the help of which the student's force is faced and the teaching objectives are achieved. Pre-planning is key to success.

4.3 Teaching Tactics

Teaching tactics means the method with which new knowledge is marked in the minds of pupils permanently. Teaching tactics are more comprehensive than the teaching strategies. In other words, under a single teaching strategy, by using one of more teaching tactics, the lesson can be made easy, clear and understandable.

5. Taxonomy

Taxonomy is a classification system that is arranged in a hierarchy. According to David J. et al (1985, p-85)"taxonomy is more than a simple division of material into groups: it involves understanding the nature of groups and their relationships to one another. He further stated that there are several advantages in using taxonomy. First and foremost, it facilitates communication. The second advantage of using taxonomy is that it is purely descriptive, without implicit value in any of its categories. It is a system that in itself is neither good nor bad. Another advantage is that taxonomy gives us a basis for selecting criteria for evaluation even self evaluation. The facilitation of planning is a third advantage. Teachers have a means of sequencing tasks, assessing relative weight given each level and evaluating congruency between goals and learning strategies selected. A final advantage to using the taxonomy is that the process of learning is enhanced. By evaluating student progress at each level of domain, learning problems can be diagnosed and remediated.

6. A brief Review of Previous Research

Yasmin, et al (1984) conducted research study on "a comparative study of the effectiveness of the inquiry and traditional methods for teaching biological sciences in laboratory at the High School Level." A sample of 400 students was further divided into 8 groups (4 experimental and 4 control). The findings of the study reveal that the inquiry approach is more effective as compared to traditional methods.

7. Statement of the Problem

The present research was designed to investigate the effective use of teaching methodologies at secondary level.

8. Objectives

The objectives of the study were to:

Investigate the effective use of methods of teaching at secondary level.

Explore advantages and disadvantages of various methods.

9. Significance of the Study

This study has great importance for the teachers in general and for secondary school teachers in particular, as this study has collected a lot of information about teaching methods. their effectiveness and appropriateness about various disciplines at secondary level. Furthermore, study will guide the head teachers in exploring proper methodologies for teaching. The significance of the study will also be for the planners and education managers in policy formulation or revision of teacher education programs at secondary level in the country. It will also help in- service teacher education institution to award or offer relevant in service i.e. training programs.

10. Delimitations

The study was delimited to all the participants of Fourth Six Week Training Workshop on "Educational leadership and institutional management" for Educational Managers at Academy of Educational Planning and Management, Islamabad.

11. Method

The following method was adopted to carry out the study:

11.1 Population

All the 25 male and female participants of the "Fourth Six Week Training Workshop on Educational leadership and institutional management for Educational Managers" served as population of the study. This included teachers and education managers all over Pakistan.

Statement	SA	A	UN C	DA	SDA	Mean Score
Teacher asks questions to see what the students knew about the new	15	7	1	2	0	
topic.	88	3%	04 %	C	08%	
A sufficient amount of material is included in the lesson.	2	15	4	3	1	
	68	3%	16 %	1	6%	3.56
Teacher states the purpose of the class session in the beginning.	8	11 6	2 8	3	1 16	3.88
Teacher presents a brief overview of the content	9	12 4	3 12	1	0 4	4.16
Teacher makes explicit the relationship between today's and the	12	8	3	2	0	
previous class session.	80	%	12 %		8%	4.2
Teacher explains new terms, concepts and principles.	11	9	4	1	0	
	80	%	16 %	4	4%	4.2
The introduction used to draw upon student's experiences.	6	15	3	1	0	
	84	.%	12 %	4	4%	4.4
The teacher arranges and discusses the content in a systematic	6	14	1	13	1	3 84
fashion.	80	%	4%	1	.6%	5.01
Teacher asked questions periodically to let students participate in	10	9	4	2	0	1.00
session.	76	76%		8%		4.08
Teacher presents clear and simple examples to clarify abstract and	11	8	3	3	0	4.00
difficult ideas.	76	i%	12 %	1	2%	4.08
Teacher uses alternate explanations when necessary.	8	10	5	1	1	2.02
	72	2%	20 %		8%	3.92
Teacher asks questions to illicit the relationships among various ideas.	5	11	7	2	0	0.54
	64	%	28 %	:	8%	3.76
Teacher periodically summarizes the important ideas.	9	12	3	1	0	
	84%		12 %	4	4%	4.16
Teacher slows the speech when ideas are complex and difficult.	10	8	6	0	4	
	72%		24 %	4	4%	4.04
Teacher does not often digress from the main topic.	5	11	8	1	0	•
		%	32 %	4	4%	3.8
The A.V. material is handled competency.	9	5	10	1	0	2.00
	56	i%	40 %	4	4%	3.88
Teacher uses questions to gain student's attention.	12	8	4	1	0	
	80	%	16 %	4	4%	4.24
Teacher pauses after all questions to allow students time to think of an	7	8	8	2	0	4.24

Results of Data Analysis (Table 01)

answer.	60)%	32 %	8%		
Teacher asks probing questions if a student's answer is incomplete.	6	11	4	4	0	
	68	3%	16 %	1	6%	3.76
Teacher repeats answers when necessary so the entire class may hear.	13	9	2	1	0	136
	88	3%	8%		4%	4.50
Teacher receives student's questions politely.	12	6	6	1	0	
	72%		24 %	4%		4.16
Teacher establishes and maintains eye contact with the class.	9	11	2	3	0	4.04
	80	80%		12%		4.04
Teacher's facial and body movements do not contradict speech or	6	14	4	1	0	
expressed intentions.	80	80%		4%		4
Teacher's voice can easily be heard.	17	6	2	0	0	16
	92%		8%		0%	4.0
Teacher speech is neither too formal nor too casual.	5	12	7	0	1	
	65	20/	28		104	3.8
	00	570	%		+ 70	
Teacher is not too stiff and formal in appearance.	4	12	8	0	1	
	64	1%	32 %		45	3.72
Teacher varies the pace of the lessons to keep students alert.	9	10	4	2	0	4.04
-	76%		16%		8%	4.04
SDA	$\Lambda = Str$	ongly	disagre	e	01 p	oints

11.2 Sample

100% male and female participants of the training workshop were taken as sample.

11.3 Instrument

Questionnaire was the major instrument of the research for the collection of data. A questionnaire was developed for the teachers and education managers of the training workshop for the purpose of data collection. The questionnaire was validated by the experts. It was further improved after pilot testing and was then finalized.

11.4 Data Collection

The questionnaire was personally administered to the respondents i.e. participants of the training workshop and collected back after their completion.

11.5 Data Analysis

Data collected were tabulated, analyzed and interpreted and presented in Table 1. Percentage was calculated by using statistical technique for analysis. The data obtained were tabulated in term of frequency. The frequencies were converted into scores by assigning the following scale value of each of five responses:

SA = Strongly agree	05 points
A = Agree	04 points
UNC = Uncertain	03 points
DA = Disagreed	02 points

12. Findings

Following findings were drawn on the basis of item analysis of questionnaire:

- 1. Majority of 88% respondents agreed with the statement that the statement the teachers ask questions to see what the students know about the new topic.
- 2. Majority of 68% respondents agreed with the statement that a sufficient amount of material is included in the lesson.
- 3. Majority of 76% respondents agreed with the statement that the teachers state the purpose of the class session in the beginning.
- 4. Majority of 84% respondents agreed with the statement that the teacher presents a brief overview of the contents.
- 5. Majority of 80% respondents agreed with the statement that the teacher makes explicit the relationship between today's and the previous class session.
- 6. Majority of 80% respondents agreed with the statement that teacher explains new terms, concepts and principles in the class room.
- 7. Majority of 84% respondents agreed with the statement that introduction of the lesson is used to draw student's experiences and attention.

- 8. Majority of 80% respondents agreed with the statement that teacher arranges and discusses the content in a systematic and organized manner.
- 9. Majority of 76% respondents agreed with the statement that teacher asks questions periodically to allow students to participate in the classroom.
- 10. Majority of 76% respondents agreed with the statement that the teacher presents clear and simple examples to clarify abstract and difficult ideas.
- 11. Majority of 72% of respondents agreed with the statement that teacher uses alternate explanations when necessary.
- 12. Majority of 64% agreed with the statement that teacher asks questions to explain the relationships among various ideas.
- 13. Majority of 84% respondents agreed with the statement that the teacher periodically summarizes the important ideas.
- 14. Majority of 72% respondents agreed with the statement that teacher pays special attention when ideas are complex.
- 15. Majority of 64% respondents agreed with the statement that teacher always stresses on the main topic.
- 16. Majority of 56% respondents agreed with the statement that the A.V aids are handled in proper manner.
- 17. Majority of 80% respondents agreed with the statement that teacher uses questions to draw students attention.
- 18. Majority of 60% respondents agreed with the statement that the teacher pauses after all questions to allow students time to think of an answer.
- 19. Majority of 68% respondents agreed with the statement that the teacher probes questions if a student's answer is incomplete or wrong.
- 20. Majority of 88% respondents agreed with the statement that teacher repeats answers when necessary so the entire class may get benefits.
- 21. Majority of 72% respondents agreed with the statement that the teacher responds student questions politely.
- 22. Majority of 80% respondents agreed with the statement that teacher establishes and maintains vigilant contact with the class.
- 23. Majority of 80% respondents agreed with the statement that the teacher's facial and body movements do not contradict speech or expressed intentions.

- 24. Majority of 92% respondents agreed with the statement that the teacher's voice can be heard easily.
- 25. Majority of 68% respondents agreed with the statement that the teacher's speech is neither too formal nor too casual.
- 26. Majority of 64% respondents agreed with the statement that the teacher is not too stiff and formal in appearance.
- 27. Majority of 76% respondents agreed with the statement that the teacher varies the pace of the lessons to keep students alert.

13. Conclusions

- On the basis of findings following conclusions were drawn:
- 1. Majority of the respondents were of the view that teachers ask questions to see what the students know about the new topic.
- 2. Majority of the respondents agreed with the statement that a sufficient amount of material is included in the lesson and the teachers state the purpose of the class session in the beginning.
- 3. Majority of respondents were of the opinion that the teacher presents a brief overview of the contents; tried to make relationship between today's and the previous class session.
- 4. Majority of respondents were of the view that introduction of the lesson is used to draw upon student's experiences and the teacher arranges and discusses the contents in a systematic manner.
- 5. Majority of the respondents were of the view that teacher asks questions periodically to enable students to participate in the classroom presents clear and simple examples to clarify difficult ideas.
- 6. Majority of the respondents were of the view that teacher pays special attention when ideas are difficult and tries not to derail from the main topic.
- Majority of the respondents were agreed that writing on board is legible and teacher uses A.V. aids vigilantly and in proper way.
- 8. Majority of the respondents were agreed that teacher uses questions to draw student's attention and the teacher probes questions if students answer is incomplete.
- 9. Majority of the respondents were of the view that the teacher repeats answers when necessary so the entire class may get benefits and teacher responds student questions politely.
- 10. Majority of the respondents were agreed that teacher establishes and maintains vigilant

contact with the students, body movements do not contradict with the speech and takes notes to respond student's curiosity.

11. Majority of the respondents were of the opinion that teacher voice can be heard easily and the teacher is neither too formal, nor too stiff in appearance and varies the pace of the lesson to keep students alert.

14. Recommendations

Following recommendations were made on the basis of conclusions:

1. Audio Visual aids should be used more vigilantly by teachers to make their teaching effective.

2. Teachers should be careful in use of words in his speech; he should neither be too formal nor too casual.

3. Teachers must pay attention to remove signs of puzzlement, boredom curiosity to make students learned in a better way.

4. Teachers should pay more attention to his personality and manners and be cooperative with students.

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Importance of Credits for Rural Women

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Abstract: One of important factor for production and occupation is capital. In developing societies that most of their exploiters are novice, lending and credit projects is suitable tool for accessing purposes such as increasing efficiency and obliterating deprivation from rural society. But at one side, we need credits to make technological changes in productive activities and at the other hand we need suitable technical technologies to use credits with optimum efficiency. 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 etc. have important role on effective activities of these credits.

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Keywords: women, rural, credit, empowerment

1. Introduction

Since women have significant role in agriculture workforce, for country (e.g. Iran) that seeking for improving rural economy and supporting of production of food products, identifying women's role in this theme is very important.

Women's agriculture activities in villages of Iran in three sections of recent history of rural improvement have been affected by developmental factors. Before land reforms (1962), according to conventional laws in Iran, women were kept apart from having farm lands. According to customs, if farmer was farming in farm system and also if he had large farms so he must used his family workforce (especially woman workforce), but if it was small, he used to work at other's fields for wage. In many cases, women had to work at other parts. Before land reforms and because of being traditional of instruments and production tools, using women's roles was often in conservation and harvesting. While cultivating, most of picking cotton, was done by women, exclusively. In same period (before 1962), women roles was remarkable in cultivating rice. While plowing and preparing fields, they took part in third time trowel, also they had significant role in preparing natural fertilizer (Changizi, 2003).

Preparing seed and preparing them for transferring to storage, were done by women. Transplanting (most important stage in cultivating rice) and weeding were done by them, completely. And finally we should remark, their roles in cultivating wheat, before land reforms. Land reform (1962) was a breaking factor for traditional capitalism relations, and it was facilitator way for renewing agriculture and making investment relations at agriculture section. Although no land was considered for women (indeed by this, major proportions of agriculture workforces was deprived from having production instruments that they work on) but, it has affected on women's roles in agriculture activity. (Banihashem, 1999).

By the way, lord-vassal relation was broken approximately, and peasant's production relation and capitalist exploitation systems were established. In peasant production, (at fertile lands) farmer's wife had to work on family land with other family members .at peasants lands; women were working out of their family farms. In capitalist exploitation systems, women workforce was considered as cheap workforce at farms. Generally, land reforms caused those women workforce is used as secondary (balali, 2005).

After Islamic revolution, although exploitation systems haven't changed lot, but developing agriculture processes on women's roles weren't affectless. in cultivating, time consuming productions (e.g. rice and tobacco) women's production roles, has remained significant and even at farms that has changed their usage to business purposes and products cash productions, women workforce is used as a cheap workforce source (as laborer). But in cultivating productions (e.g. wheat) because of using agriculture machinery in capitalist exploitation systems, we have faced with decreasing in using women workforce (same source).

Now, because independent peasant exploitation systems, women workforce is used as gratis family labors that this caused, real value of women roles being unknown in surplus economic productions of peasants family. Generally, nowadays, in minor agriculture, women role and activity is necessary.

Women activities in agriculture are more than their capacities. Because, it doesn't decrease from her home duties and consequently, and by analogy with pure housekeeper, they bear more labors. Indeed, it should be considered more importance for rural women who take part in agriculture activities and surplus economic productions, because not only they are participant in workforce market, but they play very important role in renewing economic of family (Lahsaeezade 2000)

2. Rural women's roles in economy of village

Most women, especially in developing countries are working three shifts in a day indeed, but, instead for their exhausting activities, they receive : less health care , less literacy and less wage. Compensation for them is vast sex discrimination that exists all over the worlds in various forms. For example in India, Pakistan and Bangladesh, about 1 million girls die, due to lack of proper health care.

World Health Organization estimated that women work 2times more than men averagely (Bahar, 2001). In United Nation researches, except Australia, Canada and US, women in all countries work more hours than men. But major problem here is that, work means everything that leading to financial income. So, in government statistics, women are considered as unemployed and few of female employees are counted as productive and employed forces.

In India, in one survey, and according to this perspective (Financial income) this result emerged that only 34% of women (compare to 63% of men) is counted as workforces of society. While if we also consider doing services and home productions and preparing family needs, as productive activities (without leading to Financial income), we would find different results and value of this deprived group of society, will be clear to us. By considering work and home productions in India, these results emerged: 75% of women compared with 64% of men are working (compared to 34% versus 64% of pervious statistics). (Balali, 2005)

Also in another survey in Nepal villages and according to Financial income criterion, just 20% of women are working, while by considering home production criterion, women's share of workforce, reached to 53% (Bahar, 2001). By the way researches show that women have basic role in economics of family.

About rural women in agriculture productions, some researches is done by some scholars such (Shauver, Saches, Adams and Alston) that all believe that women in activities such as cultivating (seeding) conservation (weeding, spading and sifting) and harvest (cut sugarcane branches) and they also participate in activities like doing pastoralist and caring animals, milking, nurturing poultry, gardening, fixing yard, snow removal, repairing building, handicrafts and etc (Navabakbar 1997).

So, rural women are great part of workforce, needed for agriculture and rural societies. In 1966, according to F.A.O reports to food security congress, women doing 50% of productions in agriculture part averagely and this issue in developing country has very special importance. in African desert area about 70-80%, Asia 65%, in Latin America 45% and Caribbean and in Tunes 89% of workforces are women (Varzgar 2001:217) and in Iran more than 50% of agriculture workforce are women (Banihashem 1999).

Most rural women's service work, pertain to out of house. For example: rural women not only traditionally strive for environmental protection, they also take part in maintaining forest, plant cultivation and weeding in rural regions.

So rural women, doing major part of affairs in services and doing services inside and outside the house, isn't with any risk for them. They aren't secure while cleaning stall, milking, nurturing livestock and other activities. And they are at risk of common diseases between human and livestock. In fact they haven't security against any risk of work conditions (Emadi, 2001).

Summaries of woman service activities include housekeeping duties and also service activities out. So it's necessary to revise definition and classification service activities by women. Thus, according to rural women's basic role in productive activities and even rural developing, importance of rural women's role isn't considered properly. Maybe the reason of this inattention is that rural women's productions are used inside the family. This inattention caused that no changes happen for decreasing exhausting rural women's activities, in spite of development of technology and using various new tools that leads to remarkable decrease in using human resources. In so many developing countries yet, women use traditional cultivating tools that have little efficiency and demand more activities. Further, agriculture activities accompanied their bv housekeeping duties that force them excessive efforts. One of the reasons is that rural women's role, remained unknown in economical productions. Because objective evidences at all over the world, especially in developing countries (particularly in our country) show that lost work or intangible activities is done by women that finally isn't considered as their efforts. Other reasons are: role of rural women and different productive activities remained unknown; and even lack of varying their position at different activities of village (Sadi, 2005).

3. Credits and its importance

One of important factor for production and occupation is capital. In developing societies that most of their exploiters are novice, lending and credit projects is suitable tool for accessing purposes such as increasing efficiency and obliterating deprivation from rural society. But at on side, we need credits to make technological changes in productive activities and at the other hand we need suitable technical technologies to use credits with optimum efficiency. (Fani, 1999)

So credits beside technology and skills of technical production, is complementary for each other. In order to invested credits being effective in productive activities and gain acceptable efficiency, suitable technology in that productions or activity should be provided accordant to economic and societal conditions of villagers. So from the development perspective, not only credits are used but they call it as credit program. credit program isn't just receiving and paying money but this program contains several stages in order that finally, exploiter and farmer stand in one inclusive program in this process, And educational needs , marketing services and production distribution, input and credit supply and loan be considered.

Among purposes of credit programs, it can mention to increasing efficiency in activity, job, stabilizing occupation which faced financial crisis, increasing level of income and exploiters life's level, empowering villagers and novice exploiters and also breaking deprivation cycle at society and rural families.(Rahmani 2001). So if credits, is used as a coherent and inclusive program framework and is considered base on need, power and reinforcing its proficiency level, so we can access to aforementioned purposes and also we can prepare suitable technology of production and activity with demanded credit. Finally, production and service efficiency will place at acceptable level. This systematic approach can save exploiter from deprivation and poverty cycle. conducted researches at countries like Pakistan, India and Iran showed that invested credits for productive activities was suitable if it is used with suitable technology. So credits wouldn't be effective if it is provided for exploiter exclusively and without considering his/her skill needs (Amiri, 2000).

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 (Rahmani, 2001).

4. Necessity of credits program for rural women

Women compare to men, face more cultural, social, and economic and laws obstacles, especially in developing countries and even its intensity is more for them too. In rural societies women and girls have less food, healthcare educational equipments, capital and income. thus in order to access to economic development and making optimum of using production input (especially workforce) it should pay proper consideration through emphasizing on rural women's roles in production and giving scientific approach in order to establishing cooperation between institutions to increase women contributions in productive and economic activities (Araghzade 2002).

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).

Rural occupation stirred with farming and ranching and rural economy is subsistence economy so subsistence culture is formed. In subsistence economy all of family members engage in economic activity.

Every empowering action of each member certainly affects totality of economy of family and creates remarkable evolution. One of important plan for empowering subsistence economy at village is, performing credits program through institutions that work for rural developing. But these programs are also done by private institutions that have expertise and experience in development programs. (Ghaffari, 2000)

Since, rural family members have little power to save, so saving and credits programs start with least or even no deposit. One of other features of these programs is, persuading rural family members in order to expanding initiatives and creativity for making occupation and income. But also it should be considered that various deposit practices and micro loans must be base on the need and culture of various societies and also base on current condition of market. Appropriateness or inappropriateness of deposit practice with culture and regional economic geography can affect on extent of effectiveness at credits program. And finally is that, identifying real participants need at micro credits program and also their participation in loaning and deposit managements, can affect on increasing effectiveness of program. (Rahmani, 2001)

Availability of credits in the format of inclusive program for rural women to apply at services and productive activities is necessary base on some reasons:

a- rural women's role, as productive workforce at agriculture and rural developing.

In rural regions of country, women, form major part of productive workforce that more than 40% of value of conducted activities at rural regions in various issues like handicrafts, ranching, natural sources, conversion and services industries is done by rural women. So if it is aimed to retain these activities or this part of productive human resources has active and affective role in production and activity cycle, its essential to provide them necessary funds and credits to develop and stabilize their activities inappropriate availability to credits sources, for rural women

Rural women base on different cultural, social and bureaucracy factors, couldn't have credit sources as same as they had role at agriculture and rural development.

Dominant social culture on credits system of country, rural women incapability for pledging to sources of credit provider, social and cultural limitation from family supervisor, are among those factors that prevent rural women access to credit sources (Moazami 2005)

b- level of capital efficiency at small scale Contrast to intellectual imagery, level of factors efficiency at small scale production and service unites is more than great ones. Conducted research in many Asian and European countries (specially central bank of Islamic republic of Iran researches) has shown that capital and workforce efficiency at micro productive unites, is more than those at macro scale units or agro-industry, thus it's more economical if needed capital, provided for micro scale productive units with knowledge and skills of production as "suitable Technology"

(Rahmani 2001).

c- importance of little increase in villagers income on family economy

rural society, including rural women more than others, have endured poverty and it's shortage, so performing micro scale projects by providing little credits, lead to improvement of income and life level that have high importance and more desirability at rural family economy (Moazami 2005).

d- limitation in formal credits sources (government)

In addition to lack of proper rural women access, compared to men, formal sources of countries have some limitations that can't response to credit needs of agriculture part exploiters and rural regions. results of researches show that governmental credits sources which are given to rural regions applicants by agent banks, is enough just for 50% of demands and rest of them is provided by informal sources with high costs or even applicant can't supply their requested demands and finally investment would stopped.

So this limitation is one of other factors that make, creating credits program for rural exploiter groups necessary, especially for rural women (Khazaee 2001).

5. Role of Financial empowering rural women, at sustainable rural development

If we suppose "development" as a process that whole society and social system are moving toward establishing better society and more humane in it, so base on it, preparing subsistence, honorable life, human esteem and dignity would be among most important aims of development . nowadays , about development and sustainable development, natural sources and physical capitals aren't very important and modern development theories rely on human but vet activating human capabilities itself needs investing on human resources too . Because it has proven that capable, wise, healthy and forethoughtful man, is factor of development and expansion, in one research that conducted in 192 countries, revealed that physical capital 16%, natural sources 20% and human resources 64%, share wealth averagely but we need capital and investment to foster creativity and detecting talents . (Chabokru and etal, 2005)

Now that we mentioned importance of investing on development process, we pay to varying women's Financial empowerment role, as half of active population of village in the development and sustainable development. Role of credits and credits institutions of women in improving society can be summarized as following:

a. creating esteem and human dignity in women (self reliance and confidence)

One of important goals and basic principle of development is, creating esteem and dignity for all human beings.

Financing women through credit sources can be basic step toward sustainable development and in that way; rural women grow as single-minded human and with confidence so they can feel esteem and dignity in themselves and in society. Public belief is that one success rural women, have high confidence practically and this feature creates sense of faith and belief in inner power and sense of ability to access intended goals in her. These kinds of women are not algebraic manner and believe that establishing future life and decisions making are at their own hands. women who have high confidence, have discarded one cultural deterrent factor (i.e. belief in fate) that prevent sustainable development and actively strive to establish better future (Rahimi 2001).

1- creating sense of equality with men

One of the other roles that financial credits and women financial empowering play among women is that creating their sense of equality against men. Women especially at rural societies and undeveloped countries compared with men are kept powerless from many aspects and often are not considered equal against men. For example while they do more duties compared to men, but they don't receive equal wage which men receive.

Thus if they are financed in order to participate in development freely and deliberately, so they don't fill weakness and their spirit of equality with men would increased.

On the other hand inside one active and successful woman, there are great sources of energy and power to progress. She has capacity to do constant job during the day and also she has capability to do favorite activity alongside working and she can create great evolution in economic and social life at village, if credits provided for her. (Khazaie, 2001)

b. using of women's financial power to improve social ,cultural and economic condition of village

Rural women are active at various fields of life as same as men and they accomplish their duties well. Thus undoubtedly, if we finance her as creative and active workforce and if credits sources can prepare her necessary credits, so she would be more active at different social, cultural, economic and even political areas and also would be more affective to flourish her family, village and then society. Until men and women can earn more as independently or collective, obviously they would have more lively and more healthy family; because more income leads to more purchasing and saving power and again more saving and investment cause economic, cultural and social prosperity (Fakhraee 2002)

c. creating jobs for rural family especially for women

One of the other issues that capital and investment create is that, creating job for population of one region. Undoubtedly, financing women has great role on creating job especially for rural women. Financing women and putting credit available as forms of micro and macro, would save them from lethargy and change them to creative and responsible workforce. These women want to initiate and create, and want to solve executive problems and finally to fill existing management gap and they tend to conduct flow of affairs. They strongly feel responsible and have individual and group responsibility. This is why that they are active as same as men at various areas. (kar, 2000)

d. role of credits in fighting against poverty pollution of village and protecting environment

One of the important roles that financing women do, in the form of micro credits, is to decrease poverty and fight against this great problem. women in scope of social activity that maybe even being deprived from accessing to general routs of production, have organized their foundations as they make existing sources able to invest and they earn most income from capitals and fight against poverty and deprivation of their family and society . For example, in rural societies, poor people use wood and pasture of desert for warming their house at winter.

Cutting trees and vegetation of region cause soil erosion, temperature increase, rainfall decrease and rain flood decrease, thus they putting region at risk of dangers such as drought, famine and floods.

So there isn't any doubt that the way for fighting against natural disasters such as famine and floods is depends on destroying poverty pollution. For doing that, investment is one of important methods (Bakhshude & Salami 2005).

e. corporation and sense of power in women

Women finance empowering and their corporation in different groups either mixed or women cooperatives, not only supple their economic needs but also make them powerful. But with this presupposition that established groups (institutions, cooperatives and companies and ...) have been created base on real participation principles and also base on democratic. As it was mentioned for women development in strategic statement of future of Nairobi "cooperatives can advertise and also can be origin of protecting political and economic interests of women. Financing women by credits institutions can cause their individual and groups corporation at different areas and increase their individual and groups power (kar, 2000).

f. Using of existing sources in order to develop environment

Active and successful women know that when and how establish unit by inside and outside equipments and how use needed skillful and facilitative forces to complete their goals. They especially don't think to individual success and individual activity for accessing supposed goal and they don't feel themselves needless for others help. These kinds of women as soon as being financed use existing finance sources to prepare environment and also use natural sources for developing region as much as they can. The issue is impossible without sufficient credit sources, because investment is essential principle for every action. (Chabokru and etal, 2005)

6. Conclusion and discussion:

Researches studies and surveys show that Iranian rural women that constitute 21% of whole population and half rural population have key role at production and at economic and social reproduction. Their major activities are at three parts: agriculture, handicrafts and centralized services. (Fami, 2001)

However no inclusive studies conducted About Iranian rural women's role in production and about how they participate in production process and surveying real value of their workforce , but theoretical surveys has shown their part at agriculture about 40% that proportion of their activities partly depend on cultural , social , different kind of exploiting and even kind of production and activity (farming , gardening and ranching) , culture diversity and climate condition on different regions of Iran . Nature of these activities is base on gender and social work dividing. They have complementary roles at production system.

Women largely are active at micro exploiter units and they work at larger units as form of salaried employee or receive wage.

They have not desirable conventional condition about their accessing to productive factors and sources, because of some customs and common traditions and also common norms. their limitation to access to field, bank facilities, education services, education-applied science, mechanization, modern technology and formal associations has kept their direct and independent access to production services and decision to use production sources and factors at minimum level. Women's literate proportion is less than rural men and urban men so we can explore main reason at cultural beliefs, dominant social conditions on rural society, low education scope for girls and women and lack of training importance for them (Fami, 2001).

Fortunately in two recent decades considerable efforts have been done to eradicate illiteracy that it was more remarkable about rural women so that percent of rural women literacy from 17.3% on 1355 increased to 62.4% on 1996. But 37.6% of rural women remained illiterate yet (Statistical center of Iran 2006).

Thus, what is concluded from this debate is that generally, women's economic, cultural and social conditions improved compare to past but hasn't take affective steps toward better improvement, properly. And now they aren't at proper place that they have right to be. rural women have responsibility of maternal , wife and home management in addition to associate in production and helping agriculture workforce so that they spend two third of their times to production , home management and organization , while this , reduced to one third about men (Varzgar 2001) .

At northern part of our country that rice cultivation is common, because of high workload, gender work dividing is so that women do more than 60% of production of rice, or in Gorgan, and women's proportion of harvesting cotton is reported 66%. in spite of those subjects, at Mazandaran, at most being surveyed villages, women have relative control on affaires but they haven't right to make decision and interfere in agriculture affaires and affaires of village. In many villages right of decision making about number of children belongs to men (planning and research institute of agricultural economics 1997).

But consider that aforementioned subjects, based on researches that has conducted at north of Iran that in these regions women have more active contribution from social, economic and cultural perspective.

Point that true about most of villages of Iran is that more active women economic contribution that leads to more income for family, cause that women be at higher rank for family decision making (Same source, 1997)

In spite of that, by conducted researches, rural women's roles has been surveyed very low, but another study has shown that at villages of Iran, women, especially elder, has specific dignity and have important roles at decision making.

It is important to say that by lessening literacy gap between men and women and having more public media such as radio and TV and finally by become more aware, extent of women corporation would increase in decision making. (Planning and research institute of agricultural economics 1997).

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 and Schalkwyk (1998) at their conducted research believe that now, micro credits, micro finance sources and small business units 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 effects on controlling women, and helps to family income.

In researches that conducted by Nande (2004) became clear that women participation in credits programs had positive effects 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 .

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.

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Thermo-Statically Safety Control of Dez Dam under Unexpected Lake Level Reduction

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Abstract: Dez dam stability was examined due to unexpected decrease in its reservoir level by performing a 3D finite elements analysis. The dam as well as its foundation and abutments have been modeled in a relatively exact manner. Moreover, the vertical contraction joints were simulated in the analysis. Hydrostatic, gravity and thermal forces have been taken into account as the main load combinations. 10m reduction of reservoir level from normal water level of the dam reservoir was considered in the modeling and the possibility of initiate and development of cracks in dam body was investigated by means of monitoring of principal stresses. The obtained results revealed that no serious instability would occur in Dez arch concrete dam.

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1. Introduction

In Iran, there exist some limited relatively old concrete arch dams. One of them is Dez dam located in vicinity of Dezful city in the north of Khouzestan province. This dam has an important role in the life of its neighboring inhabitants. The electricity and water for drinking and agriculture of the populated Dez river downstream villages and cities are supplied by this dam. Dam has a double curvature arch shape and constructed from concrete material during 1958 until 1963. In that time, Dez dam with 203 meter height from its base level was one of the sixth high level dams in the world. Figure 1 shows a view of the dam. The thicknesses of the dam at the crest and base level are 4.5m and 27m respectively. The crest length is about 212m and the level of the crest measured from free level of the world's oceans is about 354m.



Figure 1. View of the Dez Dam

The maximum operating level of the dam was designed 350m from free sea level which later has been increased to 352m because of the optimum operations and demands. Area of the reservoir is approximately 65 square kilometers and the minimum water level of the reservoir is 300m above sea level. The crest level of the outlets is 335m above sea level and the entrance level of the power plant tunnels designated as 275m above sea level. The electric power supplied by the dam is about 520 Mw. As usual, after a relatively long period of operation time, it is rational to think that the there is a need for Dez dam safety to be investigated under unexpected reservoir fluctuation. This unusual loading is defined here as hydro+ gravity+ thermal loads when the level of reservoir is equal to 10m below the minimum water level surface. According to literatures, after service life of several decades, a considerable percentage of existing concrete dams, illustrate some kind of deterioration. Based on the studies performed examining the causes of this phenomenon, the ASR (Alkali-Silica Reaction) and unusual extreme loading such as earthquake excitations and reservoir level fluctuations are the main reasons of this dam stiffness and strength degradation (Swamy and Al-Asali, 1988; Ahmed et.al., 2003; Pedro, 1999). Sometimes mentioned degradation is concomitant with the occurrence of local cracks in dam body which can be a threat for the dam safety. Holding this issue in mind, the main objective of this paper is to investigate the possibility of damage occurrence in Dez dam due to
unusual reduction in its reservoir level about to 10m from the minimum water level of reservoir. The same research has been done recently for another important old concrete arch dam named Karun-1 (Labibzadeh et. al., 2010, Labibzadeh and Khajehdezfuly, 2010, Labibzadeh and Khajehdezfuly, 2010).

In this investigation, as well as the hydrostatic pressure of reservoir and dam weight, the thermal loads due to air temperature changes have been assessed in the modeling. Thermal loads has a major effects in arch concrete dam stability analysis (Sheibani and Ghaemian, 2006; Ardito, et. al, 2008; Léger and Leclerc 2007; Léger and Seydou 2009; Labibzadeh and Khajehdezfuly, 2010). Furthermore, for achieving the more and more accurate in analysis, the effect of vertical contraction joints in hydrothermal simulation of the dam was taken into account. Even though the latter issue was no major challenge for dam engineers in their analyses and designs, this factor can affect the dam safety analysis results significantly (Labibzadeh and Khajehdezfuly, 2010). In the past recent years, the amount of the rainfall has been decreased considerably in Iran specifically in Dez dam water fall domain. Consequently, the reservoir volume of the dam has been reduced gradually. As the water level of the dam was decreasing, the safety controls of Dez dam became more important due to the increase in electric energy demands. Furthermore, the height of the sediment behind the dam has been progressively increased during the last four decades and this factor also reduces the volume of available water from the dam for use in drinking and agriculture. So, that would be the main reason for doing this study. The suggested research has been done by means of a relatively 3D exact simulation of the geometric, material behavior and boundary conditions of the dam. Principal stress tensors were selected as the basic stability indexes safety control and were examined. The special attention was paid on the effects of thermal strains occur in the dam due to the change in environmental temperatures during the year. It will be shown that under the reservoir level reduction up to 10m the possibility of initiate and development of cracks in downstream face of the dam exists.

2. Material and Methods

2.1. Material specifications

Table 1 summarized the material properties which have been inserted in finite elements analysis of the proposed model. These values have been derived from the authoritative reports of Khouzestan water and power organization.

	Concrete Dam	Rock Support
Density (kg/m3)	2500	2000
Young's Modulus (MPa)	25480	40000
Poisson's Ratio	0.17	0.25
Conductivity	2.62	1
Expansion (1/ C°)	10e-6	9e-8
Specific Heat (C°)	1000	2000

Table 3. Material properties library used in the analysis

2.2. Heat transfer

Heat flows from high-temperature regions to low-temperature regions. This transfer of heat within the medium is called conduction heat transfer. The Fourier heat conduction law for one dimensional system states that the heat flow Q is related to the temperature gradient $\partial T / \partial x$ by the relation (with heat flow in the positive direction of **x**), (Reddy, J. N., 1993):

$$Q = -kA \frac{\partial T}{\partial x} \tag{1}$$

Where k is the thermal conductivity of the material, A is the cross-sectional area, and T is the temperature. The negative sign in the (1) indicates that heat flows downhill on the temperature scale. The balance of energy in an element requires that

$$-kA\frac{\partial T}{\partial x} + qAdx = \rho cA\frac{\partial T}{\partial t}dx - \left[kA\frac{\partial T}{\partial x} + \frac{\partial}{\partial x}\left(kA\frac{\partial T}{\partial x}\right)dx\right](2)$$

Or

$$\frac{\partial}{\partial x} \left(kA \frac{\partial T}{\partial x} \right) + Aq = \rho cA \frac{\partial T}{\partial t}$$
(3)

Where q is the heat energy generated per unit volume, ρ is the density, c is the specific heat of the material, and t is the time. The equation (3) governs the transient heat conduction in a slab or fin (i.e., a one dimensional system) when the heat flow in the normal direction is zero. The following metric units will be used:

$$T$$
; °C (Celsius)

k; Wm^{-1} ° C^{-1} (Watts per meter per degree Celsius)

$$q$$
; Wm^{-3}

$$\rho$$
; kg m⁻³

c; $J kg^{-1} \circ C^{-1}$ (Joules per kilogram per degree Celsius or $m^2 s^{-2} \circ C^{-1}$)

It is important to note that since in this study, for the thermal analysis, the records of the thermometers installed in the different positions of the dam (mainly located in the central cantilever of the dam) are inserted directly into the finite elements model, so we have not considered the type of the heat transfer such as convection or radiation directly but in fact we have involved these effects indirectly in the analysis because that the temperatures which recorded by the thermometers have influenced by the all types of heat transfer mentioned before such as convection and radiations. This method of heat transfer analysis is called inverse (indirect) solution (Léger and Leclerc, 2007). The direct solution predicts the evolution of the temperature distributions from specified upstream and downstream temperature values. The inverse solution uses recorded temperature data at embedded thermometers to interpolate and extrapolate to the external faces the temperature field with due consideration of thermal wave attenuation and face shift with depth along a section (Léger and Leclerc, 2007).

2.3. Thermo-elastic Constitutive Relations

It is well known that a temperature change in an unstrained elastic solid produces deformation. Thus, a general strain field results from both mechanical (i.e. here hydrostatic and gravity loads) and thermal effects. Within the context of linear deformation theory, the total strain can be decomposed into the sum of mechanical and thermal components as (Sadd, M., H., 2005):

$$e_{ij} = e_{ij}^{(M)} + e_{ij}^{(T)}$$
(4)

Where e_{ij} is the total strain tensor, $e_{ij}^{(M)}$ is the mechanical and $e_{ij}^{(T)}$ is the thermal part of strain tensor.

$$e_{ij}^{(T)} = \alpha_{ij} (T - T_0)$$
 (5)

In above relation, the α_{ij} is defined as coefficient of thermal expansion tensor. This coefficient for concrete was specified in table 1.

$$T - T_0 = \Delta T \tag{6}$$

In relation (6) the ΔT

Is the thermal gradient which inserted into the model corresponding to the thermal records of the thermometers embedded in the Dez dam.

2.4. Method of using registered temperatures data

For achieving to relatively precision thermal analysis, the temperatures which recorded by thermometers thorough available period of time have been examined. The figures 2 to 11 show the variation in temperatures registered with instruments during the 1975 to 2007. The temperatures are presented in the $^{\circ}C$ (Celsius) unit and the period of each graph is equal to one year. The curves which observed from this graphs obtained through an exact regression.



figure 2. Temperature variation in 1975



figure 3. Temperature variation in 1991



figure 4. Temperature variation in 1992



figure 5. Temperature variation in 1995



Figure6. Temperature variation in 1997



figure 7. Temperature variation in 1999



figure 8. Temperature variation in 2000



figure 9. Temperature variation in 2001



figure 10. Temperature variation in 2002



figure 11. Temperature variation in 2007

This temperatures recorded by thermometers which embedded in the central cantilever of the dam. Figure 12 shows the position of thermometers in the central cantilever. As it can be realized from these ten configurations, the shape of the variation of the temperatures in Dez dam body follows the sinusoidal function. This is a rational result, because the variation of the air temperature in the site of the dam according to the available weather forecasting data also varies as sinusoidal manner. This temperature change is similar to that reported by Sheibany and Ghaemian (2006). Based on the above temperatures. for the severe thermal effects condition to be considered in the analysis, the largest existed temperature differences in our time period (1975-2007) computed and inserted as iterance data to the finite elements model. The sequence of solution has been summarized as in figure 13. The results and their interpretations are brought in the next section.



figure 12. Thermometers in central cantilever of Dez dam

3. Results and Discussions

Following the method described in previous section, the Dez arch concrete dam has been analyzed under the effect of 10m unusual reduction of its reservoir level from normal water level. The most dangerous situation which threatens the dam safety was obtained when the level of the lake came down to 290m above sea level. This means that the reservoir level reduces about to 10m from its minimum water level. The counters of dam displacements at this level have been shown in figure 14. The picture illustrates that the maximum deflection takes places in the middle of the crest of the dam and the value of this deflection is about to 4.02 cm which is accordance with the range of the records of pendulums of the dam registers the displacements of the dam. The negative sign in the values of the deflections means that these deflections have tendency towards the upstream face of the dam. This is the opposite direction which hydrostatic loads like to move the dam toward it. So it can be concluded that the effect of thermal loads in this level of the reservoir is greater than the effect of other main source of loads such as hydro and gravity forces. For investigate the possibility of occurrence of cracking phenomenon in the dam body, the maximum principal stresses are displayed in the figure 15.



Figure13. The numerical solution of proposed problem

Iteration step loop



Figure 14. The Dez dam deflections: the reservoir level= 290m



Figure 15. The Dez dam deflections: the reservoir level= 290m

In the figure 15 the color of maximum principal stresses which are greater than 3.0 MPa has been selected as grey. We can observe from this picture that only a limited area in the downstream face of Dez dam near the abutments has tensile stresses greater than 3.0 MPa (assumed the tensile strength of

dam concrete material) and there a potential for cracking. However these stresses do not penetrate through the thickness of the dam so we should only strengthen the layers of concrete near the downstream surface. So, author of this paper suggests that the operation of the dam should be organized in such a way that water level of reservoir varies between max and min water level and reduce level of water of the reservoir about to 10m below the minimum level should be avoided.

4. Conclusions

After reviewing the results of stress analysis under thermal and mechanical loadings of Dez arch concrete dam it was cleared that the unexpected reduction of the reservoir level dam about to 10m below the minimum water level can be the source of cracking on downstream face of the dam in tensile mode and such a reduction should be avoided by proper operation programming.

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The variational iteration method for exact solutions of fuzzy heat-like equations with variable coefficients

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Abstract. In this paper, the variational iteration method (VIM) and Buckley-Feuring method (BFM) are applied to find the exact fuzzy solution of the fuzzy heat-like equations in one and two dimensions with variable coefficients. Further a comparison between VIM-BFM and Seikkala solutions is provided.

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1. Introduction

Recently heat-like models for physical problems have been caught much attention. These models can exactly describe some nonlinear phenomena, for example, the most celebrated Navier-Stokes equations can be converted into various heatlike equation in some special cases. We suppose the existence of imprecise parameters in heat-like equations with variable coefficients. Since fuzzy sets theory is a powerful tool for modeling imprecision and for processing vagueness in mathematical models (Buckly 1999, Buckly 2000, Chalco-Cano2008, Nieto 2006), therefore, the purpose of this paper is using VIM and the same strategy as in Buckley and Feuring (Buckly 1999) for solving heat-like equations with fuzzy parameters.

The VIM (Abdou 2005, Shou 2008, Wazwaz 2004, Biazar 2007, Sadighi 2007, Abbasbandy 2009-64a, Abbasbandy 2009) gives rapid convergent successive approximations of the exact solution if such a solution exists without any restrictive assumption or transformation that may change the physical behavior of the problem. Very recently Allahviranloo et. al. (Allahviranloo 2009) discussed on the first order fuzzy differential equations by VIM. In this paper, we consider the iterative method for fuzzy heat-like equations in one and two dimensions with variable coefficients by VIM.

The paper is organized as follows: in Section 2, we call some fundamental results on fuzzy numbers. In Section 3 and 4, fuzzy heat-like equations and the VIM are illustrated, respectively. In Section 5, the same strategy as in Buckley-Feuring is presented for two-dimensional fuzzy heat-like equation. Some examples in Section 6 illustrated and finally conclusions are given in Section 7.

2. Preliminaries

We place a bar over a capital letter to denote a fuzzy subset of \mathbb{R}^n . So, \overline{X} , \overline{K} , \overline{C} , etc. all represent fuzzy subsets of \mathbb{R}^n for some n. We write $\mu_{\overline{A}}(t)$, a number in [0,1], for the membership function of \overline{A} evaluated at $t \in \mathbb{R}^n$. Define $\overline{A} \leq \overline{B}$ when $\mu_{\overline{A}}(t) \leq \mu_{\overline{B}}(t)$ for all t. An γ -cut of \overline{A} is always a closed and bounded interval that written $\overline{A}[\gamma]$, is defined as $\{t \mid \mu_{\overline{A}}(t) \geq \gamma\}$, for $0 < \gamma \leq 1$. We separately specify $\overline{A}[0]$ as the closure of the union of all the $\overline{A}[\gamma]$ for $0 < \gamma \leq 1$. Let E shows a set of fuzzy numbers (Ma 1999).

We represent an arbitrary fuzzy number by an ordered pair of functions $\overline{A}[\gamma] = [A_1(\gamma), A_2(\gamma)]$, $0 \le \gamma \le 1$ which satisfy the following requirements

- (a) $A_1(\gamma)$ is a bounded left continuous nondecreasing function over [0,1],
- (b) A₂(γ) is a bounded left continuous nonincreasing function over [0,1],
- (c) $A_1(\gamma) \le A_2(\gamma), 0 \le \gamma \le 1$.

Α

fuzzy set
$$A = (a_1, a_2, a_3),$$

 $(a_1 < a_2 < a_3)$ is called triangular fuzzy number with peak (or center) a_2 , left width $a_2 - a_1 > 0$ and right width $a_3 - a_2 > 0$, if its membership function has the following form

$$\mu_{\overline{A}}(t) = \begin{cases} 1 - \underbrace{(a_2 - t)}{a_2 - a_1}, & a_1 \le t \le a_2, \\ 1 - \underbrace{(t - a_2)}_{a_3 - a_2}, & a_2 \le t \le a_3, \\ 0, & otherwise. \end{cases}$$

The support of \overline{A} is $[a_1, a_3]$. We will write: (1) $\mathbf{A} > 0$ if $a_1 > 0$, (2) $\mathbf{A} \ge 0$ if $a_1 \ge 0$, (3) $\mathbf{A} < 0$ if $a_3 < 0$; and (4) $\overline{A} \le 0$ if $a_3 \le 0$. We adopt the general definition of a fuzzy number given in (Goetschel 1986).

3. Fuzzy heat-like equations

In this section, we consider the heat-like equations in one and two dimensions which can be written in the forms

(a) One-dimensional:

$$U_{t}(t,x) + p(x)U_{xx}(t,x) = F(t,x,k),$$
(1)

(b) Two-dimensional:

$$U_{t}(t, x, y) + p(x)U_{xx}(t, x, y) + q(y)U_{yy}(t, x, y) = F(t, x, y, k),$$
(2)

or

$$U_{t}(t, x, y) + q(y)U_{xx}(t, x, y) + p(x)U_{yy}(t, x, y) = F(t, x, y, k),$$
(3)

subject to certain initial and boundary conditions.

These initial and boundary conditions, in state two-dimensional, can come in a variety of forms such as $U(0, x, y) = c_1$ or $U(0, x, y) = g_1(x, y, c_2)$ or $U(M_1, x, y) = g_2(x, y, c_3, c_4), \dots$

In this paper the method is applied for the heat-like equation (2). For Eqs. (1) and (3), it is similar to (2), so we will omit them. In following lines, components of Eq. (2) are enumerated:

- $I_i = [0, M_i]$ are three intervals, which $M_i > 0(j = 1, 2, 3)$.
- F(t, x, y, k), U(t, x, y), p(x) and q(y) will be continuous functions for $(t, x, y) \in \prod_{i=1}^{3} I_{j}$.
- p(x) and q(y) have a finite number of roots for each $(x, y) \in I_2 \times I_3$.
- $k = (k_1, \mathbf{K}, k_n)$ and $c = (c_1, \mathbf{K}, c_m)$ are vectors of constants with k_i in interval J_i and c_r in

interval L_r .

Assume the Eq. (2) has a solution

$$U(t, x, y) = G(t, x, y, k, c),$$
 (4)

for

continuous $G(G_t(t, x, y, k, c) + p(x)G_{xx}(t, x, y, k, c) + q(y)G_{yy}(t, x, y, k, c)$ is continuous for $(t, x, y) \in \prod_{i=1}^{3} I_i, k \in J, c \in L$) with $(t, x, y) \in \prod_{j=1}^{3} I_j$, $k \in J = \prod_{i=1}^{n} J_j$ and $c \in L = \prod_{r=1}^{m} L_r$.

Now suppose the value of the k_i and c_r are imprecise. We will model this uncertainty by

substitute triangular fuzzy numbers for the k_i and c_r . If we fuzzify Eq. (2), then we obtain the fuzzy heat-like equation. Using the extension principle we compute \overline{F} from F where $\overline{F}(t, x, y, \overline{K})$ has $\overline{K} = (\overline{K}_1, K, \overline{K}_n)$ for \overline{K}_i a triangular fuzzy number in J_i , $1 \le j \le n$. The function U become \overline{U} , where $\overline{U}: \prod_{i=1}^{3} I_i \to E$. That is, $\overline{U}(t, x, y)$ is a fuzzy number. The fuzzy heat-like equation is

$$\overline{U}_t + p(x)\overline{U}_{xx} + q(y)\overline{U}_{yy} = \overline{F}(t, x, y, \overline{K}), \qquad (5)$$

subject to certain initial and boundary conditions. The initial and boundary conditions can be of the form $\overline{U}(0, x, y) = \overline{C_1}$ or $\overline{U}(0, x, y) = \overline{g_1}(x, y, \overline{C_2})$ or $\overline{U}(M_1, x, y) = \overline{g}_2(x, y, \overline{C_3}, \overline{C_4})$,.... The \overline{g}_i is the extension principle of g_i . We wish to solve the problem given in Eq. (5). Finally, we fuzzify G in Eq. (4). Let $\overline{Z}(t, x, y) = \overline{G}(t, x, y, \overline{K}, \overline{C})$ where \overline{Z} is computed using the extension principle and is a fuzzy solution. In Section 5, we will discuss solution with the same strategy as Buckley-Feuring for fuzzy heat- $\overline{K}[\gamma] = \prod_{i=1}^{n} \overline{K}_{i}[\gamma]$ like equation. Let and

$\overline{C}[\gamma] = \prod_{r=1}^{m} \overline{C}_{r}[\gamma].$

4. The variational iteration method

To illustrate the basic idea of the VIM we consider the following model PDE

$$L_t U + L_x U + L_y U + NU = F(t, x, y, k),$$
 (6)

where L_t , L_x and L_y are linear operators of t, xand y, respectively, and N is a nonlinear operator, also F(t, x, y, k) is the source non-homogeneous term. According to the VIM, we can express the following correction functional in t -direction as follows

$$U_{n+1}(t, x, y) = U_n(t, x, y) + \int_0^t \lambda \{ L_s U_n + (L_x + L_y + N) \tilde{U}_n - F \} ds,$$
(7)

where λ is general Lagrange multiplier (He 2004), which can be identified optimally via the variational theory (He 2006, Wazwaz 2007), and \tilde{U}_n is a restricted variation which means $\delta \tilde{U}_n = 0$. By this method, we determine first the Lagrange multiplier λ which will be identified optimally. The successive approximations U_{n+1} , $n \ge 0$, of the solution U will be readily obtained by suitable choice of trial function U_0 . Consequently, the solution is given as

$$U(t, x, y) = \lim_{n \to \infty} U_n(t, x, y).$$
(8)

According to the VIM, we construct a correction functional for Eq. (2) in the form

$$U_{n+1}(t, x, y) = U_n(t, x, y) + \int_0^t \lambda(s) \{(U_n)_s + p(x)(\tilde{U}_n)_{xx} + q(y)(\tilde{U}_n)_{yy} - F\} ds,$$
(9)

where $n \ge 0$ and λ is a Lagrange multiplier. Making Eq. (9) stationary with respect to U_n , we have

$$\lambda'(s) = 0,$$

1+ $\lambda(s)|_{s=t} =$

hence, the Lagrange multiplier is $\lambda = -1$. Submitting the results into Eq. (9) leads to the following iteration formula

0,

$$U_{n+1}(t, x, y) = U_n(t, x, y) - \int_0^t \{(U_n)_s + p(x)(U_n)_{xx} + q(y)(U_n)_{yy} - F\} ds.$$
(10)

Iteration formula start with an initial approximation, for example $U_0(t, x, y) = U(0, x, y)$. Also the VIM used for system of linear and nonlinear partial differential equations (Wazwaz 2007) which handled in obtain Seikkala solution.

5. Buckley-Feuring Solution (BFS) and Seikkala Solution (SS)

In (Buckly 1990), Buckley-Feuring present the BFS. For all t, x, y and γ ,

$$\overline{Z}(t, x, y)[\gamma] = [z_1(t, x, y, \gamma), z_2(t, x, y, \gamma)], (11)$$
d

and

 $F(t, x, y, \overline{K})[\gamma] = [F_1(t, x, y, \gamma), F_2(t, x, y, \gamma)], (12)$ that by definition

$$z_1(t, x, y, \gamma) = \min\{G(t, x, y, k, c) | k \in \overline{K}[\gamma], c \in \overline{C}[\gamma]\},$$
(13)

$$z_{2}(t, x, y, \gamma) = \max\{G(t, x, y, k, c) | k \in \overline{K}[\gamma], c \in \overline{C}[\gamma] \},$$
(14)

and

$$F_1(t, x, y, \gamma) = \min\{F(t, x, y, k) | k \in \mathbf{K}[\gamma]\}, \quad (15)$$

$$F_2(t, x, y, \gamma) = \max\{F(t, x, y, k) | k \in \overline{K}[\gamma]\}.$$
(16)

Assume that p(x) > 0, q(y) > 0 and the $z_i(t, x, y, \gamma)$, i = 1, 2, have continuous partial so that $(z_i)_t + p(x)(z_i)_{xx} + q(y)(z_i)_{yy}$ is continuous for all

$$(t, x, y) \in \prod_{j=1}^{3} I_{j} \text{ and all } \gamma \text{ . Define}$$

$$\Gamma(t, x, y, \gamma) = (z_{1})_{t} + p(x)(z_{1})_{xx} + q(y)(z_{1})_{yy},$$

$$(z_{2})_{t} + p(x)(z_{2})_{xx} + q(y)(z_{2})_{yy}],$$
(17)

for all $(t, x, y) \in \prod_{j=1}^{3} I_j$ and all γ . If, for each fixed $(t, x, y) \in \prod_{j=1}^{3} I_j$, $\Gamma(t, x, y, \gamma)$ defines the γ -cut of a fuzzy number, then will be said that $\overline{Z}(t, x, y)$ is differentiable and is written $\overline{Z}_t[\gamma] + p(x)\overline{Z}_{xx}[\gamma] + q(y)\overline{Z}_{yy}[\gamma] = \Gamma(t, x, y, \gamma)$, (18)

for all $(t, x, y) \in \prod_{i=1}^{3} I_i$ and all γ .

Sufficient conditions for $\Gamma(t, x, y, \gamma)$ to define γ -cuts of a fuzzy number are [14]: (i) $(z_1(t, x, y, \gamma))_t + p(x)(z_1(t, x, y, \gamma))_{xx} + q(y)(z_1(t, x, y, \gamma))_{yy}$ is an increasing function of γ for each $(t, x, y) \in \prod_{j=1}^3 I_j$; (ii) $(z_2(t, x, y, \gamma))_t + p(x)(z_2(t, x, y, \gamma))_{xx} + q(y)(z_2(t, x, y, \gamma))_{yy}$ is a decreasing function of γ for each

$$\begin{aligned} &(t, x, y) \in \prod_{j=1}^{3} I_{j}; \text{ and} \\ &(\text{iii}) (z_{1}(t, x, y, 1)) + p(x)(z_{1}(t, x, y, 1))_{xx} + q(y)(z_{1}(t, x, y, 1))_{yy} \leq \\ &(z_{2}(t, x, y, 1))_{t} + p(x)(z_{2}(t, x, y, 1))_{xx} + q(y)(z_{2}(t, x, y, 1))_{yy} \\ &\text{for } (t, x, y) \in \prod_{j=1}^{3} I_{j}. \end{aligned}$$

Now can suppose that the $z_i(t, x, y, \gamma)$ have continuous partial so

$$(z_i)_t + p(x)(z_i)_{xx} + q(y)(z_i)_{yy},$$

is continuous on $\prod_{j=1}^{3} I_j \times [0,1]$, i = 1,2. Hence, if conditions (i)-(iii) above hold, $\overline{Z}(t, x, y)$ is differentiable.

For $\overline{Z}(t, x, y)$ to be a BFS of the fuzzy heatlike equation we need: (a) $\overline{Z}(t, x, y)$ differentiable; (b) Eq. (5) holds for $\overline{U}(t, x, y) = \overline{Z}(t, x, y)$; and (c) $\overline{Z}(t, x, y)$ satisfies the initial and boundary conditions. Since no exist specified any particular initial and boundary conditions then only is checked if Eq. (5) holds.

 $\overline{Z}(t, x, y)$ is a BFS (without the initial and boundary conditions) if $\overline{Z}(t, x, y)$ is differentiable and

$$\overline{Z}_{t} + p(x)\overline{Z}_{xx} + q(y)\overline{Z}_{yy} = \overline{F}(t, x, y, \overline{K}),$$
(19)

or the following equations must hold

$$(z_1)_t + p(x)(z_1)_{xx} + q(y)(z_1)_{yy} = F_1(t, x, y, \gamma), \quad (20)$$

$$(z_2)_t + p(x)(z_2)_{xx} + q(y)(z_2)_{yy} = F_2(t, x, y, \gamma), \quad (21)$$

for all $(t, x, y) \in \prod_{j=1}^{3} I_j$ and all γ .

Now we will present a sufficient condition for the BFS to exist such as Buckley and Feuring.

Since there are such a variety of possible initial and boundary conditions, hence we will omit them from the following Theorem. One must separately check out the initial and boundary conditions. So, we will omit the constants $c_r, 1 \le r \le m$, from the problem. Therefore, Eq. (4) becomes U(t, x, y) = G(t, x, y, k),

so
$$\overline{Z}(t, x, y) = \overline{G}(t, x, y, \overline{K})$$

Theorem 1. Suppose $\overline{Z}(t, x, y)$ is differentiable.

(a) If

$$p(x) > 0, q(y) > 0, (x, y) \in I_2 \times I_3, \qquad (22)$$

and

$$\frac{\partial G}{\partial k_j} \frac{\partial F}{\partial k_j} > 0, \tag{23}$$

for j = 1, K, *n*, Then BFS= $\overline{Z}(t, x, y)$.

(b) If relations (22) does not hold or relation (23) does not hold for some j, then

 $\overline{Z}(t, x, y)$ is not a BFS.

Proof. It is similar to proof of Theorem 1 in (Buckly 1999).

Therefore, if $\overline{Z}(t, x, y)$ is a BFS and it satisfies the initial and boundary conditions we will say that $\overline{Z}(t, x, y)$ is a BFS satisfying the initial and boundary conditions. If $\overline{Z}(t, x, y)$ is not a BFS, then we will consider the SS. Now let us define the SS (Seikkala <u>19</u>87). Let

$$\overline{U}(t, x, y)[\gamma] = [u_1(t, x, y, \gamma), u_2(t, x, y, \gamma)].$$

For example suppose p(x) > 0 and q(y) < 0, so consider the system of heat-like equations

$$(u_1)_t + p(x)(u_1)_{xx} + q(y)(u_2)_{yy} = F_1(t, x, y, \gamma), (24)$$

$$(u_2)_t + p(x)(u_2)_{xx} + q(y)(u_1)_{yy} = F_2(t, x, y, \gamma), (25)$$

for all $(t, x, y) \in \prod_{j=1}^{3} I_{j}$ and all $\gamma \in [0,1]$. We append to Eqs. (24) and (25) any initial and boundary conditions. For example, if it was $\overline{U}(0, x, y) = \overline{C_{1}}$ then we add

$$u_1(0, x, y, \gamma) = c_{11}(\gamma),$$
 (26)

$$u_2(0, x, y, \gamma) = c_{12}(\gamma),$$
 (27)

where $\overline{C_1[\gamma]} = [c_{11}(\gamma), c_{12}(\gamma)]$. Let $u_i(t, x, y, \gamma), (i = 1, 2)$ solve Eqs. (24) and (25), plus initial and boundary conditions. If

$$[u_1(t, x, y, \gamma), u_2(t, x, y, \gamma)],$$
(28)

defines the γ -cut of a fuzzy number, for all

 $(t, x, y) \in \prod_{j=1}^{3} I_j$, then $\overline{U}(t, x, y)$ is the SS. We will say that derivative condition holds for fuzzy heat-like equation when Eqs. (22) and (23) **Theorem 2.** (1) If BFS= $\overline{Z}(t, x, y)$, then SS= $\overline{Z}(t, x, y)$. (2) If SS= $\overline{U}(t, x, y)$ and the derivative condition holds, then BFS= $\overline{U}(t, x, y)$.

Proof. (1) Follows from the definition of BFS and SS. (2) If $SS = \overline{U}(t, x, y)$ then the Seikkala derivative (Buckly 2000) exists and since the derivative condition holds, therefore, Eqs. following holds $(u_1)_t + p(x)(u_1)_{xx} + q(y)(u_1)_{yy} = F_1(t, x, y, \gamma)$, (29)

$$(u_2)_t + p(x)(u_2)_{xx} + q(y)(u_2)_{yy} = F_2(t, x, y, \gamma).$$
 (30)

Also suppose one $k_j = k$ and $\frac{\partial G}{\partial k} < 0$, $\frac{\partial F}{\partial k} < 0$ (the

other cases are similar and are omitted).

We see

$$z_1(t, x, y, \gamma) = G(t, x, y, k_2(\gamma)), \qquad (31)$$

$$z_2(t, x, y, \gamma) = G(t, x, y, k_1(\gamma)),$$
 (32)

$$F_1(t, x, y, \gamma) = F(t, x, y, k_2(\gamma)),$$
 (33)

$$F_2(t, x, y, \gamma) = F(t, x, y, k_1(\gamma)).$$
 (34)

Now look at Eqs. (20) and (21) also Eqs. (13) and (14), implies that

$$u_1(t, x, y, \gamma) = G(t, x, y, k_2(\gamma)) = z_1(t, x, y, \gamma),$$

$$u_2(t,x,y,\gamma)=G(t,x,y,k_1(\gamma))=z_2(t,x,y,\gamma).$$

Therefore BFS= $\overline{U}(t, x, y)$.

Remark 1. The Theorem 1 hold for Eq. (3) and the proof is similar to Theorem 1 in (Buckly 1999).

Lemma 1. Consider Eq. (1). Assume $\overline{Z}(t, x)$ is differentiable.

(a) If

$$p(x) > 0, x \in I_2,$$
 (35)

and

$$\frac{\partial G}{\partial k_j} \frac{\partial F}{\partial k_j} > 0, \tag{36}$$

for j = 1, K, n, Then BFS= $\overline{Z}(t, x)$.

(b) If relation (35) does not hold or relation (36) does not hold for some j, then $\overline{Z}(t,x)$ is not a BFS.

Proof. It is similar to Theorem 1 in (Buckly 1999).**6. Examples**

We consider the following illustrating examples.

Example 1. We first consider the one-dimensional initial value problem

$$U_t + \frac{1}{2}x^2 U_{xx} = k,$$
 (37)

subject to the initial condition $U(0, x) = cx^2$ and $t \in (0, M_1], x \in (0, M_2)$. Let $k \in [0, J]$ and $c \in [0, L]$ are constants. According to the VIM, a correct functional for Eq. (37) from Eq. (10) can be constructed as follows

are true.

Beginning with an initial approximation $U_0(t, x) = U(0, x) = cx^2$, we can obtain the following successive approximations

$$U_{1}(t, x) = kt + cx^{2}(1-t),$$

$$U_{2}(t, x) = kt + cx^{2}(1-t+\frac{t^{2}}{2}),$$

$$U_{3}(t, x) = kt + cx^{2}(1-t+\frac{t^{2}}{2}-\frac{t^{3}}{3}),$$

and

$$b_{n}(t,x) = kt + cx^{2}(1 - t + \frac{t^{2}}{2!} + \Lambda + (-1)^{n} \frac{t^{n}}{n!}, n \ge 1.$$

The VIM admits the use of

 \mathbf{I} $(\mathbf{A}, \mathbf{w}) =$

$$U(t,x) = \lim_{n \to \infty} U_n(t,x),$$

which gives the exact solution

$$U(t,x) = kt + cx^2 e^{-t}.$$

F(t, x, k) = kNow we fuzzify and $G(t, x, k, c) = kt + cx^2 e^{-t}$. Clearly F(t, x, K) = K so that $F_1(t, x, \gamma) = k_1(\gamma)$ and $F_2(t, x, \gamma) = k_2(\gamma)$. Also $\overline{G}(t, x, \overline{K}, \overline{C}) = \overline{K}t + \overline{C}x^2e^{-t}$, therefore,

$$z_i(t, x, \gamma) = k_i(\gamma)t + c_i(\gamma)x^2e^{-t},$$

i = 1,2, $\overline{K}[\gamma] = [k_1(\gamma), k_2(\gamma)]$ for and $\overline{C}[\gamma] = [c_1(\gamma), c_2(\gamma)]$. $\overline{Z}(t, x)$ is differentiable because $(z_i)_t + \frac{1}{2}x^2(z_i)_{xx} = k_i(\gamma), i = 1, 2$. That is, $\overline{Z}_t + \frac{1}{2}x^2\overline{Z}_{xx} = \overline{K}$, a fuzzy number. Since p(x) > 0,

 $\frac{\partial G}{\partial k} > 0$ and $\frac{\partial F}{\partial k} > 0$, Lemma (5) implies the result that $\overline{Z}(t, x)$ is a BFS. We easily see that

$$z_i(0, x, \gamma) = c_i(\gamma) x^2,$$

for i = 1,2, so $\overline{Z}(t,x)$ also satisfies the initial condition. The BFS that satisfies the initial condition may be written as

$$\overline{Z}(t,x) = \overline{K}t + \overline{C}x^2 e^{-t},$$

for all $(t, x) \in [0, M_1] \times (0, M_2)$.

Example 2. Consider the two-dimensional heat-like equation with variable coefficients as

$$U_{t}(t, x, y) + \frac{1}{2}x^{2}U_{xx}(t, x, y) + \frac{1}{2}y^{2}U_{yy}(t, x, y) = kx^{2}y,$$

$$U(0, x, y) = c_{1}y^{2} - c_{2}x,$$

which $x, y \in (0,1)$, $t \in (0,M]$, $k \in [0, J]$ and $c_i \in [0, L_i], j = 1, 2.$

Similarly we can establish an iteration formula in the form

$$U_{n+1} = U_n - \int_0^t \{(U_n)_s + \frac{1}{2}x^2(U_n)_{xx} + \frac{1}{2}y^2(U_n)_{yy} - kx^2y\} ds.$$
(38)
We begin with an initial arbitrary approximation:

We begin with an initial arbitrary approximation: $U_0(t, x, y) = U(0, x, y) = c_1 y^2 - c_2 x$, and using the iteration formula (38), we obtain the following successive approximations

$$U_{1}(t, x, y) = c_{1}y^{2}(1-t) - c_{2}x + kx^{2}yt,$$

$$U_{2}(t, x, y) =$$

$$c_{1}y^{2}(1-t + \frac{t^{2}}{2!} - kx^{2}y(-t + \frac{t^{2}}{2!} - c_{2}x,$$

$$U_{3}(t, x, y) =$$

$$c_{1}y^{2}(1-t + \frac{t^{2}}{2!} - \frac{t^{3}}{3!}) - kx^{2}y(-t + \frac{t^{2}}{2!} - \frac{t^{3}}{3!}) - c_{2}x$$

and

aı

$$U_{n}(t, x, y) = c_{1}y^{2}(1 - t + \Lambda + \underbrace{(-1)^{n}t^{n}}_{n!}) - kx^{2}y(-t + \Lambda + \underbrace{(-1)^{n}t^{n}}_{n!}) - c_{2}x, n \ge 1.$$

Then, the exact solution is given by

$$U(t, x, y) = G(t, x, y, k, c) = c_1 y^2 e^{-t} - k x^2 y (e^{-t} - 1) - c_2 x.$$

Fuzzify F and G producing their γ -cuts

$$z_{1}(t, x, y, \gamma) = c_{11}(\gamma)y^{2}e^{-t} - k_{1}(\gamma)x^{2}y(e^{-t} - 1) - c_{22}(\gamma)x,$$

$$z_{2}(t, x, y, \gamma) = c_{12}(\gamma)y^{2}e^{-t} - k_{2}(\gamma)x^{2}y(e^{-t} - 1) - c_{21}(\gamma)x,$$

$$F_{1}(t, x, y, \gamma) = k_{1}(\gamma)x^{2}y,$$

$$F_{2}(t, x, y, \gamma) = k_{2}(\gamma)x^{2}y,$$
where
$$\mathbf{k} = [k_{1}(\gamma), k_{2}(\gamma)]$$

 $\overline{K} = [k_1(\gamma), k_2(\gamma)]$

and

 $C_{i}[\gamma] = [c_{i1}(\gamma), c_{i2}(\gamma)], j = 1,2$. We first check to see if $\overline{Z}(t, x, y)$ is differentiable. We compute

$$[(z_1)_t + \frac{1}{2}x^2(z_1)_{xx} + \frac{1}{2}y^2(z_1)_{yy}, (z_2)_t + \frac{1}{2}x^2(z_2)_{xx} + \frac{1}{2}y^2(z_2)_{yy}],$$

which are γ -cuts of $\overline{Kx^2}y$ i.e. γ -cuts of a fuzzy number. Hence, $\overline{Z}(t, x, y)$ is differentiable.

Since the partial F and G with respect to k, p(x) and q(y) are positive then Theorem (5) tells us that $\overline{Z}(t, x, y)$ is a BFS. The initial condition is

$$z_1(0, x, y) = c_{11}(\gamma) y^2 - c_{22}(\gamma) x,$$

$$z_2(0, x, y) = c_{12}(\gamma) y^2 - c_{21}(\gamma) x,$$

which are true. Therefore, $\overline{Z}(t, x, y)$ is a BFS which also satisfies the initial condition. This BFS may be written

$$\overline{Z}(t, x, y) = \overline{C_1} y^2 e^{-t} - \overline{K} x^2 y(e^{-t} - 1) - \overline{C_2} x,$$

for all $x, y \in (0, 1), t \in [0, M]$. We consider the

one-dimensional heat-like model

$$U_{t}(t,x) + (\frac{1}{2} - x)U_{xx}(t,x) = -kx^{2}t^{2},$$

$$U(0,x) = cx^{2},$$
(39)

which $t \in (0,1]$, $x \in (0,\frac{1}{2})$ and the value of parameters k and c are in intervals [0, J] and [0, L],

respectively. We can obtain the following iteration formula for the Eq. (39)

$$U_{n+1}(t,x) = U_n(t,x) - \int_0^t \{(U_n(s,x))_s + (\frac{1}{2} - x)(U_n(s,x))_{xx} + kx^2s^2\} ds.$$
⁽⁴⁰⁾

We begin with an initial approximation: $U_0(t, x) = cx^2$. By (40), after than two iterations the exact solution is given in the closed forms as

$$U(t, x) = G(t, x, k, c) =$$

$$\frac{1}{12}kt^{4} - \frac{1}{6}kxt^{4} - \frac{1}{3}kx^{2}t^{3} + cx^{2} + 2cxt - ct.$$

$$\frac{\partial F}{\partial k} = -x^{2}t^{2} < 0 \qquad \text{and}$$

Since

$$\frac{\partial G}{\partial k} = \frac{1}{12}t^4 - \frac{1}{6}xt^4 - \frac{1}{3}x^2t^3 > 0, \text{ for}$$

$$0 < t \le 1 \quad and \quad 0 < x < \frac{1}{4}(-t + 4t + t^2)$$

then there is no BFS (Lemma (5)). We proceed to look for a SS. We must solve

$$(u_{1}(t, x, \gamma))_{t} + (\frac{1}{2} - x)(u_{1}(t, x, \gamma))_{xx} = -k_{2}(\gamma)x^{2}t^{2},$$

$$(u_{2}(t, x, \gamma))_{t} + (\frac{1}{2} - x)(u_{2}(t, x, \gamma))_{xx} = -k_{1}(\gamma)x^{2}t^{2},$$

subject to

 $u_i(0, x, \gamma) = c_i(\gamma) x^2,$ i = 1,2 , $\overline{K}[\gamma] = [k_1(\gamma), k_2(\gamma)]$ for and $\overline{C}[\gamma] = [c_1(\gamma), c_2(\gamma)]$. By VIM, the solution is

$$u_{1}(t, x, \gamma) = \frac{1}{12}k_{2}(\gamma)t^{4} - \frac{1}{6}k_{2}(\gamma)xt^{4} - \frac{1}{3}k_{2}(\gamma)x^{2}t^{3} + c_{1}(\gamma)x^{2} + 2c_{1}(\gamma)xt - c_{1}(\gamma)t,$$

$$u_{2}(t, x, \gamma) = \frac{1}{12}k_{1}(\gamma)t^{4} - \frac{1}{6}k_{1}(\gamma)xt^{4} - \frac{1}{3}k_{1}(\gamma)x^{2}t^{3} + c_{2}(\gamma)x^{2} + 2c_{2}(\gamma)xt - c_{2}(\gamma)t.$$
Now we denote
$$(41)$$

 $[u_1(t, x, \gamma), u_2(t, x, \gamma)],$

defines γ -cuts of a fuzzy number on area as \Re . Since $u_i(t, x, \gamma)$ are continuous and $u_1(t, x, 1) = u_2(t, x, 1)$ then we only require to check if $\frac{\partial u_1}{\partial \gamma} > 0$ and $\frac{\partial u_2}{\partial \gamma} < 0$. Since \overline{K} and \overline{C} are triangular fuzzy numbers, hence, we pick simple fuzzy parameter so that $k'_1(\gamma) = c'_1(\gamma) = b > 0$ and $k'_2(\gamma) = c'_2(\gamma) = -b$. The 'prime' denotes differentiation with respect to γ . Then, for a SS we need

$$\frac{\partial u_{1}}{\partial \gamma} = -\frac{1}{12}bt^{4} + \frac{1}{6}bxt^{4} + \frac{1}{3}bx^{2}t^{3} + bx^{2} + 2bxt - bt = b(-\frac{1}{12}t^{4} + \frac{1}{6}xt^{4} + \frac{1}{3}x^{2}t^{3} + x^{2} + 2xt - t) > 0,
\frac{\partial u_{2}}{\partial \gamma} = -\frac{1}{12}dt^{4} + \frac{1}{6}dt^{4} + \frac{1}{3}dt^{2}dt^{3} + x^{2} + 2xt - t) > 0,$$
(42)

$$\frac{1}{12}bt^{4} - \frac{1}{6}bxt^{4} - \frac{1}{3}bx^{2}t^{3} - bx^{2} - 2bxt + bt = -b(-\frac{1}{12}t^{4} + \frac{1}{6}xt^{4} + \frac{1}{3}x^{2}t^{3} + x^{2} + 2xt - t) < 0.$$

Therefore inequalities (42) hold if

 $\frac{1}{1}$ $\frac{1}$

$$-\frac{1}{12}t^{4} + \frac{1}{6}xt^{4} + \frac{1}{3}x^{2}t^{3} + x^{2} + 2xt - t > 0, \qquad (43)$$

for $x \in (0, \frac{1}{2})$ and $t \in (0,1]$. The inequality (43) holds if we have

$$0 < t \le 1$$
,

$$\frac{-12t - t^4 + 144t + 144t^2 + 60t^4 + 24t^5 + 4t^7 + t^8}{12 + 4t^3} < x < \frac{1}{2}.$$

We find that

$$-12t - t^{4} + 144t + 144t^{2} + 60t^{4} + 24t^{5} + 4t^{7} + t^{8}$$
$$12 + 4t^{3}$$
$$0 < t \le 1\} = 0.40103.$$

Hence we may choose \Re by the above assumptions in form as

and

$$\Re = \{(t, x) | 0 < t \le 1 \& 0.401031 \le x < \frac{1}{2}\},\$$

and the SS exists on \Re in form Eqs. (41). . We consider the one-dimensional heat-like model,

$$U_t(t, x) - U_{xx}(t, x) = -k \cos x,$$

$$U(0, x) = c \sin x,$$

which $x \in (0, \frac{\pi}{2})$, $t \in (0, M]$ and the value of parameters k and c are in intervals [0, J] and [0, L], respectively.

We can obtain the following iteration formula

$$U_{n+1}(t, x) = U_n(t, x) - \int_0^t \{(U_n(s, x))_s - (U_n(s, x))_{xx} + k\cos x\} ds.$$
(44)

We begin with an initial approximation: $U_0(t, x) = U(0, x) = c \sin x$. By (44), the following successive approximation are obtained

 $U_1(t, x) = c \sin x(1-t) - kt \cos x,$

$$U_2(t,x) = c \sin x(1-t+\frac{t^2}{2!}) + k \cos x(-t+\frac{t^2}{2!}),$$

and

$$U_{n}(t,x) = c \sin x(1-t+\Lambda + \frac{(-1)^{n}t^{n}}{n!}) + k \cos x(-t+\Lambda + \frac{(-1)^{n}t^{n}}{n!}), n \ge 1.$$

We, therefore, obtain

 $U(t, x) = G(t, x, k, c) = ce^{-t} \sin x + k \cos x(e^{-t} - 1)$, which is the exact solution. There is no BFS because p(x) = -1 < 0 (Lemma (5)). We proceed to look for a SS. We must solve

 $\begin{aligned} (u_1(t, x, \gamma))_t &- (u_2(t, x, \gamma))_{xx} = -k_2(\gamma) \cos x, \\ (u_2(t, x, \gamma))_t &- (u_1(t, x, \gamma))_{xx} = -k_1(\gamma) \cos x, \end{aligned}$

subject to

i = 1.2

$$u_i(0, x, \gamma) = c_i(\gamma) \sin x,$$

,
$$\overline{K}[\gamma] = [k_1(\gamma), k_2(\gamma)]$$

$$(\gamma), k_2(\gamma)$$
 and

 $\overline{C}[\gamma] = [c_1(\gamma), c_2(\gamma)]$. The solution is

 $u_1(t, x, \gamma) = c_1(\gamma) \cosh t \sin x - c_2(\gamma) \sinh t \sin x +$

 $k_1(\gamma) \cos x(\cosh t - 1) - k_2(\gamma) \cos x \sinh t$,

 $u_2(t, x, \gamma) = c_2(\gamma) \cosh t \sin x - c_1(\gamma) \sinh t \sin x + k_2(\gamma) \cos x (\cosh t - 1) - k_1(\gamma) \cos x \sinh t.$

We only need to check if $\frac{\partial u_1}{\partial \gamma} > 0$ and $\frac{\partial u_2}{\partial \gamma} < 0$, since the u_i are continuous and

 $u_1(t, x, 1) = u_2(t, x, 1)$. We pick simple fuzzy

parameter so that $k'_1(\gamma) = c'_1(\gamma) = b > 0$ and $k'_2(\gamma) = c'_2(\gamma) = -b$. Then, for a SS we require

$$\frac{\partial u_1}{\partial \gamma} = b \sin x (\cosh t + \sinh t) + b \cos x (\cosh t - 1 + \sinh t) > 0,$$

$$\frac{\partial u_2}{\partial \gamma} = -b \sin x (\cosh t + \sinh t) - b \cos x (\cosh t - 1 + \sinh t) < 0.$$
(45)

Since (45) holds for each $t \in (0, M]$ and $x \in (0, \frac{\pi}{2})$,

therefore,
$$\overline{U}(t, x)$$
 is SS and
 $\overline{U}(t, x) = \overline{C} \cosh t \sin x - \overline{C} \sinh t \sin x + \overline{K} \cos x (\cosh t - 1) - \overline{K} \sinh t \cos x$,

for all $t \in [0, M]$ and $x \in (0, \frac{\pi}{2})$.

7. Conclusion

In this paper, by the VIM, we obtain the exact solutions of various kinds of fuzzy heat-like equations. The VIM produces the terms of a sequence using the iteration of the correction functional which converges to the exact solution rapidly. Application of this method is easy and calculation of successive approximations is direct and straightforward. We using the VIM and strategy based on (Buckly 1999) introduced two type of solutions, the Buckley-Feuring solution and the Seikkala solution. If the BFS fails to exist and when the SS fails to exist we offer no solution to the fuzzy heat-like equations.

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The Zoning of Drought with SIAP model in Sapeedroud valley, Gilan-Iran, South western of Caspian Sea

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Abstract: Drought is a natural disaster and its repeatable in all climates .In each year more than half of earth is prone to drought occurrence, in Iran also, many times drought condition has happened ,such as in 25 years occurred and has influenced on crop yield and water resources. The aim of this paper is recognition of drought with Standard Index Annual Precipitation (SIAP model) in framework of research project .The results of research paper has shown in Sapeedrud valley drought has occurred in upland and lowland area(12-19% from total frequency) and the other area is 5-12 % drought from total frequency and also the focuses of drought years has located in south area in all research period.

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Keywords: Caspian Sea, Drought, Gilan, SIAP model.

1. Introduction

Despite being dominated in dry conditions in most parts of the country, drought also occurred frequently and caused water shortages in various sectors. So that in the past 25 years there have been 13 drought [1]. This phenomenon of drought in each temperature regime happening in the future and one of the major problems of humanity will be considered. Compared with other risks of climate damages, economic and social life due to drought intensity and range has enjoyed a far wider. Among the natural disasters threatening human and environment, both in terms of frequency of occurrence of drought and the amount of damages is at the top [2].

One of the major problems in drought research, defining its starting point is the simplest definition of the word is usually a period of drought drought; with below normal rainfall is defined. Of course, vet precise and comprehensive definition of scientific resources during the drought was not provided and this fits the definition differs from place to study. For example, in humid areas, rainfall distribution is uniform over the growing season and irrigation is not performed, occurrence of a period of several weeks without rain will lead to drought occurrence [3]. Instead, in dry areas, droughts only after two or more seasons without rain are identified. The significant difference between aridity and drought there. aridity, permanent feature area that is the result of inadequate rainfall amounts [4]. In contrast, temporary meteorological drought characteristics of a region just gone when the amount is below normal rainfall occurs [5].

Need to monitor and determine the degree, intensity, continuity, time, place and terminate the

dry period, spatial and temporal distribution, it has had on researchers to indicators for monitoring the dry period and ways to provide the zoning. The purpose of a drought index, a simple little three features determine the intensity, continuity and extent of its location [6]. Comparison of speech recognition and requires little or events to measure them. Therefore, to study and compare the drought at different times of drought need to be monitored [7]. Rodriguez [8] also using spatial temporal patterns, annual rainfall variability Ebarine Island for 47-year period studied for 51 stations showed. Hote [9] Analysis of Sahelian annual rainfall index over the period 1896 to 2000 statistics according to its drought began. Giddings [10] using the standard index of rainfall in drought-Mexico 50-year statistical period was specified. Pashardis [11] using SPI and RDI index to assess regional drought began in Capirus areas.

Khalili [12] using meteorological drought index in several samples from different climatic concluded that the benchmark index of annual precipitation and precipitation index decile with minimum rainfall occurred in the most severe drought occurred in all stations under study reported and of severe meteorological droughts image more efficient than other icons are the icons and other SIAP for monitoring meteorological drought is more efficient. Safdari [13] using the SPI index zoning drought frequency domain is discussed Karun watershed, results of this study show that the South East and northern areas than other areas with greater frequency of drought and the area is as areas with potential sensitivity to drought, are introduced in cases related to water resources should have special attention to it.Khorshiddost [2] using the Standardized Precipitation SPI to study rainfall fluctuations, and determine the predicted wet and dry seasons of winter in East Azerbaijan has been discussed. The results show aspects of extreme fluctuations of rainfall in all stations studied, there are some weaknesses combined with the severity and the causes of climate fluctuations represent rainfall stations this province. Stations and dry seasons of the sequences studied and further continued to enjoy the wet seasons. Mohammadi [14] also using the catchments area index SIAP in Maroon watershed years representing dry, normal, and they identify more with the zoning and drought in the region, areas with high risk of drought is introduced. Abyaneh [15] using statistical indicators of drought, dry conditions and trends in the region began to Hamadan. Ghavidel Rahimi [16] to study the drought and wet in the East Azerbaijan province has paid. Fatehi et al [17] to seasonal rainfall forecasts using links from around the lake catchment have paid. Ansari [6] zoning to the dry period using standardized precipitation index in Khorasan province have paid. Studies have shown that in recent years and the persistence of this phenomenon repeated in all parts of the province has increased, but its intensity is reduced. Dry period also occurred in the southern province of continuity and greater intensity than the central and northern provinces. Daneshvar [18] to review the regional drought in central, south and south East countries began. Soltani [19] using the SPI zoning drought began in Isfahan. Ensafee [20] using the inverse distance troubleshooting IDW basin drought climatic zoning was the Salt Lake. Khosravi [21] to review drought situation in South Khorasan province began. Karimi [22] also zoning drought with two different approaches in geographic information systems began to Yazd. Ghorbani [23] using two methods of SIAP and SPI data mining method to study the comparative profiles of meteorological drought began in the Kermanshah Province. Lshny Zand [24] to examine the possibility of drought and climate forecasts in the six basins in West and North West of Iran is discussed.

The main goal of this study attributes the frequency, intensity, continuity and extent of drought in regional and seasonal time scale annual Safeedrud Valley area to help with the Standardized Index annual Precipitation zoning maps drought during normal, dry and wet on the time scale annual and seasonal.

2. Material and Methods

Monthly rainfall data stations in period 1388-1359 was used and by statistical software were analyzed (Table 1 and Figure 1).

Table 1: profile stations studied

Station	Longitude	Latitude	Elevation
Astaneh	04 56 49	19 15 37	5-
Rasht	42 36 49	30 15 37	1
Parudbar	34 43 49	26 36 36	495
Manjil	25 23 49	54 45 36	232
Gilvan	58 07 49	42 46 36	311
Shahrbej	23 38 49	35 00 37	140
Totaki	26 52 49	08 04 37	185
Totkabo	30 34 49	49 50 36	260
Shalman	12 13 50	34 09 37	16-
Anzali	00 28 49	00 28 37	24-



Figure 1: The position of Sapeedroud Valley in Gilan and Caspian Sea.

SIAP Index

To review the process trend of droughts in the study area of the SIAP benchmark index is the annual precipitation (Khalili, 1998), was used and it is when a long period of minimum rainfall climate is at least represents a severe drought this way are identified.

SIAP=Pi- ;P/SD

Where: Pi = Precipitation in the desired ;P = mean SD = Standard deviation of Precipitation

SIAP: Standard Index Annual Precipitation That identify with descriptive measurement quality with table 2.

Then, using of Geographic Information System (GIS) software and submitted maps with Kriging methods for obtaining of zoning maps.

 Table 2: Classification criteria SIAP index classes

 and describe the status indicator value

Index Descriptive	Status
More Wet	More 0.84
Wet	0.52-0.84
Normal	0.52-(-0.52)
Dry	-0.52-(-0.84)
More Dry	Less -0.84

3. Results

Features 30-year rainfall stations within the study are described in Table 3.

Station	Mean	SD	C.V	Max	Min
	Rain				
Astaneh	8/1287	7/190	8/14	1635	5/890
Rasht	2/1307	4/167	8/12	5/1696	5/985
Parudbar	9/312	106	9/33	626	146
Manjil	1/260	9/86	4/33	484	8/97
Gilvan	2/185	8/63	4/34	8/394	4/90
Shahrbej	5/1227	3/188	3/15	1607	801
Totaki	6/1520	7/293	3/19	2233	955
Totkabo	5/526	129	5/24	37/788	5/259
Shalman	6/1178	2/171	5/14	1561	5/907
Anzali	7/1767	5/253	3/14	7/2359	7/1351

Table 3: characteristics of the study area rainfall

Features of rainfall in the range of heterogeneity within the desired time of the precipitation climate of Iran is a feature of shows . So that the maximum precipitation in the study area in the second half of the year (fall and winter) will happen and summer precipitation has the minimum required storage that this winter, and artificial feeding of underground water table shows, because fluctuations in rainfall could ultimately lead to dryness and drought is created. Maximum and http://www.americanscience.org

minimum rainfall recorded in order for the station in 1767 and Anzali amount with the amount of 185 mm Gilvan station reported. Most SD value for Tutky Station 293/7 and the lowest value for the station Gilvan 63/8 has been calculated. Maximum coefficient of variation of rainfall stations and the minimum Gilvan and Rasht station has been read. This indicates has represented of instability in Givan station and stability of precipitation of quality rainfall stations in Rasht Frequency Index SIAP according to criteria categories were extracted and subsequently with regard to values index in time series of annual stations studied area, its frequency was extracted (Table 4). The periods of drought, normal and The more specific and related to the zoning map prepared and analyzed. Map drying period (2008) According to this method (Fig. 2) shows that the region is very dry conditions.

Table 4: Frequency of many dry years in each station with SIAP method.

Frequency	More	Dry	Normal	Wet	More
Index	Dry				wet
1980	3	2	4	0	1
1981	2	0	5	1	2
1982	5	3	2	0	0
1983	0	0	3	1	6
1984	1	2	7	0	0
1985	1	3	4	1	1
1986	2	1	5	2	0
1987	0	1	7	0	2
1988	0	1	2	0	7
1989	2	1	7	0	0
1990	2	2	5	0	1
1991	5	1	4	0	0
1992	0	0	5	1	4
1993	1	0	3	3	3
1994	0	0	4	1	5
1995	3	0	2	0	2
1996	1	0	3	4	1
1997	2	3	4	0	2
1998	1	1	7	0	1
1999	7	2	1	0	0
2000	1	2	5	0	2
2001	1	1	6	0	2
2002	1	1	7	0	1
2003	0	1	3	1	5
2004	0	0	5	2	3
2005	0	0	5	1	4
2006	6	2	2	0	0
2007	0	0	5	2	3
2008	9	1	0	0	0



Figure 2: Map drought with SIAP Index in 2008. The index station for review, many obtained all the stations and converted of frequency drought classes This indicator was the percentage of the maps were presented (Table 5).

Table 5: Frequency of years with many phenomena (wet -dry) in accordance with SIAP index

Station	More	Wet	Normal	Dry	More
	Wet				Dry
Astaneh	7	4	10	2	7
Rasht	7	1	14	3	5
Parudbar	6	2	12	6	4
Manjil	6	0	13	5	6
Gilvan	4	1	16	3	6
Shahrbej	5	3	15	1	6
Totaki	5	3	13	3	6
Totkabo	6	4	13	4	3
Shalman	6	2	11	4	7
Anzali	6	3	10	5	6

Among the stations studied, with seven stations Shalman threshold and the frequency and Totkabn station with three frequency respectively the highest and lowest years are very dry. Gillvan station with the highest frequency in 16 normal and water stations and Anzali ahead with the 10 lowest frequency normal water years have been allocated to. And also the highest and lowest frequency more wet years, respectively, including the seven stations and Rasht threshold frequency and Totky Shihbijar and is the fifth frequency.

4. Discussions

Major features of rainfall stations, the coefficient of variation is studied, the highest value to Gylvan stations, and Manjil Parvdbar (above 30% by value) in the South and South West study area are located, the lack of rainfall, the quality of order In these stations shows. Lowest coefficient of variation associated with the amount of Rasht Station 8 / 12% respectively. In terms of the amount of annual rainfall stations and station Anzali Maximum Gilvan receive the least amount. Also moving average stations showed the station a decreased Manjil more tangible than any other station has. The index of the SIAP 2007 and 2008 years, dry years have been established, so that the whole region is very dry status, as well as a 1987 year period is considered normal, so that the whole region has a normal situation. Blue 1987, 1988 years was known as the wet period. Which shows that periods of dry, normal and wet years with the same index was SIAP. According to the indicators and threshold Station SIAP Shalman Totkabn highest and lowest stations in the state is very dry, also the location of the region with 18/7% equivalent to 5 years with this condition (very dry) is. Dry conditions in parts of the South, South West and North the most part is able to. In examining the relationship between height and rainfall stations were found between these two parameters is established Relation reversed, so that with increasing altitude, rainfall decreases. However, no relationship between height and occurrence of drought. The intensity and persistence of drought in the above method was determined according to the station Manjil longest continuous drought for 5 years is allocated to Shirbijar station also has a maximum value observed, and is the longest in terms of spatial continuity of the South West region and also in terms of intensity of the southern parts, South West, North, North East has the highest intensity of droughts. Investigated of graphs trend for SIAP index ,changes in the stations has showed the highest deviation from Manjil station .

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Fundamental challenges of information and communication technology (ICT) in education

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Abstract: There are Fundamental challenges about the role of information and communication technology (ICT) in education. This has led to serious skills shortages in many countries. In turn this has put increasing pressure on policy makers, universities and other training institutions to come up with approaches to inspire young students to choose ICT for their studies. There is also a strong argument for retraining many people who already have preservice and in-survive education, whether in the workforce or not, to overcome to looming ICT skills crises. This paper reports on the examination of these points. It will also explore appropriate ways to combat this problem through analysis and identification of real prospects for ICT education.

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Keywords: ICT, education

1. Introduction

Technologies (ICT) during the past two decades have had many points of contact with education and training. The development of technology is placing new demands on expertise, and it is also leading to the increased use of information technology (IT) in instruction and learning. As early as in the 1970s discussions of the future of school systems started to pay attention to the opportunities provided by ICT. Now with the approach of the new millennium, IT is playing an increasingly central role in almost all future planning of schools and instruction. (World Bank, 1999).

With the help of state and local funding, information technology has been purchased for schools ever since the 1980s. The state has also found many ways to support teacher training in the use of IT, and it has also allocated funds for the production of IT programs. Instruction in the use of IT has also played an important role in teacher training organized by local school authorities (Becker, 2000).

It is against this background that the need arose to find out how far we have progressed in the application of ICT in education and what impacts these significant economic investments have had. It is also time to start a value-oriented discussion of how strongly the future of the Iran society—and with it, of education and training— will be linked to the vision of an information society brimming over with technology (Mohseni, 2003).

Although valuable courses may around the world be learned best practices, there is no formula to

determine the best level of ICT integration in education system. The main challenges for policymakers, planners, managers, coaches and other stakeholders that should consider include, is a comprehensive educational policy and planning, infrastructure, language and capacity building and financial affairs. (Collis, 2002).

1- The challenges to educational policy and planning:

To achieve promotion and reform in education through ICT, should be considered explicit and clear objectives, guidelines, mobilize the required resources and political requirements for understanding the primary goal in all levels. Some essential elements in planning for ICT are listed below:

1-1-A correct analysis of the current state of education system. ICT impacts should be considered institutionalized as current methods, respectively, and especially "those ICT to drive forward and the barriers should be recognized, as well as those related to education and training programs, infrastructure, capacity building, language and content and finance.(Collis, 2004).

1-2-Educational objectives at different levels of education, as well as various aspects of ICT applications that can best meet these goals in the state be used. Policymakers must understand the potential of ICT in various different goals when the concepts are used. As well as may alert best practices around the world, about the priority educational needs, financial and

Human resources and capacity bottlenecks the country and how these experiences can be adapted to the specific needs of the country (Hakkarainen, 2000).

1-3- Identifying stakeholders and coordinating actions among different interest groups. 1-4- Conducting chosen model based on ICT, should are tested on a small scale, best design models or those who proved they can be used in other areas. Such guidance is essential for identifying, correcting, feasibility, etc.

1-5- Preparation of available financial resources and identify strategies to generate financial resources for strengthening the application of ICT in the long run. (Harris, 1999).

2- Infrastructural challenges in education of based on ICT:

Before any program of based on ICT to run, an Educational technology infrastructure is placed above infrastructure of information and telecommunications. Policy makers and planners should carefully take into account the following:

2-1- At first, is there suitable rooms and buildings for placing technology? Building schools in countries that they are too old, is required to ensure an extensive repair of electrical wiring system, building, cooling and heating, ventilation and safety.(Swaminathan, 2002).

2-2- are there electricity and phone? Developing countries, vast areas still lack adequate power and several miles away their nearest phone station. In some African countries are using wireless technology, although expensive approach, but other developing countries with poor telecommunications can try this solution.

2-3- Policy makers must are examined also attending a variety of ICT in the country in general and the educational system (all levels) in particular. For example, "a primary need in education of based on ICT (using a computer and via online) access to computer and Internet services at the community level, especially schools and host families (Virgo, 2008).

3- Challenges of Capacity building:

Various attempts should be occur throughout the educational system integration for success of ICT.

3-1- professional development of teachers should be have five-axis: (Dadgaran, 2002)

- Skills in specific applications
- merging in existing curriculum
- curriculum changes regarding the application of IT (including changes in instructional design)
- Changes in the teacher's role
- to support educational theories

Ideally these should be served in pre-service training of teachers and be upgraded in in-service. In some countries, like Singapore, Malaysia and England, is required to recognize the application of ICT training courses. ICT will change speedily technologies and in this regard even the most elite teachers need to promote ICT skills and are welcome the latest developments and best practices.

Although the first focus is skills with specific applications but other four focus is importance. Research on ICT application in different fields as education and uniform over the years show disability as a barrier to teachers successfully plan, understand why they should use ICT and how to properly get the best teaching aid.(Falk and Wolfmayr, 2008).

Unfortunately, most teacher professional development in ICT has been the emphasis on teaching tools and their application in education. If learning process being Student centered, anxiety of teachers from being struck by the technology or the loss of authority in the classroom, can be prevented and as a deep understanding and feeling a severe change in their role than do not have to be raised.

Whether ICT will replace teachers? Answer is "no". In fact, with promoting ICT in the classroom, teacher's role in learning process is even more important. What can and should change is the role of teacher. Likewise the role of students "developed since the ICT can be opened classroom doors to the outside world, the community could be a new role in class. (Mohseni, 2003).

Since education is transferred in model centered- teacher to centered-student model, the unique authority of teachers was low and are known more than as facilitators, observers and trainers (of the absolute ruler to guide the way).

Primary task of the teacher is teaching students how to ask questions and to discuss the issue, make hypotheses, and then if necessary to reach Information about finding the issues raised in relation to the assessment. (FAO, 2000). Because of improved ICT training a new experience, even for teachers, teachers learn educational process and new things are discovered among the students.

Plus this is not unusual to see students in a class based on ICT undertake formal and informal roles of teacher to younger friends and students and sometimes even for teachers. (Saadan, 2001).

Teachers and students from different schools, experts, parents, community and business leaders, politicians and other stakeholders are involved in the educational process areas as resource persons, critic, observer and encouraging.

They also are essential and general customers for student published work on the Web or other media. Not many teachers reluctant to use ICT are especially "computer and internet usage. Hannafin and Savenye were found several reasons for this reluctance:

- Poor design of software,
- pessimism towards Computer effects of increasing efficiency in teaching,
- lack of managerial support,
- the time and efforts to increase technology and learn how to use for training
- Fear of losing authority in the classroom, as class is centered student.

These are points that should be served in preservice training and professional development programs in in-service training of teachers. In inservice training about professional development of ICT teachers, should in the long run, be flexible and possible. (Cecchini and talat, 2002).

For many teachers lack the necessary conditions, and with less rights in developing countries, adaptation of ICT effectively subject to granting the necessary opportunities for learning things that they need to learn according to their own experience. Motivation of teachers and supporting teachers to pursue professional development plan is necessary. That can be promoted as with ICT initiatives for teachers who are classroom teachers or ensure adequate access to technology is after training.

4- Current challenges within the language and content:

English is the dominant language on the Internet. One estimate shows that 80% of online content is English. Also a large share of educational software produced in the world market is in English. A serious obstacle to maximize the use of World Wide Web in developing countries and regions outside the major cities is that English is not prevalent. (Mohseni, 2003).

Even in countries where English is a secondary language (such as Singapore, India, Philippines and

Malaysia) is essential that materials the needs of national courses and meet the local content of the curriculum, rather "to create local language be.

Must ensure that the web is a multicultural environment with people of different cultures, namely have a role and a voice in education online communities. Therefore, is essential according to the specific needs of remote and rural segments of cultural and linguistic minorities in general.

5- Challenges related to financing the cost of ICT:

One of the biggest challenges in application of ICT in education, balancing educational objectives with economic realities. ICT in educational programs requires massive investment in developing countries that should decide on what models about the current usage of ICT and be cautious and remain vigilant about keeping the economic balance. (Annan, 1997).

Finally, this issue is raised whether application of ICT value added costs to balance or not, the other for any effective ICT-based teaching strategies intended for educational purposes or not, and if there is and scale requirements that can be implemented regardless of existing human and financial resources than that, what does it support? (Dadgaran, 2002).

Whyte offers potential sources of financial and ICT applications in following:

- 1. grant aid
- 2. the public subsidies
- 3. private sector funds
- 4. Support Equipment and volunteers
- 5. community support (i.e. to putting the house without receiving rent)
- 6. Members membership fees
- 7. revenue derived from the central and main tasks:
 - a. Connections (telephone, fax, internet and web page)
 - b. direct access to computer users
 - **c.** administrative services

(photocopiers, audio-visual aids and scan)

8. Subsidiary activities income

a. Different services (word processing, preparing financial statements, the preparation, printing and adoption services)

b. Educational Services (non face to face training and educational courses)

c. social services (conference rooms, social events, local information)

- d. Works distance and consultation
- e. specific activities (telemedicine)
- f. Sale

4. 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.

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Comparison of Uniaxial Compressive Strength of Light Weight Concrete Prepared with Bagassese between Cubic and Cylindrical Specimens

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Abstract: Sugar cane had been one of the most important agricultural products in the province of Khuzestan in Iran Country. Because of that since a long time ago this region has been called "The sugar Cane Region" or "Khoozestan ". In the process of sugar cane refinery, yellow fibers called Bagasse are generated which are known as the wastes of this product. About one million tons of Bagasse is produced in the province of Khuzestan each year, which could be a source of bioenvironmental problems and environmental pollutants. In this study, this tributary product which had been used before by the author and his co-workers (labibzadeh, et. al., 2011) to generate a kind of Light Weight Concrete (LWC) was more examined in order to investigate its effect on the relationship between the compressive bearing of the proposed LWC with different shape samples (cubic and cylindrical) including Bagasse. To perform this work, at the first a constant mixing plan was considered according to ACI-211.2 standard code, then some samples were prepared according to this strategy, without adding Bagasse and after curing, they were tested. Then, samples with the mentioned mixing plan and 10,20,30,40 &50 percents of including Bagasse were designed to substitute the aggregates in the mixture and after curing these samples according to standard code manual ACI-211.2, the corresponding tests conducted and the results have been deduced and interpreted. The results showed that the ratio of compressive strength of cylindrical samples to cubic ones of the normal concrete for mass concrete is 0.89 which can be increased to 1 for 20% including Bagasse light weight concrete. Here, the point which should be considered is the noticeable reduction of this ratio for 30% Bagasse concrete which is equal to 0.988 to 0.404 for 40% Bagasse concrete.

[Hassan Tanoursaz, Mojtaba Labibzadeh, Amin Khajehdezfuly. Comparison of Uniaxial Compressive Strength of Light Weight Concrete Prepared with Bagassese between Cubic and Cylindrical Specimens. Journal of American Science 2011; 7(2):356-360]. (ISSN: 1545-1003). <u>http://www.americanscience.org</u>.

Keywords: Bagassese; Light Weight Concrete (LWC); Compressive strength; cylindrical samples; Cubic samples

1. Introduction

In concrete buildings, concrete has a very important role in the whole structural load bearing capacity and the reduction of its weight leads to significantly considerable advantages. The advantage of obtained Light Weight Concrete (LWC) has been very sensible from very ancient times. Many authors stated in their investigations that the light weight concrete has many advantages, including the high ratio of strength to weight, good tensile strength and low temperature expansion coefficient. In addition, when the concrete becomes lighter, workers will tolerate less pressure for shipping and casting it in comparison with mass normal weight concrete, and also the gross weight of materials which must be carried out will be reduced. The reduction of the dead weight of a building will result in the reduction in the cross-section of columns, beams and foundations which will be resulted in the reduction of construction cost and earthquake loadings (Liu, et al., 1995). In the recent years, considering the bioenvironmental pollutants issues and the tendency to garbage and waste materials recovery for further usage in the developing and industrial countries has

allocated a huge capital to itself. Nowadays, the common method in consuming the agricultural wastes is burning them in the open air which turns them into ash and that ash returns to the earth i.e. "from ashes to ashes". In some countries, burning these wastes in the open air has been forbidden, since it leads to polluting the environment and causes various illnesses in human beings (Tommy, et. al., 2006). Bagasse is one of these agricultural wastes. In the refining process of sugar, yellow-colored fibers called Bagasse are generated as tributary product. About 1 million tons of Bagasse are produced each year in the province of Khuzestan which brings about some bioenvironmental problems.

There exist few researches on using Bagasse in concrete making and they are limited to its usage as a substitute for cement. Gansan, et.al, (2007) have studied the effect of Bagasse ashes as a substitute for cement on the physical and mechanical characteristics of hardened concrete. They found out that the Bagasse ash is a useful mineral and the optimal substitution amount for cement is 20%. Chaslip, et. al, (2009) have studied the use of ash as a substitute for cement and have evaluated the characteristics of pressure drag, permeability and the temperature of Bagasse ash concrete and found out that the optimal amount of using Bagasse ash in the mixture as part of the cement is 20% of cement weight. When this ratio increases to 30%, the permeability and the pressure drag will be reduced.

Most of the researches on Bagasse in concrete were associated with using it as a replacement for cement in order to improve concrete mortar. In this study, based on the previous study of the authors (Labibzadeh, et. al., 2011) fibers of Bagasse are used, in a new vision, as a replacement for aggregates and we will investigate the pressure drag of cylindrical and cubic samples and their relationship with each other.

2. Material and Methods

The cement consumed in this research is the 2th brigade of Dorood-Lorestan with with density of 3.15 and specific area of about 3350 cm^2/gr . Bagasse fibers used in this study has obtained from Dezful -Hafttappeh sugarcane agro- industry factory and has 64 kg per cubic meter specific for loose specific weight kind and 129 kg per cubic meter for dense type and the moisture absorption of it about 50%. The consumed water is the drinking water of Ahvaz. The aggregates used in this research are from the fluvial Ballast of province of Dezful. The gravels have two dimensions with 50% gravel 3/8 in and 50% gravel 3/4 in with 1.16% moisture percentage and 1526 kg/cubic meter specific weight, 43% vacuum and 2.68 density. Furthermore, the sand with 5.8% moisture, 1724 kg/cubic meter specific weight, 35%vaccum and 2.64 densities has been used in the mixture of LWC.

The mix design of consumed concretes with having different percentage of Bagaase has been shown in table1. The numbers mentioned are for 1 cubic meter volume concrete.

Tab	le	l. Mix	design	(kg in	1m^3	^o concrete)
-----	----	--------	--------	--------	----------------	-----------------------	---

Bagassese	Water	Cement	Sand	Coarse	
0	160	340	840	1130	CC
32	176	340	672	904	CB20
48	184	340	588	791	CB30
63	192	340	504	678	CB40
79	200	340	420	565	CB50

It should be mentioned that Bagasse has been substituted by the aggregates in such a way so that its volume remains constant. The values in the table1 are measured in kilograms. Also, since the Bagasse absorption is 50%, surplus water consumed in the mixed design in order to avoid the moisture reduction of concrete and has been added to the aforementioned content of water in table 1. The mixtures were made manually in order to control the moisture, pour out in three layers into the cast and each layer has been compacted by 25strokes by the standard rod and its surface was level. After 24 hours, the samples are brought out of the cast and were located in the curing water basin. Totally, in this research 60 cubic and cylindrical samples have been made.



Figure 1. Dorood-Lorestan cement used



Figure 2. Bagasse used from Dezful – Hafttappeh sugarcane agro- industry factory



Figure 3. The cubic and cylindrical samples

http://www.americanscience.org



Figure 4. Fracture of the cubic sample of LWC



Figure 5. Different mode of the fracture of the cylindrical and cubic samples of LWC including Bagasse

After curing the samples, the uniaxial compressive strength of the cubic and cylindrical units made of the proposed light weight concrete including Bagasse has been investigated through performing the standard test. In the next section the results of the tests have been illustrated using tables and figures.

3. Results

The results of the compressive strength tests over 60 cylindrical and cubic samples and their specific weight have been shown in table 2. Left hand column shows the value of the specific weight of the proposed LWC. Right hand column shows the percentage of the Bagasse used in the LWC. The middle columns illustrate the compressive strength of the cubic and cylindrical samples.

		weight		
Specific	Cubical	cylindrical	Curing	
weight	compressive	compressive	time	D
ka/m^3	strength	strength		Baggase
ĸg / m	kg/cm^{2}	kg/cm^{2}	days	70
	240	205	7	
	300	270	14	0
2470	410	359	28	
	137	128	7	
	180	172	14	
2125	238	230	28	20
	103	77	7	
10.50	130	103	14	•
1950	177	150	28	30
	68	26	7	
1500	87	36	14	10
1780	119	47	28	40
	51	20	7	
1.61.0	81	29	14	
1610	110	41	28	50

Table 2. The rate of resistance loss and the specific

It should be mentioned that the 60% Bagasse concrete couldn't resist any force instead of the aggregates and it relaxes after stripping. It could be seen from the aforementioned table that all 20% and more Bagasse concretes could be considered as light weight ones. Table 3 shows the ratio of the pressure resistance changes of the samples in comparison with the natural concrete (0% Bagasse). As it is clear from the aforementioned table, the compressive strength of the Bagasse cubic and cylindrical samples in all the curing periods have a considerable reduction.

In order to compare the compressive strength of cylindrical and cubic samples and their relationship, figures 1, 2, 3, 4 and 5 have been derived and illustrated. As we see, in order to obtain the relationship between the compressive strength of cubic and cylindrical samples, linear regression has been used. The high regression coefficient of these diagrams indicates the high preciseness of this approximation. The gradient of regression lines shows the ratio of compressive strength of cylindrical samples to the compressive strength of cubic samples with a high approximation. As it could be understood from the samples, the ratio of compressive strength of cylindrical samples to cubical ones for mass concrete is 89% which increases to 1 for 20% Bagasse concrete and then this ratio will be reduced for the other percentages. But the point which should be considered in these diagrams is the noticeable reduction of this ratio for 30% Bagasse concrete from (0.988) to 0.404 for 40% Bagasse concrete. This could be the result of the change in the concrete structure from 30% to 40% and the rigid core of cubic samples which are more conspicuous than before.

Table 3.	Change in	strength	and spe	ecific w	veight due
	to chan	ge in Bag	vasse va	lues	

Specifc weight kg / m ³	Cubical compre- ssive strength kg / cm ²	cylincal compre- ssive strength kg / cm ²	Curig time days	Baggase percentae
	-43 %	-38%	7	
14.7	-40 %	-36%	14	20
-14/.	-42 %	-36%	28	
	-57 %	-62%	7	
21.7	-57 %	-62%	14	20
-217.	-57 %	-58%	28	30
	-72 %	-87%	7	
28.4	-71 %	-87%	14	40
-287.	-71 %	-87%	28	
	-79 %	-90%	7	
25.1	-73 %	-89%	14	50
-357.	-73 %	-89%	2	50



Figure 6. The relation between cylindrical and cubic compressive strength of normal concrete (0% Bagasse)



Figure 7. The relation between cylindrical and cubic compressive strength of LWC (20% Bagasse)



Figure 8. The relation between cylindrical and cubic compressive strength of LWC (30% Bagasse)







http://www.americanscience.org

Figure 10. The relation between cylindrical and cubic compressive strength of LWC (50% Bagasse)

4. Discussions

The 60% Bagasse concrete which is used instead of the aggregates could not tolerate any force and it will be relaxed after stripping.

All 20% and more Bagasse concretes could be considered as light weight concrete.

The compressive strength of concrete is reduced by the substitution of Bagasse for the aggregates which could be resulted in weakening and deleting of the main skeleton of the aggregates.

The compressive strength of the cubic samples is greater and more unified than the cylindrical samples.

The ratio of the compressive strength of cylindrical samples to cubic ones is 89% for the mass normal concrete (0% Bagasse), which increases to 1 for the Bagasse concrete (LWC) and then reduces for the other percentages of the Bagasse. The remarkable point is the conspicuous reduction of this ratio from 30% Bagasse concrete (0.988) to (0.404) for 40% including Bagasse LWC which could be due to the change in the concrete construction and the rigid core of the cubic samples which shows itself more conspicuous than before.

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Prevalence of fascioliasis among slaughter sheep in selected abattoirs in Imo State, Nigeria

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Abstract: A 12 months study on fascioliasis in sokoto gudali sheep of Hausa origin (northern part of Nigeria) was carried out in five different abattoirs in Imo State, Nigeria between 2004 and 2005. The abattoirs were Obinze, Okigwe, Afor-Enyiogugu, Afor-Ogbe, and Orlu . The objective of the study was to determine the prevalence of fascioliasis among slaughter sheep in selected abattoirs in Imo state. Five grams of feces and liver from freshly slaughtered sheep of both sexes, and from different locations and ages were collected, taken to the laboratory and analyzed for fasciola eggs and adult flukes. Direct smear, formol ether concentration and sodium chloride floatation methods were used to harvest the eggs and adult parasites. Out of 367 sheep examined, 64 (17.2%0) were infected. Infection was highest in Obinze abattoir (29.6%) followed by Afor Enyiogugu abattoir with (29.0%) Okigwe (15.6%), Orlu (12.5%) and Afor Ogbe (12.4%). Infection was highest in females than in males and was also sex dependent. Among adult sheep (>2yrs) examined , 59 (14.9%) were infected while out of 38 young goats (<2yrs), 5 (13.2%) were infected. Infection level rose with increased rainfall with the highest level observed at the peak of the rainy season and dropping during the dry season months. The number of parasites were 180, and mean worm load 281. Intensity was higher in males than in females. This result therefore calls for an improved disease control and adequate sanitation programme for sheep rearers in the study area.

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Keywords-Fascioliasis, worms, slaughter sheep, abattoirs, tropics, Nigeria.

Introduction

Fasioliasis is caused by Fasciola gigantica, a digenetic trematode which belongs to the family fasciolidae. They are very important liver parasite of cattle, sheep and goats (Ruminants). The adult inhabits the bile duct and gall bladder of liver in these animals. Inside their host, the liver flukes cause severe damage which may lead to the death of the animals (Anosike, 2005). Fascioliasis is one of the helminthic trematodes that constitute both economic and public health constraints to a profitable ruminant production in tropical Africa (Adams and Mckay, 1966, Fabiyi, 1982). Fascioliasis has been implicated as the cause of morbidity and mortality in the production of ruminants (Onwuliri 1993, Okoli 2000). These ruminants however have also been found to harbour other helminthic mixed infections as recorded by Anosike (2005). Such as Dicrobothriasis, cotylophomiasis among others. This infection brings about decrease in meat production and subsequent economic loss. The nutritional and health value of the animal deteriorates leading to poor carcass quality and loses (Shar-Fisher and Say, 1987).

Materials and Methods The Study Area

Imo State is one of the south eastern states of the Federal Republic of Nigeria. It is located within latitude 5°10' and 5°67N, and longitude 6°36 and 7°28'E. The state is bound on the North West by Anambra State, on the south-west by River State and on the eastern boarders by Abia State. The state has two main geographical regions-The coastal plain, covering the central and southern parts of the state and the Plateau and escapement zones in the northern parts of then state. The soil of the coastal plain is sand/loan and vegetation is typical rainforest, while that of the Northern-eastern geographical plain is clay with rich savannah vegetation. There are two distinct seasons, the rainy and dry season with the wet or rainy season lasting from March to October with peak rainfall occurring in July and September and short slightly drier spell in August, Popularly known as August break. Annual rainfall ranges from o.omm to 2,500mm. The mean temperatures over most of the state is 27° C, while relative humidity is about 70;80% (IMSG, 1993). The main occupation of the populace is agriculture. There are civil and public servants also, as well as fishermen and traders.

Sample Collection

A total of 367 sheep were examined for fascioliasis in five selected abattoirs in Imo State,

Nigeria namely Okigwe, Afor ogbe, Obinze, Afor Enyiogugu, and Orlu. Five grams of feces were collected from the rectum of these sheep and analyzed in the laboratory under 48hrs. Egg counts according to Fleck and Moody (1988) was done after preliminary ident ification of eggs at X10 magnification (WHO, 1991). Age of sheep was determined by estimation of dentition (Andrew et al, 1990). Sex was also observed and recorded. Prevalence was expressed as the percentage of sheep infected, while intensity was recorded as number of eggs per 5 grams of feces. The study lasted for one year (September 2004 to August 2005).

Statistical Analysis

Descriptive statistic as provided by the SPSS 17.0© and MS Exel 2010 software used to represent ensuing data. The test of homogeneity of variance in means of disease prevalence was conducted with the one way analysis of variance (ANOVA). Gender and age relatedness to disease prevalence were explored with the chi square test of significance. The influence of rainfall on disease prevalence was explored using the pearson product moment correlation coefficient(r).

RESULT

A test of variance of equality in mean prevalence of fascioliasis across the sampling location revealed significant difference F (16.24)> Fcrit (4.04) at P < 0.05.

Table 1 showed the prevalence of fascioliasis among sheep slaughtered in five selected abattoirs in Imo State Nigeria. Out of the 367 sheep examined 64 (17.2%) were infected. Infection however varied from one Abattoir to another. Highest infection was at Obinze (29.6%) followed by Afor Enyiogugu (26.3%), Okigwe (15.6%) and (12.5) and (12.4%) in Orlu and Afor-Ogbe respectively. Table 2 illustrated the sex related distribution of fascioliasis. Of 312 male sheep examined, 53 (17.0%) were infected while the 55 females gave 11(20.0%) prevalence.

Age related distribution of *F.gigantica* in sheep is shown in table 3. Out of 329 adult sheep (>2yrs) examined, 49(14.9%) were infected, while out of 38 young sheep (<2yrs), 5(13.2%) were infected.

Table 4 showed the monthly prevalence and Mean Worm Load (XWLD) of F. gigantica in the slaughter sheep. Total number of parasites was 170 and Mean Worm Load (XWLD) was 266. Infection level rose with increase in rainfall with highest level observed at the peak of the rainy season and dropping during the dry season months. Sex related egg counts were shown in table 5. Of the 6O(93.8) infected male sheep 40(66.7%) were having egg counts of 0-49, while 15(25.0%) had 55-99, and 5(8.3%) had egg counts of 100-199. In the female category, out of the 4(6.3%) infected female sheep, 2(50.0%) were having egg counts of 0-49, 2 (50.0%) had egg counts of 50-99. Total number of sheep having 0-49 egg counts were 42(65.6%), 17(26.6%) had egg counts of 50-99 while 5(7.8%) had egg counts of 100-199.

Table 1: Prevalence of	Tascionasis among sneep	p slaughtered in s	selected adattoirs in in	mo State, Nigeria.

Abattoir location	Number examined	Number infected	Percentage (%) infected
Orlu	72	9	12.5
Okigwe	90	14	15.6
Obinze	54	16	29.6
Afor-Enyiogugu	38	11	26.0
Afor Ogbe	113	14	12.4
Total	367	64	17.2%

Table 2: Sex related	distribution	of fascioliasis	among	slaughter s	sheep
			B	Serenger ver	p

Male				Female				
Abattoir	Number	Number	%	No	Number	%	Total no	Total number
location	examined	infected	infected	examined	infected	infected	examined	infected
Orlu	62	7	11.3	10	2	20.0	72	9(12.5%)
Okigwe	78	13	16.7	12	2	16.7	90	14(15.5%)
Obinze	39	13	33.3	15	3	20.0	54	16(69.6%)
Afor-Eyiogugu	30	8	26.7	8	2	25.0	38	10(26.3%)
Afor ogbe	103	12	11.7	10	2	20.0	113	14(12.4%)
Total	312	53	17.0	55	11	20.0	367	64(17.4%)

Old sheep (>2years) Young s					p (<2years)	
Abattoir location	Number	Number	% infected	Number	Number	Percentage
	examined	infected		examined	infected	infected
Orlu	100	11	11.0	13	1	7.7%
Okigwe	64	7	10.9	8	2	25.0
Obinze	84	17	20.2	6	2	33.3
Afor-Enyiogugu	49	14	28.6	5	-	-
Afor Ogbe	32	10	31.3	6	-	-
Total	329	49	14.9	38	5	13.2%

Table 3: Age related distribution of fascioliasis among sheep

Table 4: Monthly Prevalence and mean worm load of fascioliasis in sheep in relation to rainfall.

Month	Rainfall	Number	Number	% infected	Total no	Mean worm load
	in mm	examined	infected		of parasites	(XWLD)
Sept. 2004	309.1	18	6	33.5	15	250.00
Oct. 2004	322.9	5	-	-	-	-
Nov. 2004	37.0	10	-	-	-	-
Dec. 2004	0.0	17	-	-	-	-
Jan. 2005	38.3	15	-	-	-	-
Feb. 2005	84.3	10	-	-	-	-
March. 2005	103.1	15	2	80.0	29	145.0
April 2005	82.2	17	2	54.5	30	150.0
May 2005	469.8	20	12	13.3	10	83.3
June 2005	500.7	22	11	85.7	12	109.0
July 2005	260.0	25	05	52.0	19	320.0
Aug. 2005	190.5	20	14	60.0	30	214.3
Sept. 2005	490.6	20	12	60.0	35	291.6
Total		367	64	17.4	180	281

Table 5:Sex related egg counts of F. gigantica in sheep

Egg/5 grams of feces	No of males	No of females	Total no (%)
0-49	40(66.7)	2(50.0) +	42(65.6%)
50-99	15(25.0)	2(50.0)	17(26.6%)
100-149	5(8.3)	-	5(7.8%)
150-199	-	-	-
>200	-	-	-
Total	60(93.8)	4(6.3)	64

Output

1. Graphs





Discussion

Fascioliasis or Liver rot is considered an important parasitic disease of ruminants and has been implicated as the commonest disease of the liver leading to liver condemnation. The 17.2% prevalence of fascioliasis obtained in this present study agrees with the results of Alonge (1979), Okoli et al, (2000), Okoli (2001) and Okoli et al, (2002) in the north and eastern Nigeria, and in East Africa. Infection varied from one abattoir to another and may have been influenced by varying ecological and climatic parameters of the areas that this sheep that were imported from the northern part of the country must have grazed on before getting to the slaughter locations (Agbola, 1979; Aladi, 1999). Highest infection rate was recorded in Obinze (29.6%).

This abattoir plays host to most of the sheep that come from the northern part of the country. Again Obinze is a mini settlement for Hausa ethnic group, majority of who trade on sheep. This town has the physicochemical parameters and the ecological factors that favour the growth of the infecting snails. Njoku-Tony, (2007). Infection was higher in males (20.0%) than in females (17.0%). This is in line with the work of Njoku-Tony (2007) while working on fascioliasis in ruminants in Imo State. It was observed that few females were brought out to the abattoirs for slaughter. Further investigations revealed that the females are kept back for reproductive purposes and milk production. This factor actually hindered the proper assessment of the females; however the few that were examined also haboured the infection. Fascioliasis infection was therefore not gender related among slaughter sheep at the abattoirs sampled.

Prevalence of the infection also varied between the adult (>2yrs) and the young sheep (<2yrs). More adult were infected than young sheep. The variance may be as a result of method of recruitment and husbandry. However, more adult sheep were examined than young sheep. On investigation, it was discovered that meat from young sheep are not mature and so not good for consumption, and this therefore reduces the market value for the young sheep. There may also be no progressive resistance to the infection with F. gigantica, this is in line with the work of Envenihi et al, (1975). While working with nematodes and cestodes, Envinihi et al (1975) showed that there was always a close association between age and intestinal helminth. Fascioliasis infection was however not gender related. Monthly distribution, prevalence and mean worm load (XWLD) of F. gigantica revealed a gradual rise in infection with increased rainfall. Highest infection was between April and September (Fabiyi 1982, Blood et al, 1989; Shar-Fisher and Say 1989). This rise in the rate of infection during the rainy season suggest that the sheep must have picked the infection during the late dry season (LDS) and the early rainy season (ERS) these are seasons with little or no rainfall and therefore coincides with the bionomics of their snail intermediate host (Anosike 2001). Mean worm load however did not correspond with the physical manifestation as infected sheep still look healthy (Shar-Fisher and Say, 1989). The 17.2% prevalence of this infection however is of public health and veterinary interest and calls for the need for improved health and environmental surveillance and adequate control program.

Conclusion

This study therefore gives an overview on the prevalence and distribution of fascioliasis in Imo State. It furthermore suggest the need for a state as well as nationwide baseline data on the prevalence and distribution of *F. gigantica* infection.

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Geoenvironmental Study Of Groundwater Contamination In A Dual Aquifer Environment Using Earth Resistivity Imaging

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Abstract: The variation of electrical resistivity as a function of soil properties was used as a vital tool to study groundwater contamination in the vicinity of some selected solid waste disposal sites in the municipal town of Zaria. The Abem Lund Imaging system with Terrameter SAS 4000 was used for the resistivity data measurements and the Res2dinv software was used for the processing and interpretations of the data. Due to the high conductivity of the contaminant plumes it was possible to delineate their pathways into the regolith and fractured aquifer environments. Resistivity data from inverted models obtained from profiles near monitoring wells, correlated well with electrical conductivity (EC) and total dissolved solid (TDS) values of water samples taken from theses wells. The inferred water resistivity and the soil resistivity obtained from the resistivity tomosections at depths of water table revealed that the samples, which were collected from hand dug wells whose depths are within the overburden (regolith aquifer), are more polluted than the samples which were collected at deeper levels corresponding to the borehole samples (fractured aquifer). The findings of this study suggest the potentiality of the resistivity imaging technique as a pre-characterization tool for mapping subsurface contamination in the vicinity of waste disposal sites. [Samson Idowu Jegede, Isaac Babatunde Osazuwa, Nasir Khalid Abdullahi. Geoenvironmental Study Of

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1. Introduction

The evaluation of groundwater and soil properties has become increasingly important for site characterization when more industrial wastes and domestics solid refuse come into contact with groundwater and soils causing subsurface contamination. (Yoon, et, al, 2002). The variation of electrical resistivity as a function of soil properties is a vital tool for assessing the contaminated media. Electrical resistivity method is the most commonly applied geophysical method to measure the apparent resistivity of subsurface materials. Under many subsurface conditions, electrical resistivity methods can quickly and cheaply locate the general position of the contamination plumes and identify areas most feasible for sampling and monitoring. Electrical resistivity is a function of a number of soil properties, including the nature of the solid constituents (particle size distribution, mineralogy), arrangement of voids (porosity, pore size distribution, connectivity), degree of water saturation (water content), electrical resistivity of the fluid (solute concentration) and temperature. (Samouëlian, et, al, 2005). The water solution resistivity is a function of the ionic concentration, and the resistivity of the solid grains is related to the electric charge density at the surface of the constituents. These parameters affect the electrical resistivity, but in different ways and to

different extents. Electrical resistivity experiments have been performed to establish relationships between the electrical resistivity and each of these soil characteristics. Nevertheless, the electrical resistivity of bulk soil decreases as the concentration of leachate increases. Hence the higher the concentration of leachate in pore fluid, the lower electrical resistivity. The lower electrical resistivity of pore fluid can be explained. Many contaminants contain an ionic concentration considerably higher that the background level of native ground water. When such a contaminant is introduced into an aquifer, the electrical resistivity of the saturated zone is reduced. Earth resistivity imaging survey across such a suspected area can identify this reduced resistivity zone as an anomaly. But by combining knowledge of Hydrogeological and geophysical data with chemical data from monitoring wells the extent of dumpsite leachate can be delineated by geoelectrical imaging as a response to the varying electrical resistivity in the contaminated area. In this investigation five different municipal solid waste disposal sites were surveyed. These include Tsamiya dumpsite and Old cemetery dumpsite both in Samaru. Palladan dumpsite, Kapa garage dumpsite and Abubakar Iman primary school dumpsite both in Tunduwada.



Fig. 1: The settlement map of Zaria showing the surveyed locations as modified from McCurry, (1970).

2. Material and Method.

Site Description

The main goals of this study were to identify and delineate the extent of contaminated leachate plumes below surface as well as testing the efficiency of the 2-D resistivity method as a pre characterization tool for tracing the properties of the disposed waste and its severity underneath. The Samaru, Palladan and Tudun-wada areas are located in the most densely populated parts of Zaria Township (fig 1). Zaria is located approximately between Latitudes 11° 03'N and Latitude 11° 11'N to Longitude 07° 37'E and Longitude 07° 42'E. The approximate average elevation is about 670 m above mean sea level. It occurs in a dissected portion of the Zaria–Kano plains. The Zaria-Kano plains are an extensive peneplain developed on the crystalline rocks of the Nigerian Basement Complex. Zaria occurs within a semi-arid tropical continental climate, characterized by a distinct wet and dry season. Rainfall commences at about May and ends early to mid October. The mean annual amount of rainfall is about 1067mm. The eastern and western part of the study area are dissected and drained by the Basawa and Kubanni rivers and their tributaries respectively. Extensive exfoliation and chemical weathering have produced residual granitic inselbergs .The largest of such inselbergs is the Kufena Hill, just on the portion of the study area and which southern provides the main relief in the area. There are also low lying hills to the east of Kufena, around Zaria city and Tudun Wada. Fig 2 shows one of the waste disposal site located in Samaru.



Fig. 2: Tsamiya Dumpsite in Samaru. This well was initially used for domestic purpose by the indigenes but served as a monitoring well for the purpose of this research.

Hydrogeological studies undertaken in the North-central Nigeria basement complex, including Zaria revealed that the occurrence of ground water in the crystalline basement rocks occur within soft overburden, saprolite or regolith aquifer and fractures within the basement rocks. The regolith aquifer holds Geo-electrical Relationships

When an electrical current is introduced into the saturated soil it tends to move in a tortuous path through intergranular spaces following much the same path as the groundwater. In the case of groundwater flow the driving force is the hydraulic potential; in electrical flow it is the electrical potential. If the soil material itself is nonconductive, such as quartz sand, and relatively free of clay, the magnitude of the current depends primarily on effective porosity and pore-water conductivity (or resistivity). Conductive minerals such as magnetite in the matrix, as well as granular surface conductance can also affect electrical flow, but these are normally of secondary importance.

A commonly used means of expressing the relationship between pore-water ρ_w resistivity and bulk resistivity ρ_o is by formation factor F (Archie, 1942)

$$F = \frac{\rho}{\rho_v}$$
(1)

a great quantity of groundwater and most hand dug wells are located in this shallow aquifer for domestic water supply (Alagbe, 2002). At some locations, these aquifers are interconnected and form single unconfined Hydrogeological unit (Osazuwa and Abdullahi, 2008).

The formation factor is related to porosity ϕ in the following empirical equation (Winsauer et al, 1952)

 $F = a\phi^{-m}$ (2)

Where ϕ = porosity a = constant ranging from

0.47 to 2.2 m = constant ranging from 1.3 to 2.6. Combining equation (1) and (2)

 $\rho = a\rho_w \phi^{-m} \dots (3)$

In surface electrical resistivity methods, an electrical current is introduced into the ground at the surface using two electrodes, and the resultant electrical potential measured between two central electrodes as shown in the Figure 3. There are a number of electrode arrangements which have been developed for use in geoelectrical work, but only the Schlumberger and Wenner arrays are widely used. In the Schlumberger array the central electrodes are kept relatively close together as compared to the separation of the outside current electrodes, while in the Wenner the distance between all electrodes is equal. For this investigation the Wenner array was used hence further discussion in this paper will refer to that method.



Fig 3: Electrical current flow in the Earth – Wenner electrode configuration.

The configuration shown in Fig 3 is the Wenner array from which the apparent resistivity of the bulk earth can be determined by the following equation

$$\rho_a = 2\pi a \frac{\Delta V}{I} \qquad (4)$$

Where a is the selected electrode spacing,

 ΔV is the potential difference measured between the two central electrodes and I is the current imposed between the two outside electrodes and measured simultaneously with the potential difference ΔV . Two-dimensional electrical imaging/tomography surveys are usually carried out using a large number of electrodes, 25 or more, connected to a multi-core cable. In this survey, the Wenner 32SX protocol was adopted and in this, forty-two electrodes were used and adequate contacts were made with the ground. The electrode selector while measuring selects both the current and the potential electrodes based on the datum point to be measured at each time during data collection. Three stacks were used in order to ensure a reliable average data measurement. There are three basic modes of operation for any resistivity method: sounding, profiling and sounding - profiling (CVES). To obtain a good 2-D picture of the subsurface, the coverage of the measurements must be 2-D as well. As an example of the sounding profiling (CVES) resistivity method, Fig. 4 shows a possible sequence of measurements for the Wenner electrode array for a system with 20 electrodes. In this example, the

spacing between adjacent electrodes is "a". The first step is to make all the possible measurements with the Wenner array with electrode spacing of "1a". For the first measurement, electrodes number 1, 2, 3 and 4 are used. Notice that electrode 1 is used as the first current electrode C1, electrode 2 as the first potential electrode P1, electrode 3 as the second potential electrode P2 and electrode 4 as the second current electrode C2 For the second measurement, electrodes number 2, 3, 4 and 5 are used for C1, P1, P2 and C2 respectively. This is repeated down the line of electrodes until electrodes 17, 18, 19 and 20 are used for the last measurement with "1a" spacing. For a system with 20 electrodes, notes that there are 17 (20-3) possible measurements with "1a" spacing for the Wenner array. After completing the sequence of measurements with "1a" spacing, the next sequence of measurements with "2a" electrode spacing is made. First electrodes 1, 3, 5 and 7 are used for the first measurements. The electrodes are chosen so that the spacing between adjacent electrodes is "2a". For the second measurement, electrodes 2, 4, 6, and 8 are used. This process is repeated down the line until electrodes 14, 16, 18 and 20 are used for the last measurement with spacing '2a" for a system with 20 electrodes. Note that there are 14(20-2x3) possible measurements with "2a" spacing. The same process was repeated for measurements with '3a', '4a', '5a' and '6a' spacing. To get the best results, the measurements in a field survey should be carried out in a systematic manner so that, as far as possible, all the possible measurements are made. This will affect

the quality of the interpretation model obtained from the inversion of the apparent resistivity measurements (Dahlin and Loke, 1998). The 2-D electrical imaging/tomography is one geophysical development in recent years which can map even areas with moderately complex geology (Griffith and Barker, 1993).



Figure 4: The arrangement of electrodes for a 2-D ERT survey and the sequence of measurements used to build up a pseudosection. (Loke, 2004).

One technique used to extend horizontally the area covered by the survey, particularly for a system with a limited number of electrodes, is the roll-along method. After completing the sequence of measurements, the cable is moved past one end of the line by several unit electrode spacings. All the measurements that involve the electrodes on part of the cable that do not overlap the original end of the survey line are repeated (Fig. 5).



Fig. 5: The use of the roll-along method to extend the area covered by a 2-D ERT survey. (Loke, 2004).

3. Discussion of Results

The results obtained in this investigation are presented as case studies from the five selected dumpsites.

Case Study I: Old Cemetery Dumpsite Profile line H03.

This profile was taken along the western perimeter margin of the dump over the rail line. The electrode spacing was 4.0 m which gave a total length of 160 m, thus allowing depth of investigation down to 20 m Fig 6. This depth covered the depth beyond water table in the area as revealed by static water level of local wells in the area.

Resistivity Model H03. The model (Fig 6) clearly shows a contaminated topsoil of resistivity range from 22.4 - 35.0 Ohm-m, from the surface at the 136 m mark sloping down gradually to a depth of 16.0 m at the 48.0 m mark. The entire profile line has an overburden thickness of over 20.0 m at some points, narrowing down to about 17.0 m towards the northern end of the profile line. This suggests the

Fig

possibility of the leachate plume migrating into the fractured aquifer at deeper levels of the basement. Water sample analysis result from a hand dug well (HW1) very close to the 32.0 m mark of this profile, showed high electrical conductivity and high total

dissolved solid. The water is also found to be polluted with the heavy metals Pb, Cd, Cr and Ni with contaminations levels exceeding the regulated guidelines provided by the World Health Organization (WHO).



6 Inverted model of Profile line H03

Case Study II: Tsamiya Dumpsite Profile S01

Resistivity measurements were taken at this profile which defines the western margin of the dumpsite. A hand dug well at the time the resistivity data were taken was right within the dump Fig 2. The electrode spacing was 5.0 m which gave a total length of 200 m, thus allowing depth of investigation down up to 25.0 m (Fig 7); this depth covered the depth beyond water table in the area as revealed by depths of hand dug wells.

Resistivity model S01

Examining the resistivity model (Fig 7), from the 135.0 m mark to 150.0 m mark, we find a trend of decreasing near surface resistivity right from the surface down to a depth of 20.0 m. The substantial decrease in resistivity(from 82.0 - 40.9 ohm-m) obtained from the 2-D data at these depths is believed to be due to groundwater contamination as a

result of accumulation of leachate, a similar trend can be seen between the 70.0 m mark and 80.5 m mark also from the surface down to about 21.0 m. However between the 80.5 m and 115.0 m, a near surface low resistivity saturated zone appears not to be able to migrate beyond a depth of 5.0 m probably because of a non permeable material of resistivity ranging from 116-164 Ohm – m defining the subsurface topography of the dumpsite. Hence the migration pathways of the contaminant are delineated in the directions of the accumulations of the contaminants. These pathways are likely to be with materials that are highly porous and permeable. The water analysis of the hand dug well which showed elevations in concentration of organic/inorganic parameters exceeding the permissible health limits supports the fact that the delineated low resistivity portions represents accumulated leachate plumes.



Fig 7: Inverted model of Leachate accumulation at the weathered basement Profile line S01.

Case Study III: Palladan Dumpsite-Profile P03.

This profile was taken across the dump at the western flank of the dump. Eighty electrodes were planted in this profile with a spacing of 5.0 m thereby transversing a horizontal distance of 400.0 m trending N-S direction. This was made possible by the adoption of the roll along technique in this profile.

Resistivity Model P03

The model (Fig 8) shows that the top 5 -10 m of regolith has resistivity of less than 34.1 Ohm-m with the top 5 m having a resistivity of less than 16.6 Ohm-m. It indicates that within 10 m depth groundwater is strongly contaminated. The penetration of this strong contamination appears to be controlled by a layer of the subsurface with varying thickness across the entire profile. This layer is interpreted as clay layer especially as the resistivity (70.0 Ohm - m and above) is within the range of the resistivity of clay. The depth of penetration increases southwards as the clay layer thins out under the control of the subsurface topography, the strong contamination can be seen extending deeper into the weathered basement up to a depth of over 25.0 m at

the 320.0 m mark. This conclusion is supported by the result of the water chemistry analysis of water sample collected from a 27.0 m deep borehole (PBH1) located near the profile line about 5.0 m on the western side of the 320.0 m mark which showed slight elevations in concentrations of some of the heavy metals (Pb, Cd and Ni) above WHO guidelines for portable water. The inversion also reflects the bedrock at 25.0 m depth at profile position 75.0 m and 300.0 -320.0 m while the bedrock with varying moisture content and degree of weathering dominates the profile beginning from depth of 10.0 m down to 30.0 m. The degree of weathering between points B and \mathbf{B}' suggests the possibility of the interval serving as a migration pathway for contaminants into the fractured aquifer zone as was also found in profile P02. All the hand dug wells in this location had depths within the regolith aquifer and all had high TDS and showed elevations in concentrations of some heavy metals (Pb, Cd, Cr, Ni) above the WHO guidelines for drinking water. Unfortunately these wells are the major sources of water for domestic needs of the inhabitants in the environs.



Fig 8: Inverted model of Profile line P03.

Case Study IV: Kampa Garage Dumpsite- Profile T01.

Resistivity Model T01.

This profile was taken at the northern perimeter margin of the dumpsite trending E-W direction. The electrode spacing was 2.5 m which gave a total spread length of 100 m allowing an investigation depth of 15.0 m. This depth covered beyond the depth of water table in the area. Examining the model (Fig 9), from x = 0.0 m to x =27.5 m from x = 32.5 m to 92.5 m, we find a trend of decreasing near surface resistivity at depth of 0.0 m down to 6.0 m and at depth of 0.0 m down to 15.0 m, respectively substantial The decrease in resistivity(19.7 - 7.36 ohm-m) obtained from the 2-D data at these depths is believed to be due to groundwater contamination as a result of accumulation of leachate, a conclusion supported by the shallow water table in the location as revealed by hand dug well TW1 which measured 2.0 m as the depth to water table along this profile. This conclusion is also supported by the water analysis of the hand dug well which showed elevations in concentration of organic/inorganic parameters exceeding the permissible health limits. The granite outcrop observed in the location is also evident in the inverted model which reflects the bedrock at depths ranging from 0.0 m to 15.0 m at the 27.5 - 32.5 m mark. The bedrock with varying moisture content and degree of weathering was also encountered at about

6.5 m depth down to about 12.5 m between the 15.0 m mark and 27.5 m mark. The subsurface topography is controlled by the bedrock dipping in the E-W direction.



Fig. 9: Inverted model of Profile line T01

Case Study V: Abubakar Imam Primary School **Dumpsite Profile IPT04**

The model (Fig 10) shows low resistivity values ranging from 13.4 - 34.4 ohm-m from the surface layer up to a maximum depth of 6 m recorded between the first electrode (x = 0.00 m) up to the 25 m mark. This low resistivity is in the directions of hydraulic gradient where about eight electrodes were located on the dump and therefore is attributed to leachate bubbles within the refuse dump. Like in profile T04 the inversion also reflects the bedrock at 5.0m depth while the bedrock with varying moisture content and degree of weathering dominates the profile beginning from depth of 5m down to 15m. The high resistivity observed at a depth of 5m in this profile is further evidence that the bedrock is a shallow fresh basement of granite, (Plate 1). Another important feature of interest in this profile is the vertical contact between the weathered basement and the fresh basement as can be seen in the profile between 42.0m and 52.5m. This suggests the presence of fracture of possible width of 10.5m filled with material of higher porosity and permeability which could serve as a major pathway of contaminant plume into the groundwater. The high rms error is probably due to the sharp contrast in resistivity between the highly contaminated thin topsoil layers overlaying the shallow high resistivity granite For the bedrock elevation, the 2D bedrock. resistivity data for each electrical resistivity imaging line were generated into 3-dimensional representation of the subsurface topography using SURFER 8 package. Fig 11 shows one of the results obtained in the Old cemetery dumpsite.

In the study of groundwater contamination, Ebraheem et al., 1990 found that the resistivity of leachate plume is a function of the soil resistivity. The geophysical and hydrochemical data were therefore proved as follows. The specific conductivity measurements of well water samples were converted into water resistivity ($\rho_w = \frac{1}{\sigma_w}$), while soil resistivity were estimated from inverted tomography models nearest to the well at the depth corresponding to the water table depth (Ebraheem et al., 1996), to ensure that the observed values are close representative of the saturated zone. The resulting data are given in table 1. Finally, in order to obtain a relationship between water quality and soil resistivity, a plot of the water resistivity as a function of soil resistivity was done. The fitted line between soil and water resistivity (Fig

12) indicates the following empirical relationship: $\rho_w = 0.28125\rho_s + 0.98$ ------(5) where, is the water resistivity in -m and is the

soil resistivity in -m.



Fig 10: Inverted model of Profile line IPT04



Plate 1: Coarse-grained granite exposed within Abubakar Imam Primary LEA primary school dumpsite, Tudun Wada, Zaria. (Photo by Jegede, S. I.)



Fig 11: 3D mesh diagram showing the apparent Resistivity values of ERT profile H03 Depression and undulations in dark shading are good prospects of groundwater accumulations.

Nearest profile to	Name of Well	Depth of Water	EC (µS/cm)	Soil resistivity s	Water
Well		Level (m)		$(\Omega_{\rm m})$	resistivity
				()	$_{\rm w}(\Omega_{\rm m})$
H03	HW1	4.30	1340	23.70	7.46
P03	PW1	4.60	1660	16.50	6.02
P03	PW2	4.30	740	34.00	13.51
P04	PW3	4.70	1120	28.70	8.93
P05	PW4	4.60	1538	18.60	6.50
T01	TW2	2.00	1585	19.70	6.31
T02	TW1	4.50	2862	16.00	3.49
S02	SW1	5.00	1650	16.20	6.06
HO6	HBH1	25.00	238	130.00	42.02
H06	HBH2	27.00	284	150.00	35.12
P04	PBW1	27.00	280	153.00	35.71

Table 1: Geophysical and Hydrochemical data used to obtain the relationship between soil and water resistivity.



Figure 12: The relation between soil resistivity and water resistivity

This empirical relationship between soil resistivity and water resistivity reveals that the soil resistivity is strongly affected by groundwater salinity, and provides a reaffirmation of the basis for applying resistivity methods to study groundwater contamination. Figure 12 shows that the samples, which were collected from hand dug wells whose depths are within the overburden (regolith aquifer environment), are more polluted with resistivity values of soil and water samples 36.0 -m and 14.0 -m respectively than the samples which were collected at deeper levels corresponding to the borehole samples (fractured aquifer environment).

4. Conclusion

The 2-D resistivity imaging technique has been successfully used in this study to map the contamination plume and to characterize the dumpsites in terms of subsurface resistivity distribution of the waste material and soil underneath the vicinity of each dumpsite. The interpreted resistivity section which correlates well with the water chemistry result, suggests the potentiality of 2D resistivity imaging technique as a precharacterization tool for mapping subsurface contamination in the vicinity of waste disposal sites. However the complexity of subsurface conditions beneath contaminated lands requires а multidisciplinary approach combining the systematic and careful application of hydrogeological, chemical

and environmental geophysical techniques. The bedrock topography showed several basements i.e. "depressions" and "ridges", where the depression basement could be favourable zones for groundwater development. There are such depression zones in and around the dumpsites, which act as good groundwater potential zones, but they are extremely polluted especially in the regolith aquifer environment.

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Synthesis, Characterization and Biological Studies on some Derivatives of *N*-(4-Aminobenzenesulphonyl)Morpholine Carrying Amino Acid, Alkoxy and Triazole Moieties

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Abstract: The reaction of N-[4-(chloroacetyl)aminobenzenesulphonyl]morpholine (IV) in acetone or dimethylformamide with amine derivatives, 4-aminotriazoles (I,II) or sulpha drugs yielded the corresponding N-[4-(substituted glycyl)aminobenzenesulphonyl]-morpholine derivatives (V-XV). Moreover, some derivatives of N-[4-(alkoxyacetyl)aminobenzenesulphonyl]morpholine (XVI-XXI) were synthesized. The reaction of XI with the requisite aromatic aldehydes in methanol gave Schiff bases (XXII,XXIII). Coupling reaction between (III) and Phtor Tos-amino acids using the phosphorus oxychloride method furnished the corresponding N-[4-(pht- or Tosaminoacyl)aminobenzenesulphonyl]morpholines (XXIV-XXIX). All the synthesized compounds were characterized by IR, ¹H-NMR, MS spectral data and elemental analyses and investigated their antibacterial and antifungal activities.

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Keywords: Synthesis; Derivative; N-(4-Aminobenzenesulphonyl)Morpholine; Amino Acid; Alkoxy; Triazole Moieties

1. Introduction:

The present work is a report on the chemistry and preliminary microbiological study of some novel amino acid, Schiff base and alkoxy derivatives of sulfamorpholine. Our target embraced the identification of these compounds that could be important as pharmacologically active agents. The sulfonamides, and their derivatives incorporating morpholine moiety⁽¹⁻³⁾ and glycine unit⁽⁴⁻⁷⁾ have been known to display miscellaneous biological and medicinal activities. The Schiff bases have also been found to possess strong and broad spectrum pharmacological properties⁽⁸⁻¹⁰⁾.In particular, triazoles and their heterocyclic derivatives have been reported to be used as drugs and to have considerable biological activities⁽¹¹⁻¹³⁾. Keeping in view the biological importance of amino acids, and in continuation of our lab work on structure-activity relationship (SAR)⁽¹⁴⁻¹⁸⁾, the present paper has been conducted in which the previously mentioned derivatives (I-XXX) have been synthesized and evaluated their antimicrobial activity.

2. Experimental:

Melting points were uncorrected and meassured on electric melting point apparatus SMP1. Thin layer chromatography was run on plastic sheets coated with silica gel-60 (Merck) and developed with *n*-butanol- acetic acid- water (4:1:1, v/v) and detected under UV light. The infrared spectra ($_{max}$ in cm⁻¹) were

taken in KBr discs using FTIR-2000 instrument.¹H-NMR spectra were measured in DMSO-d₆ or CDCl₃ using FX90Q Fourier Transform NMR spectromrter. The mass spectra were performed using Shimadzu-GC-MS-QP 100 Ex by the direct inlet system. Elemental analysis were carried out at Microanalytical Uint, Faculty of Science, Cairo University, Cairo, Egypt. The biological activities were measured in Department of Potany, Faculty of Science, Al-Azhar University, Cairo, Egypt.

1) Synthesis of *N*-(4-amino-5-mercapto-4*H*-[1,2,4]-3-triazolylalkyl)-*p*-toluenesulfonamide derivatives (I,II)

A mixture of equimolar amounts (0.01 mol) of thiocarbohydrazide and tosylamino acid were placed in a Pyrex test tube and heated to 170-179 C in an oil bath for 30 min. During the first ten minutes the mixture was stirred. At the end of the reaction, the test tube was carefully removed and cooled until the liquid solidified. The solid material was recrystallized from ethyl alcohol. The materials were chroma-tographically homogenous when developed under UV light. I, IR(KBr):3389-3250 (broad, NH, NH₂), 3099 (CH, aro.), 2984 (CH, ali.),2298(SH), 1591(C=N), 1341, 1147(SO₂). MS m/e: 299 (M⁺, 24.46%), 284 (1.02%), 235 (8.04%), 184 (6.27%), 155 (20.03%), 128 (11.75%), 106 (13.23%), 91 (100%), 65 (33.81%). ¹H-NMR: 2.37 (s, 3H, CH₃), 2.48 (s, 2H, CH₂), 7.35-7.68 (m, 4H, Ar-H), 8.17 (hump, 2H, NH₂), 11.2 (s, 1H, SH,

canceled by D_2O). II, IR: 3321-3263(broad, NH, NH₂), 3074(CH and C=C,aro.), 2949,2874 (CH, ali.), 2308(SH), and 1592(C=N).

2) *N*-(4-aminobenzenesulphonyl)morpholine (III) was prepared using the procedure described earlier⁽²¹⁾ IR: 3436, 3366 (NH₂), 3084, 3006 (CH, aro.), 2970, 2861(CH, ali.), 1592, 1540 (C=C, aro.), 1314, 1154 (SO₂), 828 (*p*-disubstituted benzene).

3) Synthesis of N-[4-(chloroacetyl)aminobenzenesulphonyl]morpholine (IV)

A suspension of N-(4-aminobenzenesulphonyl)morpholine (III, 0.01 mol) in 30 ml of CH₂Cl₂ was treated with chloroacetyl chloride (0.011mol) gradually with stirring. The reaction mixture was refluxed for 1 h on a water bath and the solvent was then evaporated to dryness under reduced pressure. The crude product was washed several times with CH₂Cl₂ and finally recrystallized from acetone. IR: 3294(NH), 3096, 3052(CH, aro.), 2968, 2922, 2891 (CH, ali.), 1692 (C=O), 1591 (C=C aro.), 1344, 1163 (SO₂), 724(C-Cl).

4) Synthesis of *N*-[4-(substituted glycyl)aminobenzenesulphonyl]morpholine derivatives (V-X)

To a solution of N-[4-(chloroacetyl)aminobenzenesulphonyl]morpholine (IV; 0.001 mol) in acetone, excess of ammonia solution or the proper amines (0.0011 mol) was added and the resulting reaction mixture was stirred for 1 hr at r.t., refluxed for 2 h, then left to stand for overnight. The reaction solution was evaporated under reduced pressure and the residual product was washed with cold water, dried, and then recrystallized from the proper solvent. V, IR: 3366(NH₂) 3308 (NH), 3061, 3006 (CH, aro.), 2981, 2920(CH, ali.), 1694 (C=O), 1592, 1540 (C=C, aro.), 1328, 1159 (SO₂). VI, IR: 3288(NH), 3095, 3036(CH, aro.), 2971, 2860 (CH, ali.), 1705 (C=O), 1597 (C=C.aro.), 1346.1162 (SO₂). VII. MS m/e: 327 (M⁺. 241(13.43%), 2.13%). $86(C_4H_8NO,$ 63.04%). 56(C₃H₆N,100%). IX, ¹HNMR: 3.01(t, 4H, CH₂-N-CH₂), 3.74 (t, 4H, CH₂-O-CH₂), 4.01 (s, 2H, COCH₂), 7.73-7.85 (m, 4H, Ar-H) and 9.34 (s, 1H, NH). X, IR: 3336,3268(OH and NH) ,3061, 3009 (CH, aro.), 2957(CH, ali.), 1722 (C=O, COOH), 1656(C=O), 1592, 1540 (C=C, aro.), 1325 (SO₂).¹HNMR: 2.94 (t, 4H, CH₂-N-CH₂), 3.71(t, 4H, CH₂-O-CH₂), 4.30 (s, 2H, COCH₂), 7.29-7.85 (m, 8H, Ar-H) and 11.72 (s, 1H, COOH).

5) Synthesis of *N*-[4-(aminoglycyl)aminobenzenesulphonyl]morpholine (XI)

A mixture of (IV;0.001 mol) dissolved in acetone and hydrazine hydrate 85% (0.003 mol) was stirred at r.t.°C for 3 h, then left to stand for overnight. The product was filtered off, washed with cold ethanol

and then recrystallized from ethanol. MS m/e: 314 (M^{+} , 4.56%), 241 (23.4%, loss of COCH₂N₂H₃), 155 (*p*-NHC₆H₄SO₂, 4.24%), 86 (C₄H₈NO, 63.04%), 56 (C₃H₆N, 100%).

6) Synthesis of triazole, sulfamorpholine or sulfadiazine derivatives of *N*-[4-(glycyl)amino-benzenesulphonyl]morpholine (XII-XV)

A mixture of equimolar amounts (0.001mol) of triazole compounds (I or II), sulfa morpholine (III) sulphadiazine and N-[4-(chloroacetyl)aminoor benzenesulphonyl]-morpholine (IV) in 20 ml DMF containing (0.0012 mol) of triethylamine was refluxed for 2 hr. The reaction solution was evaporated under reduced pressure and the residual crude product was washed with cold water, dried and recrystallized from the proper solvent. XII, IR: 3268 (NH), 3065, 3036 (CH, aro.), 2918, 2850 (CH, ali.), 1644 (C=O), 1597 (C=C, aro.), 1316, 1159 (SO₂), 827 (p-disubsted benzene).¹HNMR: 2.93 (t,4H,CH₂-N-CH₂), 2.63 (s,2H,CH₂-triazole), 4.4 (s,2H,COCH₂), 8.52, 8.95 (s,2H,NHCO and NHSO₂), 11.12(s, 1H, SH, canceled by D₂O). XV, IR: 3275 (NH), 3099, 3041 (CH, aro.), 2969, 2846 (CH, ali.), 1694 (C=O), 1589, 1527 (C=C, aro.), 1347, 1162 (SO₂),

7) Synthesis of *N*-[4-(alkoxyacetyl)aminobenzenesulphonyl]morpholines (XVI-XXI)

A solution of N-[4-(chloroacetyl)aminobenzenesulphonyl]morpholine (IV; 0.001mol) in 30 ml of dioxane was added portionwisely to a mixture of an alcohol or phenol (0.001 mol) and sodium hydroxide (0.0011mol). The resulting reaction mixture was gently heated on a water-bath for 3-4 h. After cooling, the solution was diluted with water (10 ml) and left to stand for overnight. The crude product was filtered, washed with cold water, dried, and then purified by crystallization from the proper solvent. XVI, ¹HNMR: 2.81(t, 4H, CH₂-N-CH₂), 3.57 (t, 4H, CH₂-O-CH₂), 3.76 (s, 3H, OCH₃), 4.21 (s, 2H, COCH₂). XVII, IR: 3287 (NH), 3089 (CH, aro.), 2978(CH, ali.), 1694 (C=O), 1587 (C=C, aro.), 1257, 1065 (C-O-C), 1347, 1162 (SO₂). XVIII, MS m/e: 241(M-101, 27.7%, loss of C₅H₉O₂), 155(25.2%), 86(100%), 56(98.1%). XIX, IR: 3270(NH), 3034 (CH, aro.), 2980, 2843 (CH, ali.), 1641 (C=O), 1596 (C=C, aro.), 1254, 1105 (C-O-C), 1313, 1153 (SO₂). XX, IR: 3071 (CH, aro.), 2967(CH, ali.), 1701 (C=O), 1230 (C-O-C), 1159 (SO₂). MS m/e: 376 (M^+ , 1.32%), 299(M-77(C_6H_5), 17.23%). 226(1.03%), 155(1.56%), 86(100%), 56(76.9%).

8) Synthesis of *N*-[4-(substituted benzalaminoglycyl)aminobenzenesulphonyl]-morpholine (XXII,XXIII).

Benzaldehyde or *p*-anisaldehyde (0.005 mol) was refluxed with *N*-[4-(aminoglycyl)aminobenzene-sulphonyl]morpholine (XI,0.005 mol) in methanol for 3

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h. The solid obtained after cooling, was separated, dried and then recrystallized from ethanol. XXII, IR: 3315 (NH), 3069, 3005 (CH, aro.), 2836 (CH, ali.), 1686 (C=O), 1650 (C=N), 1601 (C=C aro.), 1326(SO₂). XXIII, IR: 3261 (NH), 3068 (CH, aro.), 2920,2857 (CH, ali.), 1697 (C=O), 1649(C=N), 1590 (C=C, aro.), 1345, 1161 (SO₂).¹HNMR: 2.98 (t, 4H, CH₂-N-CH₂), 3.80 (t, 4H, CH₂-O-CH₂), 3.91 (s,3H , OCH₃), 4.22 (s,2H,COCH₂), 7.02(s,1H,CH=N),7.45-7.90(m,8H,Ar-H).

9) Synthesis of *N*-[4-(Pht- or Tos-aminoacyl)aminobenzenesulphonyl]morpholine derivatives (XXIV-XXIX).

A mixture of Pht- or Tos-amino acid N-[4-aminobenzenesulphonyl]-(0.001mol), and morpholine (III, 0.001 mol) was suspended in 20 ml of anhydrous THF and cooled to -15 C with stirring for 15 minutes. The mixture was then treated with (0.001 mol) of purified phosphorus oxychloride and directly with (0.002 mol) of triethylamine. After the reaction mixture has stood for 1 h at -15 C; 20 ml of water was added and the mixture was evaporated in vacuo in order to remove THF. The residual material was treated with 20 ml of water and extracted two times with 20 ml portions of ethyl acetate. The combined ethyl acetate extracts were washed three times with 5 ml portions of water, several portions of 5 % sodium bicarbonate solution, and finally with water. After being dried with anhydrous sodium sulfate, the ethyl acetate fraction was evaporated in vacuo. The residual crude product was recrystallized from the proper solvent. XXIV,IR: 3301(NH), 3069 (CH, aro.),2974,2858(CH, ali.), 1756, 1702(C=O, phthalyl and amide),1598(C=C, aro.), 1158 (SO₂). ¹HNMR: 2.96 (t, 4H, CH₂-N-CH₂), 3.74 (t, 4H, CH₂-O-CH₂), 4.64 (s, 2H, COCH₂), 7.27-7.92 (m, 8H, Ar-H), 8.21 (s, 1H, NHCO). XXV, IR: 3269 (NH), 3004 (CH, aro.), 2981, 2850 (CH, ali.), 1778, 1716 (C=O, phthalyl and

amide), 1595 (C=C, aromatic). MS m/e: 443 (M⁺, 7.82%), $174(C_{10}H_8NO_2,$ 100%),159(0.98%), 46(2.64%),86(21.74%),56(47.21%). XXVI, IR: 3249(NH), 3013(CH, aro.), 2923, 2872 (CH, ali.), 1776, 1719 (C=O, phthalyl and amide), 1593 (C=C, aro.), 843 (p-disubstituted benzene). MS m/e: 469 (M-2, 10.06%), 202(5.11%), 173(19.6%), 155 (12.8%) , 146(3.67%), 86(100%), 56(61.67%). XXVII, IR: 3316(NH), 3069, 3008 (CH, aro.), 2943, 2835 (CH, ali.), 1686 (C=O), 1590,1517 (C=C, aro.), 1345, 1161 (SO₂). ¹HNMR: 2.35(s,3H, *p*-CH₃), 4.42(s,2H,COCH₂), 7.45-8.0 (m,8H,Ar-H), 8.29,9.08 (s,2H,NHCO and NHSO₂). XXIX, IR: 3307(NH), 3023 (CH, aro.), 2989, 2923 (CH, ali.), 1709, 1633 (amide I and II), 1597 (C=C, aro.), 1367(SO₂), 822 (p-disubstituted benzene). ¹HNMR: 2.41(s, 3H, *p*-CH₃), 3.02 (t, 4H, CH₂-N-CH₂), 3.16(h, 1H, isopropyl-CH), 3.80 (t, 4H, CH₂-O-CH₂), 5.01 (d, 1H, COCH), 7.27-7.62 (m, 8H, Ar-H).

10) Synthesis of *N*-[4-(succinimido) benzene-sulphonyl]-morpholine (XXX).

A mixture of (III, 0.001 mol), succinic anhydride (0.0011 mol) and glacial acetic acid (30 ml) was refluxed for 8 h at 120 C. The reaction mixture was cooled and the formed solid product was collected, washed with water and recrystallized from dil.acetic acid. IR: 3297(NH), 3032 (CH, aro.), 2972, 2865 (CH, ali.), 1762, 1711 (C=O), 1593 (C=C, aro.).

3. Results and Discussion:

Thus, *N*-(4-Amino-5-mercapto-4*H*-[1,2,4]triazol-3-yl) *p*-toluenesulfonamide derivatives (I and II) were formed by fusion of thiocarbohydrazide and tosylamino acids at 170-179 0 C⁽¹¹⁾. The products were recrystallized from ethyl alcohol. The structure was confirmed by IR spectra which revealed not only the absence of C=O band but also the presence of both strong SH and C=N stretching bands at about 2298 and 1591cm⁻¹ respectively (Scheme 1).

 Table 1: The physical data of the synthesized derivatives (I-XXX).

Compd. No.	R	$\mathbf{R}_{\mathbf{f}}$	Yield %	Cryst. Solv.*	M. P. [°C]	Mol. formula	Elemental analysis** N%
Ι	Н	0.80	75	а	193	$C_{10}H_{13}N_5O_2S_2$	23.41 23.39
Π	$-CH_2C_6H_5$	0.66	73	а	198	$C_{17}H_{19}N_5O_2S_2$	17.99 17.92
III		0.79	91	b	193-195	$C_{10}H_{14}N_2O_3S$	11.57 11.67
IV		0.74	79	b	140-143	$C_{12}H_{15}ClN_2O_4S$	8.79 8.58
V	-H	0.83	93	a	130-133	$C_{12}H_{17}N_{3}O_{4}S$	14.04 13.87

VI	-CH ₃	0.85	79	с	95-97	$C_{13}H_{19}N_3O_4S$	13.42 13.40
VII	-(CH ₃) ₂	0.69	80	с	98-101	$C_{14}H_{21}N_3O_4S$	12.84 12.82
VIII	1-piperidinyl	0.68	73	b	220-222	C ₁₇ H ₂₅ N ₃ O ₄ S	11.44
IX	4-morpholinyl	0.72	79	b	190-192	C ₁₆ H ₂₃ N ₃ O ₅ S	11.38
X	<i>p</i> -C ₆ H ₄ CO ₂ H	0.80	70	с	230-233	C ₁₉ H ₂₁ N ₃ O ₆ S	10.02
XI	-NH ₂	0.78	82	a	110-115	$C_{12}H_{18}N_4O_4S$	17.83 17.81
XII	-H	0.73	80	d	210-213	$C_{22}H_{27}N_7O_6S_3$	16.86 16.95
XIII	-CH ₂ C ₆ H ₅	0.76	82	d	249-251	$C_{29}H_{33}N_7O_6S_3$	14.60 14.56
XIV	4-morpholinyl	0.79	77	a	188-191	$C_{23}H_{28}N_3O_7S_2$	8.01 8.13
XV	2-pyrimidinyl	0.66	70	d	269-271	$C_{22}H_{24}N_6O_6S_2$	15.79 15.98
XVI	-CH ₃	0.86	88	с	145-148	$C_{13}H_{18}N_2O_5S$	8.91 8.89
XVII	-CH ₂ CH ₃	0.84	83	с	149-151	$C_{14}H_{20}N_2O_5S$	8.53 8.74
XVIII	-CH ₂ CH ₂ CH ₃	0.89	79	с	211-213	$C_{15}H_{22}N_2O_5S$	8.18 8.16
XIX	-CH(CH ₃) ₂	0.87	77	с	159-160	$C_{15}H_{22}N_2O_5S$	8.18 8.05
XX	C ₆ H ₅ -	0.81	81	а	220-222	$C_{18}H_{20}N_2O_5S$	7.44 7.41
XXI	o-ClC ₆ H ₄ -	0.83	74	а	179-182	C ₁₈ H ₁₉ ClN ₂ O ₅ S	6.82 6.79
XXII	-H	0.85	91	а	119-121	$C_{19}H_{22}N_4O_4S$	13.93 13.90
XXIII	-OCH ₃	0.82	79	а	208-201	$C_{20}H_{24}N_4O_5S$	12.96 12.91
XXIV	Pht-Gly	0.76	83	b	113-116	$C_{20}H_{19}N_3O_6S$	9.79 9.92
XXV	Pht-L-Ala	0.71	85	b	150-153	$C_{21}H_{21}N_3O_6S$	9.48 9.45
XXVI	Pht-L-Val	0.79	84	b	110-113	$C_{23}H_{25}N_3O_6S$	8.91 8.74
XXVII	Tos-Gly	0.83	69	a	184-186	$C_{19}H_{23}N_3O_6S_2$	9.27 9.46
XXVIII	Tos-L-Ala	0.74	71	с	111-113	$C_{20}H_{25}N_3O_6S_2$	8.99 9.08
XXIX	Tos-L-Val	0.83	78	а	149-151	$C_{22}H_{29}N_3O_6S_2$	8.48 8.46
XXX		0.77	91	с	213-216	$C_{14}H_{16}N_2O_5S$	8.64 8.80

*Crystallization solvent: (a) ethanol, (b) acetone, (c) acetic acid-water, and (d) dioxane-water. **All compounds gave satisfactory C and H analysis (calc./found)



Treatment of N-(4-aminobenzenesulphonyl)morpholine (III) with chloroacetyl chloride gave N-[4-(chloroacetyl)aminobenzenesulphonyl]morpholine (IV) that reacted, in acetone or DMF, with ammonia, hydrazine hydrate, substituted amines, 4-aminotriazoles (I,II) or sulpha drugs to afford the corresponding N-[4-(substituted glycyl)aminobenzenesulphonyl]-morpholine derivatives (V-XV). The products were isolated, purified and obtained in good yield. Moreover, preparation of N-[4-(alkoxyacetyl)aminobenzene-sulphonyl]-

morpholine derivatives (**XVI-XXI**) was achived by the action of sodium salt of the requisite alcohol or phenol on a solution of (**IV**) in dioxane. The products were precipitated on cooling, purified and obtained in 74-88% yield. The mechanism of formation of N-[4-(chloroacetyl)aminobenzene-

sulphonyl]-morpholine (IV) could be rationalized in terms of electrophilic attack on nucleophilic center, the acyl group of chloroacetyl chloride by the lone pair on N^4 of sulfamorpholine (III) accompained by loss of Cl^- and H^+ to afford (IV). Further, nucleophilic substitution reactions with amine or hydroxyl containing compounds produce the corresponding derivatives mentioned above. The IR spectra showed the disappearance of the absorption band noticed at 724 cm⁻¹ characteristic for C-Cl in compound (IV), and the appearance of vibrations at \sim 1257 and 1065 cm⁻¹ attributed to the strong (C-O-C) band in compounds (XVI-XXI). In addition, ¹H-NMR signals that noticed at ~ 3.76 ppm characteristic for alkoxy (OR) supports the proposed structure of these derivatives (Scheme 2).



Schiff bases, *N*-[4-(substituted benzalaminoglycyl)- aminobenzenesulphonyl]-morpholines derivatives (XXII,XXIII) were easily obtained by the condensation reaction of XI with one equivalent of benzaldehyde or *p*-anisaldehyde in abs. methanol. Complete condensation of all amino group was confirmed by the presence of strong C=N stretching band in IR at about 1649 cm⁻¹. This conclusion is also supported by the ¹H-NMR data which verified the presence of CH=N hydrogen resonance at about 7.02 ppm (Scheme 3).



Reagents: (vi) = ArCHO / CH₃OH

Some new derivatives of *N*-[4-(Pht- or Tosamino-acyl)aminobenzene-sulphonyl]morpholine (XXIV-XXIX) were prepared using the phosphorus oxy-chloride method⁽¹⁹⁾. The desired pure products were obtained upon treatment of a mixture of *N*-(4aminobenzenesulphonyl]morpholine (III), phthalyl-(Pht-) or tosyl-(Tos-)amino acids in anhydrous THF containing two molar equivalents of triethylamine at -15, with phosphorus oxychloride POCl₃. The phosphorus oxychloride method led to high yields and the products were isolated in a high degree of analytical purity prior to crystallization. The succinimido derivative (**XXX**) was synthesized by refluxing (III) with succinic anhydride in gl.AcOH for 8 h at 120 C (Scheme 4). The spectral data are found to be consistent with the formulations shown below.



Antimicrobial activities of the prepared compounds: Sensitivity of microorganisms to antimicrobial compounds:

For testing the antimicrobial activity of the prepared compounds, we used more than one test organisms as Gram positive bacteria: Bacillus subtilis (ATCC-6051), Staphylococcus aureus (ATCC-12600) and Gram negative bacteria: Escherichia coli (ATCC-11775) and Pseudomonas aeruginosa (ATCC-10415) and selscted fungi: Candida albicans, and Aspergillus niger to increase the range of antibiotic detection in the tested materials by using filter paper disc method⁽²⁰⁾. A filter paper discs must be of uniform thickness and size and containing an equal and graded amount of the agent to be tested for its antimicrobial activity. The method was performed by dissolving 5 mg of the sample in one ml. of solvent solution, N,N-dimethylformamide (DMF), then a sterile filter paper discs were dipped into this solution . After absorption, the discs were dried and placed on test organisms seeded plates to be tested for their antimicrobial activity. The inhibition zone was measured in millimeters at the end of incubation period. The activity of the compounds was compared

with the activity of N-(4-aminobenzenesulphonyl)morpholine (III) that showed a weak to moderate activity aganist B.subtilis S.aureus, P.aeruginosa and Candida albicans with inhibition zone (6, 6, 7, 12 mm respectively) and was biologically inactive against E.coli and Aspergillus niger. From the data recorded in Table 1, we could conclude that most of the synthesized derivatives (I-XXX) were found to be biologically inactive towards the test organisms except (XIII) which exhibited a moderate to high antimicrobial activity aganist B.subtilis S.aureus, E.coli, P.aeruginosa and Candida albicans with inhibition zone (12, 10, 12, 13, 13 mm respectively) and was completely inactive against Aspergillus niger. This study revealded that the incorporation of N-(4-aminobenzenesulphonyl)morpholine (III) with a triazole moiety may improve and verify the antimicrobial activity of the synthesized derivatives of such type. On the other hand, the incorporation of (III) with amines, alcohols, phenols, pht- and Tos-amino acids led to decrease or completely abolish the antimicrobial activity of the synthesized derivatives.

Compd.	(Gram –	positiv	e		Gram-negative			e Fungi			
No.	B. su	B. subtilis		ireus	E. coli P. aeruginosa		Can albi	dida cans	A. n	niger		
Ι	-	-	-	-	-	-	-	-	-	-	-	-
II	-	-	-	-	-	-	-	-	-	-	-	-
III	+	6	+	6	-	-	+	7	++	12	-	-
IV	-	-	-	-	-	+	-	-	++	10	-	-
IX	-	-	-	-	-	-	-	-	-	7	-	-
Х	+	6	+	6	+	6	+	7	+	7		-
XII	+	7	++	8	+	7	+	7	++	10		
XIII	++	12	++	10	+++	12	+++	13	+++	13	-	-
XXII	+	8	+	7	++	10	++	9	+++	15	-	-
XXIII	-	-	-	-	-	-	++	9	-	-	-	-
XXVI	-	-	-	-	-	-	-	-	+	7	-	-
XXIX	-	-	-	-	-	-	-	-	+	7	-	-

 Table 2: In-Vitro Antimicrobial Activities of Synthetic Compounds.

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12/1/2010

Identification of Cryptosporidium Species Infecting Camels (Camelus dromedarius) in Egypt.

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Abstract: *Cryptosporidium* species was investigated among 145 camels (5-8 years old) from Egypt. The prevalence of infection was 19.3%. The detected oocysts were ellipsoidal in shape with a mean length and width 7.5×5.6 um. Ten *Cryptosporidium* free mice were orally inoculated each with 350.000 oocysts (camel isolate). The prepatent period in mice was 2 days and the patent period could not be determined since they were still shedding oocysts until day 100 post- infection. The camel isolate of *Cryptosporidium* and the same isolate propagated in mice was non infective for lambs during an examination period of 3 months. Molecular characterization of the camel isolate indicated that the target gene (18SrRNA) gave positive result for *C. muris* at 435bp.

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Key words: Camels, Cryptosporidium, prevalence, morphology, PCR.

1. Introduction:

The genus *Cryptosporidium* includes a group of protozoan parasites that infect the gastrointestinal tract and other organs of mammals including human, birds, reptiles and fish (Xiao et al., 1999).

The emergency of *Cryptosporidium* as important cause of diarrheal illness and its increasing role in both localized and widespread outbreaks of disease initiated a public and animal health problems of global proportion for both developed and developing countries (Fayer and Xiao,2007).

Identification of *Cryptosporidium* species based on morphology and dimensions of oocysts is difficult since oocysts of many species lack unique features and are indistinguishable from each other. The life cycle of *Cryptosporidium* species and its variations can provide species specific information but this is also impractical. More recently gene sequence information has become the most widely applicable factor for defining *Cryptosporidium* species. Such genetic data were based primarily on slight differences in the sequence base pair within the gene referred to 18s or small subunit ribosomal (ssr) RNA. Accordingly, 16 species (Fayer and Xiao, 2007).

Previous studies on camel cryptosporidia were scanty. Molecular characterization of camel (*Camelus bactrianus*) Cryptosporidium species revealed C. muris in one study (Xiao et al., 1999) and C. andersoni in another one (Santin et al., 2007). Cryptosporidium species were reported from camels (Camelus dromedarius) in Egypt without identification of the species (El-Kelesh et al., 2009).

Therefore, this investigation was initiated to study the prevalence and molecular identification of *Cryptosporidium* species infecting camels (*Camelus dromedarius*) from Egypt.In addition to study the susceptibility of mice and lambs for infection with the isolated *Cryptosporidium* species.

2. Material and Methods

1-Collection of fecal samples:

Fecal samples were collected from 145 camels (camelus dromedarius) of various ages (5-8 years old) and sexes at El-Basatein (Cairo) and El- Warak (Giza) abattoirs (Egypt).Fecal samples were examined after preparation of smears stained with modified Zeihl Nelseen stain. Positive fecal samples were preserved in 2.5% potassium dichromate at 4°C until used for isolation of *Cryptosporidium* species.

2-Oocysts concentration and experimental inoculation of mice with *Cryptosporidium* species:

The oocysts were concentrated in order to be used for animal inoculation and molecular study. Ten, one week old *Cryptosporidium* free mice (*Mus musculus*) (10 gm weight) were purchased from private farm and each was inoculated orally with 350.000 oocysts (camel isolate). The faeces of the inoculated mice were collected and examined daily for the presence of *Cryptosporidium* oocysts for a period of 100 days post inoculation.

3-Inoculation of lambs with *Cryptosporidium* species oocysts(camel isolate):

Three, four months old *Cryptosporidium* free lambs were reared individually and conventionally in isolated pens during the period of the experiment (100 days) and fed sterilized and balanced ration. Two lambs were used for experimental inoculation with *Cryptosporidium* sp. while the 3rd lamb was used as non-infected control. One of the experimental lambs was inoculated orally with 10^6 oocyst (camel isolate) while the 2nd lamb received the same dose of *Cryptosporidium* species oocyst of camel isolate propagated in mice. Faeces of the experimentally inoculated and the non inoculated lambs were collected and examined daily for the presence of *Cryptosporidium* oocyst and for studying the prepatent and patent periods of infection.

4-Preparation of *Cryptosporidium* oocyst lysates as PCR templates:

For DNA extraction, Cryptosporidium species (camel isolate) was concentrated from faecal material of positive cases. They were washed four times by successive pelleting $(10.000 \times \text{g} \text{ for } 10 \text{ min at } 4^{\circ}\text{C})$ and resuspension in distilled water and finally suspended in 10 mM Tris (pH 8.3)-50 mM KCl. Purified oocysts were suspended at a density of 250 oocysts/µl in 100-µl aliquots of 10 mM Tris (pH 8.3)-50 mM KCl containing 0.5% (wt/vol) Tween 20. After freeze-thawing (15 cycles), samples were heated for 15 min at 100°C and then centrifuged for

2 min at $16.000 \times g$ to remove particulate matter. Supernatants were recovered and stored at -20° C until used for PCR amplification (Gobet et al., 1997).

5-PCR amplification and gel analysis of PCR products:

One-microliter volumes of the oocyst lysates were used as amplification templates in 50-µl reaction mixtures containing 75 mM Tris (pH 9); 20 mM (NH₄)₂SO₄; 0.01% (wt/vol) Tween 20; 0.2 mM each of dGTP, dATP, dCTP, and dTTP; 2 to 4 mM MgCl₂ (Table 1); 50 pM of each primer; and 1 to 2 U of Gold Star Tag DNA polymerase (Eurogentec). Reaction mixtures were overlaid with 50 µl of sterile mineral oil and subjected to denaturation, thermal cycling (Minicycler; MJ Research), and then a final elongation at 72°C. The conditions of denaturation, annealing, and elongation varied depending on the primers of the target genes (18 SrRNA gene and Hsp70 gene). PCR products were analyzed on horizontal agarose gels in TAE buffer (40 mM Tris acetate, 2 mM Na₂EDTA 2H₂O). Each amplification run included a negative control (PCR water).

 Table 1: Target genes and primers for detection of Cryptosporidium DNA

Cryptosporidium Spp.	Target genes	Primer pair	Fragment size (bp)
1-C. muris (Johnson et al., 1995).	18 SrRNA gene	5'-AAGCTCGTAGTTGGATTTCTG	435
2-C.parvum (Rochelle et al., 1997).	Hsp70 gene	5'-AAATGGTGAGCAATCCTCTG	361

bp: base pair

3. Results:

1-prevalence of the *Cryptosporidium* species in camels:

Examination of stained fecal smears from 145 camels revealed that 28 camels (19.3%) were positive for *Cryptosporidium* species The oocysts were ellipsoidal in shape 7.4-7.6 μ m (mean 7.5 μ m) in length and 5.5-5.6 μ m (mean 5.6 μ m) in width.

2-Results of experimental inoculation of mice with *Cryptosporidium* species (camel isolate):

Daily examination of stained fecal smears from experimentally inoculated 10 mice with *Cryptosporidium* species (camel isolate) revealed that the prepatent period was 2 DPI. The mice excreted *Cryptosporidium* oocysts daily until 100 days postinfection (termination of the experiment). The mean dimensions of 50 *Cryptosporidium* species oocysts excreted by mice were 6.3-7.5 $\mu m.(mean~6.9\mu m$) in length and 4.7-5.2 μm (mean 5 μm) in width.

3-Results of experimental inoculation of lambs with *Cryptosporidium* species (camel isolate)

Daily examination of stained fecal smears from lambs experimentally inoculated with *Cryptosporidium* species (camel isolate) and the same isolate propagated in mice and the control non inoculated lamb revealed negative finding during the examination period (100 days).

4-Results of PCR:

PCR gave fragment at 435 bp. using Primer pairs targeting the 18S rRNA.While the PCR showed negative result to *C. parvum* using primer pairs targeting Hsp70 gene (Fig.1).



Fig. (1): PCR products for *Cryptosporidium muris.* Lane1: negative control sample. Lane2: Camel isolate for *C. muris.* (435bp) Lane M: 100 bp marker.

4. Discussion:

Examinations of stained faecal smears from 145 camels revealed 19.3% infection rate with *Cryptosporidium* species El Kelesh et al. 2009 reported similar percent (17.5%) out of 80 camels were positive to *Cryptosporidium* species this similarity may be due to the examined camels are of the same locality, the environmental condition are nearly the same. While Alves et al., 2005 in Portugal found that, no positive cases were detected in examined camels. This difference due to the variation of localities and environmental condition.

In our study the camel isolate of *Cryptosporidium* species was infective to mice. Xiao, et al., 1999 reported that *C. muris* isolated from rodents, bactrian camel (*Camelus bactrianus*) and rock hyrax (*Procavia capensis*) were infectious to mice.

The dimensions of our camel isolate of *Cryptosporidium* were comparable with *C. muris* described by Upton and Current, 1985 (7.4x 5.6 um).

Also, Fayer and Xiao, 2007 reported that both *C. muris* and *C. parvum* were established based on differences in oocysts morphology and infection sites.

Daily examination of stained fecal smears from lambs experimentally inoculated with *Cryptosporidium* species revealed negative finding during the examination period due to the pathogencity of Cryptosporidium varries with species of parasite and species of infected host its age and immune status of the host.

In this study PCR gave the same-sized fragment of C. muris (435 bp) and primer pairs targeting the 18S rRNA gene gave positive results to C. muris. While the PCR showed negative results for C. parvum using primer pairs targeting Hsp70 gene. C. muris was detected from camel (Camelus bactrianus) and rock hyrax (Procavia capensis) at 448 bp and from cattle at 485 bp using nested PCR (RFLP) (Xiao et al., 1999). Also, Bornary-Llinares et al., 1999 detected that the C. felis specific fragment was 455 bp when compared with C. muris specific diagnostic band (431pb). Finally Champliaud et al., 1998 declared that the group which included C. muris and C. baileyi gave positive results with the two primer pairs targeting the 18S rRNA gene (Johnson, 1995, 435 bp.)

We could conclude from our morphological, biological and molecular study that the detected *Cryptosporidium* sp. oocysts from camels (*Camelus dromedarius*) were *C. muris*.

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12/2/2010

Mathematical Modelling for Radon Prediction and Ventilation Air Cleaning System Requirements in Underground Mines

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Abstract: As a part of a comprehensive study concerned with control workplace short-lived radon daughter concentration in underground uranium mines to safe levels, a computer program has been developed to calculate ventilation parameters e.g.: local pressures, flow rates and radon daughter concentration levels. The computer program (actually two parts, one for mine ventilation and other for radon daughter levels calculations) has been validated in an actual case study to calculate radon concentration levels, pressure and flow rates required to maintain the acceptable levels of radon concentrations in each point of the mine. The required fan static pressure and the approximate energy consumption were also estimated. The results of the calculations have been evaluated and compared with similar investigation. It was found that the calculated values are in good agreement with the corresponding values obtained using "REDES" standard ventilation modelling software. The developed computer model can be used as an available tool to help in the evaluation of ventilation systems proposed by mining authority, to assist the uranium mining industry in maintaining the health and safety of the workers underground while efficiently achieving economic production targets. It could be used also for regulatory inspection and radiation protection assessments of workers in the underground mining.. Also with using this model, it could be effectively design, asses and manage underground mine ventilation systems. Values of radon decay products concentration in units of working level, pressures drop and flow rates required to reach the acceptable radon concentration relative to the recommended levels, at different extraction points in the mine and fan static pressure could be estimated which are not available using others software.

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Keywords: Mathematical Modelling / Radiation Doses / Radon and Radon Daughters/Ventilation System /Underground Mines.

Introduction:

The major radiation hazard in a uranium mine originates from the short lived radon – progeny. concentrations of which mainly depend on that of the parent, radon-222, formed within the ore body. Radon enters mine atmosphere by diffusion through the rock surface. The rate of emanation is characterized by the ore grade and porosity of the rock^(1,2). Suction effect caused by lowering the atmospheric pressure is another additive factor ⁽³⁾. Although under ground water ⁽⁴⁾ and broken ore piles also contribute substantially to the radon content of a mine drift, the main supply comes from the continuous diffusion through the ore body. Ventilation plays the most effective role in reducing airborne radiation the levels underground. Knowledge of radon emanation rate is therefore essential for an efficient and economic design of a uranium mine ventilation system. Without proper air distribution and control, even the best contaminant reduction program is inadequate to maintain a healthful atmosphere.

The main objective of a Ventilation Air Cleaning system (VACs) is to provide the quality and quantity of the airflows throughout the mine and to ensure safe and health conditions for the workers to the level specified by the mining regulations. In uranium mines the planning and operation of the ventilation systems have a large impact on production due to the fact that the first engineered step to control radiation contamination is with ventilation. The techniques used to maintain good air quality in uranium mines are based on providing very large volumes of air, maintaining the residence time of the air at a minimum in all working areas, guaranteeing zero recirculation of the air and designing a highly flexible ventilation system.

Good ventilation not only safeguards employee health but also has direct bearing on mine operating cost. The effects of inadequate ventilation are easily masked, and poor ventilation is often the unrecognized cause of high accident frequency, worker dissatisfaction, and production losses. A well planned adequate air distribution system is usually less expensive to install and maintain than a slipshod or makeshift network developed through expediency. The basic criteria for judging the performance of the ventilation system in uranium mines is the level of airborne alpha activity in the working environment. Air volumes and distribution systems suitable for controlling the level of radon daughters usually are adequate for dilution of other air contaminants associated with mine operations ⁽⁵⁾.

Since radon ²²²(Rn) is of major concern for occupational health in uranium mines, and the concentration has to be maintained within allowable limits. It is necessary to ensure safe working conditions and occupational health in advance by predicting the expected radon concentrations throughout the underground network and to provide evidence of this in licensing procedure. This can be achieved by numerical modelling of the ventilation network as well as the contaminant transport. Many investigations have intended to present an assessment of radon and radon decay product exposure and to estimate the annual exposure to the workers in the underground mines ⁽⁶⁻¹⁶⁾. Also some studies have reported the effectiveness of increased air volumes in diluting radioactive air contaminants (17 and 18). However in many cases simple air volume increase does not provide a practical solution to persistent high radiation levels, and revised air distribution system must be developed. To improve existing underground ventilation or to plan the ventilation system of a projected mine, a practical computer program (actually two programs, a mine ventilation program and a radon daughter level program) has been written according to the principles outlined in the next sections.

A case study is presented where the values of radon decay products concentration in units of working level for two phosphate mines are calculated and compared with the measured values obtained by others ⁽¹⁹⁾. Also the pressure drop, the air flow rate of the mechanical ventilation required to reach the acceptable radon concentration relative to the recommended levels, fan static pressure and the approximate electrical power consumption for the two underground phosphate mines are investigated and compared with the corresponding calculated values obtained using REDES software^(20 and 21). The formula and computational method used in the mine ventilation and radon computer program are detailed in the following section.

Computational Analysis 1 Mine Ventilation Analysis 1.1 Ventilation networks

A network is a mathematical representation of a real mine ventilation system. Mines consist of

many airways interconnected in such a way that it is possible to reduce them to simple series and parallel circuits. The network of airways can, with the use of computers be analyzed to predict airflow quantities and pressures. Fig. (1) represents three possible stages for development of a mine. At first a simple series circuit, expanding when a new area is opened up creating a parallel circuit and finally the connection of the two working areas resulting in a network of airways. The airways in the system are called branches. The branches are interconnected at points called nodes. Each branch is represented by an initial node and a final node. Nodes represent the following:

i- Connections between ducts of the network.

ii- Section change in a duct. iii- Pressure data or pressure unknown.

iv- Height of any point of the network.

v- Source or sink air in any point of the network.

Airflow direction is considered positive if it, goes from initial to final node and vice versa . The flow resistance of each branch depends on its length , section geometry, rugosity, bends, and singularities (elbows, filters, valves, entrance or exit section changes, etc.).

The following data are needed to calculate the branch resistance:

i. Branch length. ii. Branch hydraulic diameter.

iii. Sum of singularities, written as a sum of "equivalent length / hydraulic diameter" of each singularity of the element.

iv. Sum of singularities, written as a sum of dimensionless pressure lost constants of each singularity of the element. V. Inside wall rugosity of the duct.

A ventilation network is very similar to an electrical diagram in which the wires are the branches (underground openings), nodes are the intersections and the calculations that follow are based on Kirchhoff's Laws ⁽²²⁾. The first Kirchhoff's Law is related to the mass flow and states that the mass flow that meets in a junction (node) is zero. The mathematical form of this law is:

$$\sum_{i=1}^{n} M = 0 \tag{1}$$

where: i, number of branches and M mass of air per unit time that is moved in a branch i, (Kg/s). If we substitute M = Q, the above formula becomes:

$$\sum_{i=1}^{n} Q_i \ \rho = 0 \tag{2}$$

where:

 Q_i is the airflow in branch i, (m³/s),

, is the air density in branch i, (Kg/m^3) and

i is the number of branches in the network



Series circuit ABCDEFGH



Compound or network system



Parallel circuits CDEF and CXYFwith common junction C and F

The construction of airway DY presents a different problem consisting of branches, junctions and meshes. Branches (connect junctions) and are ABC, CD, DEF, DY, CXY, YF and FGH (Total 6). A branch is any series of airways and may have different dimensions and must be included at least once in the group of meshes. Junction (two or more branch) C, D, Y, and F (total 4). Meshes (and closed circuit) CXYDC, DYFED, CXYFEDC and ABCXYGHA (total 4).

Fig. (1): Three possible stages for development a mine (series, parallel and network of airways)

1.2 Ventilation circuits

If the variation in density along each branch is very small, the density effect can be neglected, therefore.

$$\sum_{i=1}^{n} Q_i = 0 \tag{3}$$

The second Kirchhoff's Law states that the a lgebraic sum of the pressure drops must be zero. In

order to apply this law the mesh must be closed so the fans and the pressure differential will move the air. This law can be written as:

$$u^2/2 + z g + w = V dP + F$$
 (4)
where:

u is the air velocity, (m/s),

z is the elevation above the reference, (m),

w is the work input from fan, (J/Kg),

V is the specific volume, (m^3/Kg) ,

- *P* is the barometric pressure, (Pa) and
- F is the work done against friction, (J/Kg)

The terms z and $u^2/2$ will be approaching zero as the mesh is closed. The term - V x dP is the natural ventilating energy per unit mass.

With these two changes, the new form for this law becomes:

$$\sum_{i=1}^{n} (P_i - P_{fi}) - NVP_i = 0$$

(5) where:

 P_i is the frictional pressure drop for each branch, (Pa),

 P_{fi} is the the variation in the total pressure across the fan, (Pa) and

NVP is the natural ventilation pressure, (Pa)

1.3 Simple ventilation networks *Equivalent resistances*

The equivalent resistance calculation for simple circuits is also similar to electrical circuits and applies to branches connected in series, parallel or combination (auxiliary ventilation).

Series Circuit

The schematic of a series circuit is presented in Figure 6 $^{(23)}$. The airflow on each branch is the same while the frictional pressure varies.

$$Q = Q_1 = Q_2 = \dots = Q_b \tag{6}$$



Fig. (2) : Series circuit

The general form for frictional pressure drop on each branch is $^{(23)}$:

$$P_1 = R_1 Q_1^n \quad P_2 = R_2 Q_2^n \quad P_b = R_b Q_b^n$$
 (7)

In underground mines the flow is turbulent and therefore n = 2. The above formulas can be written for turbulent flows and their mathematical form will be:

$$P_1 = R_1 Q_1^2$$
 $P_2 = R_2 Q_2^2$ $P_b = R_b Q_b^2$ (8)
The total frictional pressure P is the algebraic
summation of frictional pressures corresponding to

each branch. $P = \sum_{i=1}^{b} P_{i}$ (9)

The equivalent resistance of a series circuit Rs, N $s^2\!/m^8$ is given by,

$$R_s = \sum_{i=1}^b P_i \tag{10}$$

where:

 $P_1, P_2, ..., P_b$ is the frictional pressure drop along each branch, (Pa),

 $R_1, R_2, ..., R_b$ is the resistance on individual branch, $(N s^2/m^8)$,

Q is the airflow volume through the branches, $((m^{3}/s),$

 R_s is the resistance for series circuit, (N x s²/m⁸) and b is the number of branches in the circuit N is the force in Newten

Parallel Circuit

The schematic of a parallel circuit is presented in the Figure 7 $^{(23)}$. The pressure drop is the same for each branch.

$$P = P_1 = P_2 = \dots = P_b$$
(11)

$$Q = Q_1 + Q_2 + \dots + Q_b$$
 (12)



Fig. (3) : Parallel circuit

The general form for frictional pressure drop on each branch is:

$$P_{1} = R_{1} x Q_{1}^{n} P_{2} = R_{2} x Q_{2}^{n} P_{b} = R_{b} x Q_{b}^{n}$$
(13)

$$Q_{1} = (P_{1}/R_{1})^{l/n} \quad Q_{2} = (P_{2}/R_{2})^{l/n} \quad Q_{b} = (P_{b}/R_{b})^{l/n} \quad (14)$$

In underground mines the flow is turbulent and therefore n = 2. The above formulas can be written for turbulent flows and their mathematical forms will be:

$$Q_1 = (P_1/R_1)^{1/2}$$
 $Q_2 = (P_2/R_2)^{1/2} \dots Q_b = (P_b/R_b)^{1/2}$ (15)

The summation of airflows from all b branches forms the total airflow in the circuit Q, m³/s.

$$Q = \sum_{i=1}^{b} Q_i \tag{16}$$

The equivalent resistance of a parallel circuit is given by:

$$1/R_p = \sum_{i=1}^{b} 1/R_i$$
 (17)

where:

 $Q_1, Q_2, ..., Q_b$ is the volume of air in each branch, $(m^3/s))$,

 $R_1, R_2, ..., R_b$ is the resistance on individual branch, $(N s^2/m^8)$,

Q _b is the air flow volume per unit time through individual branches, (m^3/s) ,

 R_p is the resistance for parallel circuit, $(N \cdot s^2/m^8)$ and b is the number of branches in the circuit

1.4 Analytical Solution

The analytical solution for simple ventilation networks is based on Kirchhoff's Laws and considers that the minimum number of meshes m needed to be selected for calculation is: m = b - j + 1, where: m is the number of meshes, b is the number of branches and j is the number of nodes. With the m number of meshes selected there will be , j - 1

junction equations (Kirchhoff's First Law) and b - j + 1 mesh equations (Kirchhoff's Second Law). The final results are coming from a system of "b" equations formed for a circuit that has "b" branches. The limitation of this method is the complexity level of calculations when it is applied to a complex mine network.

1.5 Complex Ventilation Networks (Hardy - Cross Method) $^{\left(23\right) }$

When a flow Q (m^3/s) passes through an airway of resistance R (N • s^2/m^8), the frictional pressure drop developed is given by:

$$P = R \ Q^n \tag{18}$$

In the Hardy-Cross method of analysis, any value for the airflow Q and pressure drop P can be written as:

$$Q = Qa + Q \tag{19}$$

 $P = Pa + P \tag{20}$

where:

Qa is the initial estimation of the airflow, (m³/s),

Q is the error in the initial estimation, (m³/s),

Pa is the pressure drop corresponding to Qa, (Pa) and *P* is the error in pressure drop corresponding to Q, (Pa)

Fig. 4 ⁽²³⁾ shows a flow pressure relationship applied to the Hardy - Cross analysis of networks. This figure indicates that the slope for the curve is P/ Q and at the limit dP/dQ. If one differentiates the equation P = R x Q based on Q, we get the following relation :

 $dP / dQ = n \times R \times Q^{n-1}$ For the estimated value Qa:

$$P / Q = n x R x Q_a^{n-1}$$



Fig. (4) : Pressure vs airflow variation at small increments $^{(23)}$

From Fig. 4, $P = P - P_a$, and if we substitute the values:

$$P = R Q^{n} - R Q_{a}^{n}$$

$$Q = P/n R Q_{a}^{n-1}$$

$$Q = (Q^{n} - Q_{a}^{n})/(n Q_{a}^{n-1})$$
(21)

The above equation is for one airway. In the case of an underground mine there will be branches that will form a closed mesh within the network. In this case the error in the frictional pressure is given by:

$$P = (1/b) \left[\sum_{i=1}^{b} (R_i Q_i^{ni} - R_i Q_{ia}^{ni}) \right]$$
(22)

with the mean slope of the curve given by, b

$$(1/b)[\sum_{i=1}^{b}(nR_{i}Q_{ia}^{ni-1})]$$

The general form for the mesh correction factor $Q_{\rm m}$ can be written as,

$$Q_{m} = \left[\sum_{i=1}^{b} \left(R_{i}Q_{i}^{ni} - R_{i}Q_{ia}^{ni}\right)\right] / \left[\sum_{i=1}^{b} \left(nR_{i}Q_{ia}^{ni-1}\right)\right]$$
(23)

The numerator of the above formula gives, $Pi = R_i x Q_i^n$ and represents the frictional pressure drop on the branch i. Kirchhoff's Second Law states that,

 $Q = \sum_{i=1}^{b} P_i = 0$, and, if we substitute the above

relationship in equation 23 then:

$$Q_{m} = -\left[\sum_{i=1}^{b} (R_{i}Q_{i}^{ni})\right] / \left[\sum_{i=1}^{b} (nxR_{i}Q_{ia}^{ni-1})\right]$$
(24)

The sign of these sums is very important and has to be carefully analysed. Also, in the calculation the frictional pressure drop is always positive in the direction of the flow. Mine ventilation networks have many branches and therefore the suggested rule for sign convention is that the clockwise direction on each mesh should be positive. The final relationship for Q_m has to consider fans and natural ventilation pressure, and is given by:

$$Q_m = -\left[\sum_{i=1}^{b} \left(nR_i Q_{ia}^{ni-1} - S_{fi}\right)\right]$$
(25)

where:

 $P_{\rm fi}$ is pressure of the fan that has Qia in the branch i, (Pa)and

 S_{fi} is slope of the fan characteristic in branch i, (Pa)

With the iteration process continuing, the mesh correction factor Q_m decreases in all branches. When its value approaches the accuracy level that is

considered practical (as close to zero as possible) the iteration stops. The above formulas are characterized by the index n that is a function of the type of flow. The normal values for n are considered to be between 1.8 and 2.2 (with potential to be 1.0 if the flow is laminar). For underground mines n = 2 (turbulent flows) which gives a good estimate when used in equation 25. In the process of calculations there may be a need to fix some of the air flows (fan sizing or stopping calculations) on a particular branch. In these cases the flows will be removed from the Hardy - Cross analysis.

2. Radon and Radon Daughter Level Prediction

Most of the underground operations that have to consider radiation as criteria in their ventilation planning are uranium mines and waste nuclear repositories. The main source of radiation comes from radon. Radon has three natural species, presented in Table 1.

Table	1:	Radon	species	(24)
-------	----	-------	---------	------

Radionuclide	Historical	Decay	Half
	name	Series	Life
radon - 222	Radon	Uranium	3.82
		- 238	days
radon - 220	thoron	Thorium	55.6
		- 232	sec
radon - 219	actinon	Uranium	5.96
		- 235	sec

From these three isotopes, Radon 222 is the only one that is considered to have a major impact to workers because of its half life of 3.82 days. Radon-222 is produced by the radioactive decay of radium 226, itself a decay product of uranium. Radium has a long half life of 1622 years and thus acts as an effectively constant source of radon which has a relatively short half-life of 3.82 day. The decay of radon into its four successive short lived radioactive daughter products may become trapped in the human respiratory system where they constitute a health hazard due to the ionizing alpha radiation associated with their decay. The product of the decay of radon is shown in Fig.5.



Fig. (5) : The daughter product of the decay of radon

Concentrations of the short-lived radon daughter products are measured in working levels (WL). One working level is 1.3×10^5 MeV of potential alpha energy per liter of air and it corresponds to an activity concentration of 100 pCil⁻¹ = 3700 Bqm⁻³. Exposure to radon daughters is expressed as the product of working levels and time, the recognized unit being the working level month (WLM). One WLM results from exposure to a radon daughter concentration of one WL for one working month (176 hours). The maximum permissible annual occupations exposure has been specified as 4 WLM ⁽²⁵⁾.

2.1 Radon Flux into The Mine Atmosphere

The flux of radon from ore surfaces into the mine atmosphere is determined by the rate of production of diffusing radon and the rate at which interstitial radon migrates to exterior surfaces. The radon source strength is in turn determined by the rate at which radon is produced through the decay of radium and by the fraction often termed "emanation coefficient" of the radon which escapes the rock matrix and is free to diffuse in the pore spaces. A "typical"¹ emanation coefficient of 0.2 has often been used to model radon sources from uranium mines⁽²⁶⁾

The radon production rate in a porous radium-bearing material can be expressed as⁽²⁷⁾.

$\mathbf{Q} = [\mathbf{A}_{\mathbf{R}\mathbf{a}}] \quad \mathbf{E} / \mathbf{P} \mathbf{x} = -\mathbf{m}_{\mathbf{m}\mathbf{x}} \mathbf{E} / \mathbf{P} = / \mathbf{P}$ (26) where

E is the emanation coefficient,

P is the porosity (ratio of pore volume to total volume),

Q is the radon production rate (Bq/s per m^3 of pore space),

- $[A_{Ra}]$ is the radium 226 concentration (Bq/kg), is the bulk density (kg/m³),
 - is the radon decay constant (2.1 x 10^{-6} /s) and
 - is the emanating power $(Bq/s.m^3)$

 $_{max}$ is the emanating power assuming all available radon is released to the pores. The rate of diffusion from a plane source is given by $^{(27)}$.

$$\mathbf{C} = \mathbf{C}_{\mathbf{o}} \left[\mathbf{1} - \exp\left(-\mathbf{z} / \mathbf{D} \lambda P\right) \right] \quad (27)$$

Where:

z is the perpendicular distance (m) from the source, C is the radon concentration at distance z (Bq/m³), C_Q is the radon concentration in the plane source [$C_o = q/(Bq/m^3)$] and

D is the bulk diffusion coefficient (m^2/s)

2.2 Growth of Working Levels

The radon daughter concentration in a given airway consists of two components; radon daughters resulting from decay of radon from previous airways WL_1 and radon daughters resulting from the decay of radon originating in that particular airway WL_2 . The value of radon daughters WL_1 and WL_2 can be calculated from the following equations ^(28 and 29):

$$WL_1 = K C_0 (T_{tr})^{y}$$
(28)

$$\mathbf{T}_{\mathbf{tr}} = \mathbf{T}_{\mathbf{r}} + \mathbf{T} \tag{29}$$

$$\mathbf{T}_{\mathbf{r}} = {}^{0.86} E_r / (3700K) \tag{30}$$

Where:

 $E_{\rm r}$ is the equilibrium ratio between radon and its daughters at the node 0.275,

K is the proportionality constant, $K=6.15 \times 10^{-6}$,

T is the travel time for the air $[T=V(m^3) / Q(m^3/min)]$,

 T_r is the radioactive age of the air flows through the branch (min) $[T_{r=0} \in E_r / (3700K)]$,

 T_{tr} is the total radioactive age of the air at the end of the branch (min),

Q is the branch flow (m³/min)and

V is the branch volume (m^3)

The values of radon daughter growth parameters K, y and E_r are given in Table 2 ⁽²⁸⁾.

Table 2: values of radon daughter growth parameters K, y and $E_r^{(28)}$

Time	K	у	Ratio $E_{\rm r}$
range (min)			
0.0 -	6.15 E-6	0.86	0.0 - 0.55
41.45			
41.45 -	23.15 E-6	0.50	0.55 - 0.91
112.0			
112.0 -	137.21 E-	0.12	0.91 – 0.99
220.0	6		

$$WL_2 = (60 \ K \ J \ P \ T^{1.86}) / (1.86 \ A)$$
 (31)

$$J = (C_2 - C_1) Q / (PL)$$
(32)

Where:

A is the cross sectional area of the tunnel (m^2) ,

J is the emanation rate, (Bq $m^{-2}s^{-1}$),

 C_2 , C_1 are the final and initial radon concentrations, (Bq/m^3) ,

Q is the volume of air, (m^3/s) ,

P \is the perimeter of the mine opening, (m),

L is the length between the two measuring points, (m) and

T is the travel time for the air $[T=V(m^3)/Q (m^3/min)]$ Emanation rates vary with the rock porosity and some of the values are presented below ⁽²⁸⁾:

- high porosity (sandstones): 20 (Bq m⁻²s⁻¹)

- intermediate porosity (shales): (2-5) (Bq m⁻²s⁻¹)

- low porosity (Elliot Lake conglomerates): (0.05 - 0.0) (Bq m⁻²s⁻¹)

- igneous rocks: (0.0002 - 0.002) (Bq m⁻²s⁻¹)

McPherson $^{(23)}$ proposed typical values for the case of $^{222}\mathrm{Rn}$ emanating from uranium mines of $\begin{array}{l} A_{Ra}=30 \; Bq \; kg^{-1}, \; D=1.2x10^{-6} \; m^2 s^{-1}, \; E=0.2, \; P=0.5, \\ =0.85 \; [Bq/(s^{-1}m^{-3}] \; and \; =2.65x10^3 kg/m^3. \; Using \end{array}$ the above equations, a basic computer program was written to calculate the values of radon decay products concentration in units of working level, pressure drop, air flow and fan static pressure required to reach the permissible concentration level of radon in each point in the mines. The flow chart of the general structure of computer program (RADVENTPROG) is shown in Fig. (6). Inputs to the main program come in the form of control codes. Other inputs come through the subroutines and are transferred to other subroutines through subroutine arguments. Subroutine RADCAL to calculate radon daughter concentration u, Subroutine ARBOL chooses the chords and sets up the meshes using the theories outlined in the network analysis section and the subroutine PROCA solves the mine network

problem using the Hardy Cross method. The consistency and reliability of the present predictions were evaluated by comparing the obtained results using the developed computer program with the corresponding measured values obtained by others $^{(19)}$, $^{21)}$ and with those calculated using REDES software $^{(20,\,21)}$



RADVENTPROG.

Case Study

In addition to the workers in uranium mines, the staff of other underground mines, such as workers in underground phosphate mines, can be exposed to ²²²Rn and its progeny. According to previous studies through the Egyptian national program for safety, the values of radon decay products in units of working level (WL "1WL = $1.2E-6 \text{ J/m}^3$ ") ranged from 0.67-1.28 in some phosphate mines in the eastern desert of Egypt ⁽⁶⁾. These levels are much higher than the recommended international limit for the workers which is 0.3 WL $^{(7)}$. These high levels are due to bad ventilation system in those phosphate mines. The control of radon and radon decay products in underground is mainly achieved by an efficient ventilation system. The ventilation in underground may be natural or mechanical. Depending upon a study carried out, natural ventilation was studied in some phosphate underground mines in Egypt. The values of radon decay products concentration in units of working level show higher values than that recommended internationally which means that natural ventilation is not the proper control methods ^(5,6). So mechanical ventilation is considered as the best proper control method ^(6,8) to decrease radon decay products concentration to the recommended levels ^(9,10). The proposed computer program is used to calculate the values of radon decay products concentration in units of working level, the air flow rate required of the mechanical ventilation to reach the acceptable radon concentration relative to the recommended levels, fan static pressure and the approximate electrical power consumption cost for two underground phosphate mines. The network diagrams for the two studied mines are shown in Figs. (7 and 8). The estimated pressure drop through

each branch, and the required air flow to reach the recommended level for radon concentration at each point for the two studied tunnels are tabulated in Tables (3,4,5 and 6).

From the tables it could be seen that the calculated values of radon decay products concentration in units of working level are smaller than the corresponding measured one. This could be due to the following

• The equations used in this study to model the concentrations of radon daughters in mine atmospheres are derived directly from the laws of radioactive decay and ventilation dynamics, where it is assumed that no other removal mechanisms which could influence the concentrations are operable. In a real mine however an additional variables such as the plateout of unattached (to aerosols) daughters to mine surfaces and the deposition of attached daughters also influence the ultimate airborne concentration. So a future studies and experimental measurements are required to characterize these effects

• It is difficult to predict an accurate values for radon diffusion rate science it depends on a very large number of different parameters, so an accurate prediction values are very difficult to make ⁽¹³⁾.

• .Aside from diffusion and transport directly from the rock walls, there are numerous other sources of radon in an underground mine atmosphere which are not taken into consideration, some of which may be very substantial. Blasting will cause radon to be released immediately on rock fracture, and will also affect radon influx due to stress redistribution. Piles of loose muck will emanate radon, and mucking activities greatly enhance radon production.



Fig. (7) : Schematic of the studied points, their distance from the opening as well as the duct sizing for Qusser Yonus mine (Dimensions in meter).

Bra	anch	length	Area	Q(i,j)	pressure drop
Initial	Final	m	\mathbf{m}^2	m ³ /h	(P) Kpa
node i	node j				
1	2	650	0.75	23200	0.723
2	3	50	0.4	3000	0.124
2	12	250	0.5	12400	0.238
3	4	1	0.4	1400	0.362
3	5	50	0.4	1600	0.315
2	6	100	0.5	7800	0.072
6	7	1	0.4	1700	0.243
6	8	50	0.4	6100	0.037
8	9	1	0.4	2300	0.206
8	11	50	0.4	3800	0.160
12	13	50	0.5	6300	0.035
13	14	1	0.3	3100	0.125
13	15	50	0.4	3200	0.054
12	16	100	0.5	6100	0.416
16	17	1	0.4	3000	0.018
16	18	50	0.4	3100	0.029

Table (3): The calculated air flow (Q) and pressure drop (P) required at each point to reach the recommended international limit (WL) for the workers for Qusser Yonus mine.



Fig. (8) : Schematic of the studied points, their distance from the opening as well as the duct sizing for Hamraween mine (Dimensions in meter).

Location of study point	Distance from opening point (1)	Measured WL	Calculated WL	Required flow rate (m ³ /h)
4	700	0.481	0.437	1400
5	750	0.568	0.474	1600
7	800	0.596	0.534	1700
9	850	0.641	0.567	2300
11	900	0.701	0.617	2800
14	950	0.743	0.668	3100
15	1000	0.748	0.675	3200
17	1050	0.765	0.689	3000
18	1100	0.786	0.702	3100

 Table (4): The calculated values of WL compared with the measured ones at each point and the required air flow rate required to reach the recommended international limit (WL) for the workers for Qusser Yonus mine.

 Table (5): The calculated air flow (Q) and pressure drop (P) required at each point to reach the recommended international limit (WL) for the worker for Hamraween mine.

Br	anch	Length	Area	Q(i,j) Pressure drop (
Initial	Final	m	m^2	m ³ /h	Кра
Node i	node j				
1	2	70	0.75	34500	0.143
2	3	10	0.32	6800	0.137
2	12	30	0.32	3900	0.218
2	17	40	0.75	23800	0.154
3	4	1	0.12	1000	0.209
3	5	40	0.32	5800	0.028
5	6	1	0.06	1300	0.179
5	7	20	0.32	4500	0.016
7	8	1	0.12	1400	0.171
7	9	40	0.20	3100	0.037
9	10	1	0.12	1500	0.135
9	11	40	0.80	1600	0.133
12	13	1	0.10	1100	0. 131
12	14	30	0.20	2800	0.019
14	15	1	0.10	1200	0.113
14	16	60	0.80	1600	0.112
17	18	1	0.12	1600	0.187
17	19	30	0.75	22200	0.032
19	20	20	0.24	4300	0.027
19	25	50	0.24	4100	0.129
19	28	50	0.75	13600	0.062
20	21	1	0.10	1500	0.125
20	22	40	0.12	3300	0.096
22	23	1	0.20	1600	0.033
22	24	40	0.08	1700	0.023
25	26	1	0.16	1600	0.025
25	27	90	0.12	2500	0.075
28	29	10	0.32	5000	0.045
28	32	60	0.32	8300	0.056
29	30	1	0.13	2500	0.032
29	31	100	0.24	2500	0.053
32	33	1	0,24	2500	0.051
32	34	100	0.24	5800	0.072
``34	35	1	0.24	2800	0.045
34	36	50	0.24	3000	0.055

Hann awten mine.							
Location of	Distance from	Measured	Calculated	Required flow			
study point	opening point (1)	WL	WL	rate (m³/h)			
4	60	0.358	0.328	1000			
6	100	0.381	0.351	1300			
8	120	0.384	0.354	1400			
10	160	0.402	0.367	1500			
11	200	0.421	0.397	1600			
13	90	0.375	0.348	1100			
15	120	0.387	0.353	1200			
16	180	0.417	0.383	1700			
18	100	0.393	0.361	1600			
21	130	0.393	0.373	1700			
23	180	0.421	0.371	1600			
24	230	0.425	0.383	1700			
26	180	0.398	0.362	1600			
27	270	0.458	0.426	2500			
30	180	0.441	0.401	2500			
31	290	0.489	0.445	2500			
33	240	0.478	0.435	2500			
35	290	0.512	0.473	2500			
36	340	0.572	0.572	3000			

 Table (6) : The calculated values of WL compared with the measured ones at each point and the required air flow rate required to reach the recommended international limit (WL) for the workers for Hamraween mine.

Depending upon the location of study point from the opening the air flow rate ranged from 1000 to $3000 \text{ m}^3/\text{h}$ for Hamraween mine, 1400 to $3200 \text{ m}^3/\text{h}$ for Qusser mine. Static pressure, air flow rate and the approximated electrical power consumption of the exhaust fan for each studied case are tabulated in Table 7 against the corresponding values calculated using REDES software ^(20,21). From this table it could be noticed that there is a good agreement between the corresponding values. However the estimated approximate annual electrical power consumption which may reach about 70000 kWh per tunnel in addition to initial and maintenance cost, represent a major problem for some underground work when it is not so preferable such as in case of phosphate mines. The workers health is considered as a pushing factor to develop such expensive radiation safety measure.

Table (7) : Fan static pressure, fan air flow rate and electrical running power consumption required	d to reach
the recommended international limit (WL) for the worker for the two studied mines.	

Mine	Fan air flow rate (m³/h)		Fan static pressure drop (pa)		Approximate annual electrical power consumption per tunnel kWh	
	(1)	(2)	(1)	(2)	(1)	(2)
Hamraween	34500	37000	700	750	53000	61000
Qusser Youns	23200	25000	1350	1500	60000	70000

(1) Values calculated using the present model

(2) Values calculated using REDES software

Conclusion

As a part of comprehensive study, a computer model for the calculation of ventilation parameters

has been developed and verified to applied to underground uranium mines environment. Ventilation parameters e.g.: pressures and air flow rate have been calculated to maintain the safe levels of radon daughter in underground mines for workers protection against radiation. The results of the calculations have been evaluated and compared with similar studies and good agreement is demonstrated. The present code is considered as an available Egyptian tool for regulatory evaluation of radiological safety in mines. With the present code, it is possible to calculate the safety limits of ventilation parameters at each exhaust point in the mines which is not possible in the REDES code. In spite of the important role of natural ventilation, which is sufficient to rely upon in various cases, mechanical ventilation is necessary to reduce the airborne radioactivity level to the recommended values.

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Impact of Structured Nursing Measures Pre and Post Epidural Lumbar Anesthesia on the Occurrence of Post-Epidural Anesthetic Headache

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Abstract: Lumbar puncture is a frequently performed procedure in medical emergencies and anesthesia. Headache after lumbar puncture is a common occurrence (32%) and carries a considerable morbidity, with symptoms lasting for several days, at times severe enough to immobilize. The aim of this study is to assess the impact of structured nursing measures on the occurrence of post-epidural anesthetic headache. This quasi-experimental study was conducted in El-naser Health Insurance Hospital; in Helwan city in Egypt on of 60 adult patients admitted for lower abdominal surgery using epidural anesthesia was recruited. The only exclusion criterion was pregnancy in female patients. Participants were alternatively assigned to either the intervention or control groups, ending with 30 patients in each group. The data collection tools consisted of two tools. Tool (1) was concerned with characterization of the pain and patient's personal data. The second tool was a Visual analog scale (VAS). The researchers designed a structured pre-spinal anesthetic nursing intervention to be applied to the study group. The control group received the routine nursing intervention only. The results revealed that the incidence of headache became significantly lower in the study group, reaching its lowest rate (3.3%) by the end of the third day, compared to 76.7% in the control group (p<0.001). the mean duration of headache was shorter in the study $(22.1\pm34.0 \text{ hours})$ than in the control $(111.2\pm55.9 \text{ J})$ hours) group, p<0.001. as well Patients in the study and control groups also demonstrated statistically significant differences in the experience of symptoms associated with headache (p<0.001). In conclusion, the structured nursing measures before and after the procedure was successful in decreasing the incidence and duration of this headache and its associated symptoms. Therefore, it is recommended to generalize these structured nursing measures in hospitals to be included in the routine pre-operative and post-operative nursing care for patients undergoing lower abdominal surgery with spinal anesthesia.

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Key words: Lumber puncture, post lumber puncture headache.

1. Introduction:

Lumbar puncture is a frequently performed procedure in medical emergencies and anesthesia. Headache after lumbar puncture is a common occurrence (32%) and carries a considerable morbidity, with symptoms lasting for several days, at times severe enough to immobilize the patient. If untreated, it can result in serious complications such as subdural hematoma and seizures, which could be fatal (Ahmed *et al.*, 2009).

Multiple complications can occur after dural puncture, including headache, cranial neuropathies, direct nerve root irritation, backache, infection, and spinal hematoma. Postdural puncture headache (PDPH) is the most common of these complications. It develops in 16% to 86% of the cases after attempted epidural block with large bore needles (Ghaleb, 2010). It is described as frontal or occipital pressure occurring in the upright position and decreasing or resolving when supine (Zencirci, 2010).

According to the International Headache Society, the criteria for PDPH include a headache that develops less than seven days after a spinal puncture, occurs or worsens less than fifteen minutes after assuming the upright position, and improves less than thirty minutes in the recumbent position with at least one of the following symptoms: neck stiffness, tinnitus, hypacusia, photophobia, and nausea. It should disappear within fourteen days after a spinal puncture; if it persists, it is called a CSF fistula headache (Apfel *et al.*, 2010).

These headaches are thought to result from leakage of the cerebrospinal fluid through the tiny hole created by the spinal tap needle, causing the membranes to rub painfully against the bony skull (Ghaleb, 2010). Therefore, the needle size and type are among the factors that have been shown to contribute to the risk for PDPH. Small-bore (high-gauge) needles have been shown to reduce the risk although smaller needle sizes increase the failure rate of the lumbar puncture because they are more difficult to use. As a result, needles smaller than 25 gauges are not preferred in spinal anesthesia (Shah and Thomas, 2007). A lower incidence was found when 27-gauge Quincke and 25-gauge Whitacre needles were used versus 26-gauge Quincke needles (Kuczkowski, 2004).

Patients who develop PDPH may reveal a wide range of emotional responses from misery and tears to anger and panic. It is important both from a clinical and medical points of view to discuss the possibility of headache before a procedure is undertaken that has a risk of this complication. Even so, this discussion will not prepare the patient for the sensations he/she feels should the headache develop (Lomax and Qureshi, 2008). It is important to give the patient a thorough explanation of the reason for the headache, the expected time course, and the therapeutic options available. Supportive therapy such as rehydration, acetaminophen, non-steroidal anti-inflammatory drugs, opioids, and antiemetic may control the symptoms and so reduce the need for more aggressive therapy (Ahmed et al., 2009). Caffeine, either parenteral or oral, is one of the most common treatments. As PDPH is believed to be caused by adenosine-induced cerebral vasodilatation. caffeine may act by antagonizing adenosine, thus leading to cerebral vasoconstriction (Apfel et al., 2010).

The purpose of using hydration in treating PDHD is to ensure that the rate of CSF production is appropriate. Although the degree of CSF leak does not correlate with the severity of the symptoms in a PDPH, it is assumed that improvements in the ratio of CSF production to CSF leak will improve the clinical picture. Dehydration can result in a decrease in CSF production. However, if the patient is appropriately hydrated, and the rate of CSF production is normal, there is no evidence that overhydration will increase the rate of CSF production any further. Therefore, there is no point in administering fluids to a patient who is already appropriately hydrated (Frank, 2008). If a patient develops a headache, he/she should be encouraged to lie in a comfortable position. There is

no clinical evidence to support the maintenance of the supine position before or after the onset of the headache as a means of treatment. The prone position has been advocated, although it may not be a comfortable position for the patient. This position raises the intra-abdominal pressure, which is transmitted to the epidural space and may alleviate the headache (Ahmed *et al*, 2009).

Significance of the study

Reviewing the admission rate of patients at the El-Nasr Insurance Hospital at Helwan city showed that about 200 cases required lower abdominal surgery under spinal anesthesia during the last year. In addition, around 80% of these patients complained of headache after spinal anesthesia that hindered their ability of early mobilization, eating, and/or self-caring. These factors would consequently lead to delay of the healing process of their wounds, and result in longer hospital stay and subsequent financial load on the patients and their families, as well as the healthcare system. Moreover, PDPH might give patients a bad experience with spinal anesthesia that makes them reluctant to use it again.

Aim of the study

The aim of this study is to assess the impact of structured nursing measures on the occurrence of post-epidural anesthetic headache.

Research question:

What is the effect of nursing care measures on the occurrence of post-epidural anesthetic headache?

2. Subjects and Methods:

Research design:

A quasi-experimental research design was utilized.

Setting:

The study was conducted at the El-Nasr Insurance Hospital at Helwan city.

Subjects:

A consecutive sample of 60 adult patients admitted for lower abdominal surgery using epidural anesthesia was recruited. The only exclusion criterion was pregnancy in female patients. Participants were alternatively assigned to either the intervention or control groups, ending with 30 patients in each group. The study group patients received structured nursing care measures before and after epidural anesthesia, while the control group patients were subjected to routine hospital care. The same anesthesiologist performed the procedure for all patients in both groups using the same needle caliber (needle size 25gauge Quincke) and the same anesthetic drug.

Data collection tools:

Two tools were adopted by the researchers based on related literature. The first tool was

concerned with characterization of the pain including its location (occipital, frontal, neck, and shoulder), time of start in relation to operation, duration, and associated symptoms (nausea, vomiting, blurred vision and tinnitus). The tool also included patient's personal data, past and present medical data, past history of spinal anesthesia and related headache. It also included recording of vital signs. This tool was filled out for all patients in both groups three times per day during the five days following the operation, and once after one week. The second tool was a Visual analog scale (VAS). It is an instrument used to measure the amount of pain a patient feels, according to Journal of Clinical Nursing. The visual analog scale of pain is usually a 100 mm-long horizontal line, which may contain word descriptors at each end. The patient represents their perception of the amount of pain he/she feels by marking a horizontal line between two points. The visual analog scale score is measured in millimeters from the left hand end of the line to the point indicated by the patient (Le Bach Pham, eHow Contributor, 2010) Nursing measures (the study intervention): The researchers designed a structured pre-spinal anesthetic nursing intervention to be applied to the study group. The nursing intervention consisted of pre and post anesthetic measures. The pre-anesthetic measures included psychological preparation and support to the patient through explaining what spinal anesthesia is, its importance, and correct position during lumbar puncture. They also involved improvement of patient's hydration by encouraging patients to drink more fluids as juice, tap water, and/or soup, in addition to taking two cups of coffee eight hours before the operation. The post-anesthetic measures included bed rest in prone position, improving patient's hydration by encouraging oral intake of fluids, administration of oral analgesic and caffeine, and application of an abdominal binder.

Pilot study:

A pilot study was conducted on six patients from the same setting to test the feasibility and applicability of the tools and the intervention, and few modifications were done accordingly. Data obtained from the pilot study were not included in the main study.

Study maneuver:

The study protocol was approved and an official permission to carry out the study was obtained from pertinent authorities after explanation of its purpose. An oral informed consent was obtained from every patient to participate in the study. Confidentiality and privacy were assured for each participant. The study maneuvers could not entail any harm to patients. Data collection extended over a period of one year from January 2009 to January 2010. Available patients who fulfilled the inclusion and exclusion criteria were assigned to intervention and control groups in an alternate manner. The structured nursing intervention was implemented to the study group, while patients in the control group received the routine nursing measures. Post-operatively, the post-anesthetic nursing measures were applied to the study group, whereas the control group received the routine post-operative hospital measures.

Statistical analysis:

Data entry and statistical analysis were done using SPSS 14.0 statistical software package. Quantitative continuous data were compared using the non-parametric Mann-Whitney test as normal distribution of the data could not be assumed. Qualitative categorical variables were compared using chi-square test. Statistical significance was considered at p-value <0.05.

3. Results:

Patients in the study and control groups had similar demographic characteristics with no statistically significant differences. As Table 1 shows, about half of the study (46.7%) and control (56.7%) groups were males, with mean ages 38.1 ± 10.4 and $33.8\pm$ 8.8 respectively. High percentages of them (56.7% and 53.3%, respectively) had a past history of headache. Patients in the two groups also had similar pre-operative vital signs, with no statistically significant differences between them (Table 2).

The incidence of post-anesthetic headache was similar in the two groups in the first two assessments (0 and 8 hours), with immediate rates of 53.3% in the control group and 46.7% in the study group (Figure 1). From the third assessment, the incidence of headache became significantly lower in the study group, reaching its lowest rate (3.3%) by the end of the third day, compared to 76.7% in the control group (p<0.001). One week after operation, during follow-up, 10% of the patients in the study group had headache compared to 53.3% in the control group (p<0.001).

As regards the time of start of headache, there is no statistically significant difference between study and control group patients (Table 3). However, the time of end of headache demonstrated a statistically significant difference between the two groups, with 40.0% of the study group ending in the first day, and 60% of the control group extending to follow-up (p <0.001). Also, the mean duration of headache was shorter in the study (22.1 \pm 34.0 hours) than in the control (111.2 \pm 55.9 hours) group, p<0.001.

The comparison of the intensity of headache assessed by visual analog scale indicated statistically significant differences between the two groups in the first three days and follow up measurements (Figure 2). The mean scores ranged between 2.0 to 4.4 in the study group, and 7.6 to 8.0 in the control group. Patients in the study and control groups also demonstrated statistically significant differences in the experience of symptoms associated with headache (p<0.001). As Table 4 illustrates, none of the patients in the study group who had headache had any of

these symptoms. On the other hand, all of the patients with headache in the control group had either one or multiple symptoms throughout the follow-up time.

The differences in the duration of headache between the study and control groups were still statistically significant when stratified according to their demographic characteristics and medical history. As displayed in Table 5, the duration was longer in the control group patients compared to the study group irrespective of age, gender, past history of headache, and medical history of abdominal surgery.

 Table 1. Demographic characteristics and medical history of patients in the study and control groups

		G		\mathbf{v}^2		
	Study	(n=30)	Contro	l (n=30)	A Tost	p-value
	No.	%	No.	%	Test	
Age (years):						
<40	17	56.7	23	76.7		
40+	13	43.3	7	23.3	2.70	0.10
Range	22.0	-55.0	22.0	-50.0		
Mean±SD	38.1	± 10.4	33.8	8 ± 8.8		
Sex:						
Male	14	46.7	17	56.7		
Female	16	53.3	13	43.3	0.60	0.44
Past history of:						
Recurrent headache	17	56.7	16	53.3	0.07	0.80
Abdominal surgery	14	46.7	18	60.0	1.07	0.30

Table 2. Pre-	intervention	vital signs (of patients	in the study	v and cont	rol groups
					,	

		G		Monn		
	Study (n	=30)	Control (1	n=30)	Whitney	p-value
	No.	%	No.	%	winney	
Systolic blood pressure:						
Range	110.0-180.0		110.0-1	70.0		
Mean±SD	126.3±	14.0	124.7±1	3.3	0.51	0.47
Diastolic blood pressure:						
Range	70.0-12	20.0	70.0-12	0.0		
Mean±SD	85.7±1	0.7	85.7±1	2.2	0.07	0.80
Pulse:						
Range	70.0-10	0.0	80.0-11	0.0		
Mean±SD	90.0±0	5.9	92.0±5	5.5	0.89	0.35
Respiratory rate:						
Range	22.0-2	8.0	22.0-2	8.0		
Mean±SD	22.9±	1.7	22.5±1	.3	0.92	0.34
Temperature:						
Range	38.0-39.0		38.0-3	8.0		
Mean±SD	38.1±0).3	38.0±0).0	3.11	0.08

		G	roup		\mathbf{v}^2	
	Study	(n=30)	Contro	ol (n=30)	A ⁻	p-value
	No.	%	No.	%	Test	_
Start:						
None	9	30.0	2	6.7		
Day 1	14	46.7	16	53.3	5.90	0.052
Day 2+	7	23.3	12	40.0		
End:						
None	9	30.0	2	6.7		
Day 1	12	40.0	1	3.3	26.81	< 0.001*
Day 2-5	7	23.3	9	30.0		
Follow-up	2	6.7	18	60.0		
Duration (hours):						
Range	0.0-	168.0	0.0-	168.0		
Mean±SD	22.1	±34.0	111.	2±55.9	U=27.75	< 0.001*
	0.07		(7 1) 3 5	****		

Table 3. Time of start, end, and duration of headache among patients in the study and control groups

(*) Statistically significant at p<0.05 (U) Mann-Whitney test

Table 4. Symptoms associated with headache among patients in the study and control groups throughout follow-up

	Group (with headache)					
	Stu	dy	Con	trol	A Test	p-value
	No.	%	No.	%	Test	
Day 1:						
None	12	100.0	0	0.0		
One	0	0.0	7	38.9	30.00	< 0.001*
Multiple	0	0.0	11	61.1		
Day 2:						
None	5	100.0	0	0.0		
One	0	0.0	7	30.4	28.00	< 0.001*
Multiple	0	0.0	16	69.6		
Day 3:						
None	6	100.0	0	0.0		
One	0	0.0	7	28.0	31.00	< 0.001*
Multiple	0	0.0	18	72.0		
Day 4:						
None	1	100.0	0	0.0		
One	0	0.0	6	27.3	23.00	< 0.001*
Multiple	0	0.0	16	72.7		
Day 5:						
None	1	100.0	0	0.0		
One	0	0.0	6	30.0	21.00	< 0.001*
Multiple	0	0.0	14	70.0		
Follow-up:						
None	3	100.0	0	0.0		
One	0	0.0	3	18.8	19.00	< 0.001*
Multiple	0	0.0	13	81.3		

(*) Statistically significant at p<0.05

		Group (wit	h headache)		Monn	
	Stud	у	Contro	ol	Whitney	p-value
	Mean±SD	Median	Mean±SD	Median	winney	
Age (years):						
<40	11.3±12.7	8.00	111.7±59.6	120.00	20.28	< 0.001*
40+	36.3±46.8	16.00	109.7±45.7	120.00	7.98	0.005*
Sex:						
Male	26.3±44.0	16.0	105.9±62.1	120.00	10.08	0.002*
Female	18.5±22.9	12.00	118.2±48.2	144.00	18.49	< 0.001*
History of recurrent						
headache:						
No	12.3±14.1	8.00	92.6±62.7	104.00	10.82	0.001*
Yes	29.6±42.5	16.00	127.5±45.1	144.0	18.56	< 0.001*
History of abdominal						
surgery:						
No	20.0±21.1	16.00	95.3±68.7	104.00	7.66	0.006*
Yes	24.6±45.3	12.00	121.8±44.5	132.00	16.76	< 0.001*

Table 5. Duration of headache	among	patients	in the	study	and	control	groups	adjusted	for	age,	sex,	and
medical history												

(*) Statistically significant at p<0.05









4. Discussion:

Headache following a lumbar puncture is a common and often debilitating syndrome. In this current research, the majority of patients (more than 50%) developed headache immediately after the operations. However, this high percentage persisted and even increased in the control group patients throughout follow-up. The findings are in agreement with Straus et al. (2006) and Desai et al. (2010) who reported rates of PDPH reaching as much as 70%. Moreover, Datta et al. (2009) mentioned that PDPH occurs in up to 90% of patients within two days following diagnostic lumbar puncture, which is close to the rate in the present study control group patients (83.3%). Meanwhile, much lower percentages of patients in the study group experienced headache, which points to success of the nursing intervention measures.

The needle size or bore can be a confounding factor in the study of PDPH. Studies have demonstrated a significant association between the needle gauge and the incidence of PDHD. The incidence is 40% with a 22G needle; 25% with a 25G needle; 2%–12% with a 26G Quincke needle; and <2% with a 29G needle (Apfel *et al.*, 2010). However, technical difficulties leading to failure of the spinal anaesthetic are common with needles of 29G or smaller (Lomax and Qureshi, 2008). In the present study, a needle size 25-gauge Quincke was used for all patients in the study and control groups to avoid the effect of this confounding factor.

The time of start of the PDPH was not significantly different between the study and control group patients in the current study. In both groups, about half of the patients experienced headache by the first day, and this increased to about 70% and 90%, respectively in the second day. These rates are slightly higher than those reported by Frank (2008) who found that 66% of the patients' headache starts within the first 48 hours. This difference between the two studies might be related to needle size differences or the experience of the person doing the procedure.

The present study intervention was also successful in decreasing the duration of headache among patients in the study group, compared to the control group patients. The mean duration in the study group was reduced to about one day, compared to about five days in the control group. This duration in the control group is close to that reported by Shah and Thomas (2007) who found a median of five days. Moreover, Apfel *et al.* (2010) reported that 72% of headaches lasted for seven days. Therefore, there was a true reduction in the duration of headache in the intervention group of the current study, which could be attributed to the implementation of the nursing measures. These included oral hydration, which has been recommended by Ghaleb (2010), as well as caffeine in a single oral dose, which was demonstrated to be safe, effective and recommended in the early treatment of PDPH (Smith and Hirsch, 2009; Desai *et al.*, 2010). These results of the present study are in congruence with Ahmed *et al.* (2006) who highlighted that the syndrome of postdural puncture headache may be resolve spontaneously in a few days to a week or lasts months to a year according to the provided conservative measures.

With regard to the intensity of headache, the present study findings point to significantly lower intensity scores among patients in the study group as compared to those in the control group. This can also be attributed to the effect of providing pre-and postanesthetic nursing measures for patients in the study group, which might have a positive effect on reducing the intensity of headache. This explanation is in line with the results of Kenneth et al. (2005) and David (2007), who reported the importance of conservative approaches in preventing PDPH. The higher intensity in the control group patients could also be due to the earlier start of their headache, which is usually associated with more severe and longer lasting headache as indicated by Benyamin et al. (2009).

There are certain demographic factors that seem to be associated with the risk of PDPH for reasons that are not well understood. Patient age is a risk factor, with ages between 18 and 40 years the highest risk range (Ho and Gan, 2007). The risk of PDPH at age 25 years is 3-4 times that at age 65 years. There is also significantly decreased frequency after age 60 years, which also may be related to reduce CSF pressure (Desai et al., 2010). It has been speculated that the dura mater of the elderly is less stretchable (Ghaleb, 2010). Therefore, age could be a confounding factor in the current research. However, with stratified analysis according to age, there were still statistical significant differences between patients in the two studied groups. This means that conservative intervention measures were successful in reducing the duration of PDPH regardless of patient's age.

Female sex is a debatable risk factor for PDPH (Wu *et al.*, 2006) with some studies reporting a twofold risk among women (Conn *et al.*, 2009), and others denying any gender difference (Kenneth *et al.*, 2005). Nonetheless, in the present research, the difference between patients in the study and control groups regarding the duration of PDPH remained in stratified analysis according to sex, which obviates any confounding effect of sex in the intervention study results. This adds a further confirmation of the

effectiveness of the conservative nursing measures in reducing the duration of PDPH.

Another possible confounding factor taken into account in the present study is the history of chronic or recurrent headache, which has been claimed to be a risk factor for development of future headaches (Wu *et al.*, 2006; Amorim and Valença, 2008; Benyamin *et al.*, 2009). According to the current study, the duration of PDPH was longer among patients with a past history of recurrent headache, and past history of abdominal surgery. Meanwhile, with stratified analysis according to history of headache, there were still statistically significant differences between patients in the study and control groups. This point to success of the intervention measures irrespective of past history of headache.

5. Conclusion and Recommendations:

In conclusion, a large percentage of the patients undergoing lower abdominal surgery under epidural anesthesia had post-dural puncture headache. The structured nursing measures before and after the procedure was successful in decreasing the incidence and duration of this headache and its associated symptoms. Therefore, it is recommended to generalize these structured nursing measures in hospitals to be included in the routine pre-operative and post-operative nursing care for patients undergoing lower abdominal surgery with spinal anesthesia. It is also important to be included in the curriculum of the faculty of nursing. This would decrease the incidence of complications, and reduce hospital length of stay. More research is needed to investigate the effectiveness of these measures in other types of surgery using spinal anesthesia.

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Evaluation of Some Quality Aspects in Pediatric Intensive Care Services at Benha University Hospital

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Abstract: Objective: This study was conducted to evaluate the quality practice of some aspects of pediatric intensive care services in Benha University Hospital through systematic surveillance approach for situation and gap analysis at PICU. Methods: The surveillance procedures were based on observational scoring meeting with staff and data collection by questionnaires. The surveillance activities were repeated for 9 times (from November, 2009 to March, 2010) for assurance of accuracy of collected data. Results: In the current study the overall assessment of infection control standards in all surveillance cycles (nine cycles) revealed that 6 audits were interpreted as "moderate compliance" (66.7 %) and 3 audits were interpreted as low compliance (33.3 %). As regards total sterilization standards, surveillances revealed, moderate compliance was achieved in 6 audits (66.7%) and high compliance was achieved in 3 audits (33.3%). The repeated nine audits were interpreted for personnel and structure as having moderate compliance (100%). The current study assessment of mechanical ventilation standards revealed variation through different audits that could be summarized as follows; high compliance of 6 audits (66.7%) and moderate compliance of 3 audits (33.3%). Conclusion: There are variable degrees of compliance with the national and international standards of infection control, sterilization and personnel and mechanical ventilation in Benha University Hospital PICU.

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Key words: Quality Aspects, Intensive Care Services, systematic surveillance, mechanical ventilation.

1. Introduction

Pediatric critical care medicine is unique because of the heterogeneity of critically ill patients based on age, diagnoses and treatment modalities, including type and dosing range of medications. The same factors, combined with the complexity of pediatric intensive care unit (PICU) healthcare processes, result in a system that is potentially dangerous. Thus, identification of both real and potential sources of harm to patients or of poor care quality is crucial to ensuring safe care in PICUs. The required system for PICU must describe some specific quality and safety metrics that may be of value to pediatric critical care providers and their administrative counterparts (Forest et al.,2003).

In our current clinical application in PICU at Benha University Hospital we are aware about some quality aspects like definition of quality of care and its indicators. Quality of care can be defined as 'the degree to which health services for individuals and populations increase the likelihood of desired health outcomes and are consistent with current professional knowledge (Lohr,1990).

Quality indicators are quantitative measures that can be used to monitor and evaluate the quality of important governance, management, clinical, and support functions that affect patient outcomes (JCAHO, 1989).

The use of performance improvement tools as well as indicators for performance and outcome measurement allow the quality of care and services to be measured. Quality indicators provide a measure of quality of structure, process, and outcome of care (Skews et al., 2000).

Structure can be defined as the way we organize care. Structurally, ICUs are quite heterogeneous, even within regions or countries. The main differences are how the ICU is integrated into the hospital or health care system, the ICU size, the type and amount of technologies available, and the number, roles and responsibilities of its staff (Pronovost et al., 2002).

Process is used to indicate what we do, or we fail to do, for patients and their families during their stay in the ICU. A great number of processes are normally involved in ICU patient care ranging from individual care to general procedures such as admission and discharge and maintenance of equipments (Stucke et al .,2007).

Outcome is defined as changes in the state of health of a patient that can be attributed to an intervention or to the absence of an intervention (Donabedian,2003).

All the previous definitions are included in the clinical system applied in PICU, to insure quality of service production. The system and system management are a challenge in Benha university hospital, for more than one reason. Some are related to resources, others related to resistance of change and some are related to the system itself. The national health care standards are now available and it is to be implemented for all health care sections in Egypt. These standards must be communicated properly to all health care provider national wide and the compliance will be soon the only way of accreditation.

2. The purpose of this study is to:

1- Evaluate level of pediatric intensive care in comparison to the national and international standards as regards structure, ventilator process and infection control process .

2- Evaluate potential measures of quality based on availability , safety , appropriateness, effectiveness, and the strength of evidence to support each measure and to categorize these measures as structure, process, and outcome.

3- Evaluation of the degree of compliance to national standards concerning PICU.

This allows self evaluation of our PICUs practice and facilities looking forward to better performance.

3. Patient and methods:

Patients:The study include all patients admitted to the PICU Benha university hospital through the period of the study (from November, 2009 to March, 2010).

[I] Standard assessment and classification in PICU:

Both national and international standards of health care production are tabulated in an evaluation format regarding to unit structrure and personnel, infection control, sterilization and mechanical ventilation processes.

The national standards are those generated by Egyptian MOH consultatory board in May, 2005 for accreditation of health care organization on national scale. The selected international standards are those related to structural and personnel application (David et al ., 2004), and those related to mechanical ventilation (Sean et al ., 2002). The selection was based on its critical clinical outcome impact of those standards.

Code key: IC = Infection control (includes 26 standards), ST = Sterilization (includes 16 standards), CU=Intensive care(structure and personnel) (includes 68 standards), and MV = mechanical ventilation (includes 25 standards).

Standards statement:

We used these terms for assessment: M = met: all steps are performed, P = partially met: one step or more failed. N = not met: all steps are not performed, and NA = not applicable.

[II] Group auditing:

Audit is defined as the systematic and critical analysis of the quality of clinical care. This includes unit structure and the procedures used for diagnosis and treatment.

Auditing and surviellance activities were done for all available groups.

(A) All standards were audited over the different shifts of the day:

This is adding to the personel observation of the research team and to the outcome of conversation with staff.

(B) Frequancy of auditing for each shift:

2 pm - 8 pm. (PM): 3 audits, weekend. (AM,PM): 1 audit.

8 am - 2 pm. (AM) : 2 audits, and 8 pm - 8 am. (Night): 3 audits

The auditing / surviellance duration is that of the shift time

[III] Data analysis:

For quantification of problem and proper measurement of good and bad practices.

Compliance of standards of each group, compliance of each group collectively, and compliance of all groups collectively.

Compliance was measured as low, moderate, and high compliance.

In our study we considered M = 2, P = 1, N = 0.

Compliance of standards of each group:

Each standard was audited 9 times.so the maximum score $= 9 \times 2 = 18$.

We considered: Score 0 - 6 = 100 compliance, Score 7- 12 = moderate compliance, and Score > 13 = 100 high compliace.

By the same way (according to the No of standards) we calculated the compliance of each group collectively and compliance of all groups collectively.

Statistical design: Collected data were tabulated and analyzed using suitable statistical techniques. Frequency distribution tables, Pie charts and bar charts were used.

4. Results:

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Our study evaluates some aspects in Pediatrics Intensive Care Unit through 9 audits at different shifts, times, and staff. Each audit takes a time of 2 to 3 days and each audit was re-conducted within 2 weeks interval. The preliminary instrument included 135 variable standards and this was the questionnaire that was used during the field test (November 2009 to March 2010). Our result revealed that:

Table (1):Frequancy distribution of infection control, structure & personnel, and mechanical ventilation process compliance: NB: * international standards, No: number, %: percent

Standard	Standard statement M P			þ	1	N	NA		
code	Standard statement	No	%	No	%	No	%	No	%
Infaction con	trol								
Infection con		1	1	1	1	1	1	1	1
IC.1	PICU has an active program to reduce the risks of nosocomial			8	88.9	1	11.1		
	infections								
IC.1.1	The program covers patients, staff, and visitors			8	88.9	1	11.1		
IC.2	PICUhas established a functioning infection control committee.			2	22.2	7	77.8		
IC.3	A qualified physician oversees the infection control activities			1	11.1	8	88.9		
IC.4	A qualified nurse (at least one) assists in infection control activities			2	22.2	7	77.8		
IC.5	PICU has identified those proc	edures	and pro	ocesses	associat	ted wit	h incre	ased ris	sk of
	infection. At a minimum, these in	clude tl	ne follow	ing (wh	en relev	vant to 1	the hosp	pital's	
	services):	1	1	1	1			1	
IC.5.1	Respiratory tract infections associated with intubation, ventilator support, or tracheostomy	8	88.9	1	11.1				
IC.5.2	Urinary tract infections associated with catheters.	8	88.9	1	11.1				
IC.5.3	Blood stream infections associated with intravascular devices.	8	88.9	1	11.1				
IC.5.4	Surgical wound infections	7	77.8	2	22.2				
IC 6	PICU has written infection	contro	ol polici	ies and	l proc	edures	. The	policies	and and
	procedures are followed and inclu	ide, but	t are not	limited	to, the	followiı	ıg:	1	1
IC.6.1	Hand washing.	7	77.8	2	22.2				
IC.6.2	Isolation policy, including the management and reporting of patients with suspected communicable diseases					9	100		
IC.6.3	Management of patients who are immunocompromised	4	44.4			5	55.6		
IC.6.4	Prevention of blood-borne infections among PICU staff, including disposal of sharps	5	55.6	4	44.4				
IC.6.5	Prevention of surgical sites infection							9	100
IC.6.6	Prevention of hospital-acquired respiratory tract infections	4	44.4	3	33.3	2	22.2		

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10.47	Selection and uses of antiseptics	E	667	2	22.2				
10.0.7	and disinfectant	D	00./	3	33.5				
IC.6.8	Infection control surveillance and			1	11.1	8	88.9		
10.000	data collection			1	11.1	0	00.7		
IC.6.9	Management of outbreaks of					9	100		
	Infections								
	Infection control policies and								
IC 7	all concerned departments after			1	11 1	8	88.0		
10.7	being approved by the infection			1	11.1	0	00.9		
	control committee								
	Infection control policies and								
	procedures are reviewed And								
IC.8	updated regularly by the			1	11.1	8	88.9		
	infection control committee								
	(current professional literature)								
	All relevant staff have been								
	oriented and trained in the					-			
IC.9	applicable infection control			4	44.4	5	55.6		
	policies and procedures as								
	When relevant to the hospital's								
	services there are special								
	isolation rooms in the hospital								
IC.10	including negative pressure					9	100		
	rooms, for isolating infection								
	cases								
IC 11	There are hand hygiene facilities							0	100
IC.11	in each isolation room							9	100
	The surveillance data of hospital-								
	acquired infections, and the								
IC.12	effectiveness of the program, are					9	100		
	regularly aggregated and analyzed								
	by the injection control								
	The results are disseminated to		<u> </u>						
	senior management to concerned								
IC.12.1	PICU and, when relevant, are					9	100		
	utilized by them for improving					-			
	the quality of care								
	All communicable diseases are								
IC.13	reported as required by MOHP	4	44.4			5	55.6		
	regulations.								
Sterilization:		-	[-	1		1		
	PICU has a central sterilization	0	100						
51.1	supply department (CSSD) or	9	100						
	(CSSD) is managed by a								
ST 1 1	individual who is qualified by							0	100
51.1.1	education and/or training							2	100
	The functions of cleaning								
ST.2	processing, and sterile storage			-					
~	and distribution are physically	1	11.1	8	88.9				
	separated.								
ST.3	In all areas where instruments are	1	11.1	8	88.9				

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	cleaned there must be airflow that								
	prevents contaminated material								
	from exiting the area								
	There are means of preventing								
ST 4	cross-contamination in the	1	11 1	5	55.6	3	33.3		
51.4	cleaning area	1	11.1	5	55.0	5	55.5		
	Based on the services provided								
	and the size of the hospital the								
ST.5	sterilization area has at least	9	100						
	one functioning autoclave								
	Boiling water is not used as a								
ST.6	sterilization technique			9	100				
	Whatever sterilization technique								
	is used (including chemical								
	cleaning/sterilization of								
ST.7	scopes), there is documented	1	11.1	2	22.2	6	66.7		
	evidence that complete								
	sterilization has been								
	accomplished								
	There are specific policies and								
	procedures that are followed								
ST.8	for each sterilization technique	4	44.5	2	22.2	3	33.3		
	or device used, including								
	manufacturer's manuals.								
	There is documented evidence in								
ST 9	their human resources file that	1	11.1	1	11.1	7	77 8		
N		-		-					
	stan are trained in these								
	staff are trained in these procedures.								
	stari are trained in these procedures. Policies and procedures have b	een dev	veloped	and use	ed for	all pro	cesses,	includir	ng the
ST.10	stari are trained in these procedures. Policies and procedures have b following: Receiving disinfection and	een dev	veloped	and use	ed for a	all pro	cesses,	includir	ng the
ST.10 ST.10.1	starr are trained in these procedures. Policies and procedures have b following: Receiving, disinfection, and cleaning of used items	een dev	veloped 88.9	and use	ed for a	all pro	cesses,	includir	ng the
ST.10 ST.10.1	stari are trained in these procedures. Policies and procedures have b following: Receiving, disinfection, and cleaning of used items Preparation and processing of	een dev	veloped 88.9	and use	ed for :	all pro	cesses,	includir	ng the
ST.10 ST.10.1 ST.10.2	startaretrainedintheseprocedures.Policies and procedures have bfollowing:Receiving,disinfection,andcleaning of used itemsPreparationandprocessingofsterile packs	een dev	xeloped = 88.9	and use	ed for =	all pro	cesses,	includir	ng the
ST.10 ST.10.1 ST.10.2 ST.10.3	starr are trained in these procedures. Policies and procedures have b following: Receiving, disinfection, and cleaning of used items Preparation and processing of sterile packs Appropriate inventory levels	een dev 8 8 5	xeloped = 88.9 88.9 55.6	and use	ed for a 11.1 11.1 33.3	all prod	cesses ,	includir	ng the
ST.10 ST.10.1 ST.10.2 ST.10.3 ST.10.4	starr are trained in these procedures. Policies and procedures have b following: Receiving, disinfection, and cleaning of used items Preparation and processing of sterile packs Appropriate inventory levels Emergency ("flash") sterilization	een dev 8 8 5 7	xeloped = 88.9 88.9 55.6 77.8	and use	ed for 11.1 11.1 33.3 22.2	all prod	cesses,	includir	ng the
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CU .2.1	Elevators			9	100				
CU .2.2	Operating room					9	100		
CU .2.3	Emergency room					9	100		
CU .2.4	Recovery room					9	100		
CU .2.5	Physician on-call room			9	100				
CU .2.6	Nurse manager's office			9	100				
CU .2.7	Waiting room					9	100		
CU .3	*Separate rooms available:								
CU .3.1	Family counseling room					9	100		
CU .3.2	Conference room	9	100						
CU .3.3	Staff locker room	9	100						
CU .4	Physical facility—internal:*				11				
CU .4.1	Medication station with drug refrigerator and locked narcotics cabinet	9	100						
CU .4.2	Staff toilet	9	100						
CU .4.3	Patient toilet	9	100						
CU .4.4	Hand-washing facility			9	100				
CU .4.5	Clocks					9	100		
CU .4.6	Televisions, radios, toys					9	100		
CU .4.7	Easy, rapid access to head of bed	9	100						
CU .4.8	12 or more electrical outlets per bed					9	100		
CU .4.9	2 or more oxygen outlets per bed					9	100		
CU .4.10	2 or more compressed air outlets per bed					9	100		
CU.4.11	2 vacuum outlets per bed					9	100		
CU .4.12	Heating, ventilation, and air conditioning	9	100						
CU.4.13	Fire safety					9	100		
CU .5	*Space between the beds should be 2.5 - 3 meters (7-9 feet) apart.			9	100				
CU .6	The facility ensures the presence of qualified staff 24 hours a day	4	44.4	5	55.6				
CU .6.1	The hospital has a plan of how to staff the intensive care room			8	88.9	1	11		
CU .7	All intensive care room staff are trained in CPR (cardiopulmonary resuscitation), emergency care, and the use of emergency equipment .	9	100						
CU .7.1	*Unit in facility with accredited pediatric residency program					9	100		
CU .7.2	*Unit provides clinical rotation for pediatric residents in pediatric critical care.			9	100				
CU .7.3	*Trained on PALS or advanced pediatric life support					9	100		
CU.8	The record of every patient receiv	ving int	ensive ca	re inclu	ides at l	east the	e follow	ing:	
CU.8.1	Time of arrival	9	100						
CU.8.2	Conclusions at termination of treatment	9	100						
CU.8.3	Patient's condition at discharge	9	100						
CU.8.4	Follow-up care instructions	9	100				t		

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CU.9	The hospital must have and use include at least the following:	clinical	guidelin	nes on i	ntensiv	e care.	The gu	idelines	must
CU.9.1	Emergency stabilization and treatment of chest pain	9	100						
CU.9.2	Emergency stabilization and treatment of shock	9	100						
CU.9.3	Emergency stabilization and treatment of polytrauma	9	100						
CU.9.4	Two additional guidelines for the most common diagnoses or presenting complaints	9	100						
CU.9.5	The clinical guidelines must be reviewed at least every two years and updated when indicated by current literature	9	100						
CU.10	Essential emergency equipment, as required by MOHP rules and regulations, is available and in good working order.	4	44.4	5	55.6				
CU .11	EDL medications and lifesaving drugs for emergency care must be available and secure at all times in each emergency room area.	2	22.2	7	77.8				
CU .12	Support diagnostic services are available 24 hours a day					9	100		
CU .13	All hospitals either have an ambulance or have an arrangement for ambulance services	9	100						
CU .14	The hospital ensures that the ambulance service meets the requirements of the MOHP rules and regulations.	9	100						
CU .15	The PICU should have an emer arrival of one or more seriously in	gency p njured p	olan to d patients.	leal wit The pla	h inter n shoul	nal disa d inclu	asters de the f	such a followin	s the
CU .15.1	A list of emergency responsible members, including physicians, nurses, and technicians for laboratory and radiology, and the list is posted in the critical room			5	55.6	4	44		
CU .15.2	The ability of the team to be able to reach the critical room within half an hour.	3	33.3	4	44.5	2	22.2		
CU .15.3	A list of referral centers	8	88.9			1	11.1		
CU .15.4	A plan to mobilize hospital staff and distribute responsibilities among them	1	11.1	6	66.7	2	22.2		
CU .16	PICU has a plan and process for responding to resuscitation emergencies anywhere in the hospital, Includes personnel who will respond; required emergency lifesaving drugs, including their location.types.			7	77.8	2	22.2		

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		-						
	and security; and required							
	equipment							
CU.17	Radiology services							
CU.17.1	Ease of access to other services such as X-ray	3	33.3	5	55.6	1	11.1	
CU.17.2	*Portable radiograph	9	100					
CU.17.3	*Computed tomography scan	9	100					
CU.17.4	*Ultrasound	9	100					
CU.17.5	Angiography*					9	100	
CU.17.6	Magnetic resonance imaging*					9	100	
CU.17.7	*Nuclear scanning					9	100	
CU.17.8	*Fluoroscopy					9	100	
CU.18	*Laboratory with microspecimen	capabi	lity					
CU.18.1	Blood gases Available within 15 min		-			9	100	
CU.18.2	Complete blood cell, platelet,and differential counts Available within 1 h	9	100					
CU.18.3	Urinalysis Available within 1 h	9	100					
CU.18.4	Clotting studies Available within 1 h	9	100					
CU.18.5	Chemistry profile (electrolytes, serum urea nitrogen, glucose, calcium, and creatinine) Available within 1 h			9	100			
CU.18.6	stain) Preparation available 24 h per day	9	100					
CU.19	*Monitoring equipment: Capabili	ity of co	ontinuou	s monit	oring			
CU.19.1	Electrocardiography, heart rate	9	100					
CU.19.2	Pulse oximetry	9	100					
*Mechanical	ventilation:							
MV.1	Central venous catheter infection	n Prevei	ntion of					
MV.1.1	Full barrier precautions	5	55.6	4	44.4			
MV.1.2	No routine replacement of catheters	6	66.7	3	33.3			
MV.2	Ventilator-associated pneumonia	Preve	ntion of					
MV.2.1	Gastric decontamination	6	66.7	3	33.3			
MV.2.2	No routine circuit change	7	77.8	2	22.2			
MV.2.3	Avoidance of heated humidifiers and use of heat-moist exchangers	8	88.9			1	11.1	
MV.2.4	Head of bed elevation	9	100					
MV.2.5	Educational program	8	88.9	1	11.1			
MV.2.6	Multidisciplinary team	1	11.1	6	66.7	2	22.2	
MV.3	Prevention of Vancomycin-resista	ant ente	erococci					
MV.3.1	Rectal VRE surveillance			1	11.1	8	88.9	
MV.3.2	Appropriate use of oral and intravenous vancomycin	5	55.6	4	44.4			
MV.3.3	Patient cohorting and equipment isolation	4	44.4			5	55.6	
MV.4	Prevention of Gastrointestinal ble	eeding						
MV.4.1	Appropriate use of stress	Ŭ						
	ulcer prophylaxis	8	88.9	1	11.1			

MV.5.1	Multidisciplinary team	1	11.1	4	44.4	4	44.4	
MV.5.2	Weaning protocols (nursing / respiratory therapist [RN/RT] driven)	6	66.7	2	22.2	1	11.1	
MV.5.3	Daily spontaneous breathing trial	9	100					
MV.5.4	Sedation protocols	7	77.8	2	22.2			
MV.5.5	Computer-directed weaning			2	22.2	7	77.8	
MV.5.6	Appropriate use of sedation, analgesia,		77.8	2	22.2			
MV.5.7	Time to enteral feeds	9	100					
MV.6	Prevention of Transfusion-related	l compl	ications					
MC.6.1	Appropriate transfusion trigger	7	77.8	1	11.1	1	11.1	
MV.6.2	Guideline development	6	66.7	2	22.2	1	11.1	
MV.7	Prevention of Medication errors	per ICU	J day					
MV.7.1	Average number of medications per ICU day	2	22.2	7	77.8			
MV.7.2	Computer decision support			1	11.1	8	88.9	
MV.8	Appropriate use of antibiotics via							
MV.8.1	Computer decision support			1	11.1	8	88.9	
MX7 9 2	Guidalina davalonment	5	55.6	3	333	1	111	

From this table infection control standards which are reported as low compliance (IC.2, IC.3, IC.4, IC.6.2, IC.6.8, IC.6.9, IC.7, IC.8, IC.9,IC.10, IC.12, IC.12.1, ST.7, ST.9).

Infection control standards which are reported as moderate compliance (IC.1,IC.1.1,IC.6.3,IC.6.6,IC.13,ST.2,ST.3,ST.4,ST.6,S T.8,ST.11).

Infection control standards which reported as high compliance: (IC.5.1, IC.5.2, IC.5.3, IC.5.4, IC.6.1,IC.6.4, IC.6.7, ST.1, ST.5,ST.10.1, ST.10.2, ST.10.3, ST.10.4, ST.10.6).

Structure and personnel standards which are reported as low compliance (CU.1.3,CU.2.2,CU.2.3,CU.2.4,CU.2.7,CU.3.1,CU.4.5, CU.4.6,CU.4.8,CU.4.9,CU.4.10,CU.4.11,CU.4.11,CU. 4.13,CU.7.1,CU.7.3,CU.12,CU.15.1,CU.17.5,CU.17.6, CU.17.7,CU.17.8,CU.18.1).

Structure and personnel standards which are reported as moderate compliance (CU.1.1,CU.1.2,CU.2.2,CU.2.5,CU.2.6,CU.4.4,

CU.5,CU.6.1,CU.7.2,CU.11,CU.15.2,,CU.15.4,CU.16, CU.17.1,CU.18.5).

Structure and personnel standards which are reported as high compliance (CU.3.2,CU.3.3,CU.4.1,CU.4.2,CU.4.3,CU.4.7,CU.4.1 2,CU.6,CU.7,CU.8.1,CU.8.2,CU.8.3,CU.8.4,CU,9.1,C U.9.2,CU.9.3,CU.9.4,CU.9.5,CU.10,CU.13,CU.14,CU. 15.3,CU.17.2,CU.3,CU17.4,CU.18.2,CU.18.3,CU.18.4, CU.18.6,CU.19.1,CU.19.2).

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Mechanical ventilation standards which are reported as low compliance (MV.3.1, MV.5.1, MV.5.5, MV.7.2, and MV.8.1).

Mechanical ventilation standards are which reported as moderate compliance (MV.2.6, MV.3.3,MV.7.1).

Mechanical ventilation standards which are reported as moderate compliance (MV.1.1,MV.1.2,MV.2.1,MV2.2,MV.2.3,MV.2.4,MV. 2.5,MV.3.2,MV.4.1,MV.5.2, MV.5.3, MV.5.4, MV.5.6, MV.5.7, MC.6.1,MV.6.2,MV.8.2).

There was a variation found in the compliance score regarding different shifts (audits):

At the level of infection control : AM shifts were reported as 50% low compliance and 50% moderate compliance, PM shifts were reported as 100% moderate compliance, Night shifts were reported as 66.6% low compliance and 33.3% moderate compliance, a week end shifts ware reported as100% moderate compliance.

At the level of sterilization: AM shifts were reported as 100% moderate compliance, PM shifts were reported as 33.3% moderate compliance and 66.6% high compliance, Night shifts were reported as 100% moderate compliance, A week-end shifts ware reported as 100% high compliance.

At the level of mechanical ventilation: AM shifts were reported as 100% high compliance, PM shifts were reported as 33.3% moderate compliance and 66.7% as high compliance, Night shifts were reported as 66.7% moderate compliance and 33.3% as

high compliance, And week-end shift was reported as 100% high compliance.

5. Discussion:

The systematic approach of assessing the quality of health care services is nearly a world wide accepted principle. The application variability from one place to the other remains a debatable problem either on the national or international level. This renders the presence of reference standards as well as clear auditing and surveillance procedures and accreditation mandatory all the way.

The current study was designed to evaluate PICUs at Benha University Hospitals through the national standards and some international standards of essential clinical impact.

In the current study, the overall assessment of infection control standards in all surveillance cycles (nine cycles) revealed that 6 audits were interpreted as "moderate compliance" (66.7 %) and 3 audits were interpreted as low compliance (33.3 %). As regards total sterilization standards, surveillances revealed, moderate compliance was achieved in 6 audits (66.7%) and high compliance was that achieved in 3 audits This revealed a variability of audits (33.3%). compliance probably due to different shifts and different staffs for each audit. Also we can say that PM shifts and weekend shift were better than AM shifts and night shifts. The explanation may be the over workload for AM and night shift's staff, that may lead to exhaustion and lack of concentration.. But by all the ways the presented findings are annoying, as it carries a degree of variability in performance that, for sure, will interfere with the clinical outcome of patients in PICU.

Although the rate of total infection control and "66.7%: sterilization compliance moderate compliance" may give a good impression about infection control practice in PICU at Benha University Hospital, yet the proper analysis of gained data revealed a critical situation in the practice of infection control (IC) and sterilization (ST) either in procedural or practical aspects. This is clearly demonstrated by the partial compliance of those standards related to the application of infection control program, those related to the integration of the program to cover all personnel in contact with PICU, as well as the standards related to the presence of qualified infection control physician and nurse.

Infection control guidelines are present (MOH); but there is lack of its systematic orientation, learning and application due to the absence of organizational infection control system or integrated program.

There is poor monitoring system either on the level of surveillance or data collection procedures, as

well as lack of management of infections outbreaks according to MOH national standards (Checko, 2000).

The repeated nine audits were interpreted for personnel and structure as having moderate compliance (100%). This gives the impression of certain stationary situation that may be related to structure design defect or certain system defect or absence.

The structure design, remain a point of debate, as in spite of the standard compliance yet some details are usually missing in accordance with some multinational standardization law. This was evident in facilities available in physician on-call room, nurse manager's office and hand-washing facility

The current study also could highlights many standards with poor compliance that need certain action plan for rapid remedy journey e.g, lack of room's labels, of many required facilities as clocks, televisions, radios, toys, and incomplete gas service facility, of fire and safety clear protocols and regular training of staff, and lack of some diagnostic facilities e.g. angiography, magnetic resonance imaging, nuclear scanning, fluoroscopy, and blood gases analysis is not available within 15 min (but actually available within 30-45 min).

Regarding personnel, the study declared that there is lack of training of staff including lack of staff with accredited pediatric residency program and absence of training on PALS (advanced pediatric life support).

The overall assessment of mechanical ventilation standards: The current study revealed variation through different audits that could be summarized as follow; high compliance of 6 audits (66.7%) and moderate compliance of 3 audits (33.3%) due to variation of different staff members, shifts, and audits timing.

6. Conclusion:

There are variable degrees of compliance with the national and international standards of infection control, sterilization, personnel and mechanical ventilation process in Benha University Hospital PICU.

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- 11. ABBREVIATIONS: APLS/PALS: Advanced pediatric live support, EDL: Essential drug list, ICC: Infection control committee, ICU : Intensive care unit, MOHP: Ministry of health and population, PICU: Pediatric intensive care unit, VRE: Vancomycin-resistant enterococci
- 12. WHO: World health organization.

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Detection of Community Acquired Methicillin Resistance Staphylococcus aureus among Staphylococcus aureus isolates.

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Abstract: The rates of Methicillin-resistant Staphylococcus aureus (MRSA) infections in the hospital, as well as the disease in the community, have continued to rise. Staphylococcal cassette chromosome mec (SCCmec) is a variable genetic element that contains the methicillin resistance determinant, mecA. SCCmec typing is one of the most important molecular tools available for distinction between community-acquired MRSA (CA-MRSA) and hospitalassociated MRSA (HA-MRSA) occurring on a worldwide basis. CA-MRSA has been reported to carry the loci for Panton Valentin leukocidin (PVL) in high frequency in association with the type IV SCCmec. The present study aimed to differentiate between HA-MRSA and CA-MRSA by detection of SCCmec and determination the prevalence of PVL gene among MRSA isolates. A total of 34 Staphylococcus aureus isolates were included in this study. Susceptibility of Staphylococci was determined by, Disc diffusion method including methicillin, oxacillin and cefoxitin discs. Penicillin Binding Protein (PBP_{2a}) Latex Agglutination test was done to detect the presence of PBP_{2a} responsible for methicillin resistance. In addition genotypic identification of MRSA was carried out by detecting mec gene by real time PCR. Conventional PCR was carried using different set of primers for the amplification of SCC mec for differentiating the HA-MRSA and CA-MRSA; moreover detection of PVL as virulence factor was also done. The antibiotic sensitivity of CA-MRSA ranged from 11.76% for ceftazidime to 47.06% for Imipenem, Erythromycin and Gentamycin; while the sensitivity of HA-MRSA ranged from 2.94% for Amoxicillin and Ampicillin/sulbactam to 29.41% for Amikin. Out of 34 S. aureus strains; 26(76.47%) isolates were found to be resistant to oxacillin disc, 30(88.24%) isolates were resistant to methicillin; and all strains were resistant to cefoxcitin disc. All MRSA strains were confirmed to be methicillin resistant by detection of mecA gene using real time PCR. Out of 34 MRSA strains 32 (94.12%) were PBP_{2a} producer. In the present study, though, the majority (25out of 34) of our strains were not SCC mee typable, yet among the nine typable strains the six hospital strains belonged to type II and III as reported in the literature and the three CA-MRSA belonged to the novel type V reported by other workers to be associated with CA-MRSA and the only PVL positive CA MRSA strain was untypable.

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Keywords: Detection; Community; Methicillin Resistance; Staphylococcus; aureus; Staphylococcus; aureus

1. Introduction:

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a serious threat to hospitalized patients globally and now represents a challenge for public health, as community-acquired infections appear to be on the increase in various regions and countries^(1,2) including North America ⁽³⁾, Australia ⁽¹⁾, Saudi Arabia ⁽⁴⁾ and Finland ⁽⁵⁾. Rising colonization rates lead to increased infection rates in the community and in hospitals. The consequence to the health care system is longer hospital stays and greater costs ⁽⁶⁾. Patient risks include significantly higher mortality and morbidity rates with invasive MRSA infection⁽⁷⁾.

MRSA strains produce penicillin binding protein2a, which possesses reduced affinities for binding to lactam antibiotics resulting in lactam resistance. The *mecA* gene, encoding PBP2a, is carried on a peculiar type of mobile genetic element inserted into the staphylococcal chromosome, designated staphylococcal cassette chromosome mec (*SCCmec*) elements. *SCCmec* elements typically share four characteristics: first, they carry the mec gene complex (mec) consisting of the methicillin resistance determinant mecA and its regulatory genes and insertion sequences; second, they carry the ccr gene complex (ccr) consisting of ccr genes that are responsible for the mobility of the element and its surrounding sequences; third, they have characteristic directly repeated nucleotide sequences and inverted complementary sequences at both ends; and last, they integrate into the 3_ end of an open reading frame (ORF), orfX⁽⁸⁾.

In *S. aureus*, three major mec complex classes have been described, and concerning the ccrAB locus, three major allotypes (ccrAB1 to ccrAB3)⁽⁹⁾ and one sporadic allotype (ccrAB4)⁽¹⁰⁾

have been identified. Recently, a new type of ccr gene complex, which consists of only one gene (ccrC) not closely, related to the ccrA or ccrB gene, was reported ⁽¹¹⁾. SCCmec carries other sequences that define the overall genetic organization of the resistance cassette. These regions may be used as targets for typing strategies ⁽¹²⁾, and polymorphisms within these regions, particularly in the region downstream of the ccrAB genes (the J1 region), define SCCmec subtypes or variants ⁽¹³⁾.

Based on the class of *mecA* gene complex and the type of *ccr* gene complex present, SCC*mec* cassettes are classified into seven major types⁽¹⁴⁾. The hospital-associated strains of MRSA (HA-MRSA) strains contain the larger type I, II, or III cassettes ⁽¹³⁾ , while the two smallest SCC*mec* types, SCC*mec* IV and SCC*mec* V, have been associated with community-associated MRSA (CA-MRSA)^(2,15).

Clinically, CA-MRSA usually causes skin and soft tissue infection. However, it can cause serious life-threatening conditions, which in addition to necrotizing pneumonia include necrotizing fasciitis, bloodstream infection, and septic shock (16). Reports have suggested that certain strains of CA-MRSA may be more virulent than HA-MRSA (17, 18). The expression of Panton-Valentine leukocidin (PVL), a two-component, pore-forming, cytolytic toxin that targets mononuclear and polymorphonuclear cells and causes cell death by necrosis or apoptosis, has been strongly associated with CA-MRSA ⁽¹⁷⁾. The PVL toxin consists of two synergistic proteins, LukS-PVL and LukF-PVL, encoded by the *pvl* genes *lukF* and *lukS*, which are carried on a temperate bacteriophage. (2, 19).

Clinicians are now faced with emergence of (CA-MRSA) strains that are genetically different from MRSA strains originating in the hospital. Moreover, with the recent trend and shift in epidemiology, CA-MRSA is now being found in hospitals and in some instances displacing classic (HA-MRSA)⁽²⁰⁾.

The present study aimed to differentiate between hospital acquired MRSA and community acquired MRSA by detecting different Staphylococcal Chromosomal Cassette mec types (*SCCmec*), and detecting the prevalence of Panton Valentine Leukocidin as virulence factor for CA-MRSA and determining whether its carriage could be used as a surrogate marker for CAMRSA

2. Material and Methods

I-Bacterial Isolates:

Thirty nine Staphylococcal isolates were included in this study. Data recording previous hospital admission during the 6 months ago were collected. I-Identification of staphylococcal isolates:

The staphylococcal isolates were identified morphologically and biochemically by standard laboratory procedures ⁽²¹⁾. The coagulase plasma test was performed on organisms exhibiting typical staphylococcal colony morphology to allow for discrimination of *S. aureus* from coagulase-negative staphylococci.

II-Antibiotic susceptibility testing:

Methicillin resistance (using Methicillin, Oxacillin and Cefoxitin) and susceptibility to different antibiotics were determined by the agar disk diffusion method ⁽²²⁾.

III-Detection of Penicillin Binding Protein 2a (PBP2a)

Latex Agglutination Test (Oxoid®) based on the agglutination of latex particles sensitized with monoclonal antibodies against PBP2a, was used according to the manufacturer's instructions ⁽²³⁾. Agglutination was visualized and was scored as positive, negative, or weakly positive.

IV- Polymerase Chain Reaction (PCR)

Staphylococcal DNA extraction:

Staphylococcal colonies were emulsified in 200 μ l sterile distilled water to produce a heavy suspension, and heated at 100°C for 15 min. then centrifuged at 14.000 rpm for 5 min.

PCR for *mecA* gene detection was performed using Real time (TaqMan) PCR.⁽²⁴⁾.

The primers and probe used were as follows: Forward primer, 5'TGCTAAAGTTCAAAAGAGTATTTATAACAA CA 3'; Reverse primer, 5'TGTGCTTACAAGTGCTAATAATTCACC 3'; and

Probe. 5' FAM-ATTATGGCTCAGGTACTGCTATCCACCCTCAA A-TAMRA 3'. The PCR mixture was prepared using TaqMan® Universal PCR Master Mix (2X) with final PCR mixture volume of 25 µl. Five µl of template DNA and 30 pmole of each primer and 7.5 pmol of probe were added to each test. A negative control was prepared by the addition of the same contents to the tube with water instead of the extract. Amplification was performed using MX3000P TM (Stratagene) Real Time PCR System programmed to hold at 95°C for 10 min for AmpliTag gold activation and 30 cycles of denaturation at 95°C for 15 sec and annealing and extension at 60°C for 1min with end point fluorescence detection.

PCR for detection of *SCCmec* gene and *PVL*: Polymerase chain reaction for detection of SCC*mec* and *PVL* gene was performed using genomic DNA from each MRSA isolate as template. Primers used to amplify the different *SCCmec* and *PVL* are listed in table (1). For SCCmec, 2 sets of primers were used. The first set (Oliviera primers) is designed to type *Sccmec* (I-IV) ⁽²⁵⁾ based on selected loci (A through F) upstream and downstream the *mecA* gene. Another set of primers (zhang) ⁽²⁶⁾ was used for detecting *SCCmec* type II, III and the newly described *SCC mec* type V.

PCR Conditions:

Eight μ l of DNA extract were amplified by PCR in a final volume of 25 μ l using 2x PCR master mix (Fermentas life sciences[®]) containing 0.05 units/ μ l of Taq DNA polymerase,50 picomol of each primer, PCR buffer, 2 mMMgCl2, 0.2mM of each dNTP. A negative control was prepared by the

addition of the same contents to the tube with water instead of the extract. Amplification was performed in a Perkin-Elmer 9600 thermocycler. For Oliveira primers the cycle program was performed with an initial denaturation for 5 min at 94°C, then 35 cycles of denaturation for 30 sec at 94°C, annealing for 30 sec at 53°C and extension for 1min at 72°C and final extension for 5 min. While for Zhang primers: the cycles begin with an initial denaturation step at 94°C for 5 min followed by 10 cycles of 94°C for 45 seconds, 65°C for 45 seconds, and 72°C for 1.5 min and then another 25 cycles of 94°C for 45 seconds, 55°C for 45 seconds, and 72°C for 1.5 min, and end with a final extension step at 72°C for 10 min. For amplification of PVL gene ⁽²⁷⁾: the cycle program was performed with an initial denaturation for 5 min at 94°C, then 35 cycles of denaturation for 40 sec at 94°C, annealing for 40 sec at 53°C and extension for 1min at 72°C and final extension for 10 min.

 Table (1): Primers used for amplification of SCCmec and PVL

Locus	Oliveira's	Oligonucleotide sequence (5'–3')	Amplicon	Specificity
	Primers_~~		size (bp)	(SCCmec type)
А	CIF2 F2	TTCGAGTTGCTGATGAAGAAGG	495	I
	CIF2 R2	ATTTACCACAAGGACTACCAGC		
В	KDP F1	AATCATCTGCCATTGGTGATGC	284	II
	KDP R1	CGAATGAAGTGAAAGAAAGTGG		
С	MECI P2	ATCAAGACTTGCATTCAGGC	209	II,III
	MECI P3	GCGGTTTCAATTCACTTGTC		
D	DCS F2	CATCCTATGATAGCTTGGTC	342	I,II,IV
	DCS R1	CTAAATCATAGCCATGACCG		
Е	RIF4 F3	GTGATTGTTCGAGATATGTGG	243	III
	RIF4 R9	CGCTTTATCTGTATCTATCGC		
F	RIF5F10	TTCTTAAGTACACGCTGAATCG	414	III
	RIF5R13	GTCACAGTAATTCCATCAATGC		
Zhang's P	rimers: ⁽²⁶⁾	Oligonucleotide sequence (5'-3')	Amplicon size (bp)	Specificity
Ту	pe II-F	CGTTGAAGATGATGAAGCG	398	SCCmec II
Ту	pe II-R	CGAAATCAATGGTTAATGGACC		
Ty	pe III-F	CCATATTGTGTACGATGCG	280	SCCmec III
Тур	be III-R	CCTTAGTTGTCGTAACAGATCG		
Ту	pe V-F	GAACATTGTTACTTAAATGAGCG	325	SCCmec V
Ту	pe V-R	TGAAAGTTGTACCCTTGACACC		
PVL prim	ers: ⁽²⁷⁾	Oligonucleotide sequence (5'-3')	Amplicon size (bp)	Specificity
P	VL-1	ATCATTAGGTAAAATGTCTGGACATGATCCA	433bp	PVL
P	VL -2	GCATCAASTGTATTGGATAGCAAAAGC		

Detection of PCR products was done using 2% agarose gel stained with ethidium bromide and using of the molecular weight markers (50 bp DNA ladder (Fermentas Life Sciences).

3. Results

1-Bacterial isolates and identification: Among the 39 *Staphylococcus* isolates, 34 (87.18%) were free coagulase positive by tube coagulase test.

2-Detection of methicillin resistance by Disc diffusion method: out of 34 *S. aureus* strains; 26(76.47%) isolates were resistant to oxacillin disc, 30(88.24%) to methicillin and all strains were resistant to cefoxitin disc (100%).

3-Antibiotic susceptibility: According to data regarding previous hospital admission, the 34 collected strains were divided into 18 CA-MRSA& 16 HA-MRSA strain. CA-MRSA was more sensitive, the sensitivity in CA-MRSA ranged from (22.22% for ceftazidime) to (88.89% for Imipenem, Erythromycin and Gentamycin); while the sensitivity in HA MRSA ranged from (6.25% for Amoxicillin and Ampicillin/sulbactam) to (62.5% for Amikin). All MRSA were sensitive to vancomycin.

4-Detection of *mec A gene*: All the 34 strains were *mecA* gene positive. The presence of *mecA* gene

detected by real time PCR was considered to be the gold standard in evaluating the 3 disc diffusion methods to identify MRSA.

5- Gene expression (PBP2a Production) among the 34 *mecA* positive: out of 34 MRSA strains 32 (94.12%) were PBP_{2a} producer while 2 (5.88%) were PBP_{2a} non producer. The 2 PBP_{2a} non producers isolates were CA MRSA.

6- SCC mec Typing:

Out Of the 16 HA-MRSA: 5 isolates were *SCCmec* type II (Two by Oliveira primers and three by zhang primers) and only one was detected as type III. While out of the 18 CA-MRSA Three isolates belonged to the newly described Type V reported to be associated with CA MRSA. The remaining 25 MRSA strains could not be typed.(Fig 1(A-E)

7-Detection of PVL: only one strain (CA-MRSA) out of the 34 MRSA was positive for Panton Valentine leukocidin (PVL) by PCR. (Fig 2)



Fig (1A): Ethidium bromide stained agarose gel showing a single band of 284 bp specific for SCC mec type II at lanes 3 and 7 Lane 5 shows 50 bp DNA ladder. Fig (1B): show a single band of 243 bp specific for SCC mec type III at lanes 11. Lane 5 shows 100 bp DNA ladder.



Fig (1C): Ethidium bromide stained agarose gel showing a single band of 398 bp specific for SCC mec type II at lanes 6 and 10. Lane 8 shows 50 bp DNA ladder. Fig (1D): show a single band of 325bp specific for SCC mec type IV at lanes 3 and 3 Lane 12 shows 50 bp DNA ladder



Figure 1E: SCC mec typing of the 34 MRSA

4. Discussion:

Methicillin resistance of *S. aureus* remains to be a significant problem. Rapid and accurate determination of methicillin resistance is important for initiation of appropriate antimicrobial therapy. Misdiagnosing this resistance leads to treatment failures and spread of infections with these resistant strains.

Phenotypic techniques as disk diffusion and microdilution methods are employed in routine laboratories for the detection of methicillin resistance. However, these methods are often not entirely reliable at detecting some strains that harbor the *mecA* gene ⁽²⁸⁾. Identification of the *mecA* gene remains the most reliable method of detecting MRSA isolates, however not all laboratories can include molecular biology techniques in their routine clinical practice.

In this study cefoxitin disk diffusion tests was 100% sensitive for MRSA detection. Alternatively, only 76.47% and 88.24% isolates were resistant to Oxacillin and methicillin respectively. These results were in accordance with those of several studies ⁽²⁸⁻³⁰⁾. This means that disc diffusion testing using cefoxitin disc is far superior to most of the currently recommended phenotypic methods and is now an accepted method for the detection of MRSA by many reference groups including CLSI⁽³¹⁾.

Identification of MRSA, is more accurate by either directly detect the gene encoding the methicillin resistance determinant (*mecA*) or its product (PBP2a)⁽³²⁾. MRSA-Screen test is a rapid and simple to perform method. Many studies reported that the accuracy of MRSA screen latex agglutination test for detection of PBP2a approaches the accuracy of PCR and more accurate than susceptibility testing methods with sensitivity of 97%-100% and a Fig 2: Ethidium bromide stained agarose gel showing a single band of 433 bp specific for PVL at lane 5. Lane 9 shows 100 bp DNA ladder

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two (5.88%) PBP2a non producer isolates (false negative) were identified. Other authors ^(35, 36) reported that false-negative results may occur with MRSA isolates with low oxacillin MICs (4 or $8 \mu g/ml$) due to production of smaller amounts of PBP_{2a} or the failure to express the gene phenotypically.

MRSA infection can be categorized into 2 distinct groups: HA-MRSA and CA-MRSA. The community isolates are distinctly different from the hospital strains both epidemiologically and microbiologically ⁽³⁷⁾. Both CA-MRSA and HA-MRSA are resistant to traditional anti-staphylococcal -lactam antibiotics. However, CA-MRSA isolates tend to be more susceptible to other antibiotics (including to sulfa drugs, tetracyclines) than are HA-MRSA ⁽³⁸⁾, and their narrow spectrum of resistance is solely due to determinants harbored on genetic elements present on the SCC⁽³⁹⁾. In this study, most of the 18 CA-MRSA isolates were susceptible to tetracyclines (83.3%) Erythromycin (88.9%), Gentamycin (88.9%), co-trimoxazole (77.8%), Ofloxacin (83.3%). On the other hand, the 16 HA-MRSA showed a wide spectrum of drug resistance ranging from 81.25% (ofloxacin, gentamycin and tetracyclines) to 68.7 % (erythromycin) and 56.2% (co-trimoxazole). Comparable results were obtained by various authors $^{(40, 41)}$. Kaplan $^{(40)}$ noted that most of the CA-MRSA isolates are susceptible to most antibiotics, while Anbumani *et al.*, ⁽⁴¹⁾ reported that 250 MRSA isolated from different clinical specimens were multi-drug resistant.

SCC*mec* typing is one of the most important molecular tools available for understanding the epidemiology and strain relatedness of MRSA ⁽²⁶⁾.In the present study, 2 sets of primers were used in an

attempt to type our MRSA strains. The first set was chosen according to Oliviera typing scheme designed to type *Sccmec* (I-IV)⁽²⁵⁾, and using six primer pairs only 3 of the 16 HA MRSA were typable by Oliviera primers. Two belonged SCC mec type II (amplified locus B) and one SCC mec type III. (amplified locus F), and none of the 18 CA MRSA could be typed by this scheme. As Oliveira's assay has limitations in detecting the newly described SCCmec type V, misclassifying them as type III, while failing to discriminate type IV into subtypes a, b, c, and d⁽²⁶⁾. Also, because of difficulties in assay optimization another trial of typing the SCC mec was carried using Zhang set of primers unique and specific for SCCmec types. So, another 3 out of 16 HA MRSA were classified as SCCmec type II, moreover another three belonged to SCCmec type V among 18 CA MRSA. So using both typing methods only 9 out of the 34 MRSA were typable. Similar data were noticed by others $^{(25,42)}$. Oliviera *et al*, $^{(25)}$ reported that, 8% of their isolates were non-typeable for SCCmec by their primers used in this study. Also, Shore et al, ⁽⁴²⁾ used also two typing methods the first for amplification of the ccr and mec genes, and the second method of Oliveira and de Lencastre, used in the present study and (50%) out of their 172 isolates harbored two apparently different SCCmec elements when tested by both typing methods. They suggested that PCR amplification and sequencing of the entire SCCmec element was necessary for the complete characterization of the SCCmec elements harbored by isolates with ambiguous multiplex patterns.

The differentiation between the typical HA-MRSA and CA-MRSA strains based on epidemiologic definitions becomes difficult, along with molecular distinction based on the SCCmec type is beginning to blur. ⁽⁴³⁾ In the present study, though, the majority (25out of 34)of our strains were not SCCmec typable, yet among the nine typable strains the six hospital strains belonged to type II and III as reported in the literature and the three CA-MRSA belonged to the novel type V reported by other workers to be associated with CA-MRSA

CA-MRSA has been reported to carry the loci for PVL in high frequency, and to be associated with the type IV (*SCCmec*). ⁽⁴⁴⁾ In the present study, the only PVL positive CA MRSA was untypable. This was contrary to that noted by Berglund *eta;1*⁽⁴⁵⁾ who detected PVL genes in 66% of the CA-MRSA isolates. However, Holms *etal*; ⁽⁴⁶⁾ revealed that the PVL genes are carried by a relatively low number (1.6%) of *S. aureus* isolates from their clinical laboratories, indicating an unequal distribution of the genes encoding PVL among their strains. In addition, the overall proportion of MRSA isolates carrying *pvl* was 1.8% among the 1,389 MRSA isolated from

Ireland. 7.5% of these isolates were CA-MRSA strains, of which only 6.7% carried *pvl* genes and the carriage of *pvl* was not restricted to CA-MRSA ⁽⁴⁷⁾. Similarly, 78% of CA-MRSA isolates referred to a central reference facility were *pvl* negative and 25% of *pvl* positive isolates in this group were HA. Additionally, Ko *et al*; ⁽⁴⁸⁾ was not able to detect *pvl* gene, in any of their MRSA isolates. Moreover, Kim *et al*; ⁽⁴⁹⁾ reported that none of their Korean CA-MRSA isolates contained the PVL genes. These findings agree with reports that carriage of *pvl* cannot be used as a sole marker for CA-MRSA ⁽⁴⁷⁾.

In the present study , the combination of *SCCmec* typing , in addition to the detection of PVL was not sufficient to discriminate between HA and CA MRSA due to the near absence of PVL among the CA MRSA and the limited capacity of the *SCC mec* typing methods among our strains.

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Metabolic Effects of Estrogen and / or Insulin in Ovariectomized Experimentally Diabetic Rats

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Abstract: Postmenopausal adverse metabolic changes increase cardiovascular risk and impair quality of life. This study was planned to evaluate the benefits gained by estradiol treatment alone and insulin treatment alone versus combination of these two hormonal therapies on the metabolic derangements accompanying estrogen deficiency with diabetes. Rats were divided into five groups: control sham-operated group, ovariectomized streptozotocin diabetic group (OVX-STZ), estradiol-treated OVX-STZ diabetic group that received daily subcutaneous injection of estradiol (50µg/kg) for 4 weeks, insulin-treated OVX-STZ diabetic group that received daily subcutaneous injection of insulin (10 or 20 IU/kg) for 2 weeks and combined estradiol-treated, insulin-treated OVX-STZ diabetic group. Rats in all groups were subjected to determination of body weight, body mass index (BMI), blood glucose, plasma levels of total cholesterol, triglycerides, HDL-c, insulin, estradiol, leptin and malondialdehyde (MDA). In addition, in vitro glucose uptake by the diaphragm and glucose output by both kidneys were measured. Insulin treatment alone increased peripheral glucose uptake, reduced renal gluconeogenesis, normalized blood glucose and plasma total cholesterol, decreased triglycerides, LDL-c and atherogenic index and increased HDL-c. Plasma MDA was reduced however, still higher than controls. Estrogen therapy alone lowered blood glucose although not fully normalized, increased peripheral glucose uptake and decreased renal gluconeogenesis, reduced plasma triglycerides. total cholesterol, LDL-c and MDA and elevated HDL-c as compared to untreated groups, yet, not completely normalized. Combined estradiol and insulin therapy returned all measured parameters towards control values with complete normalization of peripheral glucose uptake and blood glucose levels as well as plasma triglycerides, HDLc, atherogenic index and MDA, while BMI, gluconeogenesis, total cholesterol and LDL-c approached control values although still not fully normalized. It is concluded that either insulin or estrogen therapy provided only partial improvement of the metabolic error of estrogen deficiency with diabetes while the best cure was found with combined estradiol and insulin therapy which achieved successful optimization of weight gain, reduced adiposity, tight glycemic control, alleviated dyslipidemia and normal oxidative state. Thus, insulin therapy together with hormonal replacement therapy as a coadiuvant might be the most advisable line of treatment in postmenopausal diabetic women.

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1. Introduction:

Many postmenopausal women live with diabetes mellitus; however, little information is available about how the changes that occur around the time of menopause might uniquely affect management of diabetes mellitus⁽¹⁾.

As noticed in the literature, postmenopausal diabetic patients encountered the reality of increased atherogenic lipid profile ⁽²⁾, as well as redox imbalance ⁽³⁾, and thereby increased cardiovascular risk factors. However, the role of hormonal replacement therapy in reversing such threats remains a subject of debate.

There are conflicting data on the effect of hormonal replacement therapy in postmenopausal diabetic women. On one hand, Borissova *et al.* ⁽⁴⁾ recommended the use of hormonal replacement

therapy in diabetic postmenopausal women because of its favorable effect. On the other hand, Feher and Isaacs ⁽⁵⁾ denied the potential benefits of hormonal replacement therapy. In addition, some evidence suggests that estrogen therapy may improve insulin sensitivity ^(6,7).

It was therefore, worthwhile to investigate the effect of estrogen treatment alone and insulin treatment alone versus combination of these two hormonal therapies on modulating the metabolic error presented by altered glycemic and lipid metabolism in estrogen deficient experimentally diabetic rats.

2. Materials and Methods Experimental animals:

The present study was performed on 92 female Wistar rats. The rats were purchased from Research

Institute of Ophthalmology (Giza) and were maintained in Physiology Department Animal House, under standard conditions of boarding and given regular diet consisting of bread, vegetables and milk with free access to water.

Experimental protocol:

Rats were divided into 5 groups:

- Group 1: Sham-operated control rats (Sham) (n =16). Rats in this group were subjected to all surgical procedures of ovariectomy except for removal of ovaries. Two weeks later, they received a single i.p. injection of 0.05 M citrate buffer (1 ml/kg) and were studied 2 weeks later.
- Group 2: Ovariectomized streptozotocin diabetic rats (OVX–STZ) (n =32). Rats in this group were subjected to bilateral ovariectomy ⁽⁸⁾. Two weeks after the operation, they received a single i.p. injection of STZ (Sigma, USA) in a dose of 40 mg/kg ⁽⁹⁾ and were studied after 2 weeks.
- Group 3: Estradiol-treated ovariectomized STZ diabetic rats (E_2 +OVX–STZ) (n =16). Rats in this group were subjected to bilateral ovariectomy and on the next day, they received subcutaneous injection of estradiol (Sigma, USA) in a dose of 50 µg/kg, daily 6 days/week for 4 weeks ⁽¹⁰⁾. Two weeks later, they received a single i.p. injection of STZ then studied 2 weeks later.
- Group 4: Insulin-treated ovariectomized STZ diabetic rats (Insulin+OVX–STZ) (n =13). Rats in this group were subjected to bilateral ovariectomy. Two weeks later, they were rendered diabetic then received daily subcutaneous injection of insulin (Lilly, Egypt) in a dose of 10 or 20 IU/kg/day, 6 days/week for 2 weeks according to their blood glucose level ⁽¹¹⁾.
- Group 5: Estradiol-treated, Insulin-treated ovariectomized STZ diabetic rats (E_2 + Insulin + OVX – STZ) (n =15). Rats in this group were subjected to bilateral ovariectomy followed on the next day by subcutaneous injection of estradiol for 4 weeks. Two weeks after the operation, rats were rendered diabetic then treated with daily subcutaneous injection of insulin for 2 weeks.

Experimental Procedure

On the day of experiment, overnight fasted rats were tested for re-estimation of blood glucose level via rat tail sampling using blood glucose test strips. Then, rats were weighed and anaesthetized i.p with thiopental sodium 40 mg/kg (Sandoz, Austria). The length of the anaesthetized rat was measured from tip of the nose to the anus to calculate body mass index (BMI) according to the following equation:

BMI = Body weight (kg) / length (m^2).

A midline abdominal incision was made and blood

samples from the abdominal aorta were collected into two plastic tubes. One tube containing sodium fluoride / potassium oxalate, for immediate determination of blood glucose concentration. The other tube containing EDTA, for preparation of plasma which was stored at -20 °C for later determination of plasma leptin, estradiol, insulin, malondialdehyde (MDA) and lipid profile (total cholesterol, triglyceride and high density lipoprotein-cholesterol (HDL-c).

Immediately after blood collection, both kidneys were exposed and excised from the renal pedicle and placed separately in ice cold Krebs Ringer solution for 10 minutes after which cortical kidney slices were prepared for *in vitro* estimation of glucose output by both kidneys. Then, the diaphragm was exposed, quickly and carefully excised then immediately placed in ice cold Krebs' solution for *in vitro* estimation of glucose uptake by diaphragm.

Methods:

I. Biochemical studies

Blood glucose was determined by glucose oxidase enzymatic colorimetric technique, according to the method described by Trinder ⁽¹²⁾, using kits supplied by Stanbio, USA. Plasma lipids (triglycerides, total cholesterol and HDL-cholesterol) were measured by quantitative enzymatic colorimetric methods ^(13, 14) using kits supplied by Stanbio-laboratory, Texas, U.S.A. Plasma LDL-cholesterol and atherogenic index (AI) were calculated according to Friderwald *et al.* ⁽¹³⁾ and Grundy *et al.* ⁽¹⁵⁾ respectively as follows:

LDL-c = Total cholesterol – (triglyceride/5 + HDL-c)

Atherogenic index = Total cholesterol HDL-c

Malondialdehyde (MDA) was assayed in plasma, according to the method of Esterbauer and Cheeseman ⁽¹⁶⁾, as thiobarbituric acid reactive substance.

Plasma estradiol was estimated bv radioimmunoassay using RIA estradiol kit, supplied by Immunotech, France. Plasma insulin was measured quantitatively by immunoradiometric assay using Insulin (e) IRMA kit supplied by Immunotech, Czech Republic. The measurements of plasma estradiol and insulin levels were performed in Middle Eastern Regional Radioisotope Center for Arab Countries, Cairo. Plasma leptin was determined quantitatively by Elisa technique using leptin (sandwich) ELISA kit supplied by DRG, Germany. The measurement was performed in Oncology Diagnostic Unit, Biochemistry Department, Faculty of Medicine, Ain Shams University.

II. *In vitro* determination of glucose uptake by the diaphragm was performed according to the method described by Mohamed *et al.*⁽¹⁷⁾.

III. *In vitro* determination of glucose output by both kidneys

Cortical kidney slices from both right and left kidneys were separately used to measure glucose output by the kidney according to the method described by Randall ⁽¹⁸⁾ with few modifications.

Statistical Analysis:

Results were statistically analyzed by one-way ANOVA for differences between means of different groups. Further analysis was made by least significant difference (LSD) to find inter-groupal significance. For differences within the same group, analysis was determined by Student's't' test for paired data. Correlations and lines of regression were calculated by linear regression analysis using the Least Square Method. All data were analyzed using SPSS statistical package (SPSS Inc.) version 8.0.1. A probability of P<0.05 was considered statistically significant.

3. Results:

Body weight and body mass index (BMI) changes

In table (1), treatment of OVX-STZ diabetic rats with estrogen alone, insulin alone as well as with combined estradiol and insulin resulted in significant increase in final body weights as compared to their initial values (P<0.001).

The final body weight was significantly increased in insulin-treated OVX-STZ rats as compared to OVX-STZ, estradiol-treated and sham-operated groups (P<0.001, P<0.001 & P<0.01 respectively). Estradiol-treated OVX-STZ diabetic group demonstrated non significant difference as compared to OVX-STZ group. Combined treatment with estradiol and insulin showed non significant difference as compared to OVX-STZ and estradioltreated groups but significant decrease as compared to insulin-treated group (P<0.001).

BMI showed significant increase in insulin-treated OVX-STZ diabetic rats as compared to OVX-STZ (P<0.001) and estradiol-treated (P<0.001) groups. Combined treatment with estradiol and insulin showed significant decrease in BMI as compared to insulin-treated OVX-STZ rats (P<0.001) while non significant difference as compared to both OVX-STZ and E_2 +OVX-STZ groups.

Glycemic parameters

As shown in table (2), OVX-STZ diabetic rats demonstrated significant increase in blood glucose (P<0.001) as well as in glucose output by both right and left kidneys (P<0.001) but significant decrease in glucose uptake by the diaphragm (P<0.001) as compared to sham control rats.

Treatment with either estradiol alone or insulin alone as well as combined therapy with estradiol and

insulin resulted in significant decrease in blood glucose (P<0.001) and glucose output by right and left kidnevs (P<0.001) and significant increase in glucose uptake by the diaphragm (P<0.001) as compared to OVX-STZ rats. Compared to estradiol treatment, insulin treatment caused significant decrease in blood glucose (P<0.001) and in right and left kidneys glucose output (P<0.05 & P<0.001 respectively) but a similar effect on diaphragmatic glucose uptake. Rats receiving combined treatment with estradiol and insulin demonstrated significantly lower blood glucose (P<0.001) and glucose output of both kidneys (P<0.001) together with significantly higher glucose uptake (P<0.001) than rats receiving estradiol treatment alone as well as significantly higher glucose uptake (P<0.001) than rats receiving insulin treatment alone.

Lipid profile

OVX–STZ group showed significant increase in plasma triglycerides (P<0.001), total cholesterol (P<0.001), LDL-c (P<0.001) and atherogenic index (P<0.001) but significant decrease in HDL-c (P<0.001) as compared to sham-operated group.

In estradiol-treated OVX–STZ diabetic rats, plasma triglycerides, total cholesterol and LDL-c showed significant decrease as compared to OVX-STZ group (P<0.001) but significant increase as compared to sham control group (P<0.05, P<0.001 and P<0.001, respectively), the atherogenic index was decreased as compared to OVX-STZ group (P<0.001) but was insignificant from sham control group whereas plasma HDL-c was increased as compared to OVX-STZ group (P<0.001) but decreased as compared to sham control rats (P<0.05).

In insulin-treated OVX-STZ diabetic rats, plasma triglycerides was lower than in OVX-STZ group (P<0.001) but higher than in estradiol-treated (P<0.01) and sham control (P<0.001) rats, total cholesterol was lower than in both OVX-STZ (P<0.001) and E₂-OVX-STZ (P<0.01) rats and plasma HDL-c was higher than in OVX-STZ group (P<0.001) but lower than in sham-control (P<0.001) and estradiol-treated (P<0.001) groups. Plasma LDL-c and atherogenic index were lower than in OVX-STZ group (P<0.001) but were higher than in sham-control group (P<0.001).

In combined estradiol and insulin treated ovariectomized diabetic group, plasma triglycerides showed significant decrease when compared to non-treated OVX-STZ (P<0.001) and insulin-treated groups (P<0.001), total cholesterol was decreased as compared to OVX-STZ rats (P<0.001) but was increased as compared to sham-operated control (P<0.01) and insulin-treated (P<0.05) groups, HDL-c was increased when compared to OVX-STZ (P<0.001) and insulin-treated ovariectomized diabetic (P<0.001)

groups, LDL-c was decreased as compared to OVX-STZ group (P<0.001) but was insignificant from either estradiol treatment or insulin treatment alone and the atherogenic index was significantly decreased as compared to OVX-STZ (P<0.001) and insulin-treated (P<0.05) groups but was insignificant from estradiol-treated and sham control groups (table 3).

Plasma levels of malondialdehyde, estradiol, insulin and leptin are shown in figure 1.

Correlation studies among the experimental groups:

Correlations of plasma estradiol levels and plasma insulin levels versus other parameters in untreated ovariectomized STZ-diabetic (OVX-STZ), estradiol-treated ovariectomized diabetic (E_2 +OVX–STZ), insulin-treated ovariectomized diabetic (Insulin+OVX–STZ) and estradiol-treated, insulin-treated ovariectomized diabetic (E_2 + Insulin + OVX – STZ) groups are displayed in tables 4 & 5; figures 2 &3.

		Initial BW	Final BW	BMI
		(g)	(g)	(Kg/m²)
Sham	(16)	181 <u>+</u> 11.5	214 <u>+</u> 13.3 *	5.6 <u>+</u> 0.2
OVX -STZ	(32)	195 <u>+</u> 5.0	195 <u>+</u> 4.8	5.1 <u>+</u> 0.09 ^a
$E_2 + OVX - STZ$	(16)	174 <u>+</u> 5.7	$188 \pm 6.6 * a$	5.2 <u>+</u> 0.12
Insulin+ OVX – STZ	(13)	212 <u>+</u> 4.6	$258 \pm 5.9 * a b c$	6.3 ± 0.13^{abc}
E ₂ +Insulin +OVX – ST	Z (15)	164 <u>+</u> 5.1	$198 \pm 5.2 * d$	5.1 ± 0.06^{ad}

Number in parenthesis is the number of rats in each group. Results are expressed as means \pm SEM. *: Significance by Student's't' test at P<0.05 from respective baseline value for paired data. a: significance from sham group by LSD at P<0.05. b: significance from OVX-STZ group by LSD at P<0.05. c: significance from E₂ + OVX-STZ group by LSD at P<0.05. d: significance from insulin + OVX-STZ group by LSD at P<0.05.

	Blood glucose (mg/dl)	Glucose uptake by diaphragm (mg/g/90min)	Glucose output by right kidney (mg/g/hr)	Glucose output by left kidney (mg/g/hr)
Sham	78 <u>+</u> 2.6	6.7 <u>+</u> 0.2	3.4 <u>+</u> 0.15	3 <u>+</u> 0.2
	(16)	(16)	(15)	(16)
OVX – STZ	480 <u>+</u> 15.4 ^a	1.3 <u>+</u> 0.04 ^a	9.1 <u>+</u> 0.32 ^a	9.1 <u>+</u> 0.23 ^a
	(32)	(32)	(32)	(32)
$E_2 + OVX - STZ$	213 <u>+</u> 4.2 ^{a b}	3.9 <u>+</u> 0.18 ^{a b}	6.1 <u>+</u> 0.22 ^{a b}	6.5 <u>+</u> 0.25 ^{a b}
	(16)	(16)	(16)	(16)
Insulin + OVX –	97 <u>+</u> 4.5 ^{b c}	3.7 <u>+</u> 0.23 ^{a b}	4.9 <u>+</u> 0.19 ^{a b c}	4.8 ± 0.32^{abc}
STZ	(13)	(13)	(13)	(13)
E ₂ +Insulin + OVX-	94 <u>+</u> 2.7 ^{b c}	$6.4 \pm 0.23^{b c d}$	4.3 ± 0.11 ^{b c}	4.4 ± 0.18^{abc}
STZ	(15)	(15)	(15)	(15)

 Table (2): Blood glucose, glucose uptake by the diaphragm, glucose output by the right kidney and glucose output by the left kidney in the studied groups.

Number in parenthesis is the number of rats in each group. Results are expressed as means \pm SEM. a: significance from sham group by LSD at P<0.05. b: significance from OVX-STZ group by LSD at P<0.05. c: significance from E₂ + OVX-STZ group by LSD at P<0.05. d: significance from insulin + OVX-STZ group by LSD at P<0.05.

plasma	ow ucinsi	ty hpoprotein choic	steror (EDE c) a	na atherogenie m	dex (71) in the st	iuicu și oupsi
		TG	TC	HDL-c	LDL-c	AI
		(mg/dl)	(mg/dl)	(mg/dl)	(mg/dl)	
Sham	(16)	46 ± 1.40	85 ± 1.1	52 ± 0.93	24 ± 1.19	1.6 ± 0.05
OVX –STZ	(32)	107 ± 1.9^{a}	129 ± 1.1^{a}	26 ± 0.57^{a}	82 ± 1.33^{a}	5.1 ± 0.12^{a}
E ₂ +		56 ± 1.14 ^{a b}	98 ± 2.4^{ab}	48 ± 2.1 ^{a b}	39 ± 1.03 ^{a b}	2.1 ± 0.05^{b}
OVX-STZ	(16)					
Insulin +		69 ± 3.89 ^{a b c}	88 ± 1.4 ^{b c}	37 ± 1.19 ^{a b c}	37 ± 1.28 ^{a b}	2.4 ± 0.06^{ab}
OVX-STZ	(13)					
E ₂ + Insulin -	ł	52 ± 1.11^{bd}	94 ± 1.5 ^{abd}	49 ± 0.98^{bd}	34 ± 1.37 ^{a b}	1.9 ± 0.03 ^{b c}
OVY_ST7	(15)					

 Table (3): Plasma triglycerides (TG), plasma total cholesterol (TC), plasma high density lipoprotein cholesterol (HDL-c), plasma low density lipoprotein cholesterol (LDL-c) and atherogenic index (AI) in the studied groups.

Number in parenthesis is the number of rats in each group. Results are expressed as means \pm SEM. a: significance from sham group by LSD at P<0.05. b: significance from OVX-STZ group by LSD at P<0.05. c: significance from E₂ + OVX-STZ group by LSD at P<0.05. d: significance from insulin + OVX-STZ group by LSD at P<0.05.



Figure (1): Mean values of plasma levels of malondialdehyde (MDA), estradiol, insulin and leptin in the different groups studied.

¶: P<0.05, #: P<0.01, *: P<0.001, a: significance from sham group by LSD. b: significance from OVX-STZ group by LSD. c: significance from E_2 + OVX-STZ group by LSD. d: significance from insulin + OVX-STZ group by LSD.

Parameters	OVX – STZ + Sham				Ir OV	nsulin + /X –STZ +	E ₂ +Insulin + OVX–STZ +	
			Sham			Sham	Sham	
	r	Р	r	Р	r	Р	r	Р
Blood glucose	-0.88	< 0.001	-0.86	< 0.001	-0.57	< 0.01	-0.63	< 0.01
-	(28)		(24)		(22)		(23)	
BMI	0.22	NS	0.13	NS	-0.63	< 0.01	0.36	NS
	(28)		(24)		(22)		(23)	
Plasma TG	-0.91	< 0.001	-0.62	< 0.01	-0.54	< 0.05	-0.46	< 0.05
	(28)		(24)		(22)		(23)	
Plasma TC	-0.85	< 0.001	-0.42	< 0.05	-0.20	NS	-0.72	< 0.001
	(28)		(24)		(22)		(23)	
Plasma HDL-c	0.88	< 0.001	0.49	< 0.05	0.82	< 0.001	0.30	NS
	(28)		(24)		(22)		(23)	
Plasma LDL-c	-0.86	< 0.001	-0.76	< 0.001	-0.81	< 0.001	-0.72	< 0.001
	(28)		(24)		(22)		(23)	
Atherogenic	-0.87	< 0.001	-0.75	< 0.001	-0.82	< 0.001	-0.61	< 0.01
index	(28)		(24)		(22)		(23)	
Plasma Insulin	0.86	< 0.001	0.80	< 0.001	0.59	< 0.05	0.70	< 0.01
	(19)		(19)		(17)		(18)	
Plasma Leptin	0.76	< 0.001	0.28	NS	0.50	NS	0.06	NS
•	(18)		(15)		(15)		(17)	
Plasma MDA	-0.83	< 0.001	-0.68	< 0.001	-0.87	< 0.001	-0.34	NS
	(28)		(24)		(22)		(23)	
esis is the number	r of obse	rvations.		NS: no	ot significa	nt		

Table (4): Correlation coefficients (r) between plasma estradiol levels and other parameters in the groups of rats studied.

In parenthesis is the number of observations.

Table (5): Correlation coefficients (r) between plasma insulin levels and other parameters in the groups of rats studied.

	OV	X – STZ		$E_{2} +$	In	sulin +	$E_2 + 1$	Insulin +
	+		OV	OVX – STZ		X –STZ	OVX-STZ	
Parameters	1	Sham		+		+		+
				Sham	5	Sham	S	ham
	r	Р	r	Р	r	Р	r	Р
Blood glucose	-0.91	< 0.001	-0.90	< 0.001	-0.49	< 0.05	-0.69	< 0.001
	(28)		(24)		(22)		(22)	
Glucose uptake	0.97	< 0.001	0.89	< 0.001	0.79	< 0.001	0.47	< 0.05
by diaphragm	(28)		(24)		(22)		(22)	
Glucose output	-0.86	< 0.001	-0.74	< 0.001	-0.48	< 0.05	-0.47	< 0.05
by right kidney	(28)		(24)		(22)		(22)	
Glucose output	-0.94	< 0.001	-0.83	< 0.001	-0.61	< 0.01	-0.65	< 0.01
by left kidney	(28)		(24)		(22)		(22)	
BMI	0.19	NS	-0.09	NS	- 0.62	< 0.01	-0.03	NS
	(28)		(24)		(22)		(22)	
Plasma TG	-0.91	< 0.001	-0.66	< 0.001	-0.59	< 0.01	-0.40	NS
	(28)		(24)		(22)		(22)	
Plasma TC	-0.93	< 0.001	-0.56	< 0.01	-0.23	NS	-0.48	< 0.05
	(28)		(24)		(22)		(22)	
Plasma HDL-c	0.94	< 0.001	0.31	NS	0.62	< 0.01	0.29	NS
	(28)		(24)		(22)		(22)	
Plasma LDL-c	-0.94	< 0.001	-0.71	< 0.001	-0.54	< 0.05	-0.49	< 0.05
	(28)		(24)		(22)		(22)	
Atherogenic index	-0.93	< 0.001	-0.68	< 0.001	-0.61	< 0.01	-0.51	< 0.05
-	(28)		(24)		(22)		(22)	
Plasma Leptin	0.88	< 0.001	0.61	< 0.01	0.75	< 0.001	0.55	< 0.05
	(27)		(20)		(20)		(20)	
Plasma MDA	-0.87	< 0.001	-0.71	< 0.001	-0.65	< 0.01	-0.21	NS
	(28)		(24)		(22)		(22)	

In parenthesis is the number of observations.

NS: not significant



Figure (2): Graphs showing relationships between plasma estradiol and blood glucose in
(A) Untreated OVX-STZ diabetic group + Sham-operated controls (r = -0.88, P<0.001, n= 28).
(B) Estradiol-treated OVX-STZ diabetic group + Sham-operated controls (r = -0.86, P<0.001, n=24).
(C) Insulin-treated OVX-STZ diabetic group + Sham-operated controls (r = -0.57, P<0.01, n=22).
(D) Combined estradiol and insulin-treated OVX-STZ diabetic group + Sham-operated controls (r = -0.63, P<0.01, n=23).



Figure (3): Graphs showing relationships between plasma estradiol and plasma MDA in

(A) Untreated OVX-STZ diabetic group + Sham-operated controls (r = -0.83, P<0.001, n=28).

(B) Estradiol-treated OVX-STZ diabetic group + Sham-operated controls (r = -0.68, P<0.001, n=24).

(C) Insulin-treated OVX-STZ diabetic group + Sham-operated controls (r = -0.87, P<0.001, n=22).

(D) Combined estradiol and insulin -treated OVX-STZ diabetic group + Sham-operated controls (r = -0.34, NS, n=23).
4. Discussion:

The present work studied the extent by which either estradiol treatment or insulin treatment can improve the metabolic derangements in estrogen deficiency with diabetes and finally evaluated the gain achieved by combination of these two hormonal therapies.

Estradiol treatment was found to exert an obvious effect in improving adiposity, dyslipidemia, oxidative stress and hyperglycemia, yet still diabetic; however, with the exception of adiposity, none of the metabolic parameters were back to control values. Insulin therapy successfully normalized blood glucose, reduced dyslipidemia and oxidative stress; however, estradiol therapy was more effective in reducing oxidative stress and adiposity compared to insulin therapy. Combination of both hormonal therapies in estradiol-treated. insulin-treated ovariectomized diabetic group offered the most beneficial effect as it successfully resulted in optimization of weight gain, reduced adiposity, good glycemic control, reduced dyslipidemia and normal oxidative state.

In insulin-treated group, blood glucose was normalized, diaphragmatic glucose uptake was increased as compared to untreated group but did not differ from estradiol-treated group and was still significantly lower than the control values. Renal glucose output was decreased as compared to untreated and estradiol treated groups but still significantly higher than control values. It seems that insulin therapy exerted better euglycemia compared to estrogen therapy and this effect is probably via decreased gluconeogenesis rather than complete normalization of peripheral glucose uptake which could be due to insufficient dose or duration.

More favorable lipid profile was seen following insulin control of hyperglycemia, where it had prominent effect in lowering total cholesterol towards normal as well as improving triglycerides. HDL-c. LDL-c and AI as compared to untreated ovariectomized diabetic group but not back to control values. When compared to estradiol-treated group significant increase in triglycerides and decrease in total cholesterol as well as HDL-c was recorded together with non significant difference in LDL-c and AI. Insulin is the major hormone to inhibit hydrolysis of triglycerides (TG) in adipose cells into glycerol and free fatty acids (FFAs). Together with glucose, insulin may also play a role in the reesterification of FFAs in adipose cells, promoting TG storage ⁽¹⁹⁾. By these mechanisms, insulin lowers plasma FFA levels which are the major substrates for TG synthesis in the liver, and they stimulate apoB secretion from the liver ⁽²⁰⁾. Thus, the ability of insulin to suppress plasma FFA concentrations plays a major role in hepatic VLDL TG synthesis and secretion, apoB secretion

⁽²⁰⁾, and plasma VLDL cholesterol and apoB concentrations ⁽²¹⁾.

Following insulin treatment body weight and BMI were significantly high, this could be attributed to insulin role in promoting lipogenesis as well as its inhibitory role on protein catabolism (positive nitrogen balance) at the same time, the persisted estradiol deficiency promoted central fat deposition, so it is most likely that the increase in weight is mostly due to fat deposition as confirmed by increased BMI. The associated increase in plasma leptin level seems to be insulin-induced as insulin was reported to stimulate leptin mRNA and protein expression, due to increased activation of the leptin gene promoter ⁽²²⁾.

Suppression of oxidative stress was one of the goals achieved by insulin-mediated metabolic control. Hyperglycemia is the major causative factor of raised oxidative stress in diabetes, but in this group of rats, the insulin treated ovariectomized diabetic, estrogen deficiency also participates; SO insulin alone administration of reversed hyperglycemia with no obvious effect on estradiol values which led to partial improvement of oxidative stress, yet not back to normal values. It is worthmentioning here that, estradiol treatment showed more effective role in reducing oxidative stress than do insulin treatment although none of them succeeded to get it back to normal control values.

Unexpectedly, insulin treatment did not enhance aromatase activity and thereby estradiol production, instead estradiol values were very close to untreated ovariectomized diabetic rats. This could be attributed to the duration of insulin therapy or less than needed dose further investigations are needed to clarify this point.

Estradiol replacement was another line of treatment in the present study, the overall judgment about how much estradiol treatment was able to improve the metabolic state is that, although most of the measured parameters were significantly improved when compared to untreated ovariectomized diabetic rats, yet they were still significantly different from control values, a result that make it possible to say that hormonal replacement therapy per se is not sufficient to maintain good glycemic and metabolic control in postmenopausal women who developed diabetes.

The significant decrease in weight gain following estradiol treatment seems to be a combined effect of estradiol and diabetes, where besides the diabetic effect on lipid and protein metabolism, estradiol goes through different pathways to achieve an obesity reducing property, where it is known that estrogen decreases central adiposity ⁽²³⁾. This represents a

major health problem because abdominal visceral fat shows greater lipolytic sensitivity than femoral and gluteal subcutaneous fat due to fewer inhibitory alpha adrenergic receptors in abdominal regions and greater alpha adrenergic receptors in gluteal and femoral regions ⁽²⁴⁾.

Estradiol treatment was associated with significant rise in plasma leptin, this could be another mechanism decreasing food intake and increasing energy expenditure and thereby decreasing body weight. Our results are in agreement with Shimizu et al. (25) who experimentally proved that estradiol supplementation reversed the inhibitory effect of ovariectomy on ob gene expression and circulating leptin levels and that serum leptin concentration was higher in premenopausal women than in men and postmenopausal women; this allowed them to declare that estrogen increased in vivo leptin production in rats and human subjects. However, studies evaluating the effect of estrogen replacement therapy on leptin levels were contradictory, with some authors supporting a stimulatory effect of estrogen whereas others suggested that estrogens do not have a stimulatory action on leptin in humans (26, 27).

Estradiol relation to ghrelin hormone provides another mechanism explaining the decrease in weight gain in this group, where, estradiol was found to attenuate the orexigenic action of ghrelin ⁽²⁸⁾ and the drop of estrogen levels following ovariectomy was associated with an increase in plasma ghrelin that was associated with increased food intake, body weight, and hypothalamic neuropeptide Y ⁽²⁸⁾. From the above mentioned estradiol hormonal interactions, it could be suggested that estrogen, indeed provides protection against weight gain.

Better glycemic control was clearly demonstrated after estradiol treatment where renal gluconeogenesis was significantly decreased and skeletal glucose uptake was significantly improved and thereby blood glucose level was significantly reduced compared to untreated group, yet, their improvement was not to such an extent that their values were back to normal. This finding is consistent with results obtained from ovariectomized diabetic group that showed tendency to hyperglycemia. All these positive changes towards better glycemic control could be attributed to the roles played by estradiol both at β -cells of the pancreas as well as peripheral insulin-sensitive tissues. It was found that 17β -estradiol at physiological concentrations protects pancreatic βcells against lipotoxicity, oxidative stress, and apoptosis ⁽²⁹⁾. Estrogens and their receptors (ER) have direct effects on islet biology. The estrogen receptor ER α , ER β , and the G-protein coupled ER are present in β -cells and enhance islet survival. They, also, improve islet lipid homeostasis and insulin biosynthesis ⁽²⁹⁾. *In vivo*, estradiol treatment rescued streptozotocin-induced β -cell apoptosis, helped sustain insulin production, and prevented diabetes. *In vitro*, in mouse pancreatic islets and β -cells exposed to oxidative stress, estradiol prevented apoptosis and protected insulin secretion. Estradiol protection was through activation of ER α as it was partially lost in β cells and islets treated with an ER α antagonist ⁽³⁰⁾.

At the peripheral insulin-sensitive tissues, estradiol is known to modulate insulin sensitivity and, consequently, glucose homeostasis. Estradiol was found to counteract the effects of hyperglycemiainduced downstream of the insulin receptors, as well as modulating insulin receptors tyrosine phosphorylation ⁽³¹⁾. Some data, also, revealed a surprising role for estradiol in regulating energy metabolism and opened new insights into the role of the two estrogen receptors, ER α and ER β , in this context. New findings on gene modulation by ERa and $ER\beta$ of insulin-sensitive tissues indicate that estradiol participates in glucose homeostasis by modulating the expression of genes that are involved in insulin sensitivity and glucose uptake ⁽³²⁾. Therefore, drugs that can selectively modulate the activity of either ER α or ER β in their interactions with target genes represent a promising frontier in diabetes mellitus coadjuvant therapy.

Skeletal muscle glucose uptake is maintained by one of the isoforms of the glucose transporter family, GLUT4 ⁽³³⁾. The rate of glucose transport into muscle cells is limited by the concentration of GLUT4 at the cell surface. The enhancement of diaphragmatic glucose uptake following estradiol administration shown in this study could partly be attributed to the elevated glucose transporter–4 protein expression. It was discovered that estradiol acts on ER α and not ER β to enhance glucose transporter-4 expression ⁽³⁴⁾.

The antiatherogenic cardiovascular protective properties of estrogen emerge from its ability to direct the lipoprotein metabolism towards higher HDL-c and lower LDL-c. It is obvious from our results that treatment with estradiol gave the ovariectomized diabetic females the opportunity of lowering their plasma lipids, and this effect was prominent by the significant improvement of all the measured lipid parameters as compared to non treated group of rats.

The mechanisms of such effects are mediated through the ability of estradiol to stimulate the expression of LDL-receptor gene and increasing the number of LDL receptors. This effect was confirmed by Parini *et al.* ⁽³⁵⁾ who found that treatment of rats with ethyl estradiol for 7 days increased the hepatic LDL receptor protein and mRNA level from 3 to 4 folds. Also, Distefano *et al.* ⁽³⁶⁾ reported that the expression of LDL-receptor gene is stimulated by

estrogen *in vivo*. Also, our results came in accordance with Granfone *et al.* ⁽³⁷⁾ and Walsh *et al.* ⁽³⁸⁾ who reported that estrogen replacement is effective in decreasing LDL-c and apo B concentrations and increasing HDL-c and apo A concentrations in dyslipidemic postmenopausal women. LDL-c internalizes into the cells through the process of LDL-receptor mediated endocytosis accelerating LDL catabolism. The expression of LDL-receptor on the cell surface is a function of various hormone regulated transcription of the receptor gene; βestradiol is considered the prime hormonal regulator of LDL-receptor expression ⁽³⁹⁾.

Another protective mechanism offered by estradiol in lowering LDL-c and increasing HDL-c is through depression of hepatic lipase enzyme activity (⁴⁰⁾, thereby decreasing HDL-c catabolism. The elevated levels of HDL-c following estradiol treatment seen in our study is in agreement with Walsh *et al.* (⁴¹⁾ who demonstrated that HDL elevation following oral estradiol treatment in postmenopausal women is dose dependent. Estradiol fatty acyl esters incorporate into HDL and enhance the atheroprotective properties of HDL by mediating the initial steps of reverse cholesterol transport (⁴²⁾.

Another benefit offered by estrogen replacement therapy in ovariectomized diabetic rats was the significant decrease in plasma MDA, adding extra evidences that estrogen is more than a sex hormone and that its loss after menopause requires therapeutic intervention. It was found that lipid peroxidation is most often induced by reactive oxygen species, $O_2^$ and H₂O₂, and this damage is inhibited by superoxide dismutase and catalase. The remaining amount of damage appears to be caused by peroxyl radicals. It was documented that estradiol (E₂) alone collectively blocks 70% of such damage ⁽⁴³⁾.

This indicates that estradiol is acting as a chainbreaking antioxidant, inhibiting the effect of H₂O₂, $\cdot O_2^-$ and hydroperoxyl radicals. E₂ action in inhibiting DNA damage supports this view. E₂ prevented DNA strand breaks in a manner similar to the free radical scavengers; catalase and superoxide dismutase. E₂ might be preventing oxidative DNA damage to some extent by inhibiting the formation of superoxides ⁽⁴³⁾. Our results came in accordance with Kii et al.⁽⁴⁴⁾ who demonstrated that acute treatment with 17betaestradiol showed a protective effect against ischemiareperfusion injury through its antioxidant effects. Also, Hernández *et al.* ⁽⁴⁵⁾ showed that the lower plasma total antioxidant status, reduced thiol groups and the increase in plasma lipoperoxides presented in ovariectomized animals were reestablished with the estrogen treatment. It is to be noted here that estradiol treatment although successfully lowered plasma MDA yet it was still higher than control values, this

is attributed to that these rats are diabetic which represents other cause of oxidative stress.

Combination of estradiol and insulin therapy was last line of treatment investigated in the ovariectomized diabetic rats. Rats received combined treatment of estradiol and insulin showed normal pattern of gaining weight with lower BMI; this proves that normal hormonal state is essential to direct metabolism towards optimal balance between opposite metabolic pathways as lipolysis versus lipogenesis, glycolysis versus gluconeogenesis and positive versus negative nitrogen balance. It seems that the lowered adiposity seen in these rats with normal weight gain reflects the anabolic action of insulin on protein metabolism. The additive action of estradiol and insulin supplementation on leptin hormone was manifested by higher levels of this hormone in combined hormone-treated than in estradiol-treated alone or insulin-treated alone ovariectomized diabetic rats. Estradiol enhanced leptin gene expression ⁽²⁵⁾ and also insulin stimulated leptin mRNA and protein expression ⁽²²⁾. The resulted increase in leptin values ultimately contributes in optimization of body weight.

Combined estradiol and insulin treatment successfully normalized blood glucose through optimization of skeletal muscle uptake of glucose as well as renal gluconeogenesis. It is believed that estradiol not only increases insulin secretion from pancreatic β -cells but also enhanced insulin sensitivity in target organs ⁽⁴⁾, an effect that is most obviously seen in our study through increasing diaphragmatic glucose uptake. Also, the significant positive correlation between plasma estradiol and insulin hormones seen in untreated ovariectomized diabetic rats showed less positivity following combined estradiol and insulin treatment implying an effect of estradiol on insulin hormone and thereby glycemic control.

Insulin and estradiol teamed up to shift lipid profile towards more protective healthy picture, actually triglycerides, HDL-c and atherogenic index values were completely normalized. In addition, significant negative correlations existed between both estradiol and insulin and each of total cholesterol, LDL-c and atherogenic index, whereas, the significant positive correlation between both levels of plasma estradiol and insulin with HDL-c were abolished. These results, therefore, encourages postmenopausal diabetic women not only to control their blood glucose but also to start hormonal replacement therapy.

Moreover, marked reduction of oxidative stress was recorded, following estradiol and insulin therapy, as the lipid peroxide marker, MDA, was significantly reduced to control values. Also, the significant negative correlations between plasma levels of each of estradiol and insulin and levels of MDA, found in the ovariectomized STZ-diabetic group were abolished by combined treatment.

In explanation of these results, it could be suggested that estradiol by its antioxidant effect reduces reactive oxygen species and insulin by its hypoglycemic effect reduces blood glucose and thereby glycosylation and autooxidation of glycation products⁽⁴⁶⁾.

In view of the aforementioned data, insulin therapy alone which induced euglycemia, reduced dyslipidemia and oxidative stress yet their values were still higher than controls; while estrogen therapy in ovariectomized diabetic rats succeeded to some extent in reducing hyperglycemia, dyslipidemia and oxidative stress vet not completely normalized. Thus, from the above discussion it is clear that postmenopausal diabetic women suffer the consequences of both estradiol and insulin deficiency, and trials to reverse any of them although to some extent improved the condition yet they were not optimally successful. Combination of both estradiol and insulin therapies in ovariectomized diabetic rats showed synergistic effects and was superior in terms of optimization of blood glucose, peripheral glucose uptake and oxidative marker, plasma malondialdehyde together with alleviation of dyslipidemia. This denotes that insulin therapy together with hormonal replacement therapy as a coadjuvant might be the most advisable line of treatment in postmenopausal diabetic women. Therefore, we may advocate the use of estrogen replacement therapy side by side with insulin in postmenopausal diabetic women to achieve better glycemic control and thereby improving the general metabolic state.

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The Investigation perception of Agricultural Extension Agents about affective factors on effectiveness of Agricultural Advisory Services Companies in Iran

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Abstract: The main purpose of this study was to investigation perception of extension agents about problems that Agricultural Advisory Services Companies (AASC) faced with them and reduced their effectiveness. Statistical population of the study consisted of Agricultural extension agents (N=381). By using the formula Cochrane, sample size was determined at 179. Questionnaire was the data instrument. The appearance and content validity of questionnaire was obtained by comments of extension experts. Reliability coefficient of the questionnaire 0.83 was obtained by Cronbach alpha. The results showed that AASC Increasing farm management skills of farmers. AASC also increasing the specialty of extension services to farmers. By using exploratory factor analysis barriers are classified in four factors, including Policy-making, Socio – cultural, Infrastructural and economical factors. These factors could explain 61% of variance in reduced effectiveness of AASC Services among farmers.

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Keywords: Perception, Agricultural extension agents, AASC, Effectiveness, Iran

1. Introduction

The Human population growth and increasing urbanization are putting a massive pressure in demand for food production in developing countries. Agricultural extension service can play an important role in increasing production and improving the quality of food produced by farmers (Hosseini et *al*, 2008). Extension, in general terms, is a function that can be applied to various areas of society. It operates in the industrial, health and education sectors, as well as agricultural and rural development. Agricultural extension operates within a broader knowledge system that includes research and agricultural education (Rivera, 2001).

Shaffril et al (2010) stated that agriculture is generally used as a tool to overcome poverty and unemployment problem in the world. In Iran agriculture is one of the most important economic sectors. The agricultural sector provides about a quarter in employment human force and 33% of exports in Iran. Also Iran has advantages in producing almost of agricultural goods (Manzoralibadi, 2009; Kohansal, 2010). Despite the important role of agriculture in food production, employment and exports, unfortunately rural community is faced with numerous problems. These involves issues such as poverty, unequal income distribution, unemployment, low productivity,

unskilled labor force and lack of appropriate extension system in the agricultural sector (Merzaiy et al., 2008). To increase agricultural production level, farmers needs to have access in extension services. But despite the long term of starting agricultural extension programs, in Iran, numerous of farmers have not been covered by public extension services. Because extension agents not access on all farmers in Iran. FAO statistics in Africa show that two of every three farmers do not have access to public services. This ratio in Asia is three of every four people, Latin America six of the every seven people, and five of the six people in the Middle East (Zamanipour, 2001; Lashgarara and Peshbien, 2004; Shekara, 2001). Agricultural extension services have been widely criticized due to inability to perform assigned functions and the absence of expected effectiveness and efficiency. Therefore, major changes such as structural reform, decentralization and privatization are essential to agricultural extension (Birner et al., 2009). Rivera (2008) argues that the agricultural extension in the public sector has been seriously criticized in many countries due to its inefficiency.

Rivera (2001) described the environment of agricultural extension was changed. A large number and variety of reforms have already been put in place worldwide. Since the early 1990s, there has been large worldwide decrease trends of the public involvement in the financing and management of agricultural extension services. There are various programs for the withdrawal and changes from decentralization of public extension services to commercialization or privatization (Rivera 2000).

Increasingly privatized, agricultural information has in fact become a price commodity (Buttel 1991; Rivera 2000). The commoditization of agricultural information is a major factor to change of public sector agricultural extension and the development of private extension services. Wolf (1998) believes this change towards information commoditization reflects the privatization of information and agricultural industrialization. One result is that farmers, mainly in high-income and middle-income countries, have begun to pay for extension services.

The role of public extension in transferring of technology to farmers has been questioned (Rasouliazar and Fealy, 2008). Public extension service in Iran faced with many obstacles that influence effectiveness of its services. So that Ahmadi (2005) pointed out that negligence to capital and human factors in agriculture, lack of covering comprehensive stockholders in agricultural extension, limited resources, manpower and funds in public extension system, dearth of fitness levels of staffing and professionalism to the needs of farmers are the main problems existing in agricultural knowledge and information system of Iran (Ahmadi, 2005). Other countries have different strategies to cover reduces and weaknesses of public extension (Mandler, 2010). Policy-makers in these countries have reached an important consensus to find other alternatives to public extension. One of these alternatives is the use of private companies to provide information and transfer technologies to farmers. Privatization of extension services refers to the services that extension staff in private organizations provides for those farmers who pay the cost of services (Hanchinal et al, 2001; Saravanan, 2001; Anderson, 2004).

Amirani (2001) argues that the solution of these problems would be possible through consulting services. Privatization of extension services has been introduced as one of the suitable strategies of restructuring the public extension system obstacles (Christoplos, 2008). Application of AASC to enhances access to financial facilities and marketing would increases the production level and improves performance of farmer's production (Smith and Munoz, 2002).

Benin et al (2007) stated that the main purpose of AASC was to increase agricultural productivity by strengthening the technical skills of farmers, and to monitor their activities through delivery information and consulting services to them. Anderson (2008) believes that consulting services are critical elements which provide key information and improve the welfare of farmers. He believes that the term consulting services refers to a complete set of agricultural organizations that facilitate and support participation of farmers and solve their problems in agricultural sector with transmission of information, skills and techniques.

Transferring from public extension service to agricultural consulting services could enhance productivity in agricultural farms (Arbenz, 2004). Application of consultancy companies is meant to achieve goals such as: increased efficiency and faster economic growth, agricultural development and a decrease in government intervention in the executive of decisions (Rasouliazar and Fealy, 2008). One of the important challenges that extension planners are faced with it, that is how to increase level of effectiveness and efficiency of technical consulting services (Chipeta, 2006). Designing effective extension systems have always been indispensible to system designer and policy-makers. Sundberg (2005) asserted that effective counseling services have significant impact on performance and efficiency of farmers.

The Ministry of agriculture in 2010 reported that West Azerbaijan province has a high capacity in agriculture production (Anonymous, 2008). But due to its geographical situation (being mountainous) and scattered villages farmers have limited access to public extension services, and a large number of farmers are deprived of obtaining extension services. Accordingly, using AASC can solve many of this structural problems and bottlenecks of public extension system. Based on the statistics of Agricultural Engineering Organization over 1900 AASC have been formed and established in Iran. The largest of AASC was based in West Azerbaijan and informed in 162 AASC companies (Anonymous, 2009). Considering the important role of AASC in providing extension services to farmers, it is necessary to identify obstacles that influence the effectiveness of these companies. These obstacles will reduce the effectiveness of AAS services (Barret et al., 2005). Agricultural extension agents have good experience in delivery extension services to farmers: therefore, the main goal of this study was identify obstacles factors that influence on effectiveness of AASC in Iran.

Government plays an important role in agricultural and rural development, therefore by identifying these factors, policy-makers and extension planners could have suitable strategies to solve their problems and enhancement effectiveness agricultural advisory services.

2. Material and Methods

The methodology used in this study involved a combination of descriptive and quantitative research and included the use of correlation and descriptive analysis as data processing methods. A questionnaire was developed based on interviews and relevant literature. The questionnaire included both open-ended and fixedchoice questions. A 5-point Liker scale ranging from 1 (strongly disagree) to 5 (strongly agree) was applied as a quantitative measure. Content and face validity were established by a panel of experts consisting of faculty members and experts in the Ministry of Agriculture. A pilot study was conducted with extension workers who had not been interviewed before the earlier exercise of determining the reliability of the questionnaire for the study. Cronbach's Alpha coefficient was 0.83 which demonstrated that the questionnaire was highly reliable. The research population included extension agents that employed in public extension systems in the Provinces of West Azerbaijan (N = 381). By using a Cochran formula, sample size was determined at 179. Factor analysis statistical methods were used, with the aid of Statistical Package of social Science (SPSS).

3. Results

Table 1 shows the demographic profile and descriptive statistics. The results of descriptive statistics indicated that the all of extension agents were men. The results showed that the average age of consultants was 39 years, with 13 years work experience. The majority of them (66.5%) were Bachelor of Science (Table1).

Table 1: Descriptive statistics of Extension agen	ts
---	----

Variable		f	%	Mean	SD.
S					
Age				38.92	6.68
Age				12.63	7.40
experien					
ce					
	Diploma	34	19		
Educatio	_				
n	Bachelor of	11	66.5		
	science	9			
	Master of	26	14.4		
	Science				

Advantages of AASC from viewpoint of Extension agents

Priorities viewpoints of extension agents about advantages of advisory services companies indicated that improving farm management skills of farmers was ranked as the first advantage (CV=0.233), also increasing the specialty of extension services (CV=0.234) was ranked as the 2th, and Increasing bargaining power of farmers for acquire information and services (CV=0.250) was in the next rank. Other findings are shown in Table 2.

Table 2: Advantages of AAS from Extension
agents perception

Advantages	Mean	SD	(CV)	Rank			
Increasing farm	4.12	0.92	0.223	1			
management skills of							
farmers							
increasing the specialty	4.14	0.97	0.234	2			
of extension services							
Increasing bargaining	3.83	0.96	0.250	3			
power of farmers for							
acquire information							
and services							
Increasing participation	4.06	1.03	0.253	4			
of farmers in planning							
and decision making							
process							
Increasing	4.04	1.05	0.259	5			
responsibility of							
extension consultants							
Improving access to	4.06	1.06	0.261	6			
Demand-Driven							
extension services							
Increasing the	3.75	1.01	0.269	7			
extension services to							
farmers							
Providing rural	3.72	1.03	0.276	8			
development fields							
Increasing quality of	3.57	1.03	0.288	9			
extension services							
Reducing cost in public	3.57	1.06	0.296	10			
sector							
Strongly agree=5,Agree	=4, Inte	ermediate=	3, Disa	gree=2,			
Strongly disagree=1							

Factor analysis is a general term for some multivariate statistical methods whose main purpose to reduce the number of variables in a data set into smaller number of dimension. This method examines internal correlation in a large number of variables, and eventually is explained in the form of general operating and restricted categories. Performed calculations display that internal coherence is proportional (KMO=0.89) and the Bartlett statistics is significant (χ^2 = 1831.82 and P=0.000). To determine

the number of factors, special amount and percentage of variance was used.

Table 3 shows the classification of the factors into four latent variables using the ordinal factor analysis. The basic idea of factor analysis is to find a set of latent variables that contain the same information. The variables were named into policy-making, socio-cultural, structural and economical factors. The classic factor analysis assumes that, both observed and the latent variables are continuous variables. But, in practice, the observed variables are often ordinal. Results show that the four factors explain 61% of the total variance in reduces effectiveness of AASC (Table 3).

The first factor referred to policy-making factors with a principal component of (3.105), which is higher than other factors, explains 17.24% of the total variance. The second factor was named sociocultural factors. This factor according to the specific amount 2.94 could explain 16.37% of total variance. The third factor was named structural factors. These factors according to the specific amount 2.63 could explain 14.05 % of total variance. The fourth factor was named economical factor. This factor according to the specific amount 2.403 could explain 13.37% of total variance. Between these factors, policy-making factors can cause the most to explain the variance in the reduce effectiveness of AASC from viewpoint of agricultural extension agents. So should increasing the effectiveness of AASC and necessary will be done some practices and pointed to items mentioned by policy-making and extension planners (table 3).

	Rotation sums of squared loading							
Facto	Total	% of	Cumulative %					
r		Variance						
1	3.105	17.22	17.22					
2	2.948	16.37	33.59					
3	2.632	14.05	47.64					
4	2.403	13.34	60.98					

Table 3:	Total	variance	explained

Table (4) explained variance by each of the factors reducing effectiveness of AASC. As it can be seen policy-making factors, socio – cultural factors, structural factors and economical factors were identified as major components that effecting in the effectiveness of AASC from viewpoint of Agricultural extension agents.

Factor	Variables	Varia	%
name		nce	of
		by	Var
		facto	ian
		r (%)	ce
	Lack of services to	0.585	
	marginal farmers		
Policy-	Lack of subsidies and	0.709	
making	grants from the		
factors	government for AASN and		
	farmers		
	Lack of recognition signed	0.737	17.2
	of AASC		24
	Lack of executive power	0.564	
	of AASC		
	Lack of monitoring and	0.554	
	evaluation activities of		
	AASC		
	Unhealthy competition	0.552	
Socio-	between advisory agencies		
cultura	Lack of trust in advisory	0.543	16.3
1	services companies		7
factors	Illiteracy of farmers	0.626	
	Little attention to the needs	0.771	
-	of small farmers		
~	Lack of cooperation from	0.605	
Structu	other institutions and		
ral	organizations(public) with		
factors	AASC	0 = 1 0	110
	Lack of expert and	0.710	14.0
	technical personnel in		5
	AASC	0.705	
	Lack of coordination in the	0.735	
	activities of public and		
	private extension services	0 (9 1	
	Lack of necessary facilities	0.081	
	(venicie) by the		
	Distribute of agriculturel		
	farm		
	141111		
	High cost of consultancy	0.705	
	services	0.705	
Econo	Lack of credit and	0.842	133
mical	financial power of farmers	0.072	4
factor	interior power of farmers		Ŧ
140101	Low performance in vield	0.647	
	produce	0.017	
L	1 1	1	

Table 4: Classification of factors I	by using ordinal
factor analysis	

4. Discussions

The role of AASC in agriculture development has been the subject of intense debate among policy-makers in agriculture sector in Iran. The improving farm management skills and increasing the specialty of extension services were the main advantages of using AASN. Also increasing bargaining power of farmers for acquires information and services were identified as other advantages of counseling services. Conclusion in which there is that AASN has great capacity for agricultural development and enhancing the effectiveness of extension services to farmers. Therefore considering the cases and factors on the effectiveness of AASN is very crucial. These findings also accord with studies such (Shekara, 2001; Saravanan, 2001; Anderson, 2008; Sadighi, 2004; Rezvanfar and Arabi, 2006).

Results from factor analysis shows that some components such as policy-making, socio cultural factors, infrastructural components and economical factors influence effectiveness of AASC. The most important factors were policy-making components. There are issues such as lack of livelihood and subsistence farmers to advisory services, lack of subsidies and financial assistance from the government to provide services to marginalized groups such as women and rural youth, lack of executive power of advisory companies, and lack of credit the signing of AASC. On the other hand lack of assessment and a monitoring sector has caused many problems for AASC. Undoubtedly providing appropriate plans and programs of government can enhance AASC. Use of specialized assessment and evaluation committees to review the performance of consultants and the increase of the executive power of AASC through obtaining funding, and recognition of the sign companies could reduce the problems that are classified as obstacles factors in policy. Research findings are in line with these studies (Rezaei, 2005; Rashidpour et al., 2010; Beglarian et al., 2001).

The other component that acts on effectiveness of AASC among farmers was sociocultural factors. Unhealthy competition between AASC, Lack of trust in advisory services companies, the low educational levels of farmers and the problem of having access to women in order to deliver advisory services are considered as socio-cultural barriers. In order to solve this problem, AASC should increase their technical competences about farmers' issues in order to increase farmers' confidence and trust toward them. Also it is highly crucial that female consultants provide services to rural women. This finding is also pointed by several authors (Rasouliazar and Fealy .2008; Ahmadi, 2005; Waddington et al., 2010; Pamela t al., 2003; Rasouliazar et al., 2010).

Factors such as lack of cooperation with AASC from other organizations (public organization), lack of specialists in the AAS structure, tasks interference with public extension sector, lack of communication infrastructure (roads and ICT), and also shortage of vehicles and equipment have been identified as barriers for infrastructural factors. Therefore to increase efficiency of AASC these issues should be resolved. Therefore it is necessary that the consultants should increase their technical competences .Finally the missions and tasks of each sectors (public, private) should be explained and determined. These finding were also pointed by several authors (Arbenz, 2004; Povellato and Scorzelli, 2006; Nederlof et al., 2008; Fealy et al., 2007).

Economical factors such as high cost of consultancy services for farmers and lack of access to financial resources by farmers were identified as other barriers to the effectiveness of AASC. The extension designers and policy makers should be considering strategies to provide funding sources to farmers (such as loans), so to reduce the financial barriers. Moreover evaluation committee should be monitoring the services offered to farmers. Consultants should also use other methods to provide cost of services such contract among farmers at the end of the production process. Agricultural advisory services as a private sector were establishment to reducing problems of public extension sector and improving farm management skills of farmers. Providing information and consulting services to farmers cause the increase of quality and quantity of agricultural products (Rasouliazar and Fealy, 2008). According to these issues the following suggestions will be presented to reduce problems that faced by AASC. Some of preventing problems will be solved through reform and changes in the structure of AASC activities. Therefore acquiring professional and technical skills by consultants and employing female consultants were necessary. Furthermore the policymakers should develop facilitate mechanisms such as (providing supportive policies and infrastructure development) to AASC. In order to improve the effectiveness of AASC, agricultural extension policy makers should provide the accurate information about benefits, risks and impacts to the private sectors through variety of communication tools.

Based upon the results of this research, it is apparent that there is need to increase effectiveness of AASC and increase participation of AASC in the agricultural development. Suitable involvement will enhance the adoption of AASC among farmers and which would eventually lead to more investment in the AASC and increase effectiveness of them.

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Punjab Land use Classification, Reclassification and Redevelopment Rules: A predicament or new approach to urban management?

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Abstract: Disorganized and unsustainable patterns of land use change have seriously affected the spatial structure of cities in Punjab and Lahore is no exception to this phenomenon. Realizing the consequent problems of parking, traffic congestion and unfavourable environmental impacts, the Government of Punjab has recently enacted the new set of Rules to regulate commercialization and change of land use. This paper critically reviews the contents of the Punjab Land Use (Classification, Reclassification and Redevelopment) Rules 2009 applicable to Tehsil/Town Municipal Administrations (TMAs) and City District Governments (CDGs) since these Rules embody a system of classification of land uses for the first time in Pakistan. The study involves interviews with the architects of said Rules and a short survey of selected Town Planners working in TMAs and CDGs. The review shows that despite some of the shortcomings, these Rules provide a new approach to mapping and regulating the land use development and conversion activities in urban areas for the benefits of inhabitants. Moreover, the need to prepare Master Plans in future in the light of these Rules appears to be an attempt to introduce bottom-up approach to plan making, which also can contribute to facilitating not only their preparation but also enforcement on ground. However, results of brief survey of Town Planners show that effective implementation is likely to be constrained by lack of political will and inadequate technical and financial resources. The circumstances indicate for adoption of facilitative and motivational approach to implementation and enforcement of the said Rules.

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1. Introduction

Cities are dynamic entities and continuously keep transforming to accommodate emerging needs of the citizens. Over the last few decades urbanization has increased at an alarming pace in many cities of Pakistan. This has caused quite a lot of problems in those cities due to uncontrolled expansion and transformation processes. The problems mainly include shortage of housing, burden on education and health facilities, change of land use from residential to commercial and resultant increase in traffic congestion, and environmental degradation (Hasan, 2005; Ahmad, 2005; Cheema and Salman, 2009). This has affirmed "pressure on urban planners and managers for better urban planning and management" (Qureshi et al., 2009, p.3).

Earlier policies pertaining to commercialization failed to regulate conversion of residential buildings into commercial uses appropriately (see Nadeem and Hameed, 2005 for a critical review of the policies and issues relating to commercialization in the context of Lahore). Realizing the gravity of the problem, the urgency to manage swelling magnitude of urban land uses leading to traffic and environmental problems, and considering the directions of the Honourable Supreme Court of Pakistan, the Government of the Punjab province promulgated the three sets of land use conversion/classification rules in March 2008. These Rules were issued each for the Lahore Development Authority (LDA), for the remaining Development Authorities (DAs) of Punjab, and the City District Governments (CDGs) and Tehsil/Town Municipal Administrations (TMAs) in the Province in exercise of powers conferred by different laws of respective institution.

The 2008 Rules for LDA and DAs were restricted to regulating commercialization activity along major roads. Under these Rules both the LDA and other DAs of Punjab were required to prepare and notify a list of roads already declared commercial, and divide them into two broad categories: roads where more than 50% of the plots had already been commercialized (Category A) and roads where less than 50% were commercialized (Category B). Based on this classification, further commercialization was to be allowed only on Category A roads. Similarly, the Rules required both the LDA and other DAs of Punjab to prepare and notify a list of plots where temporary commercialization had already been allowed, and divide them into two broad categories: plots where payment of temporary commercialisation fee has been deposited (Category C) and plots where payment was due but the same has not been deposited (Category D). Based on this categorization, the LDA and other DAs were to phase out temporary commercial use of Category C plots over the next ten years, recover the outstanding dues from Category D plots, and stop entertaining and permitting temporary commercialization of properties in future.

On the other hand, the 2008 Rules for CDGs and TMAs suggested a complete system of classification and reclassification of land uses, and preparation of redevelopment schemes in built up areas and peri-urban structure plan for expanding areas in cities/towns before going for comprehensive master planning of each city/town. Interim arrangements were also suggested to deal with the issue of commercialization/conversion of land uses (LG & CD/GoPb, 2008).

But one year later, a newer version of each of these Rules was promulgated during different times in the year 2009 as follows:

- § The Lahore Development Authority Land Use (Classification, Reclassification and Redevelopment) Rules 2009 under Section 44 of the Lahore Development Authority Act 1975 (XXX of 1975) on 10th February, 2009.
- § The Punjab Land Use (Classification, Reclassification and Redevelopment) Rules 2009 under Section 191 of the Punjab Local Government Ordinance 2001(XIII of 2001) on 1st July 2009.
- § The Punjab Development Authorities Land Use (Classification, Reclassification and Redevelopment) Rules 2009 under Section 43 of the Punjab Development of Cities Act 1976 (XIX of 1976) on 14 October 2009.

A few noticeable changes that have been made through 2009 Rules include:

- § Ensuring consistency in terms of provisions thus making all three sets of Rules no longer any different from each other.
- § Reduction in the time limits for preparing land use classification map, peri-urban structure plan and list of declared commercial roads and

buildings. This was despite the fact no CDG, TMA or DA in Punjab could prepare these plans or the requisite lists of roads for regular commercialization and lists of plots enjoying temporary commercialization status for even within one year, the time limit as specified in 2008 Rules.

- § Enhancement of conversion fee from 10% to 20% of commercial value of land as per valuation table or average sale price of preceding twelve months of commercial land in the vicinity of that area (if valuation table is not available) for conversion of a land use to commercial.
- § Permitting conversion of a land use to permanent educational or healthcare on payment of 10% conversion fee.
- S Change in criteria for assessment of betterment fee.
- § Reduction of time period to phase out temporary commercialization from 10 years to 3 years in addition to increase in temporary commercialization fee.

The recommendations of two committees constituted by the Government of Punjab one after another to review the commercialization policy adopted as a result of 2008 Rules paved the way for the 2009 Rules. The committees were constituted in the wake of the observation that allowing high-rise buildings and commercial activity in different areas in Lahore where physical infrastructure of roads, sewerage, water supply etc. was not adequate to deal with the increased demand was putting tremendous strain on civic infrastructure (LG & CD/GoP, 2008). This paper critically reviews the contents of the Punjab Land Use (Classification, Reclassification and Redevelopment) Rules 2009 applicable to CDGs and TMAs, identifies conceptual ambiguities, potential difficulties in implementing these Rules, and makes some suggestions to overcome those difficulties. It is interesting to review these Rules because these:

- § are a good new addition to the array of planning rules for specifically concentrating on pressing issues of commercialization and change of land use being faced by planners in respect of urban areas in the Province.
- § introduce a system of classification of land uses for the first time in Pakistan.
- S call for preparation of several types of land use maps (e.g. land use classification map, district planning map) and plans (e.g. peri-urban

structure plan, redevelopment plan) never considered simultaneously in other laws and rules pertaining to town planning.

- § suggest phasing-out of temporary commercialization within a period of three years.
- § introduce the concept of charging betterment fee from commercialized buildings.

For the purposes of review of 2009 Rules, interviews were conducted with couple of members of the team at Urban Unit (of Planning and Development Department, Government of the Punjab) which was assigned the task of drafting the above referred Rules. A short survey of Town Planners working in TMAs and CDGs was conducted since they have a key role to play in implementing these Rules. A brief questionnaire prepared on the subject matter was mailed to 37 Officers with the request to return the filled questionnaire either through email or in self-addressed envelopes supplied for this purpose preferably within a week. Later, the time period was extended up to five weeks but despite repeated telephonic requests, the response of only 12 Officers could be received. The low rate of response (32%) was presumably because of the reluctance of the officers to put across their comments as serving officer of the Government in writing and because of the fact that with the exception of a couple of TMAs, nothing concrete has been done to implement the new Rules by other TMAs.

The next section presents a critical analysis of the key provisions of the 2009 Rules. Views of Town Planners working in TMAs and CDGs on the potential constraints in implementation of these Rules are then highlighted. A discussion on the prospects and implications of these Rules is provided in the penultimate section. The final section presents concluding remarks in the context of the preceding discussion.

2. Critical Analysis of the Land Use Rules 2009

The Punjab Land Use (Classification, Reclassification and Redevelopment) Rules 2009 comprise of 85 Sections divided into ten chapters. Although purpose is not explicitly mentioned in these rules, one can derive it from the preface of a booklet of the Local Government and Community Development Department (LG&CD) of the Government of Punjab (GoPb), as follows:

> "...to guide this [urban] transformation in the best public interests and to facilitate and ensure optimum utilization of urban land

through a comprehensive system of classification and reclassification of land use, enabling the cities to develop in a sustainable, harmonious and compact manner." (LG&CD/GoPb, 2009).

Under these 2009 Rules, all the TMAs and CDGs have to classify the area within their jurisdiction into six land uses, namely residential, commercial (including institutional), industrial, perurban, agricultural, and notified area. The residential, commercial and industrial areas have been further divided into four land use classes on the basis of plot size and abutting road width. The sub-classification of major land uses is expected to help establishing the basis for deciding, within a broad category of land use, the activities which should be allowed and the activities that may be permissible under special circumstances. It is worth mentioning that all other types of land use activities, which are neither listed in permitted nor in the permissible category, have been prohibited within the respective land use classes. However, a list of prohibited industrial uses in case of established built-up areas and industrial corridors has been provided.

These Rules also lay down detailed account of the procedures to be followed for preparation of land use classifications map, schemes for land use reclassification (preparation of which are optional but nevertheless subject to public consultation), and redevelopment plans for areas requiring improvement (preparation of which are conditional to notification of land use re-classification scheme). The Rules go on to describe the process of designating roads for legal commercial activity and phasing out the permission of temporary commercialization of buildings. A critical analysis of the subject land use Rules is presented in the following sub-sections.

2.1 Nuisance creating uses permitted within residential areas

Under these Rules, the residential area has been divided into two sub-classes i.e., approved schemes and established built-up area. Each of these is to be further divided into four categories with respect to plot size and right-of-way of roads. Within residential area, 8 types of uses are listed in permitted category and 8 have been listed as permissible uses. Of these 16 uses, only 4 pertain to various types of residential buildings/houses whereas the rest are all supporting uses like mosque, graveyard, park, dispensary etc. Ironically within the residential areas, the secondary schools and offices associated with resident professionals are also allowed despite the fact that the government's committee (as referred above) declared such uses as cancer for the living environment and against international principles of good town planning. Such conversions are usually under the made umbrella of temporary commercialization in the residential streets of planned housing schemes despite the provision of sub-neighbourhood centres. Since these Rules suggest phasing out temporary commercialization within three years, permitting schools and offices in residential areas will be on permanent basis. This will continue to affect the serenity of residential areas.

2.2 Compartmentalization of business activities within commercial areas

The commercial area of every city is required to be sub-classified into 3 categories namely, commercial area of approved schemes, established built up area and commercial corridors. Each of this sub-commercial area is further divided into 4 categories. Within any commercial area, 21 types of business activities including residential apartments and government offices are permitted whereas 19 other types of businesses including educational institutions and private hospitals are listed in the permissible uses. It is worth mentioning that compartmentalization of various business activities has been proposed on the basis of compatibility and road width. For instance, private and government offices, financial institutions and the like are grouped under the permitted uses within commercial area of approved schemes (CA2) on 1 to 2 kanal plots but the road width ranges from 30ft to 180ft. It's fine, as long as the compatibility of business activities is considered, but the road width of 30ft and even 50ft may not prove sufficient to meet the parking demand in case of above referred uses.

2.3 Review of declared roads and temporary commercialization

The Rules suggest that the District Planning and Design Committee (DP&DC) shall prepare the lists of roads or segment of roads already declared as commercial under any other law. It will then review the same and eventually decide the suitability of placing any road in a notified category of land use i.e., Category A road (where future commercial use would be permissible) or Category B road (where future commercial use would not be allowed). The DP&DC can also identify any restrictions such as type of commercial use, building height, building line, plot size etc., to be imposed on any of the Category A road. However it is not clear whether the criteria (see box 1) given in the Rules under Section 62(4) is for deciding the suitability of road as commercial or otherwise or the same criteria is meant for deciding the nature/type of commercial uses along the roads placed in Category A.

The Rules also require that a TMA/CDG should prepare a list of buildings which were granted permission for temporary commercialization under any other law and divide the buildings in to two categories viz list C (where the temporary commercialization fee has been paid), and list D (where the temporary commercialization fee was due).

Box 1 Factors to be considered for deciding future use of listed roads

- potential for up-gradation of serving road
- potential for up-gradation of existing infrastructure
- traffic impact assessment
- trend of changes in the existing land uses
- market demand for change of land use in the area
- compatibility with adjoining land uses
- consultation with the stakeholders

Source: LG & CD/GoPb. (2009).

As a policy matter, the permission for temporary commercialization of a building would be phased out within three years. Furthermore, no TMA/CDG would entertain any application for temporary commercialization presumably once the said Rules are in vogue. Category C buildings would be required to pay the fee for temporary commercialization starting from six percent till 31st December, 2009, nine percent from 1st January 2010 to 31st December 2010 and twelve percent from 1st January 2011 to 31st December 2011. On the other hand, category D buildings would be required to pay the outstanding dues within the prescribed time, failure to which would result in cancellation of the permission commercialization. for temporary However, on payment of outstanding dues, Category D buildings may continue to operate till 31st December 2011 after paying the fee at the rate as prescribed in case of Category C buildings.

2.4 Possible repercussions of fee for land use conversion

Under these Rules conversion of various land uses to commercial activities has again been

allowed. Contrary to previous commercialization policies conversion fee has also been suggested not only from any use to commercial (at a rate of twenty percent of the value of commercial land in that area) but also:

- from a land use to educational or healthcare institutional use (at the rate of ten percent of the value of commercial land in that area),

- from peri-urban or intercity service area to industrial (five percent) or residential (at the rate of one percent), and

- from industrial to residential use (five percent).

A possible repercussion of charging fee in case of conversion from a use to residential may result in price hike of residential plots in private housing schemes and take them further away from the affordability of low income people. In addition, the concerned TMA/CDG may also charge a 'betterment fee' to be determined on the basis of type of commercial activity and covered area of proposed commercial building. This will perhaps be used for upgrading the infrastructure and mitigating possible environmental impacts due to increased commercial activities, which have never been practiced effectively.

2.5 Land uses within industrial areas

These Rules suggest that in the approved industrial areas, the uses shall be allowed in accordance with the approved scheme. However in the established built-up industrial area/corridors, only cottage and light industries are permitted. Medium industries and necessary residential, commercial and education facilities for labourers may also be permissible. Provision of primary health facility which is considered the most important for industrial workers has been omitted, perhaps mistakenly. But it is good that storing, manufacturing and packing of explosives and other dangerous materials as well as casting of heaving metals etc. are not permitted. In the case of industrial corridors with large sized plots, all types of light, medium and heavy industries as well as ancillary uses are permitted.

2.6 Regulating land uses within the peri-urban areas

The subject Rules introduce a new land use class as peri-urban area and define the same as "an area that spans the landscape between contiguous urban development and rural countryside with low population density and is predominantly being use for

agricultural activity and is likely to be urbanized in the next twenty years" (LG&CD/GoPb, 2009;s2). It has been suggested in these rules that the permitted and permissible uses in such areas should be decided in accordance with an approved peri-urban structure plan of the respective TMA/CDG. However, it will be very important to determine the basis of permitting certain land uses at particular locations within periurban areas. The reason is that in most of the cities such areas are being rapidly converted to mix land uses owing to weak development control with no vision of how those will be integrated with the framework existing urban and trunk infrastructure/utility services.

2.7 Protecting agricultural areas and defining city limits

Agricultural area has been defined under these Rules as "the land immediately outside the periurban area which is predominantly used for the cultivation of crops and includes cropland, pastureland, orchards, nurseries and dairy farms" (LG&CD/GoPb, 2009, p.33). Most of the permitted and permissible uses in agriculture areas are rural in character and quite compatible with agricultural land use. But it is also a fact that the span of peri-urban areas in the cities of Punjab is short and ultimately the agricultural areas would be converted to urban land uses. These rules do not indicate any of such consideration or policy on protecting agricultural areas or putting any restriction on urban limits.

2.8 Restricting land uses within notified areas

The notified area is "an area in which special restrictions regarding its development or redevelopment have been imposed under any law for the time being in force" (LG&CD/GoPb, 2009, p.34). Such areas have been further subdivided into five categories as: historically significant, environmentally sensitive, public sector institutional, other restricted and intercity service area.

Permitted and permissible uses in historically significant, environmentally sensitive and other restricted areas have been mainly left up to the conditions suggested in any special or general law or any notification regarding such areas. The uses permitted in the institutional area are also suggested to be determined on the basis of any special or general law, but no reference to such special or general laws has been given. A list of ten uses permissible in institutional areas is, however, provided which mainly includes offices, official residences, education and health institutions. The intercity service areas located along intercity road but outside the peri-urban area have to be designated by the competent authority. Although the subject Rules permit commercial and residential uses, such areas may not be appropriate for residential purposes except hotels. Permissible uses include petrol pump/gas/service station, bus/truck terminal and allied loading unloading facilities as well as workshops which go well with such areas.

2.9 Preparation of land use reclassification scheme

The Rules suggest that a TMA/CDG may prepare land use reclassification scheme for an area under its jurisdiction after the notification of land use classification map. The criteria for the identification of urban blocks have also been given which include consideration for trends in the existing land use change/market demand, compatibility with adjoining land uses, potential for up-grading the road network serving the urban block(s) and prospects for redevelopment. The reclassification scheme may also propose improvement of slums areas, public building facades, transportation network, landscape and street furniture, and utility services. These are good suggestions and much needed for the old established urban areas of the Punjab, but doing all this would require lot of technical and financial resources. This in turn mean that only one or two Town Planners working as Tehsil Officer Planning and Coordination (TO (P&C)) may not be able to do such tasks and would need either bigger in-house team or services of planning and design consultants.

2.10 Preparation of redevelopment plan

The TMAs/CDGs are also required to prepare redevelopment plan within one year after the notification of land use reclassification scheme using the data already collected for this purpose. It is envisaged that the redevelopment plan would mainly involve renewal, reconstruction or up-gradation of infrastructure (transportation and utility services) and buildings as well as landscaping in an area (LG&CD/GoPb, 2009). In addition to above activities, the Rules suggest that redevelopment plan should undertake environmental impact assessment (EIA) or initial environmental examination (IEE), of the project area. The redevelopment plan should also take account of financial assessment plan/cost estimates, land consolidation plan, proposal for land readjustment or land pooling, implementation and monitoring framework and much more. Although these activities are not supposed to be undertaken simultaneously or at once, still each of these is a

gigantic task in itself and would require lot of commitment in terms of time and resources.

3. Views of Tehsil Officers (Planning and Coordination)

This section presents the views of the TO (P&C) concerning the possible constraints in implementing these Rules.

3.1 Lack of interest by the public representatives

About one third of the responding TO (P&C) revealed that ex-district/tehsil Nazims, and the present administrators of TMAs were not interested in implementation of these Rules mainly due to lack of understanding and awareness about the significance of these Rules. Those who know something about these Rules considered them very difficult to implement due to reasons like lack of cooperation from the general public, lack of technical and financial resources etc.

3.2 Lack of technical and financial resources

All the respondents stated that they were facing severe lack of technical and financial resources to get the assigned tasks accomplished within stipulated time-frame as suggested in these Rules. Local Government and Community The Development Department's record revealed that only one post of TO (P&C) exit in each Tehsil Municipal Administration. Every TO (P&C) is normally provided with a draftsman and 1 to 2 building inspectors who are thoroughly engaged in usual business of building control. Practically speaking, most of the TMAs may not be able to provide necessary technical and financial resources to them for accomplishing the tasks laid down in these Rules. That is why most of them are looking towards provincial government and the Urban Unit to provide the required assistance. Only two of responding TO (P&C) could manage to hire consultants for preparing mere land use classification map. It would be useful to review their experience and quality of work since given the lack of qualified town planning consultants as well.

3.3 Impracticable policy of phasing out temporary commercialization

As pointed out earlier, temporary conversion of buildings to commercial and ancillary uses is suggested to be phased out within three years under these Rules. That is why fee for temporary commercialization will gradually increase from six percent to twelve percent during this period. But surprisingly most of the responding TO (P&C) suggested that the fee for commercialization of properties (both permanent and temporary) should be reduced so as to encourage the general public to go for change from any use of land/building to commercial one. Moreover, majority of them categorically stated that it is not practicable to implement the policy of phasing out temporary commercialization as most of the converted commercial use is permanent in nature and people would resist any move requiring them to seize temporary commercial activity due to difficulties associated with shifting businesses somewhere else.

4. Discussion

Under these Rules a variety of plans/maps with rather different conceptual basis are required to be prepared by TMA/CDG at different levels of jurisdiction. Thus the built up areas are to be covered under land use classification maps and the expanding urban fringe areas under peri-urban structure plans. These maps and plans are to be combined along with the remaining agricultural area to form a planning map. As per these Rules, periodic review of land use classification map, peri-urban structure plan and district planning map should be carried out once after every five years. Furthermore, these Rules require preparation of re-classification scheme. redevelopment plans and list of commercial roads and buildings. However, several issues are likely to arise in preparation of these maps/plans.

The first and foremost would be the lack of capability of TMAs/CDGs to prepare these maps/plans owing to limited trained staff and resources. Even if these institutions decide to get the required maps/plans done from consultants, there would be a critical problem of shortage of qualified planning consultants required for the gigantic tasks of preparing the requisite maps/plans for 109 Tehsils and 05 CDGs of the Punjab province during the next few years. We think that there are no more than 15 such consulting firms having reasonable number of qualified Town Planners on their payroll. In this context, it is also a hard fact that most of the planning projects in Pakistan are undertaken by professionals other than qualified Town Planners. Resultantly, the Planners have to face criticism because of unrealistic policies and plans prepared by other professionals, ignoring social and economic forces playing in urban areas. Such forces may influence the way cities grow and how various urban activities operate in the complex net of city's interlocking systems.

If somehow the maps/plans suggested under these Rules are prepared, the task of enforcing these

would again be a great challenge for the TMAs/CDGs given the existing predominantly unplanned pattern of development. For instance, reclassification schemes for established areas would be about achieving compatibility in land uses, which in turn may require shifting of incompatible and nuisance creating uses like industry, temporary business and educational institutions etc. Such adjustments in land uses are most likely to be confronted with public resistance. To minimise such resistance, the Rules suggest direct public consultation during preparation of reclassification scheme. But if the circumstances call for shifting of some land uses anyway, then these Rules do not provide explicit guidance on how to deal with this kind of situation. It may be noted here that already existing light and medium manufacturing units, like packing of explosives, casting of heavy metals, soap manufacturing, electroplating and grinding of limestone, are mixed up with residential areas. Numerous studies have found severe social and environmental impacts as well as other hazards due to location of such units in the old established residential areas of Lahore (Hameed and Raemaekers, 1999; NESPAK/LDA, 2004; Haseeb, 2009).

These Rules also provide a list of concerned officials of TMAs/CDGs to be responsible for the enforcement of various tasks in the form of a Table D, which actually forms the only part containing some information regarding enforcement. However, by going through the said Table, it is difficult to comprehend who will do what and how. Furthermore, as very rightly pointed out by a seasoned TO (P&C), these Rules do not suggest any clear enforcement mechanism for implementation of various maps/plans and schemes once prepared. For instance, it is not clear "what penalty will be awarded against the violation and who will award under which mechanism".

The concerned TMA/CDG may also prepare Master Plans in addition to the above referred maps/plans but only in the light of these Rules. Here it can be argued that once the concerned TMA/CDG manages to get these maps/plans prepared and notified, the preparation of Master Plan may no longer be its prime consideration. The reason being that, those maps/plans could be treated as sufficient to manage city growth and development. On the other hand, if some TMA/CDG still intends to prepare a Master Plan, then these maps/plans could be made part of it, which in turn would facilitate the process of Master Plan preparation. In this case even the implementation of Master Plan can be expected to be much better since the detailed planning activities (particularly the sub-classification, reclassification of land uses, redevelopment plans, and plan for periurban areas) required for this purpose would have already been carried out. It is also safe to predict that this would lead to better management of city growth and development as compared with the past experience which suggests that Master Plans could not be implemented to achieve this very objective due to various reasons explained elsewhere (Hameed and Nadeem, 2006; Zaidi and Mayo, 2006).

Another important issue relates to bringing clarity in case of some provisions of these Rules. For instance, the Rules do provide a definition of what Master Plan is by stating "master plan means a land use plan of an area and includes a structure plan, an outline development plan, a spatial plan, peri-urban structure plan and a metropolitan plan." (LG&CD/GoPb, 2009;s2). But this definition encompasses various types of plans each having different conceptual basis, scope and approach to planning and managing city growth. In fact the way the term Master Plan has been defined in these Rules suggest its generic use for giving reference to all the above mentioned types of plans. In such a case the term "Development Plan" could have been used to serve the purpose. Similarly, some clarifications regarding various tasks to be undertaken under different provisions of these Rules like, distribution of commercial activity with respect to sub class/corridor (table B), "compatibility", and criteria to decide the future use of listed roads (see subsection 4 of section 62 of said Rules) need to be made.

Inadequate consultations with the stakeholders have lead to a general lack of comprehension and sense of custodianship of these Rules, which in fact are pre-requisites for successful implementation. This observation is based on the views expressed by a good number of Town Planners working in TMAs/CDGs of the Province during informal discussions at various occasions over the course of this research. Although, a group of 15-20 Town Planners including representatives of academia attended the consultative meetings held at the Urban Unit to discuss and obtain the feed back on the said Rules prior to their promulgation. Yet, our discussion with some of those participants revealed that their suggestions have not been given due consideration in the finalization of these Rules.

Finally, lack of coordination has been a big hurdle in timely and successful implementation of plans. This issue has been dealt with in these Rules by instituting a District Planning and Design Committee (DP&DC) and an industrial area scrutiny committee (IASC) with representation of line departments of the provincial government and other relevant local agencies. This sort of formal arrangement will certainly help in improving coordination among concerned departments/agencies.

5. Concluding Remarks

The Punjab Land Use (Classification, Reclassification and Redevelopment) Rules 2009 introduce a system of classification of land uses for the first time in Pakistan and at least explicitly give a picture of what our cities really need in terms of sensible planning. And if the suggested time bound tasks are accomplished in letter and spirit, all the TMAs in the province shall, at least, have land use classification map, reclassification schemes and documentation of commercial roads, road segment and buildings. Further, the preparation of land use maps/plans under these Rules can be expected to facilitate the implementation of Master Plans which until to date have failed, in most cases, to ensure planned development on the ground. The preparation of Master Plans in future in the light of these Rules appears to be an attempt to introduce bottom-up approach to plan making where details are to be sorted out first in bits and later synthesizing these to go for comprehensive citywide picture.

Nevertheless, the preceding analysis and discussion lead us to this conclusion that a lot of barriers would need to be surpassed to ensure successful implementation of these Rules. The foremost being lack of political will, inadequate technical capacity, and insufficient human and financial resources in the TMAs/CDGs. Furthermore. consultations with the Town Planners and administrators of TMAs were inadequate due to which there is general lack of comprehension and sense of custodianship of these rules among them. Problems in classification and re-classification of land use in existing built up areas can also be anticipated to pose serious challenges. So would possibly be the outcome of the policy of phasing out temporary commercialization within a period of three years owing to the fact that established business are difficult to shift/close down due to resistance from owners and political interventions. Similarly, the lack of clarity regarding administration and enforcement of various provisions of these Rules at the TMA and CDG levels is sufficient enough to cause delays in implementation.

It is important to recognise the limitations of TMAs/CDGs in preparing maps/plans suggested in

these Rules. Interviews with concerned staff of the Urban Unit revealed that so far it has helped in preparation of land use classification maps of areas falling under the jurisdiction of 10 TMAs. It would be a gigantic task for the Urban Unit to do the same job for all the TMAs of the Province. Instead a more appropriate approach would be to launch a campaign for generating sufficient political will and mobilizing financial resources to get the requisite maps/plans prepared in a phased manner through whatever limited number of consultants available (and offering such services) in the country. As a long term strategy, a full-fledged Planning Cell comprising of a good number of Town Planners in each TMA/CDG should be established which would be needed any way to devise implementation strategy and ensure adequate enforcement of the said Rules. A series of training workshops are badly needed at this stage not only to forge clarity and understanding of these Rules amongst the concerned officials but also to motivate them for proper implementation.

As far as the issue of temporary commercialization is concerned, it appears more realistic to allow conversion of temporary commercialization to be permanent where its location permits and where most of the properties have already been converted on permanent basis. However, declaring future use (particular criteria for commercial) of any road should be reviewed and the consideration of minimum right-of-way should form the key basis for deciding about a road to be fit for future commercialization. For the new expanding areas, the possibilities of alternative patterns to decentralize commercial activities in the form of nodal or multi-nuclei development need to be given due consideration in future planning so as to promote a culture of planned commercial centres.

This research has also pointed out the need for some clarifications in 2009 Rules. In particular, the vagueness in proposed administration and enforcement framework (as given in Table D) should be addressed so as to explicitly mention `who is responsible for what' in respect of enforcement activities. A viable mechanism for fine/penalties in case of violation of provisions of these Rules by the public should also be devised.

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Quality of Supervision of Ph.D. Program among Public Universities in Malaysia: A Rasch Model Analysis

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Abstract: This study examines Ph.D. students' satisfaction with the supervision process at four selected universities in Malaysia. In addition, the study also investigated the psychometric properties of Quality Supervision Scale (QSS); specifically the scale dimensionality, construct validity, endorsibility, and estimation of item and person score reliability of the scales. The participants were 153 Ph.D. students of these universities. The QSS includes many qualities of effective supervision such as supervisor academic competency, research methods competency, attitude towards supervisee, faculty academic and moral supports and supervisees' personal traits was distributed to the respondents. The Rasch model analysis was employed to analyze the data for reliability, fit to the model, estimation of satisfaction levels and possibility of scale to function differentially across gender. Results suggested that generally students were satisfied with the supervision processes at these universities. In addition to that, the scale satisfied psychometrics properties by maintaining unidimensionality, reliability, and internal consistency. Furthermore, Rasch analysis revealed that, for gender, differences in overall satisfaction levels between males and females were marginal. The differential item functioning showed that only 6 of 49 calibrated items function differently. This suggested that students' levels of satisfaction level across different disciplines since previous studies suggested that satisfaction different domains.

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Keywords: supervision, post-graduate studies, Ph.D. students, Rasch model

1. Introduction

In the past few decades, postgraduate studies in general and Ph.D. supervision in particular had received massive attention from scholars, practitioners, authorities and stakeholders alike (Chiang, 2003; Brew & Peseta, 2004). The main reason for this enormous attention is to refine, renovate and reenergize the postgraduate studies since the Ph.D. programme faces many challenges. The social science research on doctorial candidates and supervision has never included prolonged and systematic observation of supervision as actually happening in natural sciences, where there are more general laboratory courses, the supervision of postgraduate and post doctorate work has been observed as de facto (Hockey, 1991; 1995).

Researchers (Hockey, 1995, Burgess, 1994; Wright & Cochrane, 2000; Chiang, 2003; Marsh, Rowe, Martin, 2002; Brew & Peseta, 2004) referred the problem of the PhD programme to different opinions and views on what constitute of a Ph.D. programme. There are two major different views of what constitutes of Ph.D. The first view perceives Ph.D. process as a traditional knowledge-based in which candidates would be given a huge quantity of knowledge in various aspects of disciplines within the scope of the specialization to assist their work and to carry out independent research in the future. Conversely, there are some scholars who view Ph.D. process as a large of formal research training in which students would be trained on research without necessarily focusing on specific body of knowledge per se (Hockey, 1991). This standpoint believes that Ph.D. candidates have acquired necessary and sufficient body of knowledge in their previous studies that can enable them to carry out their project successfully. However, they need urgent resejjarch training and wide range of research methods on the way to effectively utilize their knowledge, in order to prepare them for the future research endeavours.

In 1988 in the United Kingdom, the committee of vice-chancellor and principal (CVCP) combined both views and emphasized that Ph.D. process should comprise these two understanding because it would be hard if not impossible for the candidates to successfully complete the programme without having enough knowledge in both theories and methods. Thus, the committee highlighted that the objectives of a Ph.D. programme are as follows:

- The first is to enable young people of high intellectual ability to develop and bring to fruition as far as possible the quality of originality, to contribute new and significant ideas, to make a positive contribution to knowledge and creativity in their respective disciplines.
- The second is to provide a training in research methods which makes them capable subsequently of assuming the role independent scholars and research writers at the highest levels capable of planning and carrying to completion a well-perceived plan of research directed toward a given objective without necessity in supervision from experienced people

Two and half decades ago and precisely in 1980s, higher institutions particularly in UK started to reevaluate the whole postgraduate programmes especially the Ph.D. after it became realistic that the overwhelming majority of Ph.D. candidates could not successfully complete their study within the time frame (Burgess, 1994; Brew & Peseta, 2004). Similarly in Austaralia, the proportion of the research students who fail to submit a thesis after a period of public or industrial supports has been increase (Buttery, Rithcher & Filho, 2005). In other words, low rate submission and successful completion of Ph.D. theses or dissertations had mainly instigated educationists and practitioners to devote their attention to examine the factors that might affect the problems. It was evidenced that, only 18.2 percent of total enrolled students in social science in 1980 finished their study within the allocated time. Consequently, the Economic and Social Research Council (ESRC) enacted a rule to withdraw grants for two years and sanction any institution where the Ph.D. submission is less than 10% in 1985, 25% in 1986, 35% in 198, 40% in 1988 and this percentage had increased until it reached 50% submission rate by 1993 (Burgess, 1994).

Historically, according to Wright and Cochrane (2000), a Ph.D. programme based on research is very few in Britain due to the fact that postgraduate research-based was established in Germany and spread to the United States since the nineteenth century but later adopted by Britain. Moreover, in 1960s, after the establishment of new higher institutions in UK as well as in other Western countries, and after influential Swinnerton-Dyer report (Wright & Cochrane, 2000) about the situation of postgraduate studies especially PhD, studies started to investigate the quality of research and the process of supervision. Two decades ago, Swinnerton-Dyer report recommended a series of suggestion, including: providing students with a grounding in research techniques, taking account of supervision quality, and completion within the time frame, and evaluating students' performance as well as supervisors'. The report also suggested many disciplinary acts against any institution with poor rates of submission and satisfactory completion of the research within time frame, maximum 4 years.

Harris (1996) asserted the importance of the role of supervisor in successfully completing the PhD programme. He recommended a series of guideline to energize the programme as reported in previous reports. The report emphasized on supervision, infrastructure and environment, monitoring and assessment. These reforms were considered to be an evolution to the postgraduate studies especially the Ph.D. in UK. They also transformed the UK postgraduate programme from been a traditional institutions to modern institutions in which students would acquire the necessary knowledge and sufficient research methodologies, also would be able to finish the study within the allocated time and resources.

Studies suggested that supervision is one of the major determinants of Ph.D. program succession whether in terms of efficient and effective information provides to the students in both concept of knowledge and research methodology, or in term of maintaining the student motivation through collegial stimulus and support throughout students' research training (Brew & Peseta, 2004; Seagram, Gould & Pyke, 1998). Hockey (1991) mentioned two major factors to successfully complete of Ph.D. programme; students' ability and motivation. These two factors could be formed by using two strategies; knowledge based and training in research methods. However, Blume (1995) stated that "further scientific work, leading to the title of doctor was not perceived as a training in research, but a research itself" (p.11).

However, Hockey (1991) suggested that the nature of supervision is a very crucial element in determining the completion of a Ph.D. study. In his review of literature of many studies on supervision, he found that generally there is students' dissatisfaction with supervision processes. According to him, a general trend among Ph.D. students indicated that 25% of social science students were dissatisfied with their supervision process and emphasized that they received too little supervision in the initial stage of their research. The study also revealed how bitterly students were in all stages of their studies. On the other hand, Buttery, Rithcher and Filho (2005) opined that the efficiency of Ph. D supervision process depends on various stages of thesis lifecycle. At the early stage, single supervision is considered the best as the students is in the brainstorming and planning of the study. They emphasized the best way to confused the student is by giving a conflicting advice which may detract from clear focus and research direction. However, when the research direction and questions have been identified then only it would be beneficial to discuss in group supervisions.

Interestingly, studies also suggested that the dissatisfaction of postgraduate students towards the processes of supervision differed across the disciplines (Young, Fogarty, McRae, 1987). The study indicated that social science students rated generally higher than the natural sciences in the dissatisfaction of the supervision process. The reason for the dissatisfaction of social science students might not unrelated to the nature of research in human sciences and characters of supervisors. Unlike natural sciences, social sciences' students expected to establish strong relationships with supervisors to facilitate and solve their academic problems. It was suggested that more attention should be paid to the entire Ph.D. supervision process especially in human science where completion rate and submission was poor (Hockey, 1995). The supervision relationship was found as often fraught and unsatisfactory as a result of neglect, abandonment, revenge of previous experience and disdain by the students (Johnson, Lee & Green, 2000). Due to this problem, Winfield report made some recommendations; training the supervisors should be introduced to the entire process of Ph.D., monitoring students' progress, showing more concern for the improvement and facilitating students' academic endeavours hv providing more facilities and learning equipment.

On the other hand, Hockey (1995) ascribed the problem in supervision especially in social science to lack of training for supervisors and supervisees alike. According to him, training supervisors on how to supervise showed its efficiency and effectiveness in completion rates. Moreover, Chiang (2003) also emphasized on the role of training for both supervisors and research students. This training would give both supervisors and supervisees opportunities to interact successfully, which would enhance academic stimulation and promote collegiality, as well as reduce the segregation and, diminish level of loneliness. Supervisors need to undergo a series of training especially on how the students' psyche and emotion could be handled effectively. Moreover, Hockey (1995) emphasized that the content of training for supervisors should encompass both intellectual, organizational matters and pastoral skills. He further asserted that since experienced supervisors are rare in the universities today even in well-established high institutions, providing an effective training for the supervisors is a dire need. Swinnerton-Dyer (1982) demonstrated how science students were frequently associated with their supervisors, while the chance of often meeting with supervisors was not available for

social science students. In addition to that, social sciences supervisors rarely attended any training courses to enhance their supervision ability but rather they merely relied on trail and error approach. Blume and Amsterdamka (1987) argued that the research process is totally different between natural sciences and human sciences in terms of academic culture between disciplines; when the former is laboratory-based study where things are more précised and accurately defined, the later is more complex, vague and dynamic. In addition to catering to intellectual development, both supervisors and research students should be familiar with different types of research methods not only the one that they use in their research (Chiang, 2003). It is an institutional responsibility to ensure that Ph.D. process is unique and reaches the expectation standard because the overwhelmingly majority of Ph.D. candidates return back to the institution as lecturers or academic surroundings (Chiang, 2003).

It is unjustifiable to attribute the supervisors' deficiencies as the sole factor for the students' academic deficiencies. However, supervision activities require the supervisors to develop a wide range of research relatedness and interpersonal skills. According to Wisker (2005) supervisors should be aligned with their practice and their learning behaviors with their supervisees. It is worth to note that the students are highly diverse in their academic ability, personality attributes, motivation and attitude (Zainal Abidin & West, 2007).

However, consideration of the supervision activities as the supervisors' private affair is one of the major obstacles that block supervision improvement. Supervisors are often reluctant to open their practice to criticism or benefit even from experienced supervisors. An open communication is crucial to identify the shortfall perceived by the students (Zainal Abidin & West, 2007). Factors mentioned include quality of supervision, part-time/full time status, financial situation of the candidate, and general environment. Generally, supervisors get assistance from the administration only on technical matters such as regulation and guidelines on supervision process without any concrete advice or training on how supervisors can enhance themselves especially on the body of knowledge and methodology in order to contribute significantly to the project and research (Pearson, 1996).

Supervisors should enhance themselves academically and professionally to be able to produce students with high standards and encourage them to be able to work independently. Although different supervisors have different style preference, they should put more efforts on appropriate ways to guide, direct, encourage, nurture the students' skills, foster of students' creativity, share ideas, and learn from students' experiences and knowledge if necessary. According to Acker, Hills and Black (1994), "several students indicated that infrequent supervision had taught them how to be assertive, perhaps seeking help elsewhere, or to be better organized. Some thought that their supervisor had intended this outcome" (p.494). Another researcher (Holdaway, 1997) emphasized that some students were abandoned and left alone by their supervisors during their research activity period. He reported one Canadian research administrator said that "we must get humanities faculty members to feel that students are not a nuisance in their research" (p.67). Fostering supervisors' competence aims to integrate various needs and demands innate in the learning situation such as the supervisee's needs of professional enhancement, needs of educational development in all sense of the term, and personal needs of the supervisor for academic improvement. This personal needs of supervisor could be generated through reading, personalized reading (connecting it with own world), reflection, know-how, involving in research activities, and collegiality with their own supervisees (Linden, 1999; Elton & Pope, 1989; Burgess, 1997; Marsh, Rowe, Martin, 2003). Pearson (1996) emphasized that the quality of experience and the quality of outcome of a Ph.D. program depended largely on supervision and the individualistic nature of research.

In relation to supervision across gender, Booth and Satchell (1995) stated that women are more vulnerably to withdraw from a Ph.D. programme than men across all the subject areas. Men also completed faster in all subject areas. This finding indicated that even when supervision process accounts partially for burnout and late submission of Ph.D. project, male students are still benefiting compared to the female students. However, Wright and Cochrane (2000) differentiated between genders across their specializations. They found that females in science disciplines were slightly more successful to submit their dissertations as compared to humanities. However, in general the findings reinforced the previous studies that females performed less than men.

In Malaysia, especially the research universities have been trying to promote the postgraduate studies and enhance the quality of postgraduate research. The postgraduate students especially the Ph.D. students are considered as important resources for intensification of research and publication (Krauss & Ismail, 2010). Many workshops have been conducted for postgraduate students, irrespective of their specialization to train them for their future academic challenges. The universities are working hand-in-hand with various agencies to improve the postgraduate studies, and it is also providing necessary assistance and facilities for all postgraduate students based on the philosophy that research activities are very significant in the global knowledge economy and development. It is also firmly known that to achieve the universities objectives, the postgraduate students must involve in meaningful research to improve the effectiveness and efficiency of research supervision. Unfortunately, an inefficient Ph.D. supervision often leads to an increase in the time duration to obtain the Ph.D. degree; hence students must work far beyond their financially supported period. In other cases, supervision problems may force the candidate to leave his/her research career. Thus, supervision is a major issue when talking about PhD studies and needs to be considered seriously. Unfortunately, very few empirical studies on the quality of doctorial education are driven from students' perspective (Chiang, 2003).

Despite that many institutions have been criticized for malpractice of Ph.D. process of especially supervision process, the condition persist largely unchanged in many current circumstances. Thus, this study explored the Ph.D. students' views on the supervision practice at four public universities in Malaysia and their satisfaction level experiences across gender through different item functioning. In addition, the study also investigated the psychometric properties of supervision scale constructed by Van der Heide (1994), specifically the scale dimensionality; construct validity, endorsibility, and estimation of item and person score reliability of the scales.

2. Method

2.1. Participants

A sample of 153 Ph.D students from four selected universities, participated in this study. The researchers selected third year and graduated students for this study due to the fact that they were or had engaged in the supervisor-supervisee relationships with their respective supervisors during their writing processes. Thus, the students might objectively assess their supervisor performance, academic competency, characters and faculty supports.

2.2. Instrumentation

The instrument was adopted from previous study and used to assess PhD students' views on supervision process (Van der Heide, 1994). It was initially developed by Van der Heide, (1994) to measure the extent to which PhD and postgraduate research students have satisfactory experiences in relation to the quality of their research supervision. The instrument consisted of 49 items, with five distinctive factors (supervisors' academic competency, research method competency, morals and characters towards supervisees, faculty supports, and supervisees' personal contribution to their research). The response categories were 7 for always true, 6 for almost always true, 5 for often true, 4 for sometimes true, 3 for seldom true, 2 for almost never true and 1 for never true. The internal consistency of the scale reported by Van der Heide, (1994) was ranged between .77 to .91. In the present study, the researchers tested internal consistencies of the adopted scale again employing the Cronbach's alpha and found it ranged between .96 to .98.

3. Results

Rasch model was then employed on collected data to psychometrically validate the used scale and its fulfillment of fundamental requirement to be considered sound scale to assess students' satisfaction towards supervision process. In addition to that, differential item functioning (DIF) was also used to identify items that function differently across gender.

Principal component analysis was used prior to the Rasch analysis calibration to identify the underlying factors (set of items loaded on a specific factor). The rationale behind using this technique was the fundamental requirement fulfill to of unidimensionality. After five meaningful and interpretable factors were extracted, Rasch calibrations were employed using the WINSTEPS program version 3.58.0 developed by Linacre (1991-2004). The Rasch analysis is based on assumption which derived from a basic probability that there is no relationship exists between a person's responses to different items after taking the ability into consideration (Pickard, Dalal & Bushnell, 2006). The correspondence between an individual's ability on a latent trait and the predicted response to an item is represented by an item characteristic curve which has Ogival (S-shaped) form. Item location along the continuum of the measure is expressed in log odds, or logits. Considering quality supervision, an expectation of the model for an item is that the probability of endorsing the item in the keyed direction increases as the amount of the quality supervision the individual holds increases. The Rasch analysis was selected for this study to identify the items that fit; that have equal items characteristics curve across gender. In order to prove reliability of the scale used for this analysis, the reliability of the overall and of each item were observed. Besides overall reliability of the model, WINSTEPS provides two mean square fit statistics; infit and outfit. The infit statistics "is an information-weight sum" and outfit "is based on conventional sum of squared standardized residuals" (Bond & Fox, 2001, p.176). Both infit and outfit are means square divided by their respective degrees of freedom, with an expected value of +1 and a range from 0 to positive infinity (Bond & Fox, 2001; Silver, Smith & Greene, 2001). The infit statistics are insensitive to unexpected responses to items far from a person's ability, while outfit is sensitive to unexpected ratings far from a person's ability. According to Silver et al. (2001), mean square statistics less than one (<1) suggested redundancy, dependency or constraint of data, while mean squares greater than one (>1) evidenced unexpected variability, inconsistency or extremism. Bond & Fox (2001) demonstrated that by saying infit e.g. 1.30 indicated 30% variation between the actual score and Rasch predicted score, while an outfit means square value of say 0.78 (1-0.22 = .78)showed 22% less variation in the observed score than Thus, the test of infit evaluates the modeled. consistency of item parameters across the person measured for each item. Data is combined across all items to provide an overall test of fit. On the other hand, the test of outfit shows the collective agreement for all items across persons. This is to support that item difficulties are consistent and stable (Waugh, 2001). Both item and person estimates allow researchers to determine how well an item measures a latent construct. It is worth noticing that the less variation between the actual score and the expected by the Rasch model is more desirable.

Furthermore, this study also assessed the different item functioning across gender. The Rasch model assumes that an additive structure underlies the observed data, the both participants, and items can be arrayed on a continuum, and that the items have equal discriminative power (Kan, Breteler, Van der Ven, & Zitma, 1998). Differential item functioning methods are widely used for detecting potentially comparison among quality supervision scale across gender. That means analyzing of individual differences in response tendencies as well as items discrimination (i.e. how well the item is able to discriminate between examinees holding different level of a latent construct). Thus, the Rasch model is capable to verify the fitness of the each item and person into the model spontaneously and provide the difficult level of endorsement (easy or difficult) of a person to each item. As obviously stated by Smith, ignoring or excluding misfit items from the analysis may not answer the complexity of the construct and may fail to provide an acceptable judgment. Thus, interpretation of extreme items may shed more light on how items were perceived and interpreted by an individual across the gender.

Although values range for both infit and outfit mean square fit statistics depends on testing situation and measurement purposes (Wright &Linacre, 1994), an acceptable range for this study is .60 to 1.40. The values within this range are considered relatively close enough to the perfect fit of the Rasch model. However, the more the infit mean square and outfit mean square are further from an accepted range, the more other aspects are believed to play a role in determining the pattern of responses (Bond, 2001). Furthermore, in addition to infit and outfit means square, an index of reliability, and error estimation was also provided. The infit mean-squares are used to determine the fit of the item within the construct. Advancing average measures with each category and step calibrations ensure the rating scale measure is stable and accurate. Probability curves were used to visually inspect the rating scale category function.

3.1. Quality Supervision

Rasch model was applied to investigate Ph.D students' satisfaction of quality supervision, psychometrics properties of quality supervision scale and determine equivalence across gender. The satisfaction level of the students would be determine in term of how easy for the items involved can be endorsed. The easiness and hardness of an item would be expressed through the direction and magnitude of each item estimates, while negative sign of an item indicates easy to endorse (high satisfaction), the positive sign means hard to endorse (low satisfaction) (Bond & King, 2000). Precision of the estimates would be properly identified and accurately interpreted by adding and subtracting the measurement error from the magnitude of estimate.

Due to the preliminary nature of the study, a relatively broad fit criterion was used, and item with MSQ fit values greater than or equal to 1.40 were highlighted and explained. Referring to Table 1, the Calibration of the 49 quality supervision items yielded an acceptable model fit. Accordingly, in reference of Table 2, items separation reliability was .95, while the person separation reliability was .94, indicating high level of instrument consistency, items separation along the quality supervision estimates were well dispersed along the scale. The Standard Deviation (SD) of the item calibration was .39. The mean square infit ranged between .60 to 4.54.

However, the rating scale (1-7) did not perform accordingly and the respondents haphazardly answered the questionnaires. It appears from the frequencies reported that respondents were not utilized the full range of the seven point scale, which was suspect in the overall analysis. Therefore, the researcher collapsed categories that did not act appropriately. Categories one and two were collapsed together (very strong disagree + strongly disagree) and categories six and seven (strongly agree + very strongly agree) also formed one scale. As a result, the item and person reliability slightly increased and the infit and outfit means square showed better fit.

+				 +				
I	RAW		-	MODEI	L 1	INFIT	OUT	FIT
	SCORE	COUNT	MEASURE	ERROR	MNSQ	ZSTD	MNSQ	ZSTD
MEAN	834.2	152.6	.00	.07	.99	5	1.07	1
S.D.	77.4	.6	.35	.00	.48	3.3	.71	3.5
MAX.	925.0	153.0	1.27	.08	2.86	9.9	4.00	9.9
MIN.	544.0	151.0	47	.06	.63	-3.9	.57	-3.7
REAL RI	MSE .07	ADJ.SD	.34 SEI	PARATION	4.57 ITE	M REL	JABILITY	.95
MODEL RI	MSE .07	ADJ.SD	.34 SEI	PARATION	4.88 ITE	M REL	IABILITY	.96

Table 1: Summary of 49 measured items

S.E. OF ITEM MEAN = .05

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+								
	RAW		-	+ MODE	L	INFIT	OUT	FIT
	SCORE	COUNT	MEASURE	ERROR	MNSQ	ZSTD	MNSQ	ZSTD
MEAN	267.2	48.9	.71	.13	1.08	1	1.07	2
S.D.	46.4	.5	.68	.03	.45	2.5	.74	2.6
MAX.	335.0	49.0	2.12	.26	2.57	6.0	7.93	9.9
MIN.	113.0	46.0	-1.96	.11	.10	-9.2	.11	-9.0
REAL	RMSE .15	ADJ.SD	.66 SEI	PARATION	4.37 PE	RSON REI	LIABILITY	.95
MODEL	RMSE .13	ADJ.SD	.67 SEI	PARATION	4.99 PE	RSON REI	LIABILITY	.96
			S.E. OF PERS	SON MEAN	= .06			
+								

Table2: Summary of 153 measured persons

-+

Inspection of the infit and outfit mean square scores for 49 items revealed that six items were outside the set cutoff of 0.6 and 1.40. Both infit and outfit statistics of the six items were above the set cutoff, signifying high variability of response or misfit the model. Infit statistics of item 14 (My supervisor has/had not taken over making decisions about my thesis) was above the set cutoff (infit 1.62 and outfit 2.02) Item 15 (My supervisor has/had not appreciated my work.) (infit 2.84 and outfit 3.82), item 29 (I have/had dealt with problems between my supervisor and me) (infit 2.17 and outfit 2.73) item 45 (My post graduate work (research) is like a hobby to me) (infit 1.37 and outfit 2.01), item 46 (Most of the time I have to force myself to do my post graduate study related work) (infit 1.79 and outfit 2.04) and item 47 (My post graduate study is pretty uninteresting) (infit 2.58 and outfit 3.48). These infits and outfits suggested that the

contents of the items were very hard for the respondents to endorse, in which indicating that they were less satisfied with them (Bond & King, 2000). It is obvious from the analysis that the contents of most of misfit items are negative in nature. With exception of these six items, the calibrations of the remained 43 items were within the set cutoff, indicating that the items were relatively closed enough to the perfect fit of the Rasch model.

The reliability of the items and persons was evaluated through using both the separation index (G) and reliability index (R). While the former is an estimation of how well people can be discriminated on the measured variable, the later which is conceptually similar to Cronbach's alpha is an indicator of the extent to which a different set of items measuring the same construct would reproduce the observed person scores (Bond & Fox, 2001; Hula, Doyle, McNeil, Mikolic, 2006). The examination of both separation index and reliability index yielded an acceptable level at 3.87 and R .96 for the persons while scored 4.55 and .96 for the items respectively.

Moreover, the low item estimates suggested easier endorsement or more satisfaction for the respondents. As it was shown in Table 3, the items estimation, more than half of the estimated values were negative while the positive estimate values for other items were very small in exception of departmental support items. This generally indicated that, Ph.D. students were satisfied with the supervision process's factors; supervisors' competency in both theory and methodology, attitudes towards supervisees, faculty support and practical outcomes of their research process. For example, estimate values table shows that respondents were more satisfied with the contents of item 19, "My supervisor is/was supportive", item 20 "My supervisor is/was familiar with the field of research", item 21, "My supervisor respects/respected my ideas", and item 37, "My research have/had sharpened my analytical skills" with estimate values of -.40, -.57, -.35, and -.41 with standard error ranged between .07 to .09 each respectively.

3.2. Different Items functioning across gender (DIF)

A differential item function within the Rasch analysis was employed to determine differences of respondents on quality supervision scale across gender. More precisely, to examine whether the scale is producing equivalent measures without discrimination in student satisfaction across gender that included 110 males and 43 females. The different item function occurs when different groups within the sample (e.g. males and females), despite equal levels of the underlying characteristic being measured, respond in a different manner to an individual item. Results in Table 4 revealed significant differential responses to only five items out of 49 items used in the study. The DIF contrast is the difference in difficulty of the item between the two groups, which is also significant for seven items at .62, p = .0155, -.55, p = .0007, .94, p =.0139, -.37, p = .0489, -.71, p = .0001, for item 7, 15, 20, 34, 47 respectively.

Table3: Quality Supervision of Ph.D. process input: 153 persons, 49 items measured

ENTF	RY	TOTAL			MODEL IN	IFIT OUT	FIT PT-MEA	ASURE EXACT
MATCH	Ŧ							
NUME	BER	SCORE	COUNT	MEASURE	S.E. MNSQ	ZSTD MNSQ	ZSTD CORR.	EXP. OBS%
EXP%	T.I.	EM 						
	+-				I	1	,	I.
	1	607	123	09	.10 .81	-1.3 .76	-1.6 .64	.58 50.4
44.7	1							
	2	641	126	29	.10 .60	-2.9 .65	-2.4 .68	.55 54.8
47.6	2	 613	102	_ 10	10/ 79	_1 1 72	_1 0 62	57 50 4
44.9	3		123	19	.10 .79	-1.4 .72	-1.9 .02	.57 50.4
	4	633	132	.09	.09 .71	-2.3 .72	-2.1 .69	.60 43.2
42.1	4				·		·	
	5	665	133	15	.10 .77	-1.6 .74	-1.8 .68	.56 48.1
45.8	5	(1)	1 2 1	0.5	00 70	1 6 70		
43 2	6	642 	131	05	.09 .78	-1.0 ./9	-1.5 .07	.58 45.0
	7	662	132	16	.10 .65	-2.6 .65	-2.5 .68	.57 55.3
45.7	7				I	1	I	I
Ι.	8	662	132	16	.10 .81	-1.3 .83	-1.1 .62	.57 52.3
46.1	8		101	0.4				
	9	645 I	131	04	.09 .67	-2.5 .70	-2.1 .70	.5/ 5/.3
1 10	10	1 613	125	04	.10 .67	-2.4 .59	-3.0 .74	.57 51.2
43.8	10						2.01 .71	
'	11	676	135	14	.10 .85	-1.0 .77	-1.5 .58	.56 46.7
45.7	11							

	12	650	130	14	.10 .61	-2.9 .60	-2.9	.70	.56 53.1
45.5	12 13	 618	123	20	.10 .96	2 .93	4	.58	.56 49.6
46.2	13 14	 585	139	.70	.08 1.62	4.5 2.02	6.3	.42	.61 33.8
34.5	14 15	 460	149	1.49	.07 2.84	9.9 3.82	9.9	.12	.65 11.5
31.0	15 16	 604	122	08	.10 .77	-1.6 .88	7	.66	.58 45.9
44.8	16 17	 592	117	25	.11 .65	-2.4 .68	-2.1	.67	.56 57.3
40./	18 18	 649 	129	19	.10 .65	-2.6 .61	-2.8	.73	.56 52.7
45.0	10 19 10	 625 	121	39	.11 .72	-1.9 .72	-1.8	.67	.55 53.7
49.0 50.3	20	 592 	113	53	.12 .88	7 .85	8	.63	.53 61.1
 49 7	20 21 21	 647 	125	38	.11 .84	-1.0 .79	-1.3	.65	.55 55.2
46 1	22	 661 	132	14	.10 .86	9 .78	-1.5	.65	.56 53.0
42.8	23	666 	138	.12	.09 .95	3 1.06	.4	.66	.59 49.3
49.9	24 24 24	660 	128	34	.11 .81	-1.2 .88	7	.62	.55 57.0
47.8	25 25	635 	125	24	.10 .85	9 .90	5	.64	.55 52.0
48.4	26 26	685 	134	29	.10 .68	-2.3 .65	-2.5	.67	.54 61.9
 44.4	27 27	685 	139	02	.09 .77	-1.7 .85	-1.0	.57	.57 48.2
 50.2	28 28	661 	128	36	.11 .80	-1.3 .98	1	.56	.55 51.6
 35.3	29 29	578 	136	.62	.08 2.17	7.4 2.73	9.2	.29	.63 33.8
 48.4	30 30	636 	125	24	.11 .85	9 .78	-1.4	.72	.57 56.0
 50.4	31 31	684 	132	38	.11 1.05	.4 1.03	.2	.55	.54 57.6
 37.7	32 32	637 	142	.44	.08 1.10	.8 1.08	.7	.60	.61 36.6
 39.3	33 33	638 	139	.31	.08 1.05	.4 1.00	.0	.60	.60 36.7
 42.1	34 34	673 	141	.14	.09 .89	8 .89	8	.63	.59 38.3
 49.4	35 35	716 	139	33	.10 .72	-2.0 .66	-2.4	.67	.55 61.2
 50.0	36 36	663 	128	39	.11 .89	6 .84	-1.0	.61	.55 61.7
 50.6	37 37	642 	123	48	.11 .69	-2.0 .74	-1.6	.62	.54 64.2
 50.7	38 38	644 	124	38	.11 .75	-1.6 .82	-1.1	.65	.55 62.9
 48.4	39 39	667 	131	24	.10 .83	-1.1 .83	-1.1	.62	.56 52.7

.25	.09 1.01	.1 .98	1	.63	.60 35.8
.11	.09 .95	3 1.08	.6	.57	.59 39.1
.19	.09 .94	4 .90	6	.64	.60 40.6
.33	.08 .87	-1.0 .83	-1.2	.66	.60 48.1
.36	.08 .88	9 .87	9	.66	.60 46.0
.41	.08 1.37	2.6 2.01	5.8	.42	.64 34.6
.71	.07 1.79	5.5 2.04	6.4	.36	.64 24.6
1.40	.07 2.58	9.9 3.48	9.9	.17	.63 16.8
03	.09 .85	-1.0 .92	5	.66	.59 50.7
2932	.11 .80	-1.3 .74	-1.7	.70	.55 56.6
4 .00	.10 .97	3 1.04	1		-+ 48.1
.2.42	.01 .46	2.9 .68	3.2		11.2
	34 .25 38 .11 33 .19 35 .33 37 .36 34 .41 40 .71 45 1.40 39 03 29 32 .4 .00 .2 .42	34 .25 .09 1.01 38 .11 .09 .95 33 .19 .09 .94 35 .33 .08 .87 37 .36 .08 .88 34 .41 .08 1.37 40 .71 .07 1.79 45 1.40 .07 2.58 39 03 .09 .85 29 32 .11 .80 .4 .00 .10 .97 .2 .42 .01 .46	34 $.25$ $.09 1.01$ $.1 $ $.98$ 38 $.11$ $.09 $ $.95$ $3 1.08$ 33 $.19$ $.09 $ $.94$ $4 $ $.90$ 35 $.33$ $.08 $ $.87$ $-1.0 $ $.83$ 37 $.36$ $.08 $ $.88$ $9 $ $.87$ 34 $.41$ $.08 1.37$ $2.6 2.01$ 40 $.71$ $.07 1.79$ $5.5 2.04$ 45 1.40 $.07 2.58$ $9.9 3.48$ 39 03 $.09 $ $.85$ $-1.0 $ 29 32 $.11 $ $.80$ $-1.3 $ $.4$ $.00$ $.10 $ $.97$ $3 1.04$ $.2$ $.42$ $.01 $ $.46$ $2.9 $ $.68$	34 $.25$ $.09 1.01$ $.1 $ $.98$ $1 $ 38 $.11$ $.09 $ $.95$ $3 1.08$ $.6 $ 33 $.19$ $.09 $ $.94$ $4 $ $.90$ $6 $ 35 $.33$ $.08 $ $.87$ $-1.0 $ $.83$ $-1.2 $ 37 $.36$ $.08 $ $.88$ $9 $ $.87$ $9 $ 34 $.41$ $.08 1.37$ $2.6 2.01$ $5.8 $ 40 $.71$ $.07 1.79$ $5.5 2.04$ $6.4 $ 45 1.40 $.07 2.58$ $9.9 3.48$ $9.9 $ 39 03 $.09 $ $.85$ $-1.0 $ $.92$ $5 $ 29 32 $.11 $ $.80$ $-1.3 $ $.74$ $-1.7 $ $.4$ $.00$ $.10 $ $.97$ $3 1.04$ $1 $ $.2$ $.42$ $.01 $ $.46$ $2.9 $ $.68$ $3.2 $	34 $.25$ $.09 1.01$ $.1 $ $.98$ $1 $ $.63$ 38 $.11$ $.09 $ $.95$ $3 1.08$ $.6 $ $.57$ 33 $.19$ $.09 $ $.94$ $4 $ $.90$ $6 $ $.64$ 35 $.33$ $.08 $ $.87$ $-1.0 $ $.83$ $-1.2 $ $.66$ 37 $.36$ $.08 $ $.88$ $9 $ $.87$ $9 $ $.66$ 34 $.41$ $.08 1.37$ $2.6 2.01$ $5.8 $ $.42$ 40 $.71$ $.07 1.79$ $5.5 2.04$ $6.4 $ $.36$ 45 1.40 $.07 2.58$ $9.9 3.48$ $9.9 $ $.17$ 39 03 $.09 $ $.85$ $-1.0 $ $.92$ $5 $ $.66$ 29 32 $.11 $ $.80$ $-1.3 $ $.74$ $-1.7 $ $.70$ $.4$ $.00$ $.10 $ $.97$ $3 1.04$ $1 $ $.2$ $.42$ $.01 $ $.46$ $2.9 $ $.68$ $3.2 $

 Table 4: Supervisor and supervisee relationship

DIF class specification is: DIF=\$S2W1

	+									
		PERSON	DIF	DIF	PERSON	DIF	DIF	DIF	JOINT	
	CLASS	MEASURE	S.E.	CLASS	MEASURE Number	S.E. Name	CONTRAST	S.E.	t d.f.	Prob.
-	1	14	.12	2	.04 1 1	.18 	18	.22	83 121	.4081
	1	24	.12	2	44 2 2	. 22	.19	.25	.79 124	.4325
	1	19	.12	2	19 3 3	.19	.00	.23	01 121	.9952
	1	.03	.11	2	.24 4 4	.16	21	.20	-1.10 130	.2746
	1	10	.11	2	28 5 5	. 20	.18	.23	.77 131	.4409
	1	03	.11	2	09 66	.18	.07	.21	.31 129	.7597
	1	01	.11	2	64 77	.23	.63	.26	2.45 130	.0155
	1	05	.11	2	48 8 8	. 22	.43	.24	1.76 130	.0810
	1	.01	.11	2	22 99	.20	.23	.23	1.00 129	.3176

1	.03	.11	2	26	.21	.29	.24	1.20	123	.2311
1	14	.11	2	13	.19	02	.22	07	133	.9455
1	13	.11	2	15	.19	.02	.23	.08	128	.9368
1	22	.12	2	15	.20	07	.24	32	121	.7529
1	.71	.09	2	13 13 .67	.15	.04	.17	.24	137	.8102
1	1.34	.08	2	14 14 1.89	.14	55	.16	-3.46	146	.0007
1	.03	.11	2	40	.22	.43	.25	1.73	120	.0868
1	26	.12	2	24	.21	02	.25	08	115	.9355
1	13	.11	2	39	.21	.26	.24	1.08	127	.2833
1	39	.13	2	35	.22	05	.26	18	119	.8588
1	37	.13	2	-1.31	.35	.94	.38	2.50	111	.0139
1	29	.12	2	70	.25	.41	.28	1.44	123	.1536
1	11	.11	2	23	.20	.12	.23	.54	130	.5907
1	.17	.10	2	03	.18	.20	.21	.94	136	.3466
1	30	.12	2	48	.23	.18	.26	.71	126	.4819
1	23	.12	2	27 25 25	.21	.03	.24	.14	123	.8891
1	28	.12	2	28	.21	.00	.24	.00	132	.9978
1	10	.11	2	.20 .21 .27 .27	.17	31	.20	-1.52	137	.1299
1	39	.13	2	27	.21	12	.25	50	126	.6210
1	.52	.09	2	.84	.14	32	.17	-1.91	134	.0584
1	26	.12	2	18	.20	08	.23	33	123	.7417
1	34	.12	2	50	.24	.16	.26	.61	130	.5425
1	.44	.09	2	.46	.15	03	.18	15	140	.8830
1	.25	.10	2	.45	.15	20	.18	-1.13	137	.2616
1	.03	.11	2	.40	.15	37	.18	-1.99	139	.0489
1	39	.12	2	16	.19	23	.22	-1.05	137	.2962
1	39	.13	2	37 26.26	.22	02	.25	07	126	.9430
1	45	.13	2	58 37 37	.24 	.13	.27	.48	121	.6316

1	32	.13	2	53 38 38	.23	.20	.26	.78	122	.4397
1	25	.12	2	19 39 39	.19	07	.23	29	129	.7721
1	.34	.10	2	04 40 40	.18	.38	.21	1.84	132	.0682
1	.17	.10	2	09 41 41	.19 	.26	.21	1.23	136	.2191
1	.20	.10	2	.19 42 42	.17 	.01	.20	.05	131	.9587
1	.43	.10	2	.06 43 43	.17 	.37	.20	1.89	133	.0608
1	.40	.09	2	.23 44 44	.17	.17	.19	.90	135	.3702
1	.43	.10	2	.35 45 45	.16	.08	.19	.41	131	.6839
1	.62	.09	2	.93 46 46	.14	31	.16	-1.90	136	.0593
1	1.21	.08	2	1.92 47 47	.14	71	.16	-4.41	141	.0000
1	02	.11	2	06 48 48	.19 	.04	.22	.18	136	.8568
1	31	.12	2	33 49 49	.22	.03	.25	.11	127	.9155

The different item functioning occurred for item 7 "My supervisor has/had given me constructive" where it more easier for the male students to endorse (-.01) than their female counterparts (-.64), 15 "My supervisor has/had not appreciated my work" where it very hard for female to accept; low satisfaction (1.89) than their male counterparts (1.34). The different in response also found in items 20 "My supervisor is/was familiar with the field of research" where female respondents found it easier to accept (-1.31) than male respondents (-.37). Discrimination also occurred with item 34 "I received appropriate assistance in locating a supervisor", and item 47 "My post graduate study is pretty uninteresting" in where they were very difficult for females to endorse (low satisfaction) while were quite easy for male students to endorse (high satisfaction) .40, .03; 1.92, 1.21 females and males for both items respectively. This can be visually seen in the different item function plot displayed in Figure 1 as considerable differences across gender in the aforementioned items is obviously clear. (See figure 1)

4. Discussion

The Rasch model analysis was used to investigate students' level of satisfaction in the supervision process. The analysis was carried out firstly to validate the scale, secondly to test the satisfaction level of respondents and finally to assess the possibility of the items to function differently across gender. The results are largely in consistent with previous studies regarding students' satisfaction of supervision process (Hockey, 1991; 1995; Young, Fogarty, & McRae, 1987).

Interestingly, the present results generally support the construct and content validity of quality supervision scale (QSS) and provided evidence for the unidimensionality of the scale since the majority of the infit and outfit statistics of the items fall within an acceptable set cutoff of .60 - 1.40. It suggested for high level of scale consistency and item separation along the quality supervision scale continuum. However, the Rasch analysis revealed low level of satisfaction for faculty social and academic supports.

On the other hand, it was found that only 5 items of 49 were flagged for differential item functioning across gender. As also noted through the analysis and DIF plot, the Rasch-derived person scores are sufficiently precise to differentiate satisfaction level across gender. This finding should be treated with caution due to small sample size which can be interpreted as less representative of the population and also measure might also not mean the same thing across gender. Nevertheless, six items of QSS were fall out of set cutoff and they were highlighted and explained the possible reason for their misfitting. As suggested by, Buttery, Rithcher and Filho (2005) the efficiency of Ph. D supervision arrangements depends on various stages of thesis lifecycle. Since different faculty has different supervisions styles, it is strongly recommend that future studies should also take to account quality supervision across different disciplines (Swinnerton-Dyer, 1982; Young, Fogarty, & McRae, 1987), as the researcher attempted to do initially, however, due to the lack of equal size across disciplines impede the objective. It is worth mentioning that was suggested that at least minimum of 30 respondents for each domain is required before different items function could be meaningfully employed (Linacre, 1994).



5. Conclusion

Generally, this study found that the Ph.D. candidates and graduates satisfied with the process of supervision especially with supervisors competent factors either in body of knowledge or in research methodology competency. They were also satisfied with their supervisors' moral interactions and their personal involvements and responsibilities towards their programs. However, although they study found general satisfaction of supervisees towards supervision process, the academic stage of the respondents is very significant element to direct their responses. More precisely, it was noted that respondents would naturally respond in positive tone and trivialize difficulty faced during their PhD process if they eventually and successfully completed their program irrespective of challenges. Thus, graduated study usually rated the supervision process positively even if otherwise happened.

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Cultivation and Detection of Sulfate Reducing Bacteria (SRB) in Sea Water

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Abstract: Sulfate-reducing bacteria (SRB) represent a class of anaerobic microorganisms that conduct dissimulatory sulfate reduction to obtain energy. The present study aimed to detect and control SRB activities using a very rapid detectable culture medium and reduction of potential economic loss in the petroleum sector. This study is an attempt to isolate SRB from sea water by rapid and sensitive culture media and to control their effect using eight commercial biocides (Aldehydes and quaternaries). The present work studies the effect of composition of four recommended culture media (Postgate medium B, Starkey's, Baar's and API media), besides, the presence of metal coupons in these media to enhance the growth of SRB. Furthermore, the present study evaluates the efficiency of filtration of these culture media on the growth of SRB. The results revealed that modified Postgate medium B was the recommended medium for SRB growth. In addition, the results showed that rapid and abundant growth of SRB when the metal coupons were immersed in the culture media which were deficient in iron. The unfiltered culture media improved the SRB growth. The growth of SRB was depressed by 15 ppm of the commercial quaternaries rather than 20 ppm of the aldehydes.

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Key words: SRB, Culture media, Biocides, Minimal inhibitory concentration

1. Introduction:

One of the important practical problems is the control of SRB growth in economically important situations in the petroleum sector. Consequently, considerable research has been devoted to testing various potential micro biocides and the results have been displayed throughout the scientific literature (Kumaraswamy et al. 2010). SRB, which generate large amounts of toxic hydrogen sulfide in aquatic ecosystems, are important not only for ecological reasons but also economically. The activities of SRB in natural and man made systems are of great concern to engineers in many different industrial operations (Gibson, 1990; Odom, 1990; Odom and Singleton, 1992). Oil, gas and shipping industries are seriously affected by the sulfide generated by SRB (Battersby, 1988; Hamilton, 1994; Peng et al., 1994; Okabe et al., 1995 and Cullimore, 2000). In the oil industry most monitoring of microbiologically influenced corrosion (MIC) has in the past only been conducted on sulfate-reducing Bacteria (SRB) carried out by cultivation based techniques. (Jan Larsen, 2010).

Sulfate reducing bacteria (SRB) are a group of genetically similar anaerobic organisms that were first discovered by Hamilton (1994). The SRB form an integral part of a group referred to as "sulfur bacteria" and are sometimes considered to be nuisance bacteria in a number of ways, (Tiller, 1990 and American Water Works Association, 1995). These bacteria are seldom isolated because of their slow growth. Colonies appear after more than three days of incubation and are generally not noticed, being overgrown by the accompanying flora. Accordingly, their isolation requires specific or selective growth medium (Julien Loubinoux *et al.*, 2003).No growth takes place in media rendered "biologically free" of iron, (Postgate, 1984; Widdel, 1988; Parkes, *et al.*, 1989).

The present study was conducted to show the efficacy of the impure (turbid) media on the detection of SRB growth.

2. Material and Methods:

2.1. Organisms

A stabilized mixed culture of sulfate reducing bacteria (SMC-SRB) was isolated from the failure shipping pipe line for treated oil (Esh El-Mallaha. Petroleum Company).

2.2. Culture media

Four recommended media of the most commonly used ones were evaluated for SRB growth. The compositions of these media were nominated in table (1). Thioglycollic and Ascorbic acid were added to all media to increase their reducing power. Saline water was used in replacement of tap and distilled water.

2.3. The electrode

The electrode used was derived from mild steel sheets with the following composition:

0.09 % (C), 0.07 % (Si), 0.37 % (Mn), 0.017 % (S), 0.028 % (P), 0.005 %(Al), 0.015 %(Ni), 0.11 %(Cr), 0.004 % (Mo), 0.006 % (Cu), and 0.007 % (V). The electrodes were polished with emery papers 200.400, 600, grade for fine polishing . They were washed with distilled water then degreased with acetone and finally dried till use.

2.4. Pipe line description by ESHPETCO

Length 7 Km (above the ground surface); diameter 18", construction date since 1982; fluid was crude petroleum oil with water content 0.05 % Vol. sulfur content 4.5 % wt, pH value was 6-6, 5 and temperature 20-30°C and the pipe line grade was API–42.Pipe line operation data was as following pressure was 14 bar, stagnant fluid periods since operation were at 1^{st} time (9 months in 2006) the 2^{nd} time and 6 months in 2007.

2.5. Pipe line failure

The pressure dropped and cured oil shortage delivered at point was noted at rapid date. Pipeline track was surveyed and spelled oil was found in one of the train depression along 7 Km train, the pipes lie directly on the ground according to its natural topography of elevations and depressions.

One pipe was found ruptured open to a perfect longitudinal line extending to about 2 meters, slight bulging is clear in the middle of the opening. Huge amounts of crude oil were spilled due to the failure estimated by 10.000 barrels. The opening was in the position 6 o'clock in contact with the ground.

2.6. Field Inspection

Field inspection was done by Central Metallurgical Research and Development Institute (CMRDI). The inspection result reported that the pipe line failure is due to badly fabricated welded pipe. Internal pitting corrosion due to sour oil (along 25 years of service) was a trigger of cracking in the infused weld grooves which ended to complete rupture of the pipe. Two other factors contribute to the failure; a) positioning of the weld line (seam) at the bottom (6'oclock) where water can best accumulate, and b) stagnant long (shut down) periods. They did not ignore the possible malfunction of the treatment plant with respect to water and salt content in the treated oil.

2.7. Isolation and enrichment of SMC-SRB

SMC-SRB were obtained by transferring 1ml of the received internal sludge sample of the failure shipping pipe line into sterile screw capped vials (1.5×5 cm) containing modified Postgate medium B as mentioned in table (1).The bottle was incubated for 7 days at 30°C. Blackening of the bottle meant a positive growth for SRB. This step was repeated at least 10 times to obtain a SMC-SRB (figure1).

Chemical ingredient	Postgate B	API	Starkey	Baar's
KH ₂ PO ₄	0.5	0.01	0.5	0.5
NH ₄ Cl	1.0	-	1.0	1.0
Na ₂ SO ₄	1.0	-	1.0	-
CaCl ₂ .6H ₂ O	0.1	-	0.1	-
MgSO ₄ .7H ₂ O	2.0	0.2	2.0	2.0
Sodium Lactate (60 –70%)	5 ml	4	5 ml	5
Yeast extract	1.0	1.0	-	-
Ascorbic acid	0.1	0.1	0.1	0.1
Thioglycollic acid	0.1	0.1	0.1	0.1
FeSO ₄ .7H ₂ O	0.5	-	-	-
NaCl	26	26	26	26
Fe(SO ₄) ₂ (NH ₄) ₂ .6H2O	-	0.2	0.5	0.5
CaSO ₄	-	-	-	1.0
Sea water	500 ml	500 ml	500 ml	500 ml
Distilled water	500 ml	500 ml	500 ml	500 ml
рН	7-7.5	7-7.2	7-7.3	7-7.5

Table (1) Chemical composition of the modified culture for SMC-SRB growth in g/L



Figure (1): Gram negative stabilized mixed culture SRB

2.8. Physicochemical analysis of saline sea water The water sample was completely analyzed according to APHA (1989) as recorded in table (2).

Table (2): Physical chemical analysis of sea waterreceived from ESHPETCO.

6.73
1.017
0.227 Ohm. m
4904 ppm
130
1231
1763
0.02
0.09
0
26
0
0
13000
2400
117
0
23601

2.9. Evaluation of the culture media on the SRB growth

Four different recommended culture media (Postgate B, API, Starky and Baar's) were prepared according to their compositions as shown in table (1). Thioglycollic and ascorbic acid were added to increase the reducing power of the medium. All media were autoclaved at 121°C for 20 min. Observation of the culture media was recorded in table (3). API medium was the only clear one. These media were distributed in 10 ml sterile screw capped vials (9ml) in each one of them. Enriched SMC-SRB 4 day's old culture was inoculated into the previous culture vials. Then all vials were incubated at 30°C for 7 days and observed by naked eye (table 4). The sulfide production was determined via the SRB activity during 7 days to record the time course of the sulfide production by SMC-SRB (figure 2). Sulfide was determined iodometrically according to APHA (1989).

Table	(3):	Observati	ions of	the	modified	culture
media	for S	SMC-SRB	in sea `	Wate	er.	

Media Properties	Postgate (B)	API	Starky	Baar's
Color	Yellow with faint gray	Yellow	White	White
Turbidity	Turbid	Clear	Turbid	Turbid
pH before autoclaving	7.3	7.2	7.2	7.2
pH after autoclaving	6.7	6.4	6.4	6.4
E _h (mV)	-345	-260.3	-200.7	N.D

N.D Not determined.

Table (4) Naked eye observation of SMC-SRB growth using the modified culture media in sea water during 7 days incubation at 30° C

Time (days)	Media						
Zero Time	Postgate (B)	API	Starky	Baar's			
1	-	-	-	-			
2	+	-	-	-			
3	++	-	-	-			
4	+++	-	+	±			
5	+++	-	++	+			
6	+++	- +	+++	+			
7	+++	+	+++	+			

- No growth

+ Moderate growth

++ Good growth

+++ Severe growth



Figure (2): Time course of sulfide production by SMC-SRB during 7 days incubation at 30°C in different culture media

2.10. Effect of metal coupons on the enhancement of SRB growth

The mild steel coupons of $0.2 \times 1 \times 5$ cm were prepared using emery papers with very fine grade as mentioned before. The prepared clean mild steel coupons were immersed in the screw sterile vials which contained the previous culture media. One ml of the enriched SMC–SRB was inoculated into the previous vials. The vials were incubated and observed (table 5) for 3 days at 30°C. Then the sulfide

produced was determined as mentioned before (figure 3).

Table (5): Naked eye Observation of SMC-SRB growth in the presence of metal coupon in the culture media after 3 days incubation at 30° C

Media Time (days)	Postgate (B)	API	Starky	Baar's
7	++++	++	++++	+++





2.11. Effect of the weight loss measurements

Weight loss measurements were carried out in screw capped vials containing the previous sterile culture media. These vials were inoculated with 1 ml of enriched SMC-SRB. The clean weight mild steel coupons (W1) were immersed completely in the medium and incubated for 7 days at 30 °C. After the incubation period ended the coupons were picked up and immersed in a washing solution (1 % HCl + 0.5 % Thiourea) for 5 min to remove the corrosion product layer. Then the coupons were washed by distilled water and dried, then reweighed and the weight loss was recorded to calculate the corrosion rate as showed in (table 6) according the following equation.

MPY = (Area factor)* X (Wt.loss in mg)/ (Days exposed)

*(The area factor is computed from the exposed surface area and density)

Table (6): Effect of different culture media on the corrosion rate of the mild steel. Coupon after 7 days of incubation at 30°C by weight loss technique (MPY)

Post gate (B)	API	Starkey	Baar's	
0.87	5.2	14.8	0.534	

2.12. Effect of filtration of the culture media on the SRB growth

The turbid culture media Postgate B, Starky and Baar's were filtered through filter paper before autoclaving. After that the culture media were distributed in sterile screw capped vials and then inoculated with 1 ml of enriched SMC-SRB and incubated at 30°C for 7 days. Sulfide production was determined as mentioned before (table 7).

Table (7):	Effect	of clear	medium	on	the	SMC-
SRB growt	th after	3 days ir	ncubation	at	30°C	using
different ci	ulture n	nedia				

Media	Sulfide concentration mg S/L	Without filtrate after 3-days
Postgate B	115	288
API	167	99
Starkey	11	135
Baar's	13	213

2.13. Biocide test

The Baar's medium as mentioned in table (1) dispensed in 9.0 ml amounts into a series of 10 ml capacity screw capped glass vials. These vials contained various concentrations ranged from 5 to 20 ppm of commercial biocides (four quaternaries and four aldehydes) coded as Q1, Q2, Q2, Q3, Q4, A1, A2, A3, and A4, respectively. The vials were autoclaved at 121°C for 15 min .After cooling the enriched SMC-SRB was inoculated and the SRB growth was detected after 7 days incubation calorimetrically by measuring the absorbance at 580 nm (table 8). Control vial was inoculated with sterile H₂O. The efficiencies (E %) of biocides (table 9) were calculated according to the following equation: E % = E uninhibited – E inhibited/Euninhibited × 100.

Table (8): Determination of the mic of the tested biocides against SMC-SRB by using colorimetric measurement at 580 nm.

Cone Prm	Tested samples							
Conc. I pin	Q1	Q2	Q3	Q4	A1	A2	A3	A4
Control	0.230	0.230	0.230	0.230	0.230	0.230	0.230	0.230
5	0.209	0.200	0.229	0.205	0.211	0.203	0.213	0.219
10	0.107	0.103	0.119	0.120	0.073	0.088	0.079	0.086
15	0.078	0.055	0.066	0.060	0.011	0.012	0.011	0.013
20	0.011	0.009	0.012	0.013	0.006	0.006	0.004	0.007

Table (9) Efficiencies (%) of the tested biocides against SMC-SRB

Cone Ppm	Tested samples							
Conc. I pin	Q1	Q2	Q3	Q4	A1	A2	A3	A4
Control	0	0	0	0	0	0	0	0
5	9	13	4	11	30	38	45	45
10	53	55	48	48	68	62	65	63
15	66	76	71	74	95	95	95	94
20	75	80	79	80	97	97	98	97

3. Results and Discussion:

Physicochemical analysis of saline sea water as recorded in table (2) revealed that it was a very good source for SRB due to the sulphate contents (2400 ppm). This was confirmed by the field inspection done by (CMRDI); they did not ignore the possible malfunction of the treatment plant with respect to water and salt content in the treated oil. Thus the pipe line failure might be due to biological effect in addition to physical one. Besides the Blacking of modified Postgate medium B revealed the positive growth of SMC-of SRB.The results detected an improvement for SRB growth due to addition of supplied sea water in comparison with the previous studies .Figure (1) showed that SRB was a pure Gram negative short rods. SRB are present in most soils and water, but are outnumbered by other types of microbes except in special environments. Accordingly enrichment of the needed environments with these bacteria is usually necessary before isolation is attempted (Widdel, 1988). On the other hand the presence of reductants in culture medium makes isolation a much less formidable task (Postgate, 1984).

Postgate medium B is a multi purpose medium for detecting and culturing Desulfovibrio and Desulfotomaculun. Most of the ingredients can be prepared and held as a stock, but the thioglycollate and ascorbate, which may be omitted if the inoculua are fresh, flourishing culture, should be added and the pH adjusted just before autoclaving. The medium should then be used as soon as it cools down because the reductants deteriorate in air at neutral pH values. This process accompanied by a transient purple color. The precipitate in medium B aids growth of tactophilic strains. This medium is recommended for long term storage of strain. In cultivation of the SRB in pure culture, the major prerequisite is simple. The redox potential $(E_{\rm b})$ of the environment must start around -100 mV. This means that mere exclusion of air is not sufficient to ensure growth (a boiled-out Lactate + sulfate medium would have an E_h of about +200 mV under N₂ be about with 5-m M Na₂S the value would be about (-220 mV) (Postgate, 1984). This was mentioned in tables (4) which showed that modified Postgate medium B was the recommended medium for SRB growth followed with Starky then Baar's one because the precipitate in these media aids growth of tactophilic strains and this was confirmed by the effect of filtration of the culture media on the SRB growth as showed in table (7) ,the filtration of the turbid medium reduced the SRB growth . In addition the results in table (5) and figure (3) revealed that rapid and abundant growth of SRB after 3-days incubation when the metal coupons were immersed in the culture media which were

deficient in iron. This due to the utilization of the hydrogen evolved when metallic iron immersed in mineral medium provides additional evidence for the presumed role of SRB in anaerobic corrosion of ferrous pipes namely that the depolarizing of cathodic elements of electrochemical systems on the surface of the metal. Weight loss measurement recorded that Starky medium was the most aggressive medium to mild steel. This might be due to the anodic dissolution of mild steel and cathodic depolarizer's effect of FeS film which formed due to SRB activity. So for diagnostic purposes media often prescribed are those which contain about 0.5 % of a ferrous salt. This forms a black precipitate of FeS when sulfide is formed, so blacking of the medium as a whole, or the zone round a colony, is an evidence for bacterial sulfate reduction as shown in equation (1) (RzecZycka and Blaszczyk, 2005).On the other hand API medium showed a high mpy corrosion rate in comparison to modified Postgate B and Baar's media. This might be due to metal exposure to chemical dissolution in that medium (general corrosion) not for SRB activity. This illustrated that the growth of SRB by (using API medium) conventional methods is very time consuming, (Iverson, 1987; Taylor and Parkes. 1983).

SO_4^{2-} + Organic matter = $HS^- + H_2S + HCO_3^{-}$ ⁽¹⁾

Due to the economic losses as well as environmental health and safety hazards caused by the activity of stabilized mixed culture containing reducing bacteria,(SMC-SRB)in sulfate many industrial sector such as the oil and gas industry, it was important to minimize the risks resulting from SRB activity. These bacteria are mainly sulfate reducers, and their growth frequently causes severe corrosion problems in oil well pipes. One of the simplest ways to measure the effect of biocides on an organism is by determining the Minimal Inhibitory Concentration (mic) which just prevents growth in a suitable medium. The antibacterial agent is serially diluted in the medium and standardized inocula of the test strain are added. After incubation for a predetermined growth, the cultures are examined and the mic for the biocide is detected (Sharma, et al., 1986). The mic of microbicides and bacteriostatic substances are usually governed by the nature of the medium in which the substance is tested and also by the size of the inocula. Iron salts can increase the apparent resistance of cultures to inhibitors, and in case of Desulfovibrio species the presence or absence may influence inhibition (example a of NaCl quaternary biocide) (Bessems, 1983). In the present work the effect of tested biocides on a cell is normally dependent on its concentration and it's can

be seen from the slight increase and leveling of A_{580} at growth of SRB was depressed by 20 ppm of the commercial quaternaries rather than 15 ppm of the aldehydes. The results in tables (8&9) showed the biocidal activity and biocidal efficiencies and recorded that up to 97% for commercial aldehydes and up to 75% for commercial quaternaries at 20 ppm.The cell membrane of microorganisms is composed of several lipids and protein layers arranged together in a specific arrangement called the bilayer (or multi layer lipoprotein structure). The presence of the lipids as a building unit in the cell membrane acquires them their hydrophobic Character (Bessems, 1983). The selective permeability of the lipoprotein membrane represents the main function, which control the biological reaction in the cell. Hence any factor influences that permeability causes a great damage to the microorganisms, which leads it to die.

4. Conclusion:

The isolation of SRB by conventional methods is very time consuming. The present work study recommended modified Postgate B and Starkey's media because the precipitate in these two media aids growth of tactophilic strains. Also the use of the supplied water sample by 50% of the total volume of the culture medium improved the SRB growth. Besides, the presence of metal, improved the SRB growth.Ontheotherhand the growth of SRB was depressed by 15 ppm of the commercial quaternaries rather than 20 ppm of the aldehydes. The present study aimed to detect and control SRB activities using a very rapid detectable culture medium .In addition to reduction of their economic loss in the petroleum sector.

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Effective Factors on Discontinuance of Sprinkler Irrigation Systems among Farmers in West Azerbaijan Province of Iran

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Abstract: The purpose of this study was to determine effective factors on discontinuance of sprinkler irrigation systems (SIS) among farmers in West Azerbaijan Province of Iran. A causal-comparative design was used and data was collected by means of questionnaire and interview with farmers who had used SIS and at least produced and harvested one agricultural crop in West Azerbaijan Province, as the target population. The sample was obtained through proportional stratified sampling (n=124). Instrument validity was established by a panel of experts and reliability analysis yielded an alpha value of 0.81. Study results showed that approximately 30% (n=36) of farmers discontinued use of SIS. The findings indicated that there was a statistically significant difference between adopters who continued SIS regarding some dependent variables including respondents' personal and farming characteristics and respondents' viewpoints about installing and keeping SIS). The result of discriminate analysis showed that "use of river as water source", "use of Gun system to farm irrigation", and "system design", were identified as the most discriminative factors (99.20% of population), affecting discontinuance of SIS.

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Iran has an average annual rainfall of 252 millimeters, and 90 percent of its area is considered as arid and semi-arid. In order to respond to increase in demand for water in this country, most politics are focusing on supply water management includes constructing dams, irrigation channels, and Make full use of groundwater that usually whole of this increasing demand with more extraction is provided by traditional methods from groundwater sources. On the other hand, the use of traditional irrigation methods with on-farm irrigation efficiencies ranged between 23 and 32% causes that the balance between amount of using groundwater sources (55 billion m³) and extraction of groundwater (45 billion m³) eliminates (U1-Hassan et al., 2007). Thus, it is essential to pay more attention to demand for water management politics such as using modern irrigation systems. many authors have investigated Many effective factors on adopting SIS Kebede et al., 1993; Noruzi and Chizari, 2006; Skaggs, 2001; Caswell and Zilberman, 1985; Stevens, 2006; Xue et al., 2007; Tollefson et al., 2002; Barja1, 2002; Shrestha and Gopalakrishnan, 1993; Caswell, 1991) or have assessed different irrigation methods at specific places (Tecle and Yitayew, 1990; Karami, 2006). These studies show that farmers choose the best system based on their knowledge and information, economic and social conditions, and amount of society and government support that they expect to get these consequences such as good control of water application, rapid germination, saving labor and energy expenditures, applying nutrients through the irrigation system, and decreasing plant diseases on level of farm by using (Skaggs, 2001; Qassim, 2003). If these phases aren't done very well in this process, it will cause dissatisfaction and discontinuance using of SIS as an innovation.

Few studies have been conducted in discontinuance of an innovation, so that there is little information about this important aspect of behavior. Karami (2006) stated that discontinuance of an innovation is a decision to reject it after having adopted it. Rogers (1995) believed that this discontinuance may occur because of a better idea or dissatisfaction from innovation performance. Leuthold (1967) believed that in determining the extent of adoption of an innovation, the rate of discontinuance is as important as the rate of adoption. Researches among the USA and Canadian farmers indicated that innovators and early adopters, and laggards have the least and the most of extend of innovation discontinuance, respectively. Available data from theses researches showed that rate of discontinuance ranges from 14 to 40 percent of adopters (Bishop and Coughenour, 1964; Leuthold, 1967). Sofranko et al. (2004) used the term "deadoption" to describe using discontinuance of previously adopted innovation and identifying nonprofitability as the main reason for innovation discontinuance in farmers of Illinois. Oladele (2005) research showed that lack of extension contacts are the main reason for innovation discontinuance in farmers of Nigeria. Kulecho and Weatherhead (2005) identified three reasons for micro-irrigation discontinuance among farmers in Kenya including: lack of maintenance, irrelevant cultural background and unreliable water supply. Kolawole et al (2003) identified three forms of immediate, gradual and rapid discontinuance in terms of innovation nature and farmer conditions in Ekiti state of Nigeria. Kulecho and Weatherhead (2005) found out in their research that inappropriate keeping, lack of social support, and unstable water sources were the main reasons for SIS discontinuance among farmers in Nigeria. Report of Ul-Hassan et al (2007) shows that rate of using SIS has a slow rate in Iran. So, only 2 percent (250 thousand hectares) of farm lands is under cultivation with these systems. In many cases, even after installing these systems, a number of adopters end up with disenchantment discontinuity and return to traditional methods. Now, this study attempts to reply this question: what reason is considered, in spite of expensive cost to install system' farmer X continues its use and farmer Y discontinues it after a while and returns to traditional irrigation system?

The main purpose of this study was to examine the effective factors on discontinuance of SIS among farmers in West Azerbaijan Province of Iran. The specific objectives were:

1. To determine personal and farming characteristics of farmers;

2. To determine viewpoints of farmers about Usefulness and;

3. To compare the selected independent variables between two groups of farmers, those who continued use of SIS and those who discontinued use of SIS;

4. To identify the major components of independent variables for discriminating farmers who continued

2. Materials and methods

A casual-comparative study was conducted to achieve research objectives. Farmers, who used SIS and at least produced one agricultural crop in the West Azerbaijan Province of Iran, were the target population of this study. Sample size was determined a Krejcie and Morgan (1970). Therefore, a sample of (n=124).West Azerbaijan Province is located in northwest of Iran, and produces many agricultural crops such as wheat, alfalfa, sugar beat, corn, and barely. This province is located between 36° and 39° E and 44° and 47° N. It has an area covering 37590 square kilometers and has a population of 2873459, out of which, 1148505 live in rural areas. Its annual rainfall average is 300-400 mm.

A questionnaire was to collect data. The instrument was divided into three sections. Section one was designed to gather data about farmers' personal and farming characteristics such as age, experience in farming, education level, farm acreage. Section two was designed to gather data about farmers' viewpoints regarding system design (11 items), and system's economic consequents (12 items). Third focused on farmers' viewpoints regarding the amount of social (7 items), organization (7 items), after sales-services (7 items) and extension-education (6 items) supports For using SIS. six-point Likert-type Scales ranged from 0=not at all, 1=very low, 2=low, 3=medium, 4=high, and 5=very high), were developed to measure variables in section 2 and section 3.. It should be noted that, although discontinues of SIS depends on local climate and soil characteristics in a complex way, local climate and soil characteristics may not vary much from field to field within a location. Therefore, these variables were not investigated in this research.

Face validity was established by a panel of experts consisting of faculty members at Tarbiat Modares University, Department of Agricultural Education and Extension, and Irrigation Sciences. The reliability for each of the section within the study were: system design=0.91; system's economic consequences=0.85; social supports for installing and using system=0.79; organizational supports for installing and using system=0.89; after salesservices=0.87; and extension and education supports for installing and using system=0.92

Data was collected through interview with farmers on their farms from June to August 2009. The data was coded and analyzed using the Statistical Package for the Social Science (SPSS 14). Descriptive statistics (frequencies, means, standard deviations, range, minimum, and maximum) were used to describe data. Independent sample t-test, Mann-Whitney test, Chi-Square test, and Stepwise Logistic Regression were employed to analyze the relationships and differences among variables.

3. Results and discussion

Finding for each objective will be presented in this section in the order outlined in the purpose and objective section, and will be discussed as follows. *Personal and farming characteristics of farmers:*

Data analysis showed that all farmers who used SIS for irrigating were male. The mean age of farmers was 48 years old and the majority of them (50.6%) Were between 46 - 56 years old (Table 1). While 4.8% of farmers (n=6) were illiterate, 44.40% (n=55) had a primary school education. About 25.80% of farmers (n=32) had guidance level education and 25% (n=31) had high school or post secondary education. Respondents had, on average, 30 years of experience in agriculture and majority of them (36.3%) had an experience between 30 - 40 years. A majority of respondents (68.5%) farmed from 2 to 12 hectares of farm land. In other words, farmers owned 13 hectares of agricultural land that 7 hectares of which was irrigated by using of SIS. With regard to SIS type used by farmers, about 41.1% of the respondents (n=20) used Solid-Set, and approximately 11% (n=14) of them used Continuous Move. Regarding water resources,, about 16.1% of the farmers (n=20) used rivers and approximately 62% (n=77) of them used exclusive water well.

Farmers' viewpoints on system design, its economic consequences, and supports for installing and using it: viewpoints on installing and using SIS was investigated as described in the methodology section. For the purpose of characterization, the scores were labeled as: "weak", "mediate", "good", and "excellent". Based on means and standard deviations of the view point score, the four categories were determined by scores that within two standard deviations to the left of the mean on a normal curve, and two standard deviations to the right of the mean (Sadighi and Mohammadzadeh, 2002).

A = weak: A<Mean-SD

B = mediate: Mean-SD<B<Mean

C = good: Mean < C < Mean + SD

D = excellent: Mean + SD<D

Farmers' view points about system design ranged from 19 to 42 (M=34.63 and SD=5.92). Table 3 shows that a majority of farmers had "mediate (n=22 & f=17.70%) and good (n=78 & f=62.90%)" viewpoints about system design in their farms. Farmers' view points about system economic consequents ranged from 13 to 58 (M=43.70 and SD=11.32). Table 3 shows that a majority of farmers had "mediate (n=33 & f=26.60%) and good (n=62 & f=50%)" viewpoints on the system economic consequents. Farmers' view points about social supports installing and using it ranged from 12 to 32 (M=19.40 and SD=4.98). Table 3 indicates that a majority of farmers had "mediate (n=34 & f=27.40%) and good (n=11 & f=9%)" viewpoints about social supports for installing and using SIS. Farmers' view points about organization supports for installing and keeping SIS ranged from 3 to 21 (M=17.07 and SD=3.13). Table 3 indicates that a majority of farmers had "mediate (n=34 & f=27.40%) and good (n=67 & f=54%)" viewpoints about organization supports for installing and keeping SIS. Farmers' view points about after-sales services of SIS keeping ranged from 4 to 21 (M=11.67 and SD=3.62). Table 3 indicates that a majority of farmers had "mediate (n=43 & f=34.70%) and good (n=42 & f=34%)" viewpoints about after-sales services. Farmers' view points about extension-education supports for SIS ranged from 2 to 25 (M=15.11 and SD=6.54). Table 3 indicates that a majority of farmers had "mediate (n=47 & f=37.90%) and good (n=16 & f=13%)" viewpoints on extension-education supports for installing and keeping SIS.

Table 1:	Respondents'	personality	and farming
	characteris	tics (n=124)	

Variables	Items	f	%
	24-34	14	11.30
	35-45	28	22.60
Age	46-56	64	51.60
	57-67	18	14.50
Land under	2-12	85	68.50
cultivation	13-23	20	16.10
(Hectare)	24-34	13	10.60
	35-45	6	4.80
	2-4	18	14.50
Size of land	5-7	46	37.10
under	8-10	33	26.60
cultivation	11-13	25	20.20
using of SIS	14-16	2	1.60
(Hectare)			
	~		.
T	Gun	43	34.70
Type of	Solid-Set	51	41.10
irrigation	Continuous	14	11.30
system	Move	1.6	12 00
	Semi-Portable	16	12.90
Europianas in	0 10	24	10.40
forming (Voor)	0-10 10-20	24 42	19.40
farming (Tear)	19-29	45	26 20
	30-40 41 5 1	43	0.70
	41-31	12	9.70
water	River	20	16 10
resource	Spring	17	13.70
10000100	Participatory	10	8.10
	water well	10	0110
	Exclusive	77	62.10
	water well		
	Illiterate	6	4.80
	Primary school	55	44.40
Education	Guidance	32	25.80
level	High school	28	22.60
	Post secondary	3	2.40

mistaning and Keeping 515 (n=124)			
Variables	Items	f	%
	Weak	18	14.50
system Design	Mediate	22	17.70
	Good	78	62.90
	Excellent	6	4.90
	Wool	24	10.40
Social supports for	Modiato	24	27.30
installing and keeping SIS	Good	55	27.30
instanting and keeping 515	Evallant	11	44.40 8 00
	Excellent	11	8.90
after-sales services	Weak	19	15.30
	Mediate	43	34.70
	Good	42	33.90
	Excellent	20	16.10
	Weak	17	13.70
System economic	Mediate	33	26.60
Consequents	Good	62	50
	Excellent	12	9.70
Organization supports for	Weak	13	10.50
installing and keeping SIS	Mediate	34	27.40
	Good	67	54
	Excellent	10	8.10
Extension-education	Weak	24	19 40
supports for installing and	Mediate	47	37.90
keeping SIS	Good	16	12.90
more and and	Excellent	37	29.80
	LACCHEIR	51	27.00

Table 2: Farmers'	view	point	about	supports	for
installing an	d kee	eping S	SIS (n=	=124)	

Comparison of selected independent variables in two groups of farmers, those who continued use of SIS and those who discontinued use of SIS:

- Comparison of personal and farming *characteristics of farmers who continued use of the system* and those who did not.

independent-samples An t-test was conducted to evaluate the differences between these two groups of farmers. As shown in table 4, there was a statistically significant difference between two groups in terms of their age. The findings indicated that farmers who are still using the system are older that those who discontinued. This finding contradicts the results of various published literature (Rogers, 1995; Bishop and Coughenour, 1964; Leuthold, 1967). An independent-sample t-test was conducted to evaluate the differences between two groups. As shown in table 4, there was a statistically significant difference between two groups of farmers in terms of land under cultivation and irrigated farm Using SIS. The findings indicated that farmers who still use SIS had larger farms and larger irrigated farms using SIS, than those who discontinued. This supports Karami (2006) study. His study showed that using sprinkler irrigation was an inappropriate decision for farmers who had not good economic condition and they should not have used it.

- Comparison of the viewpoints of two groups of farmers on system design, system economic consequences, and supports for using SIS.

Mann-Whitney tests were conducted to evaluate the differences between two groups as shown in table 3; there were statistically significant differences in the viewpoints of two groups on system design and economic consequences of the system. The findings indicate that design of system and system economic consequents in continued users were better and more than discontinued users. This finding is consistent with the results of prior research (Sofranko et al., 2004; Kolawole et al., 2003).

As shown in table 3, there was a statistically significant difference in the viewpoints of two groups on Extension-education supports for installing SIS. The finding indicated that farmers, who are continuing the use of SIS, received stronger extension and educational supports. This finding supports the findings in Oladela (2005) study. The results implied that extension agents should continue extension-education supports after adoption of innovation, because in many systems Farmers often receive negative messages about adopted innovation.

Table 3: Comparison of independent variables in
two groups of farmers

Variables	Continued users (n=88)	discontinued users (n=36)	t-test	P- value
	Mean	Mean		
Age	49.27	45	2.35^{*}	0.020
Experience in farming	28.39	26.83	0.93	0.353
Size of land under cultivation	14.55	8.83	3.88***	0.000
irrigated farm using SIS	8.04	6.27	2.89**	0.004

Note: $P \le 0.001^{***}$, $p \le 0.01^{**}$, $p \le 0.05^{*}$

As shown in table 4, there was a statistically significant difference in the viewpoints of two groups on social supports for SIS. The finding indicated that social supports for using SIS in farmers who are still using SIS was stronger compared to the supports received by those who discontinued SIS. This confirms also pointed by Kulecho and Weatherhead (2005).

Variables	Mean Rank	Mean Rank	Z	U	p- value
Education level	60.06	68.46	-1.25	1369.50	0.209
system design	75.14	41.94	- 4.69 ^{***}	736	0.000
System economic consequents	72.84	37.22	5.02***	674	0.000
After-sales services	59.93	55	-0.70	1458	0.484
Organization supports or using SIS	63.05	61.15	-0.97	1460	0.783
Extension- education supports or using SIS	61.18	51.06	-2.27*	1172	0.023
Social supports for using SIS	67.76	49.64	-2.56*	1121	0.010

 Table 4: Comparison of independent variables in two groups of farmers

Note: $P \le 0.001^{***}$, $p \le 0.01^{**}$, $p \le 0.05^{*}$

The major components of independent variables for two groups of farmers

A forward stepwise logistic regression analysis technique was employed to identify the major components of independent variables for discriminating continued users from discontinued users of SIS. The statistically significant dependent variables in (an independent) t-test (table 3). Mann-Whitney test (table 4), and Chi-square test (table 4) were used as independent variables in logistic regression analysis. The findings indicated that the logistic regression stopped on the third step, and variables such as "use of river as water source" (Dummy variable), "use of Gun system for irrigation" (Dummy variable), and "system design " were found as the most important discriminative components of discontinuance of SIS. Table 5 shows the detail analysis of the logistic regression test. These factors made a valuable distinction among 99.20% of population. The variability of Chi-square shows high magnitude and effect of discriminative variables (variable components) on discontinuance of SIS (table 5).

For predicating framer's decision on continuance or discontinuance of SIS, the legit of f(x) function was calculated (Table 6). Based on statistically significant variables in the logistic regression analysis, and constant values, the logistic regression equation could be derived as follows: $F(x) = 6.774 + 0.503 (X_1) + 0.109 (X_2) - 0.089 (X_3)$ The magnetite of f(x) could be predicted by determining the value of each major variable in this equation. The positive values of beta in this equation indicate that Increase in the value of these two variables (use of river as water source and use of Gun system for irrigation), raises the possibility of discontinuance.

anecting discontinued user of 515					
Step	Variable	Correct	Chi-	df	р-
		Class%	square		value
1	Use of river as water source (X ₁)	87.10	60.106***	1	0.000
2	Use of Gun system for irrigation (X ₂)	96.80	121.847***	2	0.000
3	Appropriate and engineering design of system (X ₃)	99.20	136.889***	3	0.000

 Table 5- Discriminative dependent variables

 affecting discontinued user of SIS

Note: $P \le 0.001^{***}$, $p \le 0.01^{**}$, $p \le 0.05^{*}$

Table 6: Variables in Logistic Regression Analysis

Variable	Beta	SE	d f	P- value	EXP(B)
Use of river		0.87	1	0.007	
as water	0.503	0.87			0.001
source (X_1)		0			
Use of Gun	0.100	0.02	1	0.007	1.005
system for	0.109	2			1.085
irrigation (X_2)		0.21	1	0.000	
System design	-	0.31	I	0.002	1.118
(X_3)	0.089	0.00		0.007	
Constant	6.774	0.03 5	1	0.007	1.221

Note: -2 Log likelihood= 12.516; Cox & Snell R Square= 0.668; Nagelkerke R Square= 0.995

4. Conclusions

Continuance of innovation is another side of adoption of innovation that has been investigated in many studies. This study aimed at determining the factors influencing farmer's decision to continue or discontinue SIS. The result of this study showed that economic factors such as size of cultivated land, appropriate engineering design, system economic consequents, extension-education supports for SIS, social supports for use of SIS, type of system had an impact on continuing or discontinuing SIS. A model was developed to predict who will continue SIS. Three variables including "use of river as a water source", "use of Gun system for irrigation" and "system design "were included in the model. The results of this study could help Government and donor agencies to predict who will continue SIS, and in which farms this system could be more effective. This prediction will result in more cost recovery and infrequent failure

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Reduction of Alfatoxin in Clarious lazara Catfish By Ginseng Extract and Nigella sativa Oil

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Abstract: Aflatoxine the major toxic metabolites of fungi which are able to induce chronic liver damages. The antioxidant and hepatoprotective effects of Ginseng extract and Nigella sativa Oil 1% on Alfatoxin was investigated. Alfatoxicosis causes significant increase in liver enzyme SGOT and SGPT, Alkaline phosphatase activity and an increase in the level of cholesterol total lipid, decrease the level of total protein and hemoglobin and P.C.V. Moreover the liver exhibited some clinicopathological changes and decreased body weight. Both Ginseng extract and Nigella sativa Oil 1% reduced the development of hepatotoxicity by Aflatoxin. Nigella sativa showed more improvement of all enzymes of kidney and liver, and also total lipid and cholesterol were reduced and dody weight increased.

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Keywords: Aflatoxin toxicity. Nigella sativa oil effect. Ginseng extract effect. Clarious lazara Catfish

Introduction:

Aflatoxin is a toxic compound produced by Aspergillus flavus and A. parasiticus. The molds can grow in improperly stored feeds and feeds with inferior quality of ingredients. Aflatoxins represent a serious source of contamination in foods and feeds in many parts of the world . These toxins have been incriminated as the cause of high mortality in livestock and in some cases of death in human beings (Muriani.2003).

Aflatoxin is a potent hepatocarcinogen, strong mutagen and a potential teratogen.(Canton, et al. 1998; Bulter and Clifford, 1985). There are four main Aflatoxins:B1, B2, C1,C2. Aflatoxin B1 is known to be the most significant form that causes serious risk to animals and human health. The carcinogenic effect of aflatoxin B1 has been studied in fishes such as salmonid, rainbow trout, channel catfish, tilapia, guppy and Indian major carps (Jantrarotai and Lovell, 1990; Lovell, 1992; Tacon, 1992; Wu, , 1998; Chavez et al., 1994; Murjani, 2003). Aflatoxins inhibited RNA synthesis and DNA in liver. (Jindal et al. 1994).

Nigella sativa is a spicy poten belonging to ranunculacea seeds oil showed antibacterial fungicidal (Akguil, 1989). Nigella sativa inhibited chemical carcinogensis. Some investigators reported that its antioxidants effect inhibited Chemical carcinogenesis. Ascorbic acid and Nigella sativa could reduce Aflatoxin induced liver cancer (Newperne et al., 1999). Panax ginseng C.A. Mayer is an herbal root that has been used for more than 2000 years throughout Far Eastern countries including China, Japan and Korea. Its beneficial effects have bee nanalyzed by extensive preclinical and epidemiological studies (Yun, 2003). Recently, 20-O-(h-D-glucopyranosyl)-20(S)protopanaxadiol (IH-901), a novel ginseng saponin metabolite, formed from ginsenosides Rb1, Rb2 was isolated and purified after giving ginseng extract p.o. to humans and animals (Hasegawa et al., 1996).IH-901 has been shown to enhance the efficacy of anticancer drugs in cancer cell lines previously resistant to several anticancer drugs (Lee et al. 1999).

Aim of present work

This study was conducted to evaluate the effect of Aflatoxin and ginseng with Nigella sativa oil 1% on some nutritional status and clinicopathological changes in Catfish toxicated with Aflatoxin and treated with ginseng and Nigella Sativa oil 1%.

Material and Methods

Experimental conditions: 60 catfish clarious lazera were obtained from Abbassa and were acclimatized to laboratory conditions. They were kept in glass aquaria supplied with dechlorinated tap water at a rate of one liter for each cm of fish body. They

were fed commercial fish diet were supplied by Aflatoxin contaminated ration with corn 80ug toxin/kg ration, as shown in Table (1). A total number of 60 cutfish were used in this experiment: 20 Fish each group, 20 cotfish control, 20 fed Aflatoxin and 20 treated with Faxatation Nigella Sativa.

The third group Aflatoxin contaminated ration + 0.2 ginseng + Niegella sativa oil injected daily 1/p. the fish were fed by hand twice daily and feed consumption in all groups was recorded daily, also mortality and body weight due to Aflatoxin were recorded.

Samples: serum were collected 3 times at 3 months interval and sera were frozen at-20. Tested kits

supplied from biomerieux, France were used for determination of the activity of serum glutamic pyurvic transaminse and glutamic oxalocetic transaminase as described by Reitman and Frankel (1956), serum creatinine was determined according to Henery, (1968). Enzymatic determination of urea was done according to King (1965).

Blood hemoglobin was assessed by cyame hemoglobin method Hematocrit value was carried out by using microhematacrit capillary tubesrentri fuged at 2000P.M. for 5min according to the method of Drabkin (1946) serum cholesterol according to the method Flegg(1973), total lipids according to the method of Siesta (1981), andstatistical analysis according to the method of Gad and Weil (1986).

Ingredient	Control		Provimate	chemical
			composition	
Hah meal	30	Crude protein Pg%	35.87	
Meas meal	8	M.E/kg	2297.21	
Bone meal	1	Ether extract g%	2.78	
Soya bean	5	Crude liber g%	3.91	
Skimnied milk	3	Ash g%	8.735	
Wheat bran	20	Calcium mg&	2.069	
Wheat flour	20	Lysine mg%	2.105	
Yeast	10	Methionine mg%	0.562	
Codliver oil	1			
Mincral and premix	2			

Table(1). Ingredients and proximate chemical composition of diets used in the experiments.

Mineral and vit. Premix perlkg of pellet food

Vit. A 8000 IU, vit. D 900 IU, vit, E 2 IU, vit, K4mg, B2 3.6mg, niacin 20mg, choline chloride 160mg, pantothenic acid 7mg, pyridoxine 0.2mg, vit, B12, 5ug, Mn 70mg, Zn 60mg, Fe 20mg, Cu 2mg, Co 0.2mg.

N.B.: we added 80Ug polluted corn with Aflatoxin B1, in this ration.

Results

Aflatoxicosis produced a significant decrease in body weight if compared with control group as shown in Table2. statistical analysis revealed effect of Aflatoxin, B1 on erythrogram. There is a significant decrease in P.C.V. Hemoglobin (P<0.01) as shown in Table2. there is a significant decrease in mean of total protein and a significant increase in SGOT, SGOT, Urea, creatinine, total lipid, cholesterol and alkaline phosphatose (P<0.01).

Post treatment with ginseng and Nigella sativa oil injection 1% of body weight for 3 months. All this parameters return to normal level as shown in Table3 and 4 if compared with control group.

Table(2). (Effect of Aflatoxin after 1-2 months on clinicopathological changes in catfish after treatment with ginseng and Nigella sativa 1%

Parameters	Control N=(20)	Aflatoxin 1month N=(20)	Alfatoxint + ginseng +Nigella sativa 1% N=20	Control N=20	2months group N=20	Aflatoxint + ginseng +Nigella sativa 1% N=20
AST U/L	82±0.23	133±0.06**	103±0.05	84±1.27	121±2.4**	946±0.09
ALT U/L	17±0.67	27±0.72**	22±0.74	18±0.72	31±0.89**	21±0.18
Urea mg/dl	2.87±0.27	4.6±0.64**	5.2±0.27*	2.7±0.74	5.3±912**	3.3±0.20
Creatininemg/dl	0.72±5.4	0.8±0.23**	0.88±0.34	0.83±0.26	1.3±0.50**	0.83±0.28
Total protein mg/dl	46±0.17	3.5±0.72**	4.4±0.70	5.7±0.22	3.3±0.14**	4.4±0.60
Total lipidscholesterol mg/dl	88±0.99	143±0.23**	104±0.27*	97±0.14	184±1.2**	101±0.74
Cholesterol	178±0.79	212±2.8**	197±0.39*	188±0.64	244±3.6**	191±2.1
Alkaline phosphates mg/dl	16.9±0.37	28.8±0.33**	23±0.18	18.7±0.18	34.8±0.27**	21±0.12
Hemoglobin mg/dl	7.2±0.23	5.4±0.74**	7.1±1.60	8.6±0.29	4.8±0.72**	7.3±11.75
P.C.V%	38±0.63	33±0.05	33±0.05	42±0.71	28±0.02**	37±0.27

P<0.01

Table(3). Effect of Aflatoxin after 3 months on clinicopathological changes in catfish after treatment with gensing and Nigella sativa 1%

Parameters	Control 3 months	Aflatoxin 3 months	Aflatoxin plus ginseng + Nigella sativa 1% 3 months
AST U/L	81±0.14	133±6.2**	82±0.27
ALT U/L	182±0.20	25±0.37**	18.3±0.07
Urea mg/dl	2.88±0.22	5.3±0.18 ^{**}	2.64±0.39
Creatinine mg/dl	0.81±0.46	1.5±0.54**	0.81±03.2
Total protein mg/dl	5.7±0.24	3.1±0.45**	5.4±0.74
Total lipid mg/dl	98±0.78	191±1.4**	94±0.82

Cholesterol mg/dl	184±0.94	254±2.3**	182±0.73	
Alkaline phosphatose U/L	18.8±0.27	36.4±0.91**	18.2±0.32	
Hemoglobin %	8.7±0.44	4.6±0.72**	8.6±0.37	
P.C.V. %	37±0.21	23±0.15**	40.3±0.24	
P<0.01				

Table(4). Effect of Aflatoxin on body weight of catfish during the course of experiment

Group	1month	2months	3months
Control 20 fish	68±0.21p	98±0.16*	121±0.72
Aflatoxin group (20fish)	92±0.10	81±0.2*	74±0.13
Aflatoxint + gensing + Nigella sative (20fish)	86±0.06	104±0.73*	134±0.64

*P<0.01

Discussion

Aflatoxins are hepatotoxins (Pier. 1987, 1999) and also impair immunity which ultimately led to increased susceptibility to disease (Zaki, 1999).the present work demonstrated a severe necrosis in liver of catfish. The liver is the primary site of metabolism of ingested Aflatoxin. (Butler and Clifford, 1985; Ali etal., 1994). The pathological changes of liver observed in the present investigations may be due to primary site of metabolism o ingested Aflatoxins as well as the primary laceratian laceratian of residues and lesions. Similar finding reported by Newperne (1999). The increase of enzyme Urea, creatinine. These changes due to necrosis of kidneys reported by Jindal and Mahipal (1994), Mansfeld (1989), Pier (1987). The lipid metabolism was altered during Aflatoxicosis as judged by increase of total lipid content. In the present experiment, here is a highly elevation of total lipid and cholesterol in serum which agree with Sipple, et al. (1983), Sisk et al. (1988). It is obvious that administration of ginseng and Nigella sativa oil injection 1% of body weight reduced the Aflatoxin in liver, kidney, of infected fish and may protect liver from free radical reactions due to Aflatoxin, also total lipid, cholesterol return to normal level Mona,et al.(2002).

The present study showed a significant decrease in P.C.V., HB concentration in the affected fish that was proportionally correlated with the severity

of Aflatoxicosis. This result is in accordance with Robert(1989). El-Bouhy et al., (1993). They found similar results in broilers chickens common carp Fish and this indicates that the toxin causes a deleterious effect on the hemopoeitic system.

Regarding the biochemical serum analysis, the noticed decreased in T.P. may be attributed to the improved protein synthesis as a result of liver function due to Aflatoxicosis. (Ali et al., 1994, A kguil 1989, Edds, 1993). The increase in ALT and AST activities recorded by Jassar and Balwant (1993), Rasmassen et al., (1986), Sisk et al., (1988), due to liver affection in case of Aflatoxicosis the elevation of ALP activity comes in consistence with mentioned by Jassar and Balwant (1993), Svobodava et al. (1999), in chicken due to degenerative changes in the liver causing leakage of enzymes into serum and cause the highest concentration of alkaline phosphates. The great increase of alkaline phosphates activity due to damage of liver. The detection of Aflatoxin in the liver tissues explain the liver degeneration. Similar results were described by Kubena et al., (1990), who used ginseng for preventing the absorption of Aflatoxins from gastrointestinal tract.

In conclusion, the metabolism of Aflatoxin result in the alteration of various metabolic process within hepatocytes which leads to severe serum biochemical alterations and serious pathological changes which affect fish production but treatment with ginseng and Nigella sativa give an excellent of results.

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Influence Of Gender And Self-Esteem On The Organisational Commitment Of Civil Servants In Ekiti-State, Nigeria

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Abstract: This study examined the influence of gender and self-esteem on the organizational commitment of civil servants in Ekiti State. Two hundred civil servants drawn from five ministries in Ekiti State responded to a battery of instruments (Self- Esteem Scale, Index of Self-Esteem and Organizational Commitment Scale). Four hypotheses were tested in the study. Results showed that there was no significant gender difference in employees' level of self-esteem in ministries in Ekiti State [t (198) = 0.41; p>.05], significant gender difference in employees' level of perceived organizational commitment was also observed [t (198) = 2.18; p<.05]. Further revealed was a significant main effect of gender [F (1, 199) = 3.99; p <.05] and self-esteem [F (1, 199) = 101.96; p <.05] on organizational commitment among civil servants in Ekiti State. Findings from hypothesis four showed that gender [B = 0.18, t = 2.78; p<.05] and self-esteem [B = 0.60, t = 10.49; p <.05] had significant independent prediction on perceived organizational commitment of civil servants. The implications of these findings were discussed in light of the literature. [Adebayo Sulaiman Olanrewaju¹, Olowookere Funmilola Kansola. **Influence Of Gender And Self-Esteem On The Organisational Commitment Of Civil Servants In Ekiti-State**, Nigeria. Journal of American Science 2011; 7(2):597-603]. (ISSN: 1545-1003). <u>http://www.americanscience.org</u>

Keywords: Gender, Self-esteem, Organisational commitment, Civil Servants, Ekiti State, Nigeria.

1. Introduction

As the world becomes a global village, one prominent challenge facing human resource professionals is finding how to gain organizational competitive advantage in the rapidly changing environment. Having an appropriate manpower structure and inducing in the workforce job and organizational commitment is one approach (Jones, 2007).

Hitherto in Nigeria, job security was associated with employment in public sector organizations because, for the most part, they were thought to be protected from the vagaries and instability characteristic of private sector firms by profit objectives and competitive forces of globalization. In fact, many who chose to work for and commit to public sector organizations did so because it provided job security. Therefore, the level of commitment was perceived to be usually high.

It is no secret any longer that securing a job in the public service is no more an achievement in Nigeria. This is not unconnected with the poor remuneration and low level of activity in the ministries. Delay in the payment of salaries, lack of motivation, and the merger of state politics with work schedules are some of the factors that have made civil service work unattractive and unenviable in Nigeria. In spite of the above civil servants already on the job seem to show evidence of job involvement and organization commitment. It is of theoretical and practical significance importance to investigate factors that may be responsible for organization commitment of the Nigerian civil servants. Organizational commitment, according to O'Neil (1989), is individual psychological bond to the organization. This includes a sense of job involvement, loyalty and belief in the values of the organization. Organizational commitment from this point of view is characterized by employee acceptance of the organizational goals and their willingness to exert efforts on behalf of the organization (Miller & Lee 2001).

Commitment is a psychological state that characterizes the employee organization relationship and which has implication for the employee's decision to continue or discontinue membership in the organization. While employees with a strong affective commitment remain with an organization because they want to, those with a strong continuance commitment and strong normative commitment remain because they have to and because they feel they ought to respectively (Meyer, Allen and Smith (1993).

Organizational commitment often reflects certain behavioral patterns. These patterns of behavior are guided by internalized normative pressures to act in a way that meets organizational goals and interest (Wiener, 1982). Wiener and Gechman (1977) suggest that commitment behavior should meet three important criteria:

reflection of personal sacrifices made for the sake of the organization; demonstration of persistence that is, the behaviors should not depend primarily on environmental controls such as reinforcements punishment; indication a personal or preoccupation with the organization, such as devoting a great deal of personal time to organization-related actions and thoughts. In this sense, organizational commitment is viewed as (1) willingness of an individual to identify with and the desire not to leave an organization for selfish interest or marginal gains; (2) willingness to work selflessly and contribute to the effectiveness of an organization; (3) willingness to make personal sacrifice, perform beyond normal expectations and to endure difficult times with an organization-- low propensity to "bail-out" in difficult times (4) acceptance of organization's values and goals the internalization factor. This study adopted the organizational commitment behavior-related approach.

It has been persuasively argued that due to the high degree of situational strength characterizing most organizational contexts, personality exerts relatively little influence in the workplace. But one personality attribute that consistently predictably and enhances understanding of organizational behaviour is 'selfesteem' (Folre, 2007). Researchers have distinguished among several types of esteem, including global self-esteem (an individual's overall evaluation of worth), role-based selfesteem (worth derived from incumbency in a particular position), and task-based self-esteem (worth based on self-efficacy). Within the last five years, an additional form, "organization-based self-esteem" (OBSE), has appeared in the literature. OBSE reflects the degree to which employees selfperceive themselves as important, meaningful, effectual, and worthwhile within the organizational setting (Sunmola, 2006; Ottu, 2005; Mark 2007).

The antecedents of OBSE are, to some extent, controllable at the organization level. For example, OBSE is diminished by structural factors such as a mechanistic form, interpersonal factors such as lack of managerial consideration, and design factors such as the creation of positions that induce role conflict and ambiguity (Rosenburg, 1990). Other indicators, such as environmental instability and lack of an articulated strategy and mission, are also known to depress OBSE. Hence, when individuals are employed in similar positions within the same organization, one might predict that reported OBSE levels would be comparable. This is not necessarily so, however, as there are variations in OBSE among individual incumbents that may be reflective of their perceptions of selfworth in general.

This research is geared towards examining the effect of gender and Self-esteem on organizational commitment among workers in the ministries.

2. Material and Methods

200 participants drawn from five ministries in Ekiti State were used using the purposive sampling technique. The ministries are Health; Justice; Agriculture and Rural Dev.; Lands, Housing and Environment; and Work and infrastructure. The participants consist of 100 males and 100 females with an average age of 35years and age range of 30-55years. From the participants, 25% are SSCE holders, 20% are OND holders, 15% are HND holders, 25% are university first degree holders and 15% are the remaining postgraduate degree holders. 15% of the participants have spent 1-3years in service, 25% have spent 4-6years in service, 20% have spent 7-10years in service, 10% have spent 10-15years in service and 30% have spent above 15 years in service.

Measures

The questionnaire consists of three sections. Section A measured the demographic information of the respondents. These include: age, gender, educational level, tenure, marital status and religion. Section B measured the self esteem of the respondents using Hudson Index of Self Esteem. Section C measured the organizational commitment of the respondents.

Organizational commitment was measured by using Organizational Commitment Scale. This 23-item inventory was developed by Buchannan (1974) to assess the extent to which a worker is effectively attached to the achievement of the goals and values of an organization with particular emphasis on the role the worker selflessly plays in the process of the achievement. The inventory assesses three components on commitment which are: identification (1-6), job involvement (7-12) and loyalty (13-23).

Buchannan (1974) reported coefficient alpha of .86, .84, .92 and .94 fir identification, involvement, loyalty and overall test respectively. Cook and Wall (1980) correlated OC with overall job satisfaction by Warr, Cook and Wall and obtained a concurrent validity coefficient of .62.

Self-esteem was measure using Hudson's (1982) Index of Self Esteem Scale. This scale is designed to measure the self perception and self evaluation component of the self esteem which are

the sum totals of the self perceived and other perceived views of the self held by the person. According to Hudson (1982), ISE has a valid coefficient alpha of 0.93 and a two hour test-retest coefficient of 0.92. It has a concurrent validity coefficient of 0.87 when correlated with Hare Self Esteem Scale.

Design

Although, the study was concerned with the influence of gender and self-esteem on organizational commitment, attempt was also made to know whether variables in the organization like tenure, position and personal variables like age, educational qualification, marital status and religion can also influence organizational commitment. To this extent, independent t-test, one-way ANOVA and regression were employed.

Scoring

The two scales that were used had both direct and reverse scoring. The direct scores are obtained by adding the values of the number in the direct score items and the reverse scores were obtained by adding the values of the number in the reverse score items. And the overall scores were obtained by adding the two scores together. The higher the score, the higher the organizational commitment and self-esteem and the lower the score, the lower the organizational commitment and self-esteem.

3. Results

<u>Table 1</u>: Summary of independent t-test showing difference in the mean scores of females and males on self-esteem.

Variables	Ν	Mean	SD	t	df	Р
MALE	100	79.68	40.18			
				0.41	198	>.05
FEMALE	100	77.32	41.78			

Table 1 above revealed that there is no significant gender difference in civil servants' level of self esteem (t (198) = 0.41; P > .05). Thus, hypothesis 1 which states that females would exhibit significantly greater self esteem than males is rejected.

<u>**Table 2**</u>: Summary of independent t-test showing difference in the mean scores of females and males on organizational commitment.

Variables	Ν	MEAN	SD	t	df	Р
FEMALE	100	73.55	23.02			
				2.18	198	< .05
MALE	100	66.09	25.29			

Table 2 above revealed that there is a significant gender difference in employees' level of perceived organizational commitment in ministries in Ekiti state (t (198) = 2.18; P < .05). From the table, female employees (mean= 73.55) perceived a significantly higher organizational commitment than males (mean = 66.09). Thus, hypothesis 2 which stated that females would perceive a significantly higher organizational commitment than males is accepted.

<u>**Table 3:**</u> Summary of 2 X 2 sowing the main and interaction effect of gender and self esteem on organizational commitment

Source	Sum of square	Mean square	Df	F	Р
GENDER (A)	1464.89	1464.89	1	3.99	< .05
SELFETSEM (B)	37424.60	37424.60	1	101.96	< .05
A X B	6437.71	6437.71	1	17.54	< .05
Error	71942.23	367.05	196		
Total	118543.52		199		

Table 3 above revealed that there were significant main effects of gender [F (1, 199) = 3.99; P<.05] and self esteem [F (1, 199) = 101.96; P< .05] on organizational commitment among of employees of ministries in Ekiti state.

The table also revealed that there was a significant interaction effect of gender and self esteem on organizational commitment among of employees of ministries in Ekiti state [F (1, 199) = 17.4; P< .05]. Thus, hypothesis 3, which stated that there would be significant main and interaction effects of gender and self esteem on organizational commitment is accepted.

Predictors	β	t	Р	R^2	F	Р
Age	0.10	1.36	>.05			
Educational level	0.09	1.41	>.05			
Length of service	0.08	1.26	>.05			
Marital status	0.03	0.42	>.05	0.40	21.27	<. 05
Gender	0.18	2.78	<.05			
Self esteem	0.60	10.49	<.05			

Table 4: Regression table showing the influence of the predictor variables on Organisational Commitment.

 $n = 200, \beta = Standardized regression weight computed at the end of each step, R² = Adjusted R²$

Table 4 above shows the hierarchical multiple regression analyses. It was hypothesized that Age, educational level, length of service, marital status, gender and self esteem will significantly independently and jointly predict organizational commitment. The results showed that age (β =0.10, t= 1.36; P>.05); educational qualification (β =0.09, t= 1.41; P>.05); length of service (β =0.08, t= 1.26; P>.05) and marital status $(\beta=0.03, t= 0.42; P>.05)$ did not have significant independent prediction on perceived organizational commitment among employees in the ministries in Ekiti state. The table also revealed that gender (β =0.18, t= 2.78; P<.05) and self esteem (β =0.60, t= 10.49; P<.05) had significant independent prediction on perceived organizational commitment among employees in the ministries in Ekiti state. Altogether, all the predictor variables of age, educational level, length of service, marital status, gender and self esteem accounted for 40% of the total variance in perceived organizational commitment among the staff of the selected ministries. $[R^2 = 0.40, F (6,$ 199)= 21.27, P<.05].

Thus, hypothesis four, which stated that age, educational level, length of service, marital status, gender and self esteem would significantly independently and jointly predict organizational commitment was partially rejected.

4. Discussions

Four hypotheses were tested with various statistical methods. Hypothesis one which stated that females would exhibit significantly greater self-esteem than males is rejected. Findings from this study revealed that there was no significant gender difference in employees' level of self esteem in ministries in Ekiti state. Contrary to the findings of the present study, SarAbadaniTafreshi, 2006 found out that there is a significant difference in self esteem between males and females. Similarly, Zareh (1994) studied the relationship between achievement motivation, self-esteem and gender among high school students and reported a significant relationship between self esteem and gender.

However, in line with the findings of this study, Hossaini (2002), found out that gender is not a predictor of self -esteem of pre-university students. Gender, is generally asserted to impact upon the growth, demonstration and manifestation of self-esteem, but in the present study it is not found to be so. Generally, in the civil service in Ekiti State, workers are not usually enthused about their job; poor pay, lack of functioning schedules and politicization of key job functions are usually the bane among civil servants . Thus, both male and female staff do not seem to feel different with their self esteem.

Results from testing hypothesis 2 female revealed that workers perceived significantly higher organizational commitment than male workers. This finding seems to be in line with the general pattern in the literature which appears to contend that women as a group tend to be more committed to their employing organizations than their male counterparts (Cramer, 1993; Harrison & Hubbard, 1998; Mathieu & Zajac, 1990; Mowday et al., 1982). Loscocco (1990) found that women were more likely to report that they are proud to work for their organization, that their values and the company's values are similar, and that they would accept almost any job offered to them in order to remain with their current employer. Several explanations have been offered to account for the greater commitment of female employees. Mowday et al. (1982) maintain that women generally have to overcome more barriers to attain their positions within the organization. They concur that the effort

required to enter the organization translates into higher commitment of female employees. Harrison and Hubbard (1998) similarly argue that women display greater commitment because they encounter fewer options for employment.

Numerous researchers have, however, failed to find support for a relationship between gender and organisational commitment (Billingsley & Cross, 1992; Ngo & Tsang, 1998; Wahn, 1998). It may, thus, be concluded that a growing body of evidence appears to support either no gender difference in organizational commitment or the greater commitment of women.

Hypothesis 3 which was confirmed by the findings of the present study revealed that there were significant main and interaction effects of gender and self esteem on organizational commitment. Studies abound (e.g. Ashford & Mael, 1989; Dutton et al., 1994; Herrbach & Mignonac, 2004; Maignan & Ferrell, 2001a; Peterson, 2004; O'Reilly & Chatman, 1986; Van Knippenberg & Van Schie, 2000) supporting this finding.

Hypothesis 4 which stated that age, educational level, length of service, marital status, gender and self esteem would significantly independently and jointly predict organizational commitment ispartially rejected. Findings revealed that age, educational qualification; length of service, and marital status did not have significant independent prediction on perceived organizational commitment among employees in the selected ministries in Ekiti state civil service, however, there was a significant joint effect of these demographic factors on perceived organizational commitment. This result negates that of Dunham, Grube & Castaneda (1994) who a significant relationship found between organisational commitment and age. Similarly, in related studies, (e.g. Meyer & Allen, 1997; Cramer, 1993; Lok & Crawford, 1999; Loscocco, 1990; Luthans, 1992; Mowday et al., 1982; Sekaran; 2000) significant positive relationships between age and organizational commitment were also reported. Some authors argued that, as individual's age increases, alternative employment opportunities become limited, thereby making their current jobs more attractive (Kacmar et al., 1999; Mathieu & Zajac, 1990; Mowday et al., 1982). Other proponents hypothesized that older individuals may be more committed to their organisations because they have a stronger investment and a greater history with the organisation than do younger employees (Harrison & Hubbard, 1998; Kacmar et al., 1999). Therefore, younger employees are generally likely to be more mobile and to have lower psychological investments in the organization. The older employees become, the less willing they are to sacrifice the benefits and idiosyncratic credits that are associated with seniority in the organization (Hellman, 1997).

However, the findings of this study that showed no significant effect of age on commitment may be attributable to the general low morale of the Nigerian civil service that cuts across age, education marital and length of service strata.

Also, researchers were of the opinions positive relationship exists between that organisational commitment and length of service (Allen & Mowday, 1990; Dunham et al., 1994; Gerhart, 1990; Larkey & Morrill; 1995; Malan, 2002; Meyer & Allen, 1997; Mowday, et al., 1982). Research overwhelmingly indicates that tenure has a positive influence on organisational commitment (Loscocco, 1990; Luthans, 1992; Luthans, Baack & Taylor 1987; Mowday et al., 1982). One possible reason for the positive relationship between tenure and commitment may be sought in the reduction of employment opportunities and the increase in the personal investments that the individual has in the organisation. This is likely to lead to an increase in the individual's psychological attachment to the organisation (Harrison & Hubbard, 1998; Lim et al., 1998; Luthans, 1992; Mowday et al., 1982). Sekaran (1992) maintains that tenure is associated with some status and prestige, and that this induces greater commitment and loyalty to the employing organisation.

However, researchers such as Luthans, McCaul and Dodd (1985 cited in Vorster, 1992) supported the findings of the present study that there was no relationship between lenght of service and organisational commitment. Kinnear and Sutherland's (2000) research did not find support for the relationship between organisational commitment and tenure. This is further substantiated by Cramer (1993) who contends that longer tenure is not associated with greater commitment.

In contrast to the findings of this study, a number of researchers maintain that the higher an employees level of education, the lower that individual's level of organisational commitment (Luthans et al., 1987; Mathieu & Zajac, 1990; Mowday et al., 1982). The negative relationship may result from the fact that highly qualified employees have higher expectations that the organization may be unable to fulfill. Chusmir (1982 cited in Voster, 1992) maintains that there is a positive relationship between commitment and educational qualifications, and level of education may be a predictor of commitment, particularly for working women. It is however not so in the present study.

In line with the findings of this study, however, the level of education does not seem to be consistently related to an employee's level of organizational commitment (Meyer & Allen, 1997). Higher levels of education are postulated to enhance the possibility that employees can find alternative employment which may reduce their levels of commitment. McClurg's (1999) research found that highly educated employees had lower levels of organisational commitment. This is supported by other research findings (Luthans et al., 1987; Mowday et al., 1982; Voster, 1992).

More educated individuals may also be more committed to their profession. As a result, it would become difficult for an organisation to compete successfully for the psychological involvement of these employees (Mowday et al., 1982). This is because, according Mathieu and Zajac (1990), more highly qualified individuals have greater number of alternative work opportunities. Billingsley and Cross (1992) further corroborated this in their failure to find support for a relationship between education and commitment.

Conclusion

This study examined the influence of gender and self esteem on the organizational commitment of civil servants in Ekiti State. It can be said based on the findings of this research that females would perceive a significantly higher organizational commitment than their male counterparts.

Also, there were significant main and interaction effects of gender and self esteem on organizational commitment among employees of ministries in Ekiti state.

Furthermore, gender and self esteem had significant independent prediction on perceived organizational commitment among employees in the ministries in Ekiti state.

The study also revealed that all the predictor variables of age, educational level, length of service, marital status, gender and self esteem had joint influence on organizational commitment among employees of ministries in Ekiti state.

Recommendations

Based on the findings of this study, the following recommendations were suggested.

There should be no gender discrimination in the nature of assignment given to workers in the ministries. This study revealed that female workers were more committed than their male counterparts. Yet, female workers are usually faced with discrimination, gate ceiling, harassment etc.

Training will boost workers level of self esteem and enhance their competencies. Since government always desire productivity and creativity from workers, good training programmes tailored to meet workers needs will not only boost their level of esteem but also prepare them to meet up with various job challenges. This will enhance workers level of organizational commitment.

It is also recommended that workers salaries should not only be increased to measure up with their counterparts in the ministries, but also be paid promptly. Workers get committed when their demands could be catered for by the income they earn from their job.

It is also recommended that further studies involving other variables not considered in the present study but that may influence organizational commitment of civil servants in Ekiti State should be carried out.

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Cultivation and Detection of Sulfate Reducing Bacteria (SRB) in Sea Water

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Abstract: Sulfate-reducing bacteria (SRB) represent a class of anaerobic microorganisms that conduct dissimulatory sulfate reduction to obtain energy. The present study aimed to detect and control SRB activities using a very rapid detectable culture medium and reduction of potential economic loss in the petroleum sector. This study is an attempt to isolate SRB from sea water by rapid and sensitive culture media and to control their effect using eight commercial biocides (Aldehydes and quaternaries). The present work studies the effect of composition of four recommended culture media (Postgate medium B, Starkey, Baar's and API media), besides, the presence of metal coupons in these media to enhance the growth of sessile SRB. Furthermore, the present study evaluates the efficiency of filtration of these culture media on the growth of SRB. The results revealed that modified Postgate medium B was the recommended medium for SRB growth. In addition, the results showed that rapid and abundant growth of SRB when the metal coupons were immersed in the culture media which were deficient in iron. The unfiltered culture media improved the SRB growth. The growth of SRB was depressed by 15 ppm of the commercial quaternaries rather than 20 ppm of the aldehydes.

[E. A. Ghazy, M.G. Mahmoud, M. S. Asker, M. N. Mahmoud, M. M. Abo Elsoud and M. E. Abdel Samie. Cultivation and detection of sulfate reducing bacteria (SRB) in sea water. Journal of American Science 2011; 7(2):604-608]. (ISSN: 1545-1003). http://www.americanscience.org

Key words: SRB, Culture media, Biocides, Minimal inhibitory concentration

1. Introduction:

One of the important practical problems is the control of SRB growth in economically important situations in the petroleum sector. Consequently, considerable research has been devoted to testing various potential micro biocides and the results have been displayed throughout the scientific literature (Kumaraswamy et al. 2010). SRB, which generate large amounts of toxic hydrogen sulfide in aquatic ecosystems, are important not only for ecological reasons but also economically. The activities of SRB in natural and man made systems are of great concern to engineers in many different industrial operations (Gibson, 1990; Odom, 1990: Odom and Singleton, 1992). Oil, gas and shipping industries are seriously affected by the sulfide generated by SRB (Battersby, 1988; Hamilton, 1994; Peng et al., 1994; Okabe et al., 1995 and Cullimore, 2000). In the oil industry most monitoring of microbiologically influenced corrosion (MIC) has in the past only been conducted on sulfate-reducing Bacteria (SRB) carried out by cultivation based techniques. (Jan Larsen, 2010).

Sulfate reducing bacteria (SRB) are a group of genetically similar anaerobic organisms that were first discovered by Hamilton (1994). The SRB form an integral part of a group referred to as "sulfur bacteria" and are sometimes considered to be nuisance bacteria in a number of ways, (Tiller, 1990 and American Water Works Association, 1995). These bacteria are seldom isolated because of their slow growth. Colonies appear after more than three days of incubation and are generally not noticed, being overgrown by the accompanying flora. Accordingly, their isolation requires specific or selective growth medium (Julien Loubinoux et al., 2003). No growth takes place in media rendered "biologically free" of iron, (Postgate, 1984; Widdel, 1988; Parkes, et al., 1989).

The present study was conducted to show the efficacy of the impure (turbid) media on the detection of SRB growth.

2. Material and Methods:

2.1. Organisms

A stabilized mixed culture of sulfate reducing bacteria (SMC-SRB) was isolated from the failure shipping pipe line for treated oil (Esh El-Mallaha. Petroleum Company).

2.2. Culture media

Four recommended media of the most commonly used ones were evaluated for SRB growth. The compositions of these media were nominated in table (1). Thioglycollic and Ascorbic acid were added to all media to increase their reducing power. Saline water was (50% of the total volume) used in replacement of tap and distilled water. 2.3. The electrode

The electrode used was derived from mild steel sheets with the following composition:

0.09 % (C), 0.07 % (Si), 0.37 % (Mn), 0.017 % (S), 0.028 % (P), 0.005 %(Al), 0.015 %(Ni), 0.11 %(Cr), 0.004 % (Mo), 0.006 % (Cu), and 0.007 % (V). The electrodes were polished with emery papers 200.400, 600, grade for fine polishing .They were washed with distilled water then degreased with acetone and finally dried till use. 2.4. Pipe line description by ESHPETCO

Length 7 Km (above the ground surface); diameter 18", construction date since 1982; fluid was crude petroleum oil with water content 0.05 % Vol. sulfur content 4.5 % wt, pH value was 6-6, 5 and temperature 20-30°C and the pipe line grade was API-42.Pipe line operation data was as following pressure was 14 bar, stagnant fluid periods since operation were at 1st time (9 months in 2006) the 2nd time and 6 months in 2007.

2.5. Pipe line failure

The pressure dropped and cured oil shortage delivered at point was noted at rapid date. Pipeline track was surveyed and spelled oil was found in one of the train depression along 7 Km train, the pipes lie directly on the ground according to its natural topography of elevations and depressions.

One pipe was found ruptured open to a perfect longitudinal line extending to about 2 meters, slight bulging is clear in the middle of the opening. Huge amounts of crude oil were spilled due to the failure estimated by 10.000 barrels. The opening was in the position 6 o'clock in contact with the ground.

2.6. Field Inspection

Field inspection was done by Central Metallurgical Research and Development Institute (CMRDI).The inspection result reported that the pipe line failure is due to badly fabricated welded pipe. Internal pitting corrosion due to sour oil (along 25 years of service) was a trigger of cracking in the infused weld grooves which ended to complete rupture of the pipe. Two other factors contribute to the failure; a) positioning of the weld line (seam) at the bottom (6'oclock) where water can best accumulate, and b) stagnant long (shut down) periods. They did not ignore the possible malfunction of the treatment plant with respect to water and salt content in the treated oil.

2.7. Isolation and enrichment of SMC-SRB

SMC-SRB was obtained by transferring 1ml of the received internal sludge sample of the failure shipping pipe

line into sterile screw capped vials $(1.5 \times 5 \text{ cm})$ containing modified Postgate medium B as mentioned in table (1).The bottle was incubated for 7 days at 30°C. Blackening of the bottle meant a positive growth for SRB. This step was repeated at least 10 times to obtain a SMC-SRB (figure1). 2.8. Physicochemical analysis of saline sea water

The water sample was completely analyzed according to APHA (1989) as recorded in table (2). 2.9. Evaluation of the culture media on the SRB growth

Four different recommended culture media (Postgate B, API, Starky and Baar's) were prepared according to their compositions as shown in table (1). Thioglycollic and ascorbic acid were added to increase the reducing power of the medium. All media were autoclaved at 121 °C for 20 min. Observations of the culture media were recorded in table (3). API medium was the only clear one. These media were distributed in 10 ml sterile screw capped vials (9ml) in each one of them. Enriched SMC-SRB 4 day's old culture was inoculated into the previous culture vials. Then all vials were incubated at 30 °C for 7 days and observed by naked eye (table 4). The sulfide production was determined via the SRB activity during 7 days to record the time course of the sulfide production by SMC-SRB (figure 2). Sulfide was determined iodometrically according to APHA (1989).

Table ((1)	Chemical	composition	of th	e modified	culture	for S	SMC-	SRB	growth in	σ/Ι
Lanc	,	Chemicar	composition	UL UL	ic mounicu	culture	IUI N		DID	SIOW III III	

Chemical ingredient	Postgate B	API	Starkey	Baar's
KH ₂ PO ₄	0.5	0.01	0.5	0.5
NH ₄ Cl	1.0	-	1.0	1.0
Na ₂ SO ₄	1.0	-	1.0	-
CaCl ₂ .6H ₂ O	0.1	-	0.1	-
MgSO ₄ .7H ₂ O	2.0	0.2	2.0	2.0
Sodium Lactate (60 –70%)	5 ml	4ml	5 ml	5ml
Yeast extract	1.0	1.0	-	-
Ascorbic acid	0.1	0.1	0.1	0.1
Thioglycollic acid	0.1	0.1	0.1	0.1
FeSO ₄ .7H ₂ O	0.5	-	-	-
NaCl	26	26	26	26
Fe(SO ₄) ₂ (NH ₄) ₂ .6H2O	-	0.2	0.5	0.5
CaSO ₄	-	-	-	1.0
Sea water Distilled water	500 ml 500 ml	500 ml 500 ml	500 ml 500 ml	500 ml 500 ml
pH	7-7.5	7-7.2	7-7.3	7-7.5



Figure (1): Gram negative of stabilized mixed culture SRB

pH at 20°C	6.73
Sp. Gr. at 20°C	1.017
Resistivity at 20 °C	0.227 Ohm. m
Sodium (Na ⁺)	4904 ppm
Potassium (K ⁺)	130 ppm
Calcium (Ca ²⁺)	1231 ppm
Magnesium (Mg ²⁺)	1763 ppm
Iron (Fe ⁺⁺)	0.02 ppm
Manganese (Mn ²⁺)	0.09 ppm
Barium (Ba ²⁺)	0 ppm
Strontium (Sr ⁺⁺)	26 ppm
Zinc (Zn ⁺⁺)	0 ppm
Lead (Pb ⁺⁺)	0 ppm
Chloride (Cl ⁻⁾	13000 ppm
Sulphate(SO ₄)	2400 ppm
Bicarbonate(HCO ₃ ⁻)	117 ppm
Carbonate ($CO_3^{}$)	0 ppm
Total dissolved Solids	23601 ppm

 Table (2): Physical chemical analysis of sea water received from ESHPETCO.

 Table (3): Observations of the modified culture media for

 SMC-SRB in sea Water.

Media Properties	Postgate (B)	API	Starky	Baar's
Color	Yellow with faint gray	Yellow	White	White
Turbidity	Turbid	Clear	Turbid	Turbid
pH before autoclaving	7.3	7.2	7.2	7.2
pH after autoclaving	6.7	6.4	6.4	6.4
E _h (mV)	-345	-260.3	-200.7	N.D

N.D Not determined.

Table (4) Naked eye observation of SMC-SRB growth using the modified culture media in sea water during 7 days incubation at $30^{\circ}C$

Time (days)	Media						
Zero Time	Postgate (B)	API	Starky	Baar's			
1	-	-	-	-			
2	+	-	-	-			
3	++	-	-	-			
4	+++	-	+	±			
5	+++	-	++	+			
6	+++	-	+++	+			
7	+++	+	+++	+			

- No growth + Moderate growth ++ Good growth +++ Severe growth

2.10. Effect of metal coupons on the enhancement of SRB growth

The mild steel coupons of $0.2 \times 1 \times 5$ cm were prepared using emery papers with very fine grade as mentioned before. The prepared clean mild steel coupons were immersed in the screw sterile vials which contained the previous culture media. One ml of the enriched SMC–SRB was inoculated into the previous vials. The vials were incubated and observed visually (table 5) for 3 days at 30 °C. Then the sulfide produced was determined as mentioned before (figure 3). Figure (2): Time course of sulfide production by SMC SRB during 7 days incubation at 30°C in different culture media



Table (5): Naked eye Observation of SMC-SRB growth in the presence of metal coupon in the culture media after 3 days incubation at 30° C

Media Time (days)	Postgate (B)	API	Starky	Baar's
7	++++	++	++++	+++



Figure (3): Effect of presence of metal coupon on the SMC-SRB growth using culture media after 3 days incubation at 30°C.

2.11. Effect of the weight loss measurements

Weight loss measurements were carried out in screw capped vials containing the previous sterile culture media. These vials were inoculated with 1 ml of enriched SMC-SRB. The clean weight mild steel coupons (W1) were immersed completely in the medium and incubated for 7 days at 30° C. After the incubation period ended the coupons were picked up and immersed in a washing solution (1 % HCl + 0.5 % Thiourea) for 5 min to remove the corrosion product layer. Then the coupons were washed by distilled water and dried, then reweighed and the weight loss was recorded to calculate the corrosion rate as showed in (table 6) according the following equation.

MPY = (Area factor)* X (Wt.loss in mg)/ (Days exposed)

*(The area factor is computed from the exposed surface area and density)

Table (6): Effect of different culture media on the corrosion rate of the mild steel. Coupon after 7 days of incubation at 30°C by weight loss technique (MPY)

Post gate (B)	API	Starkey	Baar's	
0.87	5.2	14.8	0.534	

2.12. Effect of filtration of the culture media on the SRB growth

The turbid culture media Postgate B, Starky and Baar's were filtered through filter paper before autoclaving. After that the culture media were distributed in sterile screw capped vials and then inoculated with 1 ml of

enriched SMC-SRB and incubated at 30° C for 7 days. Sulfide production was determined as mentioned before (table 7).

 Table (7): Effect of clear medium on the SMC-SRB growth after 3 days incubation at 30°C using different culture media

Media	Sulfide concentration mg S/L	Without filtrate after 3-days
Postgate B	115	288
API	167	99
Starkey	11	135
Baar's	13	213
0.10 D' '	1	

2.13. Biocide test

The Baar's medium as mentioned in table (1) dispensed in 9.0 ml amounts into a series of 10 ml capacity screw capped glass vials. These vials contained various concentrations ranged from 5 to 20 ppm of commercial biocides (four quaternaries and four aldehydes) coded as Q1, Q2, Q2, Q3, Q4, A1, A2, A3, and A4, respectively. The vials were autoclaved at 121°C for 15 min .After cooling the enriched SMC-SRB was inoculated and the SRB growth was detected after 7 days incubation calorimetrically by measuring the absorbance at 580 nm (table 8). Control vial was inoculated with sterile H₂O. The efficiencies (E %) of biocides (table 9) were calculated according to the following equation:

E % = E uninhibited – E inhibited/Euninhibited × 100

Table (8): Determination of the mic of the tested biocides against SMC-SRB by using colorimetric measurement at 580 nm.

Conc. ppm	Tested samples									
	Q1	Q2	Q3	Q4	A1	A2	A3	A4		
Control	0.230	0.230	0.230	0.230	0.230	0.230	0.230	0.230		
5	0.209	0.200	0.229	0.205	0.211	0.203	0.213	0.219		
10	0.107	0.103	0.119	0.120	0.073	0.088	0.079	0.086		
15	0.078	0.055	0.066	0.060	0.011	0.012	0.011	0.013		
20	0.011	0.009	0.012	0.013	0.006	0.006	0.004	0.007		
Table (9) Efficiencies (%) of the tested biocides against SMC SRE										
Conc. ppm	Tested samples									
	Q1	Q2	Q3	Q4	A1	A2	A3	A4		
Control	0	0	0	0	0	0	0	0		

Control	0	0	0	0	0	0	0	0
5	9	13	4	11	30	38	45	45
10	53	55	48	48	68	62	65	63
15	66	76	71	74	95	95	95	94
20	75	80	79	80	97	97	98	97

3. Results and Discussion:

Physicochemical analysis of saline sea water as recorded in table (2) revealed that it was a very good source for SRB due to the sulphate contents (2400 ppm).This was confirmed by the field inspection done by (CMRDI); they did not ignore the possible malfunction of the treatment plant with respect to water and salt content in the treated oil. Thus the pipe line failure might be due to biological effect in addition to physical one. Besides the Blacking of modified Postgate medium B revealed the positive growth of SMC-of SRB.The results detected an improvement for SRB growth due to addition of supplied sea water in comparison with the previous studies .Figure (1) showed that SRB was a pure Gram negative short rods. SRB are present in most soils and water, but are outnumbered by other types of microbes except in special environments. Accordingly enrichment of the needed environments with these bacteria is usually necessary before isolation is attempted (Widdel, 1988). On the other hand the presence of reductants in culture medium makes isolation a much less formidable task (Postgate, 1984).

Postgate medium B is a multipurpose medium for detecting and culturing Desulfovibrio and Desulfotomaculun. Most of the ingredients can be prepared and held as a stock, but the thioglycollate and ascorbate, which may be omitted if the inoculua are fresh, flourishing culture, should be added and the pH adjusted just before autoclaving. The medium should then be used as soon as it cools down because the reductants deteriorate in air at neutral pH values. This process accompanied by a transient purple color. The precipitate in medium B aids growth of tactophilic strains. This medium is recommended for long term storage of strain. In cultivation of the SRB in pure culture, the major prerequisite is simple. The redox potential (E_h) of the environment must start around -100 mV. This means that mere exclusion of air is not sufficient to ensure growth (a boiled-out Lactate + sulfate medium would have an $E_{\rm h}$ of about +200 mV under N_2 be about with 5–m M Na_2S the value would be about (-220 mV) (Postgate. 1984). This was mentioned in tables (4) which showed that modified Postgate medium B was the recommended medium for SRB growth followed with Starky then Baar's one because the precipitate in these media aids growth of tactophilic strains and this was confirmed by the effect of filtration of the culture media on the SRB growth as showed in table (7) ,the filtration of the turbid medium reduced the SRB growth . In addition the results in table (5) and figure (3) revealed that rapid and abundant growth of SRB after 3-days incubation when the metal coupons were immersed in the culture media which were deficient in iron. This due to the utilization of the hydrogen evolved when metallic iron immersed in mineral medium provides additional evidence for the presumed role of SRB in anaerobic corrosion of ferrous pipes namely that the depolarizing of cathodic elements of electrochemical systems on the surface of the metal. Weight loss measurement recorded that Starky medium was the most aggressive medium to mild steel. This might be due to the anodic dissolution of mild steel and cathodic depolarizer's effect of FeS film which formed due to SRB activity. So for diagnostic purposes media often prescribed are those which contain about 0.5 % of a ferrous salt. This forms a black precipitate of FeS when sulfide is formed, so blacking of the medium as a whole, or the zone round a colony, is an evidence for bacterial sulfate reduction as shown in equation (1) (RzecZycka and Blaszczyk, 2005).On the other hand API medium showed a high (mpy) corrosion rate in comparison to modified Postgate B and Baar's media. This might be due to metal exposure to chemical dissolution in that medium (general corrosion) not for SRB activity. This illustrated that the growth of SRB by (using API medium) conventional methods is very time consuming, (Iverson, 1987; Taylor and Parkes, 1983).

 SO_4^{2-} + Organic matter = HS^- + H_2S + HCO_3^- (

Due to the economic losses as well as environmental health and safety hazards caused by the activity of stabilized mixed culture containing sulfate reducing bacteria,(SMC-SRB)in many industrial sector such as the oil and gas industry, it was important to minimize the risks resulting from SRB activity. These bacteria are mainly sulfate reducers, and their growth frequently causes severe corrosion problems in oil well pipes. One of the simplest ways to measure the effect of biocides on an organism is by determining the Minimal Inhibitory Concentration (mic) which just prevents growth in a suitable medium. The antibacterial agent is serially diluted in the medium and standardized inocula of the test strain are added. After incubation for a predetermined growth, the cultures are examined and the mic for the biocide is detected (Sharma, et al., 1986). The mic of microbicides and bacteriostatic substances are usually governed by the nature of the medium in which the substance is tested and also by the size of the inocula. Iron salts can increase the apparent resistance of cultures to inhibitors, and in case of Desulfovibrio species the presence or absence of NaCl may influence inhibition (example a quaternary biocide) (Bessems, 1983). In the present work the effect of tested biocides on a cell is normally dependent on its concentration and it's can be seen from the slight increase and leveling of A580 at growth of SRB was depressed by 20 ppm of the commercial quaternaries rather than 15 ppm of the aldehydes. The results in tables (8&9) showed the biocidal activity and biocidal efficiencies and recorded that up to 97% for commercial aldehydes and up to 75% for commercial quaternaries at 20 ppm. The cell membrane of microorganisms is composed of several lipids and protein layers arranged together in a specific arrangement called the bilayer (or multi layer lipoprotein structure). The presence of the lipids as a building unit in the cell membrane acquires them their hydrophobic Character (Bessems, 1983). The selective permeability of the lipoprotein membrane represents the main function, which control the biological reaction in the cell. Hence any factor influences that permeability causes a great damage to the microorganisms, which leads it to die.

4. Conclusion:

The isolation of SRB by conventional methods is very time consuming. The present work study recommended modified Postgate B and Starkey media because the precipitate in these two media aids growth of tactophilic strains. Also the use of the supplied water sample by 50% of the total volume of the culture medium improved the SRB growth. Besides, the presence of metal, improved the SRB growth.Ontheotherhand the growth of SRB was depressed by 15 ppm of the commercial quaternaries rather than 20 ppm of the aldehydes. The present study aimed to detect and control SRB activities using a very rapid detectable culture medium .In addition to reduction of their economic loss in the petroleum sector.

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Identification of Cryptosporidium species infecting camels (Camelus dromedarius) in Egypt.

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Abstract: Cryptosporidium sp was investigated among 145 camels (5-8 years old) from Egypt. The prevalence of infection was 19.3%. The detected oocysts were ellipsoidal in shape with a mean length and width 7.5×5.6 um. Ten Cryptosporidium free mice were orally inoculated each with 350.000 oocysts (camel isolate). The prepatent period in mice was 2 days and the patent period could not be determined since they were still shedding oocysts until day 100 post- infection. The camel isolate of Cryptosporidium and the same isolate propagated in mice was non infective for lambs during an examination period of 3 months. Molecular characterization of the camel isolate indicated that the target gene (18SrRNA) gave positive result for C. muris at 435bp.

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Key words: Camels, Cryptosporidium muris, prevalence, morphology, PCR.

Introduction

The genus *Cryptosporidium* includes a group of protozoan parasites that infect the gastrointestinal tract and other organs of mammals including human, birds, reptiles and fish (Xiao et al., 1999).

The emergency of *Cryptosporidium* as important cause of diarrheal illness and its increasing role in both localized and widespread outbreaks of disease initiated a public and animal health problems of global proportion for both developed and developing countries (Fayer and Xiao,2007).

Identification of Cryptosporidium species based on morphology and dimensions of oocysts is difficult since oocysts of many species lack unique features and are indistinguishable from each other. The life cycle of cryptosporidia and its variations can provide species specific information but this is also impractical. More recently gene sequence information has become the applicable widely factor for defining most Crvptosporidium species. Such genetic data were based primarily on slight differences in the sequence base pair within the gene referred to 18s or small subunit ribosomal (ssr) RNA. Accordingly, 16 species of Cryptosporidium were defined as valid species (Fayer and Xiao, 2007).

Previous studies on camel cryptosporidia were scanty. Molecular characterization of camel (*Camelus bactrianus*) *Cryptosporidium* sp. revealed *C. muris* in one study (Xiao et al., 1999) and *C. andersoni* in another one (cited by Santin et al., 2007). Cryptosporidia were reported from camels (*Camelus dromedarius*) in Egypt without identification of the species (El-Kelesh et al., 2009).

Therefore, this investigation was initiated to study the prevalence and molecular identification of *Cryptosporidium* spp. infecting camels (*Camelus dromedarius*) from Egypt. The susceptibility of mice and lambs for infection with the isolated *Cryptosporidium* sp. was also investigated

Materials and Methods

1-Collection of fecal samples:

Fecal samples were collected from 145 camels of various ages (5-8 years old) and sexes at El-Basatein (Cairo) and El- Warak (Giza) abattoirs (Egypt).Fecal samples were examined after preparation of smears stained with modified Zeihl Nelseen stain. Positive fecal samples were preserved in 2.5% potassium dichromate at 4°C until used for isolation of *Cryptosporidium* sp.

2-Oocysts concentration and experimental inoculation of mice with *Cryptosporidium* spp:

The oocysts were concentrated in order to be used for animal inoculation and molecular study. Ten, one week old *Cryptosporidium* free mice (*Mus musculus*) (10 gm weight) were purchased from private farm and each was inoculated orally with 350.000 oocysts (camel isolate). The faces of the inoculated mice were collected and examined daily for the presence of *Cryptosporidium* oocysts for a period of 100 days post inoculation.

3-Inoculation of lambs with *Cryptosporidium* sp. oocysts:

Three, four months old lambs were reared individually and conventionally in isolated pens during the period of the experiment (100 days) and fed sterilized and balanced ration. Two lambs were used for experimental inoculation with *Cryptosporidium* sp. while the 3rd lamb was used as non-infected control. One of the experimental lambs was inoculated orally with 10^6 oocyst (camel isolate) while the 2nd lamb received the same dose of *Cryptosporidium* sp. oocyst

of camel isolate propagated in mice. Faeces of the experimentally inoculated lambs were collected and examined daily for the presence of *Cryptosporidium* oocyst and for studying the prepatent and patent periods of infection.

4-Preparation of oocyst lysates as PCR templates:

For DNA extraction of *Cryptosporidium* sp. (camel isolate) was concentrated from faecal material of positive cases. They were washed four times by successive pelleting $(10.000 \times \text{g} \text{ for } 10 \text{ min at } 4^{\circ}\text{C})$ and resuspension in distilled water and finally suspended in 10 mM Tris (pH 8.3)-50 mM KCl. Purified oocysts were suspended at a density of 250 oocysts/µl in 100-µl aliquots of 10 mM Tris (pH 8.3)-50 mM KCl containing 0.5% (wt/vol) Tween 20. After freeze-thawing (15 cycles), samples were heated for 15 min at 100°C and then centrifuged for 2 min at 16.000 × g to remove particulate matter. Supernatants were recovered and stored at -20°C until used for PCR amplification (Gobet et al., 1997).

5-PCR amplification and gel analysis of PCR products:

One-microliter volumes of the oocyst lysates were used as amplification templates in 50-µl reaction mixtures containing 75 mM Tris (pH 9); 20 mM (NH₄)₂SO₄; 0.01% (wt/vol) Tween 20; 0.2 mM each dGTP, dATP, dCTP, and dTTP; 2 to 4 mM MgCl₂ (Table 1); 50 pM each primer; and 1 to 2 U of Gold Star Taq DNA polymerase (Eurogentec). Reaction mixtures were overlaid with 50 µl of sterile mineral oil and subjected to denaturation, thermal cycling (Minicycler; MJ Research), and then a final elongation at 72°C. The conditions of denaturation, annealing, and elongation varied depending on the primers of the target genes (18 SrRNA gene and Hsp70 gene). PCR products were analyzed on horizontal agarose gels in TAE buffer (40 mM Tris acetate, 2 mMNa₂EDTA 2H₂O). Each amplification run included a negative control (PCR water).

Results

1-prevalence of the Cryptosporidium sp. in camels:

Examination of stained fecal smears from 145 camels revealed that 28 camels (19.3%) were positive for *Cryptosporidium* sp. The oocysts were ellipsoidal in shape with a mean dimensions 7.5×5.6 um.

2-Results of experimental inoculation of mice with *Cryptosporidium sp.* (camel strain):

Daily examination of stained fecal smears from experimentally inoculated 10 mice with *Cryptosporidium* sp. (camel isolate) revealed that the prepatent period was 2 DPI. The mice excreted *Cryptosporidium* oocysts daily until 100 days postinfection (termination of the experiment). The mean dimensions of 50 *Cryptosporidium* sp. oocysts excreted by mice were $6.9 \times 5 \mu m$.

3-Results of experimental inoculation of lambs with *Cryptosporidium* sp. (camel isolate)

Daily examination of stained fecal smears from experimentally inoculated lambs with *Cryptosporidium* sp. (camel isolate) and the same isolate propagated in mice revealed negative finding during the examination period (100 days).

4-Results of PCR:

PCR gave the same-sized fragment of *C. muris* (435 bp). Primer pairs targeting the 18S rRNA gene gave positive result for *C. muris*. While the PCR showed negative result to *C. parvum* using primer pairs targeting Hsp70 gene (Fig.1).



Fig. (1): PCR products for *Cryptosporidium muris*. Lane1: negative control sample. Lane2: positive camel sample for *C. muris*. (435bp) and lane M: 100 bp marker
Discussion

Examinations of stained faecal smears from 145 camels revealed 19.3% infection rate with *Cryptosporidium* sp. El Kelesh et al. 2009 reported 17.5% out of 80 camels were positive to *Cryptosporidium* spp. Alves et al. (2005) in Portugal found that, no positive cases were detected in examined camels.

In our study the camel isolate of *Cryptosporidium* sp. was infective to mice. Xiao, et al., 1999 reported that *C. muris* isolated from rodents, bactrian camel (*Camelus bactrianus*) and rock hyrax (*Procavia capensis*) were infectious to mice and *Cryptosporidium* oocysts from cattle were not infective to mice.

The dimensions of our camel isolate of *Cryptosporidium* were comparable with *C. muris* described by Upton and Current (1985) (7.4x 5.6 um). Also, Fayer and Xiao, 2007 reported that both *C. muris* and *C. parvum* were established based on differences in oocysts morphology and infection sites.

In this study PCR gave the same-sized fragment of *C. muris* (435 bp) and primer pairs targeting the 18S rRNA gene gave positive results to *C. muris*. While the PCR showed negative results for *C. parvum* using primer pairs targeting Hsp70 gene. *C. muris* was detected from camel (*Camelus bactrianus*) and rock hyrax (*Procavia capensis*) at 448 bp and from cattle at 485 bp using nested PCR (RFLP) (Xiao et al., 1999). Also, Bornary-Llinares et al., 1999 detected that the *C. felis* specific fragment was 455 bp when compared with *C. muris* specific diagnostic band (431pb). Finally Champliaud et al., 1998 declared that the group which included *C. muris* and *C. baileyi* gave positive results with the two primer pairs targeting the 18S rRNA gene (Johnson, 1995 (435 bp)).

We could conclude from our morphological, biological and molecular study that the detected *Cryptosporidium* sp. oocysts from camels (*Camelus dromedarius*) were *C. muris*.

Table 1: Target genes and primers for detection of Cry	otosporidium DNA
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Cryptosporidium Spp.	Target genes	Primer pair	Fragment size (bp)
1- <i>C. muris</i> (Johnson et al., 1995).	1-18 SrRNA gene	5'-AAGCTCGTAGTTGGATTTCTG	435
<i>2-C.parvum</i> (Rochelle et al., 1997).	2-Hsp70 gene	5'-AAATGGTGAGCAATCCTCTG	361

bp: base pair

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Effect of Ultrasound Radiation on the Aqueous Humor of Rabbits' Eye

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Abstract: The present work aimed to evaluate the protein rabbit aqueous humor changes after exposure to ultrasound. Sixteen New Zealand rabbits (male and female) weighing 2.0-2.5 Kg, divided into four groups, group I served as control and the other three groups exposed to ultrasound of power intensity 3W/cm² at frequency 10.8MHz for 10, 20 and 40 minutes exposure time. Estimation of protein content, gel filtration chromatography and Sodium Dodecyl Sulfate-Polyacrylamide gel electrophoresis (SDS-PAGE) were carried out to aqueous humor for all the studied groups. The results showed a significant decrease of protein content of rabbits aqueous humor of all groups reached to maximum decrease (-41.3%) at 40 minutes of exposure. A change in the molecular structure of aqueous humor protein was observed in the shift of the protein fractions to high molecular weight and decrease in the mobility of all peaks in the electrophoretic pattern. It is concluded that aqueous humor protein is sensitive to the ultrasound exposure as a function of time of exposure and may lead to denaturation of proteins.

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Key words: Ultrasound, Aqueous humor, Column chromatography, Electrophoresis, rabbits.

1. Introduction:

Diagnostic ultrasonography is now widely accepted and used technique which employs low intensity ultrasound to image the eye and the $orbit^{(1,2)}$. The basic methods are A-scan. B-scan. Doppler techniques, and three dimensional approaches⁽³⁾.Unique for ophthalmology is newly invented, highly resolving equipment utilizing ultrasound frequencies of 50 MHz and higher socalled ultrasound biomicroscopy⁽⁴⁾. The therapeutic use of ultrasound by hyperthermia has gained much interest in ophthalmology. The therapeutic applications of ultrasonic energy are also being studied for the treatment of glaucoma, ocular tumor ,retinal detachments, coagulations of lens proteins disruption of vitreous membranes and vitreous hemorrhages⁽⁵⁾. Exposure to high power density ultrasound may produce adverse biological effects. High power ultrasound characterized by high intensity out puts (20-100 KHz) which has a wide range of applications throughout industry ⁽⁶⁾. Doses of ultrasound (3W/cm²) reduce the electroretinograph-b wave amplitude and induce irreversible destructive change in cells of all retinal layers while therapeutic doses (0.6 W/cm²) of ultrasound increased the bdeflection amplitude and a decrease in the osmiophlicty of rods and cons⁽⁷⁾. The degree of corneal endothelial damage caused bv phocoemulsification depended on ultrasonic duration ultrasonic power, the distance of phacotip and the angle of phagotip ⁽⁸⁾. The morphology of rabbit eye encleated in various periods after ultrasound exposure illustrated two mechanisms of focused ultrasound

effects, inhibited secretion of intraocular fluid and creation of transscleral route of chamber fluid discharge ⁽⁹⁾. The heating capability of ultrasound beams of lens and cilliary body was studied and measuring of the mean temperature rise in human lens and cilliary body using the maximum exposure settings of ultrasound scanner and they were 2.27°C and 1.93°C, respectively⁽¹⁰⁾.

In the present work the effect of ultrasound at power intensity of 3W/cm² at 10.8Hz for 10, 20 and 40 minutes on the molecular structure of aqueous humor protein of rabbits were studied.

2. Material and Methods

Animals

Sixteen male and female NewZealand rabbits of either sex weighing 2.0-2.5 Kg were used for this study. The animals were divided into four groups, group I (8 eyes) served as control ,groups II,III and IV exposed to ultrasound (US) of power intensity 3 W/cm^2 at frequency 10.8 MHz for 10,20 and 40 minutes, respectively.

Insonification

The rabbits were anesthetized by injection of 1ml/Kg Xylazine (Rompun manufactured by Bayer AG Leverkusen, Germany) intravenously as muscle relaxant at the beginning and after 15 minutes 1ml/Kg were administered by Ketalar[®] (Ketamine supplied by Ayerst Labs Ontario, Canada) intramuscularly. The eyes were covered after anesthetization of the rabbit, and eye lid was opened with a stainless steel speculum. For preventing

impedance coupling, a gel was used between the eye of rabbit and ultrasound prop. Sonification of rabbit's eyes was carried out with continuous ultrasound waves from ultrasound generator and transducer which is consists of a piezoelectric crystal (type SVHSP 101). The transducer is calibrated in Faculty of Science, South Valley University, Egypt.

Sample preparation

After decapitation, the eyes of rabbits were enucleated then the aqueous humor was collected by direct aspiration through transcorneal puncture of the anterior chamber using a 27-gauge needle with 1ml sterile syringe.

Quantitative analysis of total protein

The total protein content of all samples of aqueous humor was measured with the Bio-Rad protein microassay (Bio-Rad Chemicals, CA) according to Bradford (1976) ⁽¹¹⁾ and Tripathi *et al.* ⁽¹²⁾.Twenty μ L of each sample was diluted to 0.8 ml with bidistilled water and combined with 0.2 ml dye reagent (Coomassie blue G-250). The spectrophotometric reading was taken at 595 nm with spectrophotometer (type UV-visible Recording 240 Graphical, Shimadzu, Japan). Standard bovine albumin was used for generating of the standard curve.

Gel chromatography

A volume of 0.2 ml of sample was injected on column (1.6 cm diameter and 30 cm height) loaded with sephacryl S-300 (Pharmacia Fine Chemicals AB Uppsala, Sweden). The elution was carried out by phosphate buffer with pH 7.4. Fractions of 1 ml/min were collected using fraction collector (type Haake Buchler Instruments, Inc. Saddle Brooke, USA). Gel filtration calibration kits (Pharmacia Fine Chemicals) were used in setting up the calibration curve by which the approximate molecular weights of the protein peaks in the aqueous humor chromatogram can be evaluated.

SDS polyacrylamide electrophoresis

Aqueous proteins were separated according to their molecular weights by Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli ⁽¹³⁾ using 5% stacking gel and 12% separating gel. The data represented graphically with an automatic scanner (model R-112, manufactured by Beckman).

Statistical analysis

All values were statistically analyzed by a computerized statistical package SPSS and expressed as mean \pm S.D. Comparison of the values was

performed using one way ANOVA test. Statistical significance was set at 95% confidence level.

3. Results:

Table(1) represents the concentrations of aqueous humor proteins for normal rabbits and those exposed to ultrasound of power 3W/cm at 10.8 MHz for 10,20 and 40 minutes. The total protein content of normal aqueous humor was 55.4 ±2.0 mg/100 ml. The results showed a significant decrease (p 0.001) in total protein content for all groups exposed to ultrasound for the different times of exposure, reached the maximum decrease for group exposed to US for 40 minutes (32.5 ± 6) . The percentage decrease of total protein content were 15.5%, 35.6% and 41.3% for groups exposed to US for 10, 20 and 40 minutes, respectively. Figure (1) summarizes the obtained proteins values for all the studied groups in comparison with control to indicate the direct proportional relationship between the time of exposure to US and the total protein content, as the time of exposure increase the total protein content of aqueous humor decrease.

Figure (2) illustrates the chromatographic elution pattern of aqueous humor proteins of normal and exposed to US (3W/cm at 10.8 Hz) for 10 min. The normal pattern of aqueous humor was eluted in 3 peaks; the first peak had a molecular weight of about 170KDa, the second peak was the highest peak in the pattern and had a molecular weight of about 141KDa and the third peak had a molecular weight of about 105KDa. There was a shift of all peaks to high molecular weight (199,145,117KDa, respectively).

Figure (3) represents the chromatographic elution pattern of aqueous humor exposed to 3W/cm for 20 min as compared to the normal. There was a shift of the first and the last peaks to high molecular weights (209 and 129KDa, respectively). In the same time, the second peak was fractionated into two peaks 170KDa and 151KDa.

By increasing the time of exposure to 40 min, all peaks showed high molecular weights (Fig. 4). This shift was appeared clearly in the first peak which contain high molecular weight proteins and partially excluded from the gel. Also, the fractionation of the second peak was increased and had molecular weights of 178 and 162KDa, respectively. The last peak had a molecular weight of 135KDa which was higher than its corresponding peak in normal rabbits.

Figure (5) shows the electrophoretic patterns of aqueous humor for control rabbits and exposed group to ultrasound radiation (3W/cm at 10.8 Hz) for 10 minutes. The control pattern was characterized by the presence of 9 peaks, which reflect the different protein fractions with specific intensities and broadening that covered the molecular weight range 40-220 KDa. The patterns of aqueous humor of the animals exposed to ultrasound for 10 minutes revealed a shift of all peaks to high molecular weight indicating decrease in the protein mobility and covered the molecular weight range 50-230KDa. Also intensity of most peaks was slightly decreased in comparison with the control.

Figure (6) shows the electrophoretic patterns of aqueous humor of animals exposed to ultrasound for 20 minutes compared to the patterns of the normal aqueous humor. Decrease in the mobility

of all proteins fractions was observed and the intensity of all peaks was decreased after exposure to ultrasound. The molecular weight range for this group was 55-235KDa.

Figure (7) shows the electrophoretic patterns of aqueous humor for rabbits exposed to ultrasound for 40 minutes compared to the normal. The most important observation in this group was continuing of the decrease the mobility and the intensity of all peaks fractions till the last peak began to diminish. The molecular weight range is 65-245KDa.

Table 1: Effect of ultrasound of power 3W/cm²at frequency 10.8MHz on aqueous humor proteins of rabbit eyes after different times of exposure

	Total protein (mg/100ml)	Decrease %
control) (Group I	55.4±2	
Group II (10 min)	$46.8\pm3^{\dagger}$	15.5
Group III (20 min)	$35.7\pm2^{\dagger}$	35.6
Group IV (40 min)	[†] 32. 5±6	41.3

[†] Statistically significant, P 0.001











4. Discussion:

After the end of World War II, advances in ultrasound technology brought improved possibilities for medical applications. The first major efforts in this direction were in the use of US to treat diseases. Medical studies were accompanied by experiments with laboratory animals and other model systems to investigate basic biological questions and to obtain better understanding of mechanisms. Also, improvements were made in methods for measuring and controlling acoustical quantities such as power, intensity and pressure. When diagnostic US became widely used, the scope of biological and physical studies was expanded to include conditions for addressing relevant safety matters^(14,15). Two primary forms of ultrasound include diagnostic and therapeutic. Diagnostic ultrasound is used for medical imaging while its therapeutic counterpart is used in the treatment of various physical ailments; the combined. Diagnostic involves the emission of pulsed waveforms of less than 1 W/cm²intensity while therapeutic US typically employs incident waves of either 1 or 3 MHz, transmitted as either pulsed or continuous waveforms depending on the desired physiological effect⁽¹⁶⁾.

Aqueous humor provides optical transparency, structural, integrity and nutrition of the eye $(^{17,18,19})$. There have been numerous studies about the protein structure of aqueous humor in normal and diseased eyes $^{(20,21)}$.

In the present study, the effect of continuous wave ultrasound with intensity 3W/cm² at frequency 10.8 MHz for different duration (10, 20 and 40 minutes) on aqueous humor protein of rabbits was investigated. The protein content in the normal aqueous humor is in agreement with data of Waters et al.⁽²²⁾. The results of electrophoretic separation are in agreement with data of Tripathi *et al.*⁽¹²⁾. Litin *et al.*⁽²³⁾ also reported that human aqueous humor contains many high and low molecular weight bands which are common to serum. The decrease that appeared in aqueous humor protein of the exposed animals is timedependant and propagated with time increase to 40 minutes. This decrease may be due to temperature rise and this is in agreement with Cuvenic et al. (10) who reported that the main effect of ultrasound exposure for long time lead to rise in the temperature and known as thermal effect. These changes in aqueous humor content is supported by the molecular weight distribution where shift of all peaks for all groups to high molecular weight and the degradation and the aggregation of protein molecules. Moreover, it was noticed from the electrophoretic studies decrease of the mobility of all protein fractions due to either loss of surface charge or increase in the molecular weight. This finding is in agreement with Putilina et al.⁽²⁴⁾ who reported that sonification may alter the confirmation of the protein subunit at or near its surface. Also other effects of ultrasound are due to non thermal mechanisms mediated by a process called cavitation. Cavitation essentially describes the biophysical interaction of gaseous inclusions (bubbles) within tissues when exposed to an incident waveform as ultrasound. Typically, bubbles will expand and contract as the peak positive and negative pressures propagate through the tissue which causes protein denaturation, affecting the ion permeability that induces biophysical effects⁽²⁵⁾.

In conclusion ultrasound can produce a variety of bioeffects through thermal and non thermal effects. Aqueous humor protein is sensitive to ultrasound of power intensity 3W/cm²at 10.8Hz as a function of time of exposure and may lead to denaturation of protein.

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A New Pool Market Method for Generation Expansion Planning in Restructured Power System

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Abstract: The issue of generation expansion planning (GEP) is more complicated in the restructured and modern power systems rather than traditional and monopoly systems. In Modern power systems, each Generation Company (Genco) invests in the section of generation in order to get to his own maximum profit. This paper presents a new mixed method to solve the GEP problem in Power Pool Market. This method is formed of two levels: local level and national level. In local level, each of Gencos declares his own generation level to Independent System Operator (ISO) aiming maximize the profit just with respect to local constraints. In national level, first the competition between Gencos will be modeled by game theory and Nash-Cournot equilibrium. Then, due to the generation level of each of Gencos, the system national constraints will be checked. If these constraints would be satisfied, problem-solving would be completed but if each of these constraints won't be satisfied, their relevant coefficients will be changed in problem and this procedure would be repeated again and again until problem was converged to accepted solutions which satisfy local and national constraints.

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Keywords: Generation Expansion Planning, Pool Market, Game Theory, Uncertainty

Nomenclature

T: Time horizon of planning *m*: Number of generation technologies p(t): Price of power sale in year t_{th} q(j,t): Quantity of that power which is generated by technology *j* in year t_{th} *B_i*: Sum of profit of i_{th} Genco in generation planning C(j,t): Cost of power which is generated by technology *j* in year t_{th} EM(j): Emission coefficient of technology *j* and EML(t): Allowable emission margin in year t_{th} MaxExp(t): Maximum level of expansion in year t_{th} $C_{inv}(t)$: Cost of investment per year MaxCap(t): Maximum rate of investment of each investor in year t_{th} r_{min}^{t} : Down reserve margin

 r_{max}^{t} : Up reserve margin

h: Allowable level of LOLE

1. Introduction

In Generation Expansion Planning (GEP), the generation level of each of generation company (Genco) in market would be specified in a determined time horizon. The Gencos will also regulate their own investment plans with respect to this planning. The establishment of restructuring in Power Industry has greatly influenced GEP. In a traditional system, the main objective is to minimize the total of generation costs, but in a modern power system (restructured environment), Gencos as players in power market just want to maximize their own profit and they accomplish their generation planning in order to achieve this aim (Kagiannas et al., 2001). In such an environment, ISO has a duty to control market in generation, transmission and distribution sections. In generation section, ISO applies the system constraints in GEP problem (Murphy and, Smeers, 2001).

In this paper, in the first stage, ISO takes into consideration the local constraints of system like emission constraint, fuel constraint and etc for each Genco. Having applied these constraints, each Genco will specify his own generation level in a planning horizon. In the second stage, after competing Gencos in a game and achieving Nash-Cournot equilibrium, ISO checks the national constraints of system like LOLE and reserve constraint with respect to the sum of Genco's generation in pool market (Torre, et al., 2003). Finally, the problem would be converged to some solutions which satisfy local and national constraints.

2. Material and Methods

In a traditional power system, the aim is to minimize the sum of system costs but in a modern power system, the objective function is in the form of total of profit gained by generation for each of Gencos and this profit must be maximized (Lin et. al., 2004). The problem's constrains are different as compared with traditional case, because some constraints like financial limitation of investment and the maximum constraint of generation level will also be applied in problem per year for every Genco, in order to prevent creating the market power, in addition to traditional environment's constraints like emission constraint, fuel constraint and etc.

1- Objective function

For *ith* Genco, the objective function will be written as follows:

$$Max \ B_i = \sum_{t=1}^{T} \sum_{j=1}^{m} [p(t).q(j,t) - C(j,t)]$$
(1)

Here, *T* is the time horizon of planning, *m* is the number of generation technologies (thermal, Gaseous, nuclear, etc.), p(t) is the price of power sale in year *t*-th, q(j,t) is the quantity of that power which is generated by technology *j* in year *t*-th and *Bi* is the sum of profit of *i*-th Genco in generation planning. C(j,t) is the cost of power which is generated by technology *j* in year *t*-th and is expressed by equation (2).

$$C(j,t) = \frac{1}{2}a.q^{2}(j,t) + b.q(j,t) + c$$
(2)

Here, a, b and c are cost function coefficients.

2- Local constraints

I) Emission constraint: this constraint controls the emission level of various technologies of generation.

$$\sum_{j=1}^{T} q(j,t).EM(j) \le EML(t)$$
(3)

Here, EM(j) is the emission coefficient of technology j and EML(t) is the allowable emission margin in year *t*-th.

II) The constraint of maximum expansion level will be specified for each Genco per year by ISO and it causes to prevent creating market power and competition-escaping.

$$\sum_{j=1}^{m} q_i(j,t) \le MaxExp(t) \tag{4}$$

In this equation, MaxExp(t) states the maximum level of expansion in year *t*-th.

III) The financial constraint of investment is related to each investor.

$$C_{inv}(t) \cdot \sum_{j=1}^{m} q_i(j,t) \le MaxCap(t)$$
(5)

In this equation, $C_{inv}(t) C_{inv}(t)$ is the cost of investment per year and MaxCap(t) states the

maximum rate of investment of each investor in year *t-th*.

3- National constraints

I) Reserve constraint: the sum of generation per year must between up and down reserve margin.

$$(1 + r_{\min}^{t})D(t) \le \sum_{i=1}^{n} q_{i}(t) \le (1 + r_{\max}^{t})D(t) \quad (6)$$

Here, r_{\min}^{t} is the down reserve margin and r_{\max}^{t} is the up reserve margin.

II) *LOLE* Constraint: In year *t-th*, *LOLE* rate must be lower than its allowable level.

LOLE (t)
$$h (day/year)$$
 (7)

Here, *h* is the allowable level of *LOLE*.

In a GEP problem with n candidate for expanding generation, generating quantities for each Genco (qi) should be specified in planning horizon (*T* years):

$$q = \begin{bmatrix} q_{11} & q_{12} & \cdot & \cdot & q_{1T} \\ q_{21} & q_{22} & \cdot & \cdot & q_{2T} \\ \cdot & & & & \cdot \\ \cdot & & & & \cdot \\ q_{n1} & q_{n2} & \cdot & \cdot & q_{nT} \end{bmatrix}$$

Game theory is used in order to solve the problem of GEP in national level and modeling the competition among Gencos. In this algorithm, the necessary data like Market Clearing Price (MCP), fuel limitation, Reserve Margin and the index of reliability will be declared by ISO to every Genco. In local level, Each Genco accomplishes its own generation planning aiming maximize its profit. The planning results of every Genco will be declared to ISO which is including the total of new added capacity by various generation technologies. ISO declares those results which are relevant to planning of every Genco to all other Gencos.

In next stage, in local level, each of Gencos will solve the GEP problem more accurately with respect to the latest results of other Genco and they make their results update and then send results to ISO. This procedure is like a game to Nash-Cournot equilibrium (Chaung, et. al., 2001). This game goes on until none of Gencos Wouldn't like to change their own generation level in planning horizon. Since decision-making of Gencos happens simultaneously and competition between Gencos is based on quantity, these conditions state that they have achieved the Nash-Cournot equilibrium (Cournot, 1897). Then, in national level ISO will check the system national constraints (Equations 6 & 7) with respect to output results of Gencos. If these results would satisfy the national constraints of system, ISO confirms the results corresponding to planning and the problem-solving will be completed, otherwise when each of national constraints wouldn't be satisfied as well as disapproval of planning results, the problem should be resolved applying some changes.

These changes will be applied in problem using some coefficients and which are related to reserve constraints and *LOLE*, respectively.

Having used these coefficients, Equations (6 & 7) will be corrected as Equations (8 & 9):

$$(1 + r_{\min}^{t})D(t) \le (1 \pm \alpha) \sum_{i=1}^{n} q_{i}(t) \le (1 + r_{mac}^{t})D(t)$$
(8)

$$(1 - \beta)LOLE(t) \le h (day / year)$$
⁽⁹⁾

If the sum of existing and added generation would be higher than up reserve margin, coefficient should be gradually decreased in equation (8) and if the sum of existing and added generation is lower than down reserve margin, coefficient should be gradually increased in equation (8). In equation (9), in order to achieve the considered level of reliability in national level changes in coefficients is used, Gencos have to gradually decrease the considered LOLE indices (Kirschen, and Strbac, 2004).

The problem will be resolved for every Genco by applying these changes (Javadi, et. al., 2009). If each of national constraints would be disregarded, Each Genco applies equations (8 & 9) with new and changed coefficients in their planning problem. For example, if equation (8) wouldn't be satisfied and the sum of generation is lower than down reserve margin, ISO forces each of Gencos to increase their generation in order to achieve the considerable degree while it increased coefficient because in normal condition, none of Gencos have no inclination to change their generation level in planning horizon while they are achieved their premium planning and maximum profit.



Figure1. Pool Market method flowchart for GEP

This procedure goes on until all national constraints would be satisfied. In this stage, planning has Nash-Cournot equilibrium and none of constraints check would be disregarded. Therefore, these results will be taken into consideration as final results of GEP. The flowchart of GEP in a Power Pool Market is given in Figure.1.

3. Results

In this paper, 3 Gencos who had various generation technologies will compete together in order to expansion the generation in a pool market. The data related to these technologies are given in Table 1 (Su et. al., 2000).

Generation Technology	No. of Units	Capacity (MW)	Max. Expansion in Year (no. of units)
Nuclear	2	650	1
Coal 1	2	400	2
Coal 2	2	200	3
Oil	2	300	3
Comb. Tur.1	2	50	5
Comb. Tur.2	4	25	5

Table 1. The data related to generation technologies

These 3 Gencos have 6 choices for type of technology of generation unit altogether which includes nuclear, coal (2 types), oil and combustion. Turbine (2 types).The number of generation units, maximum of expansion level for every unit per year and generating capacity of these units are determined in this table. Some technical and economical data of these generating units such as maintenance, fuel and investment cost and Forced Outage Rates (FOR) are given in Table 2. The horizon of planning is considered for 5 years.

Table 2. Technical and economical data of candidate units

	Maintenance	Fuel	Capital	
Unit Type	Cost	Cost	Cost	FOR
	(\$/MW)	(\$/KWh)	(\$/KW)	
nuclear	113.75	2.41	625.5	0.05
Coal 1	516	4.21	635	0.09
Coal 2	450	4.21	595	0.15
Oil	195	11.3	255.75	0.36
Comb.Tur.1	235	12.16	152	0.015
Comb.	145	12.5	100	0.007
Tur.2	143	12.3	100	0.007

It is supposed that information related to 10 years from 1999 to 2008 such as load peak and the average of electricity sales price are available and the planning of generation expansion has to be executed in this time horizon while we used the forecasted information related to 5 next years from 2009 to 2013.

Table 3. Load peak and average of electricity sales price in past 10 years

Year	Load(MW)	Average Price(\$/MW)
1999	17465	9
2000	18821	10
2001	19805	11
2002	21347	12
2003	23026	14
2004	24750	15
2005	27107	15.5
2006	29267	16
2007	32200	18
2008	34200	19

The amount of load peak and its price in past 10 years is according to Table 3. With respect to information of Table 3 using (Auto Regressive Integrated Moving Average (ARIMA) method (Abraham and Ledolter, 1986), the level of load peak and the average of electricity sales price will be forecasted in next 5 years. The forecasted values are given in Table 4.

	of electricity sales price				
Year	Load (MW)	Average Price (\$/MW)			
2009	36652	19.5			
2010	38765	20			
2011	41120	21			
2012	43242	22			
2013	45462	22.5			

 Table 4. Forecasted values of load peak and average of electricity sales price

Three modes are taken into consideration for load:

- Peak load mode: load is equal to values of table 4.
- Average load mode: load is equal to %90 values of load peak values.
- Base load mode: load is equal to %80 values of load peak.

The capacity which is installed in 2008 is equal to 35000 MW. The rate of LOLE is 5 (days/year) and reserve margin is between 5% -15 %. The value of initial coefficients is related to reserve constraint () and confidence capability () is 25 MW and 0.001.

Due to the flowchart of this model in Figure 1, Gencos will compete together in order to maximize their profit firstly. As it is mentioned before, this competition is modeled by game theory and since decision-making of Gencos would be simultaneously happened, the Nash-Cournot balance point will be created in this game. The change of total generation for each Genco and achieving Nash-Cournot equilibrium in first year of planning is shown in Figure 2.

Load has no definitive mode and it is mixed of three peaks, average and base modes and a coefficient-giving to three modes of load will be performed in order to achieve the load rate in these conditions with respect to seasons of year. As we know electricity consumption is in peak mode in summer usually and it is in average mode in fall and spring seasons and it is in base mode in winter, i.e.: the lowest value.

Therefore, we can consider the consumption load in a year with an appropriate approximation like the following:

The year consumption load = peak load of year $\times 0.25$ + average load of year $\times 0.5$ + base load $\times 0.25$

By using represented method in this paper, the results related to generation expansion plans in a planning horizon are in the form of Table 5 and Figure 3.



Figure 2. The change of total generation for each Genco and achieving Nash-Cournot equilibrium in first year of planning

4. Discussions

In represented model in this paper for solution GEP problem in pool market, new electricity market concepts such as forecasting, game theory and etc are combined with GEP problem in modern system. This combination bring about the comprehensive and accurate model for solution GEP problem. In our study, this concept is never used synthetically. Besides using these concepts in the represented model, a simple method is used for convergence the problem answer to the answer that maximizes the Gencos benefit. In this model, ISO leads the solution problem to a direction that the Genco should oblige to exist from its production optimized manner a little to satisfy the overall constraints which are as the security constraints of network by exertion some coefficients in the constraints and finally the overall constraints are satisfied. In this model GEP problem is simulated in addition to applying new concepts of electricity 2/9/2011

market with a simple but useful method and present an optimal and reasonable answer for the GEP problem.

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An Empirical Study of Awareness in Web Based Cooperative Writing Applications

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Abstract: As part of any groupware that supports people achieving a common goal, it is required to provide information about structured shared objects and the activities of participants, as well as an efficient communication service and effective coordination mechanism. Thus, this paper presents an empirical study of the trade-off concerning awareness functionality with in fifteenth well know cooperative writing applications (CWAs), evaluated on the basis of present and past awareness elements, these applications are classified taking into account time and work place parameters.

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1. Introduction

Current computer technologies like network communication made possible the cooperative work in a distributed way, independent of geographical location of hardware resources, software, and presence of users. In addition to study and understand the working of computer technology like engineering disciplines, it is also essential to comprehend how people use a groupware application when they produce whether individually or in group (social and cognitive sciences). Both domains give roots to the Computer Supported Cooperative Work (CSCW). This kind of work is related to the technology whose goal is to support groups of people collaborating to reach a common goal or perform a common task [15].

An infrastructure that supports a group of people to carry out shared tasks to achieve a common goal is named a groupware [6] like instant messenger, email service, or cooperative writing applications. A groupware considers issues like how people can produce in group (social aspect), how to share available resources like working memory, storage media, including hardware (computer support).

Analyzing groupware applications based on data collection techniques, software development cycle, and conclusion drawn for their evaluation [3], it is realized two main characteristics of a groupware [15]: a) the synchronous vs. asynchronous interaction mode, for instance conferencing vs. email, b) the remote versus co-located processing nature, for instance meeting rooms, and argumentation system. Despite of synchronous/asynchronous execution mode, a groupware should provide information about user activities and status of common production [10]. This knowledge is provided by means of group awareness functionality, whether integrated into the groupware application or developed separately to be plugged into it. Awareness concerns with present and past actions executed by group members on commonly produced objects, providing a way to communicate and coordinate user activities [13].

In [15], a survey that evaluates about 45 papers from ACM CSCW conferences. These papers are analyzed in five aspects: data collection techniques, software development cycle and conclusion drawn from the evaluation. Almost onethird of the analyzed groupware system were not evaluated in a formal way and only about onequarter of the articles includes evaluation in real world and wide variety of evaluation techniques are available. This survey concluded that there should be some new techniques for the evaluation of groupware which should be simple and low in cost.

The hypothesis of an empirical study on collaborative writing [14] is that people use collaborative writing editors only if they are sure that their partners also use the same. Thus, an inquiry placed on WWW and related to the use of collaborative editors, was filled out by 41 persons. This inquiry analyzes "How people work when they write a document in a collaborative way? What kind of tools do they use? In particular, do they resort to groupware for this task?" etc. As a result of this inquiry, it seems that people like a word processor instead of using specialized collaborative writing. New communication technologies such as chat as instant messaging are hardly to be used. They are more familiar with email, face-to-face meetings, and telephone (Section 3). People give highlighted importance to functions like tracking, version control, and synchronous work. Section 4 describes our study of CWAs based on present and past awareness elements. Section 5 draws conclusions.

2. Group Awareness

Cooperative Writing Application, CWA offers an environment support to users working in group, like individual/group editing, uploading/ downloading productions, and annotation. In addition, awareness, communication service, and coordination mechanism are also incorporated. The hardware part comprises input/output resources including, shared catalogs and primary/secondary storage.

There are two major types for viewing a groupware [6]. The first one is based on the combination of time-space variables: - same place and time likes decision support systems; - different place but on same time e.g. video conferencing, games; - same place but different time e.g. shared office systems; and - different place and time e.g. email services. The second type irrespective of location work, based only on execution time, a groupware can be synchronous or asynchronous. In real time, changes are visible to all group members when each participant allows his/her changes to others. It means users produce at the same time a common document and they receive or view the updated content of the production from other coauthors. For instance, REDUCE [16]. In asynchronous groupware, users produce and upload their production on a designated document storage site. Later, this production is available to his/her colleagues. For instance, BSCW [1] and EquiText [2].

No matter, a CWA application is synchronous or asynchronous, distributed or centralized; it should provide awareness information to users working in groups.

Awareness elements: Awareness concerns with "an understanding of the activities of others, which provides a context for your own activity" [12]. Information from the workspace comprises awareness elements related to present and past user activities, realized within the workspace (see Table 1).

The present awareness elements are [7]:

- Who element like presence, showing who is currently in the workspace? Identity or authorship of original idea.
- What element includes actions, information about executed operation, intention (what is the outcome of a particular operation), artifact (on which object a user is working).
- Where element involves location where user are working, gaze (where are users looking?), view (where can a user see?), reach (where can a user reach?).

The past elements are divided into five categories:

- **How** element indicates action history (how did an operation happen?), history (how did this object come to be in this state?).
- When shows event history (when did an event occur?).
- Who demonstrates presence history (who was present and at what time?).
- Where present location history (where was a user during a particular time?).
- What includes action history (what has a user been doing?).

On the basis of above elements, awareness can be:

- Workspace awareness concerns with all elements [7],
- Presence awareness provides who (past and present) information [13],
- Situation awareness deals with who, how, and where [12],
- Contextual awareness integrates where element [4],
- Action awareness gives information about how, when, and what elements [6].

To better collaborate, group members require a communication service to exchange their view points, as well as an efficient coordination mechanism to resolve conflicts, after having information either about user activities, product evolution, or artifacts.

3. Communication and Coordination

Normally, users at different physical locations need to share their ideas using a communication service, the second important issue in cooperative work. Group communication started from the development of Delphi method, allowing communication about complex problems among experts. Emergency Management Information System and Reference Index (EMISARI) derived from Delphi Conference System which is used for monitoring purposes from long time. The Delphi systems are famous for their features such as quantitative communication structures, content and indirect based communication, roles, and notification. Computer-mediated Communication (CMC) uses capabilities of computer network, processing, and storage to support communication within group [9].

In recent times, several communication modes are available: message posting, chat, conferencing, email. Instant messaging (IM) is a quasi synchronous communication, convenient for discussing activities irrespective of location work. Recently, IM provides facility of video and voice conferencing, along with awareness functionality like users status, management of members of different groups (friends, co-workers, ...). User presence could be a constraint, because not all the time, participants are able to establish a chat session, due to different time zones, variable office/lab working hours, and other social/professional engagements.

Email is also a popular asynchronous mode of communication. An email message is composed of two major sections: header and body. Coordination of users activities could be a major issue in email communication since group members have to exchange so many long textual messages and concerned users have to wait until recipient responds is comprehensible. Absence of context could also be the cause of exchange of long text messages.

Short Messaging Service (SMS) is an asynchronous communication, used for mobile devices like cell phones and PDAs. Initially, SMS supported simple textual communication sending short messages to group members. Recently, audio and video information is also sent from mobile to mobile, web to mobile, and mobile to web. The limited size display and the messages size are the main limitations of SMS messaging. The use of unknown abbreviations may be another restriction.

The implementation of an appropriated kind of communication is guided by the work domain and the groupware application. For instance, instant or SMS messaging are suitable for mobile device whereas emails for office/lab works.

Working in group implies many constraints: limited resources available, changes in roles, priorities of task, user interests, management of dependencies among user activities to share resources. and perform effectively the collaborative/cooperative work. The support of these constraints gives rise to the coordination. In the context of CSCW, the coordination is defined as "the support for the activity of managing dependencies and possible conflicts between collaborative entities (users and their roles) involved in common and inter-related tasks of a collaborative activity (actions performed in the shared workspace)" [8].

We explore CWAs in order to investigate the kind of awareness integrated, the way group members communicate, and mechanism they coordinate their activities.

4. Awareness in Cooperative Writing Applications

CWA is accessed through web browser over network. Normally, a web application is built using standard HTML/XHTML format [17]. In such case, data from server is downloaded into browser buffer and then displayed on the user screen allowing users to interact with server or with other clients thus, any modification in the shared resource on server side is easily reflected on the client side. Three types of architecture are used in developing CWA [11]: a) Centralized architecture allows collaborators to store and process data on central server, b) Fully distributed architecture copies all components and shared resources on the different site servers so that each provide same functionality, and c) Hybrid architecture replicates processing component on the local sites and shared data on the central server. To perform any action on hybrid architecture, first collaborators download data from the central server and introduce the lock feature to avoid inconsistent/incoherent shared production, modify it locally, and upload it on central server, and unlock the data.

Following, we describe the working/architecture of each studied CWA, and then evaluate its awareness system, communication service, and coordination mechanism.

4.1 Basic Support for Cooperative Work (BSCW).

BSCW [1] built on centralized architecture, supports either synchronous cooperation, providing tools for planning and organizing meetings, or in asynchronous mode, providing shared workspace for storing, managing, jointly editing, and sharing information.

BSCW functionality are related to personal objects: trash option to prevent unauthorized/unintentional deletion of objects, address book to invite members to join workspace, calendar helps to manage appointments, bookmark option to provide quick and easy access objects, task list to be carry out, briefcase option to synchronize documents from local computer to the workspace, adding option to upload documents, discussion option to propose ideas.

In addition, BSCW provides - view and reach by viewing user's role, - action and event history is launched by clicking 'history' option (presence and location history information) (see Table 1), the last activity performed by user on a particular location of the workspace is indicated by color-code; - monitor option provides information about authorship, action, intention, and artifact; identity (who are the participants and their roles in a particular activity) is provided by clicking on the shared icon.

The communication among users depends upon server configuration: - instant messaging, -SMS when users mobile phone numbers are included in address book, or - computer conferencing when users are currently running on the same computer conferencing program (see Table 2).

The BSCW users coordinate their activities through set lock and freeze option in the workspace. An owner can temporarily locks a document, so it cannot be modified or replaced by other one and a lock-icon appears on the shared object. When a document is frozen, it cannot be modified until it is unfrozen by owner or by the system administrator. The freezing document is also shown with the same icon.

4.2. Equitext

Equitext[2] is an centralized asynchronous collaborative writing editor. Two options are offered: start a new activity or work on the existing one. To create a new theme, users contact the administrator, who gives them a password to access a theme or create a new one. Users can select from already registered texts to contribute.

Information about when a collaborator has performed operations on a particular object is provided by clicking on the record option. Event, presence, and action history by viewing date and time of any performed operation or comment on an object. Artifact history is provided through partial draft option (see Figure 1).

When collaborators implicitly exchange their point of view by making comments on a paragraph, an asterisk appears against it. Users cannot coordinate their activities in Equitext. Minor change in architecture of product (software) can imply major modification in task dependencies and when these changes do not reflect towards collaborators, who are coordinating the product development, cause severe consequences, such as diverting from the modified goal.

4.3. Thinkfree

Thinkfree [18] is asynchronous web and desktop based cooperative writing editor built on

hybrid architecture. Users can also work on offline mode. The offline file manager keeps track of local files present on the computer. When Internet is available, collaborators can synchronize all documents that were modified during offline mode with the respective online version stored on the Thinkfree Web Server. Thinkfree manager is used by users who use desktop version.

Users are able to create projects and sends invitation through email service to other members to join the project. Online storage facility called MyOffice. Event history is provided about tasks, issue, or comment made by collaborators. Actions history information is limited to make comments. Presence history is achieved on limited scale from event history, who collaborator was present and when (past).

After naming and describing a new task, it is possible to include the email of all users who join the project. A message is send to concerned users when creator selects the notification action. Thus, communication is done through email notification and by posting comments. In Thinkfree, there is no way to coordinate collaborators activities, implying possible redundant results and inconsistent document production.

4.4 Writely.

It [19] built on hybrid architecture. It is quasi-based synchronous web based writing editor as updates itself after two minutes. The word document, presentation, or spread sheet is published on the web browser. The file organizer is very simple, provides a very usable interface for uploading, downloading, and creating new files in any of the suite's three applications. Users can produce while they are disconnected, and then synchronize their changes automatically when they are reconnected.

Writely provides information about identity through user name list, authorship through owner, viewer, and collaborator fields. Information about action, artifact, event, presence, and action history comes through 'revision function' of the last two revisions, displayed on three columns: revision column specifies an integer which is the revision number, - last edited column displays the time of the revision and the author, - changes column describes the modification, date, and time.

Users who want to share a document with other collaborator send an invitation by email, the service is available when any shared document is opened in the workspace.



Figure-1. Awareness elements in EquiText

4.5 Project & Course Forum.

Course Forum is an asynchronous web based writing editor used for remote learning [20] and Project Forum a multiple project in office [21], whose architecture are centralized. Course makes instructor contact, course information, and lectures on which student could make comments at any stage and post assignments on them. Teacher and students can interact conveniently to create posts and share/discuss course content. Project Forum makes meeting notices, to-do list, provides information about group members, share, discuss, and review ideas.

The awareness in Course and Project forum constitutes with information about the identity of the group member who posts comments and event history (who and how elements). If at the time of posting comments, they are authoring, the author identification is always displayed. The document displays the date of the last modification (when element). On every page of Course/Project Forum there is a box for writing and posting comments, the authorship is optional. While a page is locked, users clicking on the lock option, a password is asked to enter who is allowed to post comments. This is a way to coordinate user's activities.

4.6 Synchroedit.

Synchroedit [22] is synchronous system, developed on hybrid architecture. Each collaborator and his/her modifications are identified by a color. This feature helps collaborator to inform about authorship of realized actions. Document Object Module (DOM) tree keeps track of each user modifications by means of an event handler.

Synchronoedit provides awareness about presence (connected users), showing different color. All performed writing action is highlighted by the specified authorship color. Also, the user current position is marked with an author flag. Chat environment communication is provided by just clicking on the log in session, so users can start talk session with collaborators online. This is the way to coordinate their activities.

4.7 DocReview/Quick DocReview

[23][24] is Unix based asynchronous editors, implemented on centralized architecture. Users are allowed to make comments or to review a document published on Web. Firstly a document is uploaded and a form is filled then the document is converted into HTML format. Additional options can be added, for instance, assign titles, make a directory.

DocReview maintains event history (when element) by comments and last modified field (respectively Quick DocReview by viewing last modified and viewing comments). Presence history (who element) is provided by the last modified field (resp. by viewing comments text field, authorship name, date, and time).

Communication is achieved by posting comments or emails when a collaborator uploads a file, and sends emails invitation with the link of uploaded document to others. This is the way to

coordinate activities among collaborators.

Awareness Elements		Cooperative Writing Applications
	Durannas	BSCW, Synchroedit, EtherPad, Gobby REDUCE,
	Presence	MoonEdit, AllianceWeb
	Idontity	BSCW, Writely, Synchroedit, EtherPad, Gobby,
	Identity	REDUCE, MoonEdit, AllianceWeb
	Authorship	BSCW, Writely, Synchroedit, REDUCE,
_	Action	BSCW. REDUCE. AllianceWeb
Present	Intention	BSCW. AllianceWeb
Awareness	Artifact	BSCW. Gobby. REDUCE. MoonEdit. AllianceWeb
Element	Location	REDUCE, AllianceWeb
-	Gaze	
	View	BSCW, AllianceWeb
	Reach	BSCW, AllianceWeb
	Action History	BSCW, Equitext, Thinkfree, Writely, REDUCE,
		AllianceWeb
	Antifact History	BSCW, Equitext, Writely, Etherpad, REDUCE,
	Afulact History	AllianceWeb
		BSCW, Equitext, Thinkfree, Writely, Course &
	Event History	Project Forum, Doc Review, Quick Doc Review, Wiki
Deat Among and	-	wiki web, REDUCE, AllianceWeb
Past Awareness		BSCW, Equitext, Thinkfree, Writely, Course &
Element	Presence History	Project Forum, Doc Review, Quick Doc Review,
	2	REDUCE, AllianceWeb
	Location History	BSCW REDUCE Alliance Web
	Location mistory	
	Action History	BSCW, Equitext, Writely, REDUCE
		1

Fahle	1.	Awareness	Elements	in	CWAs
Lanc	1.	Awareness	Liements	ш	CWAS

4.8 Etherpad

Etherpad [25] is a real time synchronous collaborative writing editor, built on centralized architecture. Each author has colored identification along with his/her name, without any sign in procedure to enter into the collaborative writing environment. Authors invite others by sending invitation and information about who are joined (identity) and who is present (presence). By saved revision function, coauthors acknowledge the artifact history (how).

Communication chat is provided. There is a separate chatting box present on the Etherpad workspace. No mechanism has been provided explicitly for coordination.

4.9 Wiki Wiki Web

[26] is a web based asynchronous editor having centralized architecture. Users can edit a topic without permission from other author and hence the document consistency cannot be guaranteed. Event history (when) is possible by viewing "last modified field" at the end of every page. This field contains comments, name of user who makes comments, date, and time on which comments are written. Name of user reflects in the last modified field if collaborator set user name in wiki editor before starting work, otherwise IP address is shown instead of user name. Mechanisms do not exist for coordination neither for communication.

4.9 Gobby

[27] implemented on hybrid architecture, is a synchronous cross-platform that enables developers to import source code or a document to edit at the same time. Gobbly application fixes bugs and can be used by more than one user. Each user is identified by a color code that enables to see changes made by others. Presence awareness (who), identity, and authorship (who) are the information about who is present on workspace along with file name on which users are working on, as well as artifact information (what)(see Figure 2). Users are able to chat with others and also work on more than one document from any location.



Figure 2: Awareness elements in Gobby

4.10 REDUCE

[16] is a synchronous editing application, developed on distributed architecture. Collaborators are notified with coloring scheme about different writing activities. For instance, red color is used to show active tasks, and green color indicates the idle tasks. REDUCE is not a free software. We evaluate awareness, communication service, and coordination functions by analyzing its published work.

Presence awareness is provided by showing who has authority to modify or view a particular document. Present users highlighted by their identity through user name list. REDUCE provides awareness location and authorship by toggle multi-user scroll bar. The user position is displayed within the document, indicating who is producing the document. REDUCE also provides knowledge about action and artifact by splitting window view showing users view and their working sections.

Action, event, presence, and action history (past element) are provided through modification director which notifies by flashing icons when user's contribution is update.

Collaborators coordinate their activities through set lock and freeze option. Communication service is done through email and by its own chat environment.

4.11 Moon Edit

[28] is a synchronous editing system, works on hybrid architecture. Awareness about presence and identity through user name list is provided. Information about artifact is provided through menu bar. A user can modify a document locally by using "host" and remotely by "join" functionality. Users can exit from the shared space whenever they desire. Communication is done through own chatting environment. However, coordination mechanisms do not exist.

4.12 Alliance Web

[4] is developed with hybrid architecture, running on offline mode in case of network failure. User manager role assigns on each partition document, the role of each user (manager, writer, reader or null). This assignment is dynamic, so the manager has right to change the user role at any time. Writing action information is presented through event notification on the basis of nature of the cooperative production. External Call Facility (ECF) of Alliance Web helps in tracking authoring action such creation, open, saving a document, selection, copy, and paste writing operation, annotation, etc. Writing actions are represented by events and handled through a Distributed Event Management Service (DEMS)[5].

Presence awareness is achieved through user name list and session starting and termination alert message, when user signs in or/off, this action is notified to all members in session. Identity awareness is obtained by the help of user id, user name, working site or storage site id. Authorship knowledge is achieved by user role assignment manager. Intention comes to know through request of change role from co-worker to the manager.

		Cooperative Writing Application
	Email	BSCW
	Chatting	BSCW
Communication	Computer Conferencing	BSCW
	Message Posting	BSCW, Equitext, Thinkfree, Writely, Course & Project Forum, Doc Review, Quick Doc Review, REDUCE
	Set Lock	BSCW, REDUCE
Coordination	Freeze	BSCW, REDUCE
	Exclusive Writing	Alliance Web

 Table 2: Communication and Coordination in CWAs

Artifact information arrives by viewing chronological event history. Location awareness is achieved through working site id. Information about view and reach also come through role assignment manager. Action/event awareness, artifact history knowledge, presence history, and location information is achieved through event notification alert message

Communication service is provided through email and chatting. The context-based/work-focus synchronous communication allows users to communicate with each other when their focus of discussion is simultaneously present on their displays[4]. The awareness system takes advantage of unique identifier associated to each object that is present in the shared document.

Coordination mechanism is maintained through exclusive writing and work proximity. Exclusive writing refers to production of document part when it cannot be accessed by any other coauthor. Work proximity concerns with changes in objects like figures, tables which are referred or produced by more than two authors. Concerned users are notified each time an actualization is made on these objects.

5. Discussion on Survey

A synchronous CWA lies in the same timeplace region, while asynchronous one is placed in different time-place region (see Table 3). A real-time synchronous CWA implies maximum number of awareness functions as it creates illusion of face to face meeting that is why present awareness is more focused rather than past awareness elements. Whereas, asynchronous groupware application emphasizes those awareness elements which concern with shared production. In asynchronous nature of production more emphasize should be given to production evolution i.e. past awareness elements.

The classification helps a group to select a CWA according to its nature of production and mobility/availability of users. In case, users want to see their own modification or that of their colleagues,

synchronous CWA should be chosen. Otherwise, asynchronous type application be a good choice[29].

From the studied CWAs, BSCW and Alliance Web have maximum number of awareness elements: 14 out of 16, implying more effective performance. Whereas, Equitext has five awareness elements and Wiki Wiki Web presents least awareness function (only one). BSCW contains all type of communication means whereas Wiki Wiki Web does not offer any one. Coordination facilities are present in BSCW (set lock), REDUCE (freeze), and Alliance Web (exclusive writing and proximity).

6. Conclusion and Future Work

The purpose of this study is to assist people in selecting a Web based cooperative writing application (CWA). In addition to user friendly interface, convenient shared object management and efficient mechanism for processing, other essential features like awareness, good communication service, and efficient coordination mechanism should also be considered for evaluation of cooperative applications. CWA operating in real-time mode must have awareness information, and synchronous communication service i.e. instant messaging, video conferencing. In contrast, a non-real time CWA should have asynchronous communication service. email, message posting, e.g. etc. Efficient coordination mechanism is also necessary to maintain consistency in shared production. For this, we argue that "set lock" feature is more suitable as a user independently locks a document fragment and releases it when updated. In exclusive writing, a member asks the manager to get right of changing the document.

We plan to define an evaluation criterion for graphical and mathematical groupware. Such works need to include multiple objects like line, poly lines, ellipses, texts, formulae. The criterion will help to decide the expertise level of coworkers.

Table	3:	Time	and	Space	based	classification	of
CWA							

	Same/Different places
Same	REDUCE, Syncroedit, Gobby,
time	Etherpad, Writely, Moon Edit
	Wiki Wiki Web, Course/Project
Different	Forum, Doc Review, Quick Doc
time	Review, ThinkFree, EquiText,
	AllianceWeb, BSCW

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Preventive Measures to Reduce Post - spinal Anesthesia Hypotension for Elective Cesarean Delivery

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Abstract: Aim: To explore the effect of wrapping and/or raising of the legs as a Preventive Measures to Reduce Post - spinal Anesthesia Hypotension for Elective Cesarean Delivery.Setting: The study was conducted in the operating room (cesarean section) at Ain Shams maternity hospital .Study design: An experimental design. Type of Sample: - purposive sample. Methods: 120 parturients were undergoing elective Caesarean section randomly scheduled to four groups: Group (I) (n=30) parturients legs wrapped immediately before injection anaesthesia and elevated immediately after anesthesia administration. Group (II) (n=30) parturients legs wrapped, tightly wrapping was achieved after leg elevated to 45 degree for 2 minutes, with an elastic bandage applied from ankle to mid thigh, immediately before anesthesia administration. Group (III) (n=30) parturients legs elevated to 20 degree immediately after anesthesia administration. Group (IV) (n=30) no intervention. Tools of data collection consisted of 1) Demographic data,2)Automated monitors for measurement of blood pressure,3)Graphic flow sheet to record blood pressure, and 4) neonate assessment sheet to record Apgar score at 1and 5minutes. Results : The findings revealed that, This study showed that, there is no inter group's differences regarding their age, body mass index & baseline. Mean systolic arterial pressure MSAP. Meanwhile, a significant difference was noticed among the groups, whereas GI (wrapping & elevation) had a higher MSAP, lower percent of hypotension women late onset time of hypotension and a lower percent of babies with bad outcome the Neonatal outcome was excellent and similar in both groups. Conclusion: wrapping and elevation of the legs for parturients at spinal block for Cs had more effective measures to prevent hypotension. Recommendations: wrapping and elevation of the legs should be used in addition to traditional measures to prevent post-spinal hypotension as a non-pharmacological technique.

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Keywords: Lower limbs, hypotension, cesarean section, spinal anesthesia, wrapping, elevation.

1. Introduction

Pamela.J.et al., (2011).Stated in their results that, the common clinical practice of volume expansion with crystalloid is not uniformly effective in reducing the incidence of maternal hypotension after spinal anesthesia for cesarean delivery. Colloid administration is more consistent but is associated with additional risks and costs. Mechanical means of central blood volume expansions also appear to be effective but have not come into widespread use. Greater emphasis is being placed on increased utilization of regional rather than general anesthesia, whenever possible Spinal anesthesia is easy, has a rapid onset, is reliable and provides good surgical conductions (Lewis et al., 2000 & Smeltzer, Bare.2000).

In women's with spinal Hypotension occurred frequently during spinal anesthesia (80%) and with an incidence similar to that in other reports. It result from the decrease of arterial blood pressure after spinal anesthesia is thought to be the result of sympathetic block, which could cause pooling and redistribution of blood into the lower extremities. Hypotension after induction of spinal anesthesia for cesarean section is remains a common clinical problem and a potentially serious complication despite acute expansion of intravascular volume (Sun .HL, et al. 2004). It is often associated with bradycardia, nausea, vomiting and if sever: unconsciousness &pulmonary aspiration(Emmet et al.,2002 and Hartmann et al.,2002).Hypotension can threaten the wellbeing of the unborn child and compromise Fetal circulation, causing hypoxia, neurological injury and fetal acidosis in the unborn baby (A. Herdan , R. Roth, D. Grass & M. Hessen 2010).

In a recent study, carried by Ngan. K & Warwick. D(2010), they found that risk factors for hypotension include increased sympathetic tone, increasing age, obesity, higher blocks and higher birth weight. Prevention of hypotension is more desirable than correction after it has been occurred, as uterine blood flow has been shown to remain impaired for some minutes even after blood pressure has been restored (burns et al., 2003). Post - spinal appear to be difficult to avoid by traditional preventive measures (fluid preload, positioning & vasoconstrictors drugs) (Klasen et al., 2003).

In another study conducted by (Singh .J et al.(2010)they performed study to explore the Efforts to prevent hypotension have been attempted like preloading with crystalloids, colloids or use of vasopressor. During spinal anesthesia, 16 - 20R% of the total blood volume is in the legs. Bagaert (1998) & (Iwama et al.(2002) stated in their study that preventive methods (wrapping and /or elevation) of legs would decrease incidence of post – spinal hypotension by venous pooling in the legs it appears as a theoretically attractive technique counteracting undesirable physiological changes of spinal anesthesia hypotension.

On the other hand (van .B, 1998 & B. S, et al. (1990)in their study to prevent these complications they have been attempted like wrapping, and elevation of legs they founded that, Wrapping of the legs was a nonpharmacological, prophylactic method to reduce hypotension during regional anesthesia for caesarean sections.

Considering prophylactic methods for compressing the lower limbs to suppress redistribution and augment venous return have been used, with varying degrees of success in preventing hypotension after spinal anesthesia.(Bjornestad.E., Iversen.O., Raeder.J.2009)t hey suggested that ,Wrapping of the legs significantly reduces hypotension during regional anaesthesia for caesarean sections. However, most obstetric anesthetists prefer to use vasoconstrictors for reducing hypotension.

Justification of the problem:-

Spinal anesthesia is commonly used for elective cesarean delivery. Associated hypotension is caused by an increase in venous capacitance and a reduction in systemic vascular resistance. Because uterine blood flow is dependent on perfusion pressure, hypotension results in reduced uterine blood flow, with a potential compromise in fetal oxygenation. Maternal nausea and vomiting may also occur.

Most previous study showed that, these prophylactic measures were clinically useful to reduce Hypotension after conduction of spinal anesthesia for caesarean sections but have not come into widespread use in operating room for cesarean section most obstetric anesthetists prefer to use vasoconstrictors for reducing hypotension. so this study was designed to apply prophylactic measures such as" wrapping and elevation of legs versus pharmacological methods in parturient during cesarean section to prevent hypotension associated with spinal anesthesia.

Aim of the study:

To explore the effect of wrapping and/or raising of the legs as a Preventive Measures to Reduce Post - spinal Anesthesia Hypotension for Elective Cesarean Delivery.

Hypotheses:

Based on other research studies, it was hypothesized that: 1)Wrapping and elevation of legs as a non – pharmacological technique in addition to traditional measures have a positive effect on preventing or reducing post – spinal hypotension . 2) Nurses have unsatisfactory level of knowledge regarding post – spinal hypotension.

2. Subjects and Methods:

Hypotension was defined as a decrease in any mean arterial pressure (MAP) measurement by more than 20% of the baseline MAP. Systolic (SAP), MAP and diastolic (DAP) arterial pressure, pulse pressure (PP), and heart rate (HR) were noted at baseline and every minute after the spinal block until delivery.

Design: an experimental design was used in the conduction of this study.

Setting: This study was conducted in the operating room at Ain Shams maternity hospital (cesarean section).

Subjects: 120 and twenty parturients undergoing for elective cesarean section under spinal anaesthesia and one 120 babies were studied.

Criteria of selection: Inclusion criteria parturients under spinal anesthesia and with the same level of sensory block.

Exclusion criteria: Obesity ,diabetes, hypertension, heart disease, multiple gestation, pre-term parturient, age less than 18 or more than 40years and height less than 152cm.

Tools of the study: four tools were used for data collection:

- Demographic data (age, height, weight &body mass index).
- Automated monitors for measurement of blood pressure.
- Graphic flow sheet to record blood pressure.
- Neonate assessment sheet to record Apgar score at 1 and 5 minutes.

Pilot study:

It was conducted on 10% of the total study sample to measure the feasibility of the study settings, clearty, reliability of tools and time required for completion of each study tools.

Procedure

Immediately after the approval for the conducting the study, sampling started and completed within 6months.

The cases were allocated randomly to the following: **Group I** (n=30): parturients legs wrapped immediately before injection anaesthesia and elevated immediately after anesthesia administration. **Group II** (n=30): parturients legs wrapped. Tightly wrapping was achieved after leg elevated to 45 degree for 2 minutes, with an elastic bandage applied from ankle to mid – thigh, immediately before anesthesia administration.

Group III (n=30): parturients legs raised to 20 degree by tilting foot – end of operating table, immediately after anesthesia administration.

Group IV (n=30): As control group no intervention.

Baseline SAP determined by calculating the mean of 3 blood pressure measurements at 3 minutes interval in preoperative holding area by using auscultatory method. The SAP was recorded 30 minutes before anesthesia administration.

The SAP was monitored every 5minutes during first 30 minutes of anesthesia administration then every 10 minutes for later 30 minutes by automatic blood pressure monitor. Among the four groups when hypotension occurred, it was rerated with vasoconstrictor drugs. Assessment of baby Apgar score at 1 and 5minutes post delivery was recorded, normal score ranged from 7-10.

3. Results

Table (1) reveals that demographic characteristic among parturients, there is no significant differences regarding their age and body mass index.

Table (2) Demonstrates that, there is no significant differences pre anesthesia (baseline). Meanwhile, post anesthesia a significant difference was noticed, whereas GI (wrapping & elevation) had a higher mean. As obviously, there is a gradual decrease in MSAP over 30 minutes of observation.

Table (3) Exposes that women apply leg wrapped were reduce Incidence of Hypotension about the no intervention group.

Table (4) Displays that incidence of hypotension was significant among studies and control groups

Table (5) Shows that, Leg wrapping resulted in a significant reduction in the incidence of post spinal hypotension in comparison to the control group.

Table (6) Exhibits MSAP and time onset of hypotension among parturients. As observed, G1 (Wrapping & elevation) Followed by G2 (Wrapping) had a higher percent SAP and late onset time of hypotension. The above table shows that there was significant difference in the time of onset of hypotension between the groups.

Table (7) Exposes Neonatal Outcome, Occurrence rate of Apgar score <7 at 5 minutes was lower in G1. (Wrapping & elevation) than G4 (control), 6.7% and 20.0% respectively.

Table	(1)•	· demogran	hic char	acteristics	of	narturient	include	d in	the study
Lanc	(1).	. uemograp	me chai	acteristics	UI.	parturient	muluuc		the study.

Variables	G 1(n=30)	G 2(n=30)	G 3(n=30)	G 4(n=30)
	$X \pm SD$	$X \pm SD$	$X \pm SD$	X <u>+</u> SD
Age (years)	27.5 <u>+</u> 6.1	28 <u>+</u> 7.1	25.3 <u>+</u> 6.7	26.7 <u>+</u> 5.8
Body mass index (kg/cm2)	27.4 <u>+</u> 3.4	29.17 <u>+</u> 3.8	28.5 <u>+</u> 5.7	28.7 <u>+</u> 3.8

Table (2): Pre & Post Spinal anesthesia for Mean Systolic Arterial Pressure among the Parturient.

Grou	ps		Time o	of MSAP obs	servation	
		Base line	15 min.	30 min.	45 min.	60 min.
		$X \pm SD$	$X \pm SD$	$X \pm SD$	$X \pm SD$	$X \pm SD$
	G 1:	92.5 <u>+</u> 4.1	91.6 <u>+</u> 3.5	84.7 <u>+</u> 5.8	86.4 <u>+</u> 5.4	88.7 <u>+</u> 7.3
es	Legs wrapped immediately before					
easur	anesthesia & Elevated Immediately after anesthesia					
Ň	G 2:	92.2 <u>+</u> 4.0	90.8 <u>+</u> 3.6	83.6 <u>+</u> 5.9	86.0 <u>+</u> 5.5	88.4 <u>+</u> 7.2
tic	Legs Elevated for 2min.& wrapped with					
/lac	elastic bandage immediately before					
chq	anesthesia					
rol	G 3:	91.5 <u>+</u> 3.5	89.5 <u>+</u> 3.7	81.5 <u>+</u> 6.1	84.9 <u>+</u> 85.0	86.8 <u>+</u> 7.4
ц	Legs elevated immediately after					
	anesthesia					
rol	G 4:	91.8 <u>+</u> 3.7	82.9 <u>+</u> 6.0	75.0 <u>+</u> 5.1	78.0 <u>+</u> 6.5	80.2 <u>+</u> 6.3
Cont	No intervention					
0						

Table (3): Incidence of Hypotension among Parturients

	Groups	(n=120)				
		No.	%				
ic	G 1:Legs wrapped immediately before anesthesia & Elevated Immediately after	8.0	26.7				
act	anesthesia						
hyl asu	\overrightarrow{s} \overrightarrow{g} G 2: Legs Elevated for 2min& wrapped with elastic bandage immediately before						
Me	anesthesia						
Pr	G 3:Legs elevated immediately after anesthesia	9.0	30.0				
atr I	G 4: No. Intervention	14.0	46.7				
Col Col							

Table (4): Number and Percent Distribution of Parturients who had not suffered from Hypotension Among Studied and Control Groups

	Groups	(n=120)	
		No.	%
ic	G 1:Legs wrapped immediately before anesthesia & Elevated Immediately after	22.0	73.3
act res	anesthesia		
hyla	G 2: Legs Elevated for 2min& wrapped with elastic bandage immediately before	22.0	73.3
op Ve:	anesthesia		
Pr	G 3:Legs elevated immediately after anesthesia	21.0	70.0
rol	G 4:	16.0	53.3
ont	NO Intervention		
C			

Table (5): Number and Percent Distribution of Present or Absent Hypotension among Parturients.

	Groups	(n=120)				
		Present	-	Absent		
		No.	%	No.	%	
	G 1:Legs wrapped immediately before anesthesia & Elevated	8.0	26.7	22.0	73.3	
i C.	Immediately after anesthesia					
act	G 2: Legs Elevated for 2min& wrapped with elastic bandage	8.0	26.7	22.0	73.3	
hyl	immediately before anesthesia					
op Ne						
Pr	G 3:Legs elevated immediately after anesthesia	9.0	30.0	21.0	70.0	
tro	G 4:	14.0	46.7	16.0	53.3	
l	NO Intervention					
\circ						

Table (6): Presentation of MSAP and Onset Time of Hypotension among Groups.

Items			Studie	ed Groups			Control Group		
		Intervention (Preventive Measures)						No Intervention	
	G 1	(n=30)	G 2	G 2(n=30)		G 3(n=30)		G 4(n=30)	
	No.	X <u>+</u> SD	No.	X <u>+</u> SD	No.	X <u>+</u> SD	No.	X <u>+</u> SD	
MSAP of Hypotension	8.0	77.8 <u>+</u> 1.8	8.0	77.1 <u>+</u> 2.3	9.0	76.6 <u>+</u> 2.5	14.0	75.8 <u>+</u> 3.8	
Time Onset of Hypotension	8.0	28.7 <u>+</u> 0.9	8.0	28.2 <u>+</u> 1.0	9.0	21.1 <u>+</u> 0.8	14.0	20.5 <u>+</u> 0.7	

Table (7): Number and Percent Distribution of Neonatal Outcome Among Studies and Control Group

Item	Studied Groups					Control group		
	I	nterver	ntion (Pre	eventive l	Measures	5)	No Intervention	
	G 1(n=30)		G 2(n=30)		G 3(n=30)		G 4(n=30)	
	No.	%	No.	%	No.	%	No.	%
Apgar Score <7 at 5 min.	2	6.7	3	10.0	4	13.3	6	20.0

4. Discussions

Klohr.S.et al. (2010) reported that Spinal anaesthesia for caesarean section may cause hypotension, jeopardizing the fetus and its mother .Spinal anaesthesia is often selected for elective or emergency Caesarean section. The advantages are simplicity, rapid onset, reliability, dense motor block, and avoidance of the potential airway complications associated with general anaesthesia. However, hypotension occurs frequently following spinal block. The incidence of hypotension during spinal anaesthesia for Caesarean section is reported to be as high as 80 %(Farruk.M.A, Aneela.P&Vigar.A.2008).

Klohr.s. et al. (2010) agree with the result of this study they reported that , a decrease below 80% baseline and the combined definition of a blood pressure below 100 mmHg or a decrease below 80% baseline were the two most frequent definitions, found in 25.4% and 20.6% of the papers, respectively. When applying the spectrum of definitions to a prospective cohort. In addition (Klohr . S. et al. 2010) they reported that the incidences of hypotension varied between 7.4% and 74.1%. The incidence increased from 26.7% to 38.5% when using a value below 75% of baseline instead of below 70% of baseline .The lower extremities during central neural block results in peripheral vasodilatation, decreased venous return and hypotension (bagaert, 1998).

Concerning the preventive methods were provide a rich ground for developed holistic care, it is easy and inexpensive(Al-Sharkawi et al.,2002 and Ahmed et al.,2003).There has been a marked interest in complementary interventions as a non – pharmacological measure(Salama, 2001).Currently, several strategies are used to prevent or minimize hypotension but there is no established ideal technique (Emmett et al.,2001).Regarding parturient characteristics, no significant differences were noticed regarding their age and body mass index. This finding may be attributed to the criteria of sample selection.

Emett et al., (2002) supported the finding of the current study and they reported that in their study, the highest risk factors for hypotension were age greater than or equal to 50 years and body mass index greater than or equal to 30. Hartman et al. (2002) they added that, aging increases risk of hypotension because of the decrease in cardiac reserve or changes in autonomic functions which may play a role. Concerning MSAP, there is no intergroup significant difference pre- anesthesia regarding baseline MSAP. This result was helpful for the researchers on assessment of MSAP post – anesthesia. Buggy et al (1998) was consistent with the previous interpretation

and added that, higher baseline blood pressure provides a margin of safety in that systolic pressure can decrease 30 - 40 % post- anesthesia and still remain greater than 90 mmHg.

Post- anesthesia, all groups had a gradual decrease in MSAP over the first 30 minutes. This result was supported by Knoerl et al. (2001), who concluded that, there is no intervention that reliable prevents hypotension during spinal anesthesia. The result of this present study showed that incidence of hypotension was in the leg-wrapped group (26.7%) compared with the control group (46.7%) in table 5.

Van. Bogaert.L.J(1997)supported the result of this study ,he mentioned that , The SAP remained significantly higher with wrapping; elevation did not add any benefit. The number of episodes of severe hypotension (defined as a SAP decrease 20% of baseline and <100 mmHg) was significantly reduced by wrapping (15.8% of cases) as compared to controls (45.5%)(2=11.02; P=0.012). Elevation alone did not prevent hypotension (2=0.76; P=0.38). Conclusion: Wrapping of the legs at spinal block for Cs is recommended to reduce hypotension. In the same context, a significant difference was shown among the four groups regarding incidence of reduction in MSAP, whereas G1 (wrapping & elevation) followed by G2 (wrapping only) had a lower incidence. This finding could be interpreted as wrapping and elevation of the legs would somehow decrease the magnitude and prevalence of hypotension by venous pooling in the legs.

Ghabash et al.(1997), wrapping and elevation of the legs might prevent the sudden decrease in arterial blood pressure during spinal anesthesia. Van Bogaert (1997) mentioned that, tightly wrapping the legs is safe and efficient in preventing hypotension. Also Iwama (2002) stressed that, wrapping reduced usage of vasopressor agents. In addition. Rout.C, Rocke.D, Gouws.E (1993) reported that, the use of leg compression post spinal provides a simple means of reducing the accompanying hypotension and should be used more widely. Also Pamela.J.et al., (2011), they reported that, several factors accounting for the success of leg wrapping in increasing central blood volume at cesarean delivery. First, there is approximately 150 mL of blood in the legs of nonpregnant subjects without spinal anesthesia. During pregnancy, venous blood volume increases in the lower extremities, particularly after the 30th week of gestation, with spinal anesthesia further increasing the volume of blood in the legs by induction of a sympathectomy. The effect of leg wrapping on the central volume has not yet been measured in parturients. In addition they showed in their study that Leg wrapping, was the most effective means of reducing the incidence of hypotension compared with the control groups (no intervention or leg elevation alone). In each case, the incidence of this complication, as defined by the authors was reduced to less than 20%.

In relation to onset time of hypotension among parturients. It was noticed that at first 30 minutes post anesthesia G1 (wrapping & elevation) followed by G2 (wrapping) had a late onset time. Meanwhile, G4 (control) had a rapid onset. The previous finding was supported by Kohler et al. (2002). Also, Mendonca et al. (2003) claimed that the greatest magnitude of decrease in blood pressure was noted to occur 15-30 minutes after initiating spinal anesthesia. In the study conducted by Hartman et al (2002), he found that mean time of hypotension occurrence was 28+34 minutes after anesthesia. He also added that these mean times should not mislead, however hypotension occurred at all times during spinal anesthesia in patients who had been stable for hour or longer, thus observation may be required during spinal anesthesia.

As clear from this study, percent of babies with bad outcome was lower in G1 (wrapping & elevation) followed by G2 (wrapping only). This result could be interpreted as wrapping& elevation of legs in addition to the vasoconstrictor drugs during spinalanesthesia decrease the prevalence of hypotension, which affect negatively on the fetus. In the same line, percent of babies with bad outcome was higher in G4 (control).Jorgensen et al. (1996) was in agreement with the previous finding and stated that, hypotension Carries potentially serious consequences for both mother and fetus. Nishikawa et al (2000) claimed that, prolonged or severe maternal hypotension can cause serious adverse fetal and neonatal effects. Ngan kee et al.(2001)clarified that transient decreases in blood pressure, rapidly treated with drugs, do not usually affect fetal acid – base status. Apgar score <7 at 5 min were recorded among groups.

5. Conclusion:

Wrapping and elevation of the legs for parturients at spinal block for Cs had more effective measures to prevent hypotension.

Recommendations:

Wrapping and elevation of the legs should be used in addition to traditional measures to prevent post-spinal hypotension as a non-pharmacological technique.

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Preliminary Horticultural Studies To Describe And Identify Of Two New Egyptian Mango Strains Using DNA Fingerprint.

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Abstract: Selection of some strains is considered the first step in improving mango production. So, this study was done for a three successive seasons (2003 – 2005) on two Egyptian mango strains "Hania" and "Aml" to describe them, horticulturally and identification those genetically utilizing DNA fingerprint. The vegetative characters (leaf shape, length, width, etc) and histological characteristics (number of stomata per mm (stomatal density), stomata length and width) showed great variation between the two studied strains. The fruit weight was 581 gm for Hania strain and 1020 gm for Aml strain. The two strains fruits had good characters as shape, net weight ratio, firmness, SSC, TA, Vit. C and total sugars. In generally, physical and chemical properties of Aml strain fruits were better than Hania strain. These differences of horticultural aspects due to genetic variances, which were determined by using SSR markers, of the 42 primers screened, 36 primers gave reproducible polymorphic DNA amplification patterns. 60.7 % of the scored fragments are considered putative genotypes-specific markers in both strains. The polymorphic information content (PIC values) ranged from 0.25 to 0.75, with a mean value 0.51 for all loci. The heterozygosity level was 0.68 and 0.53 for Hania and Aml strains, respectively. By banding patterns obtained from these 36 primers, each strain in this study could be distinguished from the other, indicating that, PCR by using SSR primers was an efficient method for genotype identification.

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Key words: Mango, Histological, Morphological, fruit, DNA, Fingerprint, SSR.

1. Introduction

Mango (*Mangifera indica* L.) is a diploid fruit tree (2n = 40). The mango is considered as one of the oldest cultivated trees in the world.

Mangoes are an important fruit crop in Egypt. According to the latest statistics provided by the Ministry of Agriculture and Land Reclamation of Egypt (2007), indicated that, a total of 184204 Feddan are planted by mangoes. While, only in Ismailia Governorate the total area was about 92557 Feddan (50.24 % from total area in Egypt), about third of this area was cultivated by local seedling trees. Each seedling tree can be considered as a separate type exhibiting wide variation in different characters. Many of them are still unknown and the information on their ecology, morphology and productivity is limited. Mango cultivars are commonly divided into two groups based on their mode of reproduction from seeds and their origin; monoembryonic (Indian types) and polyembryonic (Indo-Chinese types). Monoembryonic mango seeds contain a single zygotic embryo, mostly are subtropical and the fruit skin is highly colored (mixes of red, purple and yellow). While, polyembryonic seeds (Southeast Asian types) contain several nucellar embryos, mostly tropical with skin is not highly colored (green to light green to yellow) (Iyer and Degani, 1997). There is considerable confusion in mango cultivars nomenclature because many clonally propagated mango cultivars have unique local and regional names and the spelling and name variants have been translated to the Roman alphabet (Viruel *et al.*, 2005), as presently several mango cultivars have many synonyms in different regions, which makes identification difficult.

However, selection and correct identification of genotypes is essential for any breeding and improvement effort, is difficult, inefficient and inaccurate when based on morphological traits only. Even though a high number of descriptors are used (Thomas *et al.*, 1994), this due to some phenotypic traits are difficult to describe, and phenotypic data may be influenced by environmental factors and growing conditions, in addition to quantitative inheritance, or partial and complete dominance often confound the expression of genetic traits.

Recently, as in other fruit tree species molecular identification of mango cultivars has been carried out with different molecular systems as isozymes, minisatellites (Adato et al., 1995; Eiadthong et al., 1999), AFLPs (Eiadthong et al., 2000) While DNA profiles based on polymorphic band patterns from Random Amplified polymorphic DNA (RAPD) analysis have been described for several fruit species including mango (Schnell et al., 1995; Lopez-Valenzuela et al., 1997 and Hemanth Kumar et al., 2001). RAPD is a dominant marker, does not require target sequence information for design of amplification of primers. RAPDs are easy, cheap and fast and detect genetic differences between organisms, but their reproducibility is low. Different thermocyclers, Taqpolymerases, DNA primer concentrations and even the skill of the experimenter can influence the results, which makes comparisons of results between laboratories unreliable (Sefc et al., 2001). However, DNA-fingerprinting based on simple sequence repeats (SSRs) or microsatellites, in addition to their usefulness in mapping and breeding (McCouch et al., 1997), has become the marker of choice, because of their widespread occurrence throughout the genomes of all eukaryotic species, their co-dominant inheritance and the high level of polymorphism observed due to variations in repeat lengths. The high discriminatory power of SSRs is also important for analyzing variation in gene pool of crops (Powell et al., 1996).

DNA fingerprinting using SSRs has been applied to accession identification programmes and for genetic analyses of a broad range of agricultural and horticultural crops (Pedersen, 2006).

The mango industry in most countries based on a few commercial cultivars, due to numerous problems with most of these cultivars like poor fruit quality, a narrow maturity window and physiological disorders, so the need is for new cultivars to replace them.

Thus, the present work was carried out to describe morphological, horticultural, histological characteristics of two new mango genotypes (Hania and Aml), and identification of them utilizing DNA fingerprint.

2. Material and Methods

The present work was carried out during three successive seasons (2003 – 2005) on two local mango strains namely "Hania" and "Aml". The two strains are grown at private orchard of Ismailia Governorate, Egypt. The trees are about 25 years old.

2.1. Horticultural aspects:-

2.1.1. Physical and chemical properties of fruits:-

Samples of ripe fruits were taken randomly from each tree (strain) at harvest time in each sample, average fruit, peel, seed weight and volume, fruit and seed dimensions were recorded. Moreover, the skin firmness (kg/cm²) by using effegi pentrometer, fruit length (cm), width (cm), fruit shape index (length/width), fruit thickness (cm), pulp/fruit ratio (net ratio), soluble solids content (SSC %) by hand refractometer, fruit acidity, vitamin C and total sugars were determined as described by A.O. A. C. (1995).

2.1.2. Leaf characters:-

Samples of maturity leaves were outlined on paper, then length and width of blades were measured in cm and the area was estimated by planimeter (cm^2) .

2.1.3. Histological study (Number, length and width of stomata):-

The stomata length, width and density of two strains were measured using eye-epiece micrometer (10x eye-piece and 40x objective piece lenses). A fully development leaves from two strains. The stomata were measured from an imprint of the lower leaf surface obtained by painting clear finger-nail polish on the leaf, allowing it to dry and peeling it of (Hamill *et al.*, 1992). The imprint was laid on a microscope slide with a drop of water to allow the analysis of the guard cells. The stomata were measured at 400x magnification. The length, width and density of stomata were measured for each strain using eyepiece graticule in a microscope at 400x magnification.

Stomatal densities were calculated using the following formula:

Stomatal density (SD) = NS / A

Where: NS = number of stomata in the microscope field, and A = area of the microscope field.

The obtained data were statistically analysed using T test according to Snedecor and Cochran (1967).

2.2. DNA fingerprints.

2.2.1. Plant material

Young leaf samples of the two mango strains studied in this research were collected in early spring, lyophilized at -20°C for 48 h and ground to powder.

2.2.2. Total genomic DNA extraction

Total genomic DNA was extracted according to the basic DNA extraction protocol (Dellaporta et al., 1983) with slight modifications by Porebski et al. (1997) and adapted to mango, for obtaining good quality total DNA, 500 mg. of ground, lyophilized leaves tissue were extracted by the addition of 10 ml preheated (65°C) from cetylhexadecyl-trimethyl ammonium bromide (CTAB) extraction buffer [3% CTAB (w/v), 100 mM Tris- HCl, pH 8.0, 20 mM EDTA, 1.4 M NaCl, 2% (w/v) PVP (Polyvinyl pyrrolidone)], and then 1% (v/v) of β -mercaptoethanol (15 mM) with further grinding.. The mixture was incubated at 65°C for 60 min, followed by two extractions with chloroform/isoamyl alcohol (24:1). After which the nucleic acids precipitated with Cold isopropanol, and the pellet was dissolved in 1 mL TE 0.1X (Tris-EDTA) buffer. RNA was removed with RNaseA 4 μ l (10 mg/mL). The DNA was purified further by 300 μ l phenol: chloroform: isoamyl alcohol (25:24:1), then overnight at (-20 °C) using 1/10 vol. from 2 M Na acetate (pH 8.0) and one volume of cold isopropanol alcohol. The precipitated was washed twice, and the pellet was dissolved in 0.1X TE buffer. The purified total DNA was quantified by gel electrophoresis, and its quality verified by spectrophotometry.

2.2.3. PCR (SSR) amplification and product electrophoresis

The thirty eight SSR primer pairs used for PCR amplification, previously described by Viruel et al. (2005), Duval et al. (2005), Honsho et al. (2005) and Schnell et al. (2005). These primers were synthesized by VBC-Biotech, Vienna, Austria (www.vbcbioch.com). PCR reactions were performed according to published procedures by Viruel et al. (2005) with some modifications using fluorescent fragment detection on a LI-COR 4200 DNA dual-dve sequencing system. For this method either one of SSR primer had a M13 tail as a third primer of a fluorochrome labeled M13-30 oligo (5' CCC AGT CAC GAC GTT G 3') was add to the PCR reaction. Microsatellites amplification was performed in 10 µl volume contained: 0.02 µM forward primer (M13-30 sequence at the 5' end), 0.18 µM M13-30 oligo infrared fluorescence dye (IRD 700 or IRD 800 labeled), 0.2 µM reverse primer, 0.2 mM of each dNTP, 1.8 mM MgCl2, 0.05 U Taq polymerase, 1X PCR buffer, and 10 ng of template DNA.

The amplification was performed on a 'Primus' 384 well thermocycler (MWG Biotech, Germany) using the following temperature: after a first denaturation step at 94 °C for 2 min, the reaction went through 30 cycles with (94 °C for 1 min, 0.5 °C/sec to 51 °C, 51 °C for 30 sec, 0.5 °C/sec to 72 °C, 72 °C for 1 min) followed by a final extension step of 5 min at 72 °C. The analyses were repeated at least twice to assure the reproducibility of the results.

PCR products were detected by electrophoresis on 7 % Polyacrylamide non-denaturing gels to exact allele sizing of the SSR loci, then the products were visualized using fluorescent fragment detection on a LI-COR 4200 DNA dual-dye sequencing system. Quantity-one software was used to estimate the sizes of the products.

2.2.4. Data analysis

Ninety two reproducible bands from selected primers were scored as 1 (presence) or 0 (absence) for the two accessions tested. Allelic composition of each accession and the number of total alleles was determined for each SSR locus. Putative alleles were indicated by the estimated size in bp. The genetic information was assessed for single locus SSRs using the following parameters: number of alleles per locus (A), observed heterozygosity (Ho, direct count), and polymorphic information content values for each locus (PIC) were calculated as follows:

He or PIC = $1 \cdot \sum pi2$ where pi is the frequency of the ith allele, and summation extends over n alleles (Nei, 1973), Wright's fixation index (F) = (1-Ho/He) (Wright, 1951), and heterozygosity level of the two genotypes assayed.

The computations were performed with the programs, GENEPOP version 1.31 Raymond and Rouset (1995), Quantity one, Irfanview and Microsoft Excel.

3-Results

3.1. Leaf characteristics:-

3.1.1. Morphological characteristics:-

Data in Table (1) revealed that there were significant differences between the two studied strains in both length, width, length/width ratio and leaf area, "Aml" strain showed greater figures than "Hania" one.

3.1.2. Histological characteristics:-

Results pertaining to stomata characteristics in the same table and figure (1) illustrated that, the average number of stomata per mm² for the lower epidermis of "Aml" mango strain was (677.5) compared to (384) with "Hania" strain. In the other hand, the average stomata length and width of "Hania" strain were 0.89 and 0.72 μ m whereas that of "Aml" strain were 0.68 and 0.55 μ m, respectively. These increased were found to be statistically highly significant with t_test.

3.2. Fruit properties:-

Regarding physical characteristics data in Table (2) showed that, significant differences in fruit weight, volume in all seasons. It can notice that "Aml" strain fruit weight and volume were higher than "Hania".

Concerning fruit dimensions, from results of same table it is clear that significant increments were obtained from "Aml strain for fruit length, width, thickness and length/width ratio (shape fruit) compared to "Hania" strain in all seasons. It can concluded that "Hania" fruit shaped is oval to rounded, full while "Aml" fruit shaped is oval, oblong, plump and thick.

In comparing peel and seed weight of "Aml" and "Hania" strains, it noticed that "Aml" fruit was significantly greater in fruit peel and seed weight than "Hania" one and the opposite trend was observed in pulp/fruit ratio (net ratio).

From results of the same table it clear that, significant differences were found between two strains in seed length, width and thickness. In generally, the values of seed length and width of "Aml" strain were greater than "Hania" one, while the opposite trend was obtained in seed thickness.

Fruit firmness is one of the most important parameters, the greater the firmness, the greater, the quality of the fruit. It was significantly differed in the two evaluated strains and ranged from 1.45 to1.27 kg/cm2 as average of three years for "Hania" and "Aml" strains, respectively.

As regard titratable acidity the highest values (0.68 and 0.74) were obtained with "Aml" compared to (0.47 and 0.66) "Hania" in both seasons, respectively.

Fruits of "Aml" strain contain high percentage of SSC (18 and 15.20 %) comparatively with (14.4 and 15.25 %) for "Hania" strain in both seasons, respectively. Thereat, it can suppose that the values of SSC/acid ratio took against trend of acidity for two studied strains in all seasons. With respect to vitamin C in all seasons, "Aml" fruits had ascorbic acid higher than "Hania" one. At last, as fruit coloration it could be concluded that "Hania" strain had attractive fruit yellow colour, while "Aml" strain had greenish yellow (fig. 2).

3.3. SSRs polymorphism and molecular fingerprinting:-

Of the 42 SSR primer pairs screened, 38 loci generated fragments for the two genotypes. Two loci were monomorphic, each locus showed one fixed band in both genotypes.36 loci (Table 4) were selected in our analysis for their reproducible and polymorphic DNA amplification patterns among genotypes (an example of the amplification pattern obtained is shown in Fig.3 ,with 12 primer pairs only). Analysis of the variability parameters for the 36 SSRs in the two mango strains are shown in (Table 4), detected a total of 92 scorable bands with an average of 2.55 Band/SSR, ranging from 2 to 4 bands/SSR. This is lower than those reported by Viruel et al. (2005) in their work with 16 primer pairs among 28 mango genotypes, probably due to the lower number of analyzed samples, as well as due to the less diverse genotypes analyzed. According to the banding patterns obtained with 36 selected primer pairs, one or two bands were present in each genotype; the amplification pattern seems to indicate the detection of a single locus. Mango has been described as allopolyploid (Mukherjee, 1997) and these results suggest a complete depolarization in this species. The strains studied were considered homozygous and heterozygous when one or two fragments were present per locus, respectively (Callen et al., 1993). Consequently, the heterozygosities of the two strains

under study were 0.72 for strain "Hania" and 0.52 for strain "Aml", with a mean value of 0.62, and the two strains showed heterozygosities higher than 0.50 (Table 4). The great heterozygosity for the two mango strains can be attributed to the mating system of this species that is normally out cross pollination with some self pollination. The higher level of heterozygosity observed in the present study has been also reported by Shiran et al. (2007). From a total of 92 scorable alleles, 66 were polymorphic bands and 26 monomorphic. These results indicate that any 66 out of the 92 (60.7 %) fragments are considered putative genotypesspecific markers in both strains. Strain "Hania" showed the presence of 36 genotype-specific fragments and strain "Aml" was present 30 genotype-specific fragments.

Observed heterozygosity, calculated from direct counts, for the loci identified by each primer pair ranged from 0.1 at most loci, indicating high diversity of the two mango strains to zero with a mean for all the loci of 0.62 (Table 4). The polymorphic information content (PIC values) ranged from 0.25 to 0.75, with a mean value of 0.51 for all loci. Based upon discriminating power (DP) (Table 4). The PIC value provides an estimate of the discriminatory power of a marker by taking into account not only the number of alleles at a locus but also the relative frequencies of these alleles. Expected and observed heterozygosity values were compared using the fixation index (F), the F values indicated the global behavior of these strains, similar to a random mating collection. This could be due to the fact that mango cultivars are the result of selection from open-pollinated seedlings, most of them from chance seedlings from natural cross-pollinations.

SSR profiles, combined over the thirty sex loci, were compared to determine if both strains were genetically identical and through the high average of observed and expected heterozygosity in this work, as well as low number of fixed alleles or monomorphic fragments (26 in 92) compared to the high number of genotype-specific alleles (66 in 92) indicate that a high variability is detected in the strains tested and could be distinguished from each other. Microsatellites are becoming the marker of choice for fingerprinting and genetic diversity studies in a wide range of living organisms (Shiran et al., 2007). Consequently, the approach described in this work shows that microsatellite analysis is a powerful tool also for the characterization and identification of mango strains by comparing the 92 alleles, which were detected using the 36 selected primers (Table 4).

Stuain]	Leaf	Stomata				
Strain	Area (cm ²)	Length (cm)	Width (cm)	Length/Width	Density (mm ²)	Length (µm)	Width (µm)	
Hania	104.39	23.95	6.53	3.67	384.00	0.89	0.72	
Aml	126.63	25.89	7.14	3.63	677.50	0.68	0.55	
Т	*	*	*	ns	*	*	*	

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Table (2): Some fruit characteristics of two (mono embryonic) seedling mango strains (Hania and Aml) from2003-2005.

				Fruit				Т.	Peel	Seed	NLA		
Strain	Weight (g)	Volume (cm ³)	Specific gravity	Length (cm)	Width (cm)	Shape	Thickness (cm)	(kg/cm ²)	weight (g)	weight (g)	Net ratio		
	First season												
Hania	620.00	624	0.99	13.90	9.60	1.45	8.75	1.43	64.70	45.20	82.27		
Aml	1012.5	988	1.05	19.05	9.80	1.95	8.95	1.19	145.70	139.55	71.84		
Т	*	*	ns	*	*	*	ns	*	*	*	*		
	Second season												
Hania	658.85	700	0.94	13.25	9.90	1.34	9.30	1.44	36.55	46.80	87.30		
Aml	1077.8	1110	0.97	21.25	10.50	2.02	9.65	1.50	82.95	89.30	84.01		
Т	*	*	ns	*	*	*	*	*	*	*	ns		
					Т	hird seas	on						
Hania	465.40	460	1.01	12.71	8.65	1.47	7.60	1.49	49.40	59.25	76.63		
Aml	969.75	1010	0.96	19.00	10.30	1.85	9.10	1.13	107.75	71.55	81.33		
Т	*	*	ns	*	*	*	*	*	*	ns	*		
					Av	erage sea	sons						
Hania	581.42	594.67	0.98	13.29	9.38	1.42	8.55	1.45	50.22	50.42	82.07		
Aml	1020.02	1036.00	0.99	19.77	10.20	1.94	9.32	1.27	112.13	100.13	79.06		
Т	*	*	ns	*	*	*	*	*	*	*	ns		

Table (3): Some fruit characteristics	of two (mono	embryonic)	seedling m	ango strains	(Hania	and Aı	ml) from
2003-2005.							

	Seed						Total	VC		
Strain	Length	Width	Thickness	SSC (%)	TA (%)	SSC/TA	sugars	vC (mg/100g)		
	(cm)	(cm)	(cm)				(%)			
	First season									
Hania	11.20	3.90	2.25	14.40	0.47	30.98	11.71	25.60		
Aml	16.50	4.50	2.20	18.00	0.68	26.68	12.98	41.60		
Т	*	*	ns	*	*	*	*	*		
	Second season									
Hania	10.18	4.25	2.60	15.25	0.66	23.29	15.34	28.40		
Aml	16.64	5.10	2.40	15.20	0.74	20.55	15.60	33.60		
Т	*	*	ns	ns	*	*	*	*		
	Third season									
Hania	10.70	4.10	2.65	-	-	-	-	-		
Aml	15.10	4.45	2.35	-	-	-	-	-		
Т	*	*	ns	-	-	-	-	-		
	Average seasons									
Hania	10.69	4.08	2.50	14.83	0.57	27.14	13.52	27.00		
Aml	16.08	4.68	2.32	16.60	0.71	23.62	14.29	37.60		
Т	*	*	ns	*	ns	*	*	*		

Locus name	Size range (bp)	n	Но	F	PIC
MIAC_3	185-193	2	0.5	-0.33	0.375
mMiCiR_5	171-182	2	0.5	-0.33	0.375
mMiCIR_16	240-242	2	0.5	-0.33	0.375
LMMA_7	200-212	2	1.0	1.0	0.05
LMMA_1	199-208	3	1.0	-0.6	0.625
LMMA_15	199-221	3	1.0	-0.6	0.625
LMMA_8	255-267	3	1.0	-0.6	0.625
LMMA_9	171-179	2	0.5	-0.33	0.375
LMMA_11	232-239	2	0.5	-0.33	0.375
LMMA_13	179-198	3	1.0	1.0	0.05
mMiCIR_8	150-166	3	1.0	-0.6	0.625
MiSHRS_4	118-124	3	1.0	-0.6	0.625
MiSHRS_1	190-205	2	0.0	1.0	0.05
MIAC_4	93-112	4	1.0	-0.33	0.75
MIAC_5	117-124	3	0.5	0.2	0.625
MiSHRS_32	205-231	3	0.5	0.2	0.625
mMiCiR_9	158-163	3	1.0	-0.6	0.625
mMiCiR_14	149-152	2	0.5	-0.33	0.375
mMiCiR_10	282-298	3	0.5	0.2	0.625
mMiCiR_22	141-170	4	0.5	1.0	0.25
MIAC_2	139-163	2	0.0	1.0	0.5
MiSHRS_29	175-179	2	0.5	-0.33	0.375
MiSHRS_39	350-369	3	0.5	0.2	0.625
LMMA_4	231-241	2	1.0	1.0	0.5
LMMA_14	161-169	2	1.0	1.0	0.5
LMMA_10	143-170	3	0.5	0.2	0.625
LMMA_5	280-282	2	0.5	-0.33	0.375
LMMA_12	201-205	2	0.5	-0.33	0.375
mMiCiR_3	314-320	2	0.0	1.0	0.5
mMiCiR_18	202-232	3	0.5	0.2	0.625
mMiCiR_25	212-214	2	0.5	-0.33	0.375
MiSHRS_48	206-218	2	0.0	1.0	0.5
MIAC_6	270-307	3	1.0	-0.6	0.625
mMiCIR_36	249-263	3	1.0	-0.6	0.625
mMiCIR_29	175-195	3	0.5	0.2	0.625
mMiCIR_30	186-196	2	0.5	-0.33	0.375
Mean All loci		2.55	0.62	1.64	0.51

 Table (4): Locus name, size range, number of alleles (n), observed (Ho) and polymorphic information content (PIC) of microsatellite data for two mango genotypes.


Fig (1): Stomata characteristics in leaf of two strains of mango (left) Hania and (right) Aml (bar 2 µm)



Fig (2): Fruit of Hania strain (left) and Aml strain (right).



Fig. (3): Heterozygosity level of the two mango strains $\{(18) | (Hania) = 0.68 \text{ and } (19) | (Aml) = 0.53\}$.

4. Discussion

The two mango strains "Aml" and "Hania" differed in leaf characteristics (length, width and area) and stomata characteristics (length, width and stomatal density (ND)). It concluded that, there are relationship between the number of stomata and it size, consequently decrease the number of stomata when stomata dimensions increased. Also, it can noticed that, clear relationship between increment of leaf and stomata dimensions and fruit quality (weight, size...etc). These results, refereed to evident Genetic variances which found between two strains under study. Where, each seedling tree (strain) can be considered as a referate type exhibiting wide variation in different characters (Singh, 1990).

It can conclude that, the highest pulp (Net ratio) percentage was up to 70% for two strains during three seasons. In general the pulp percentage more than 70% was desirable for fresh consumption and processing. (Naglaa, 2010).

SSC percentages reflect the richness of the studied strains in this regard and consequently excellent taste.

These results, referred to evident genetic variances which found between two strains under study. Where, each seedling tree (strain) can be considered as a separate type exhibiting wide variation in different characters (Singh 1990). Thirty six loci from forty two SSR primer pairs screened, generated fragments for the two strains. Any 66 out of the 92 (60.7 %) fragments are considered putative genotypes-specific markers in both strains. Hania strain sowed the presence of 36 genotype-specific fragments and Aml strain was present 30 genotypespecific fragments. The heterozygosities of the two strains under study were 0.72 for Hania strain and 0.52 for Aml strain with a mean value of 0.62, and the two strains showed heterozygosities than 0.50. The greatest heterozygosities for the two mango strains under study can be attributed to the mating system of this species that is normally out cross pollination with some self pollination (Shiran et al., 2007).

5. Conclusion

Two mango strains "Hania" and "Aml" differed in vegetative characters (leaf shape, length, width, etc)

and histological characteristics (number of stomata per mm (stomatal density), stomata length and width). It can be concluded that these two new strains had a good fruit properties. The fruit weight was 581 gm for Hania strain and 1020 gm for Aml strain. The two strains fruits had good characters as shape, net weight ratio, firmness, SSC, TA, Vit. C and total sugars. In generally, physical and chemical properties of Aml strain fruits were better than Hania strain. These differences of horticultural aspects due to genetic variances, which were determined by using SSR markers, of the 42 primers screened, 36 primers gave reproducible polymorphic DNA amplification patterns. 60.7 % of the scored fragments are considered putative genotypes-specific markers in both strains.

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Effect of some chemicals on growth, fruiting, yield and fruit quality of "Succary Abiad" mango cv.

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Abstract : The present investigation was carried out in two successive seasons of 2007 and 2008 on mango cv. "Succary Abiad", at Abou Swear region, Ismailia Governorate, Egypt in a sandy soil and irrigated with immerged irrigation system, to study the effect of some chemicals and growth regulators on growth, leaf mineral contents, fruiting, yield and fruit quality. The trees were subjected to eleven treatments using urea 2%, NAA 40 and 60 ppm, Ca Cl₂ 2%, GA₃ 20 and 40 ppm and water spraying as control. The results revealed that, spraying with urea, NAA and GA₃ at all concentrations significantly increased shoot length, number of leaves per shoot and leaf area higher than control while urea showed the superiority effect. Nitrogen and Potassium content in leaves significantly increased within urea, NAA and GA₃higher than control. Calcium content in the leaves showed fluctuated values during the two seasons within the different treatments although Ca Cl₂ 2% sprayed at two months after full bloom showed the highest values in the two seasons of study. All treatments had significantly higher yield than control. Fruit firmness and SSC were increased within all treatments with significantly increments than control. Vitamin C was significantly increased higher than control within all treatments except GA₃ 20 ppm added at one month after full bloom.

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Key words: Mango, Urea, NAA, CaCl₂, GA₃, yield, fruit quality

1. Introduction

Mango (*Mangifera indica* L) is considered the king of fruits in many countries (Purseglove, 1972). In Egypt, mango cultivated area reached 184204 fed. (Ministry of Agriculture, 2007). More than 40% of this areas exists in Ismailia, which the main cultivar planted is "Succary Abiad". Mango yield worldwide are generally poor, ranging from 4 to 9 t/ha in the major production countries (Oosthuyse, 1993). This is attributable to wide tree spacing malformation, alternate bearing, environmental factors and fruit drop (Jana and Sharangi, 1998).

In spite of adequate flowering, low fruit yield in mango orchards have been experienced because of low initial fruit set and subsequently higher fruitlet abscission (Singh and Singh, 1995). Fruitlet abscission is a very complex physiological process, occurs in many cultivars of mango and at all stages of development, but it is particularly high during the first 3-4 weeks after pollination and accounts for over 90 % loss of set fruitlets (Bains, *et al.*, 1997 and Wahdan and Melouk, 2004).

Several factors affect fruitlet abscission and some of the reasons suggested are the lack of pollination and failure of fertilization, ovule abortion, and embryo degeneration, hormone content, climatic factors (day length, temperature and wind), inadequate soil moisture and low photosynthate level (Whiley, 1986 and Bains, *et al.*, 1997).

The use of growth substances and some chemical compounds may regulate fruit set in many fruit crops. Many investigators found that spraying mango trees with NAA at different concentrations (20, 25 and 40 ppm) increased fruit set percentages and fruit retention (Oksher et al., 1980 and Singh and Ram, 1983). Auxin is well known as inhibitors of ethylene action in a number of plants (Beyer, 1976). Moti-Singh et al., (1987) with Langra and Dashehari cvs stated that NAA or GA3 each 5-25 ppm once sprayed at full bloom or twice at full bloom and at pea stage or thrice plus at marble stage increased fruit retention. Singh et al. (1991) found that the highest fruit retention and yield/tree were recorded on mango cv. (Amrapali) by spraying urea with 3 % at pea stage.

The main objective of this study was to investigate the effect of some chemicals and growth regulators on growth, leaf mineral contents, fruit set, yield and fruit quality.

2. Materials and Methods

The present study was conducted throughout two successive seasons of 2007 and 2008 on mango cv. "Succary Abiad". The trees were grown in private orchard at Abou Swear region, Ismailia Governorate, in a sandy soil and irrigated with immerged irrigation system. Trees were 30-year-old, planted at 7×7 m space, grown under the same common agricultural practices. Thirty-three healthy trees were selected nearly similar in vigour and size.

The work in this experiment aimed to study the effect of some chemicals and growth regulators on growth, leaf mineral contents, fruiting, yield and fruit quality of the previously above-mentioned mango cultivar. The experimental treatments were as follow:

- 1- Control treatment was sprayed with tap water.
- 2- Urea 1% sprayed once at full bloom.
- 3- Urea 1% sprayed once at one month after full bloom.
- 4- NAA 40 ppm once at one month after full bloom.
- 5- NAA 60 ppm once at one month after full bloom.
- 6- Ca Cl_2 2% once at one month after full bloom.
- 7- Ca Cl_2 2% once at two months after full bloom.
- 8- GA3 $\overline{20}$ ppm once at one month after full bloom.
- 9- GA3 40 ppm once at one month after full bloom.
- 10- GA3 20 ppm once at two months after full bloom.
- 11- GA3 40 ppm once at two months after full bloom.

Thus, eleven treatments were investigated, where all treatments were arranged in a complete block randomized design and each treatment was replicated three times with one tree per replicate. So, thirty three trees were used (11 treatments \times 3 replicates).

Studying parameters:

1. Vegetative growth:

At the beginning of the first growth cycle, ten shoots per tree were tagged.

Shoot length (cm), number of leaves per shoot and leaf area (cm²) by using Electronic Digital Planimeter (HAFF com. Germany) were measured at November.

2. Leaf mineral contents:

Sample of twenty leaves per tree were picked from the 3rd and 4th node below panicle after two months of full bloom. The samples were washed, dried, ground and digested using sulphoric acid and hydrogen peroxide according to Chapman and Pratt (1961). N, P, K and Ca were determined in the digested solution as follows:

- a) Total nitrogen was determined using the micro-Kjeldahl method as described by Pregl (1945).
- b) Phosphorus was estimated colorimetrically by the stannous chloride method according to Truog and Meyer (1929).
- c) Potassium content was determined by Flame photometer according to method of Jackson (1958).

d) Calcium was determined by titration against versenate solution (Na-EDTA) according to Chapman and Pratt (1961).

3. Fruit set and fruit retention:

The number of fruits per panicle was counted after 15 days of full bloom to determine the initial number of fruits per panicle. The initial fruit set was calculated as a percentage. After recording the initial fruit set, the number of fruits per panicle was recorded at mature stage. The percentage of retained fruits at harvest time was calculated.

4. The yield:

In each season, at harvest time, the numbers of fruits per panicle and per tree were counted for each treatment. Tree yield in kilograms was estimated by multiplying the number of fruits per tree and the average fruit weight.

5. Fruit quality:

At harvest time, samples of 5 firm ripe (commercial stage) fruits were taken from each replicate to study the average of fruit, skin and stone weight (g), fruit length (cm), width (cm), fruit shape index (length/width), fruit thickness (cm), pulp/fruit ratio (net ratio), fruit volume (cm³), fruit firmness (kg/cm²) by using effegi pentrometer, soluble solids content (SSC %) by hand refractometer, fruit acidity, SSC/Acid ratio, vitamin C, total carotene, total, reducing and non-reducing sugars, and total phenols were determined as described by A.O. A. C. (1995).

6. Statistical analysis:

Data were subjected to the analysis of variance and a complete block design was used (Steel and Torrie, 1980). Analysis of variance and mean comparison (LSD, at 5%) were done by MSTAT-C program version 7 (1990).

3. Results

3.1. Effect of spraying Urea, NAA, CaCl₂ and GA₃ on vegetative growth:

All the treatments at all concentrations significantly increased the shoot length compared with control, (Table, 1). The highest values (22.1 and 16.0 cm) were obtained from 20 ppm of GA_3 at one month after full bloom and 2% of urea at one month after full bloom, while the control gave the lowest values (5.3 and 2.3 cm) in the first and second seasons, respectively.

In the same table data showed that, all concentrations of Urea, NAA, $CaCl_2$ and GA_3 at any date of applications had significant effects on the number of leaves per shoot. The highest values reached 16.6 and 10.2 leaves per shoot with 20 ppm of GA_3 applied at one month after full bloom and 2 % of $CaCl_2$ applied at one month after full bloom compared with control (7.1 and 4.5 leaves per shoot) in the first and second seasons, respectively.

Results in Table (1), reveal that leaf area significantly increased by using Urea, NAA, $CaCl_2$ and GA_3 at all concentrations and at any date of applications. GA_3 at 20 ppm when sprayed on mango trees at one month after full bloom gave the highest values (85 and 81.1 cm²) compared with control (69.9 and 67.1 cm²) in the first and second seasons, respectively.

content in all treatments was higher than it in control. The higher value of nitrogen in leaves (1.19 %) was prove with 2 % of CaCl₂ at month after full bloom followed by (1.15 and 1.16 %) 2 % of CaCl₂ applied at two months after full bloom and 2 % of Urea sprayed at one month after full bloom, respectively, compared with 1.06 % of control (Table, 1).

Data in the same table show no clear trend for the effect of all treatments on leaf phosphorus content in both seasons.

As for potassium content, all tested treatments at all concentrations recorded significantly higher percentage of leaf potassium contents than those of control in both seasons. Data in Table (1) illustrated that the highest values of leaf potassium content (0.67 and 0.70 %) were obtained from 20 ppm of GA₃ applied at two months after full bloom and 2 % of CaCl₂ sprayed at one month after full bloom compared with control which gave the lowest values of leaf potassium content (0.52 %) in both seasons, respectively.

As for leaf calcium content data presented in the same table show clear trend in this respect, in both seasons the application of 2 % $CaCl_2$ at two months after full bloom gave the highest values of leaf calcium content (1.44 and 1.31 %) in the first and second seasons, respectively.

3.3. Effect of spraying Urea, NAA, CaCl₂ and GA₃ on fruit retention and yield:

Concerning, fruit retention percentage data in Table (2) illustrated that all treatments in both

obtained from trees treated with Urea 2 % at one month after full bloom in both seasons, respectively.

3.4. Effect of spraying Urea, NAA, CaCl₂ and GA₃ on fruit characteristics:

Data in Table (3) indicated the variances fruit characteristics throughout the two seasons of study. Fruit weight was the highest within all treatments with significantly increments than control except the application of GA_3 40 ppm at two months after full bloom in the first season. But in the second one the highest value (413.8 g) of fruit weight was obtained within the Urea 2 % at one month after full bloom

3.2. Effect of spraying Urea, NAA, CaCl₂ and GA₃ on leaf macro elements content:

In the first season, all treatments increased leaf nitrogen content compared with control. The highest value (1.28 %) was obtained with 2 % of Urea sprayed at full bloom followed by (1.23 %) which obtained with 2 % of Urea applied at one month after full bloom and 2 % of CaCl₂ at both dates compared with control treatment which gave 1.12 % nitrogen leaf content. In the second season, nitrogen leaf seasons significantly increased fruit retention percentage. The highest values (25.68 and 25.42 %) were obtained with Urea 2% at one month after full bloom compared to lowest values (12.91 and 11.57 %) with control in both seasons, respectively.

Data in Table (2) indicate that in both seasons, number of fruits per tree, were the highest on trees treated with 2 % of Urea at one month after full bloom (213.7 and 194.3 fruits per tree). While, the lowest values were on untreated trees (130 and 118 fruits per tree) in the first and second seasons, respectively. Generally, all treatments significantly had higher in this parameter than control except the treatment of GA₃ 40 ppm applied at two months after full bloom which showed no significant differences compared with control in both seasons. In the second season, the fruit number per tree showed that clear trend was detected but all values were lower than it in the first one. This was due to mango trees being characterized by alternative bearing. So the higher fruit number in the first season means that most trees were under on year bearing while in second one, the trees were under off year bearing.

The yield per tree (kg/tree) in the first season in all treatments was higher than it in the second one. This increase could be due to the number of fruits per tree as shown in the same table. The lowest yield per tree (42.7 and 38.8 kg/tree) was obtained from control trees in both seasons which could be attributed to the lowest fruit number per tree. While, the highest yield per tree (73 and 80 kg/tree) was

treatment, while the lowest values (321.5 and 296.9 g) were obtained within Ca Cl_2 treatments compared with control (328.6 g). In the same table, date tabulated that, fruit pulp weight in the first season, showed no significant response to treatments except treatment of Urea 2 % at full bloom and NAA 40 ppm at one month after full bloom. On the other hand in the second one all treatments increased pulp weight than control except calcium treatments which gave lowest values of pulp weight (255.7 and 231.2 g) compared with control (265.5 g).

With respect to fruit net ratio, data presented in Table (3), also show that no clear trend was detected.

Whereas, all treatments significantly decreased fruit net ratio in the first season. In the second one, the treatments of Urea at two dates, NAA 40 ppm at one month after full bloom and GA₃ 20 ppm at one month after full bloom only gave values higher than control.

In the same table it is clear that, all treatments at all concentrations significantly increased fruit length in the first season except the treatment of GA₃ 40 ppm at two dates, but in the second one it is no clear trend except the treatments of Urea 2 % at one month after full bloom and GA₃ 20 ppm at one month after full bloom which gave highest values (11.8 and 11.4 cm) compared to control and GA₃ 40 ppm at two months after full bloom which gave lowest values (10.7 and 10.0 cm), respectively. Data also, revealed that, spraying with Urea 2 % at one month after full bloom, NAA 60 ppm at one month after full bloom, CaCl₂ 2% at two months after full bloom and GA₃ 20 ppm at one month after full bloom significantly increased fruit width compared with another treatments in the first season, while in the second one, the highest value (8.2 cm) was obtained with Urea 2% at one month after full bloom and GA₃ 20 ppm at one month after full bloom compared to lowest values (7.5 and 7.6 cm) which obtained with CaCl₂ 2% at two months after full bloom and GA₃ 40 ppm at two months after full bloom, respectively.

Regarding to fruit shape (length/width ratio) it is clear from the data presented in Table (3) also, that no significant differences between the control and other treatments except the treatments of NAA 40 ppm at one month after full bloom and CaCl₂ 2 % at two months after full bloom which gave highest values (1.38 and 1.40) compared with control (1.33) in the first season. While, in the second one also, no significant differences obtained between control and other treatments in fruit length and width ratio except the treatment of Urea 2 % at one month after full bloom which gave highest value (1.43) and treatments of GA₃ 40 ppm at two dates which gave lowest values (1.32 and 1.32) in comparison with control (1.38).

Data in Table (3) also, revealed that skin firmness was the highest within all treatments with significantly increments than control in both seasons. In the first season, the highest values (1.59, 1.50 and 1.43) of firmness were obtained with GA₃ 20 ppm at one month after full bloom followed by CaCl₂ 2 % at two months after full bloom respectively. While, in the second one, the treatments of GA₃ 20 ppm at one month after full bloom, GA₃ 40 ppm at two months after full bloom, GA₃ 40 ppm at two months after full bloom and CaCl₂ 2 % at two months after full bloom and CaCl₂ 2 % at two months after full bloom and CaCl₂ 2 % at two months after full bloom gave the highest values (1.37, 1.33 and 1.29), compared with control treatment which gave the lowest values (1.02 and 1.00) in both seasons respectively.

3.5. Effect of spraying Urea, NAA, CaCl₂ and GA₃ on chemical properties:-

Data in Table (4) show the effect of Urea, NAA, $CaCl_2$ and GA_3 spraying on the fruit chemical properties. Results tabulated in Table (4) indicated that SSC in the first season responded to all treatments, so significant differences were observed among almost treatments and control. In the second season, all treatments appeared more SSC than control (14.3) except treatment of GA₃ 20 ppm at one month after full bloom (13.7) in comparison with highest value (17.1) which obtained with Urea 2 % at full bloom.

Fruit acidity in the two seasons had no clear trend. Anyhow, in the first season, fruit acidity increased as a result of Urea and GA₃ at one month after full bloom spraying compared with control and other treatments. While, in the second one, the acidity value for control fruits was significantly increased in comparison with all treatments. SSC/acid ratio in the first season was the highest in the fruits harvested from trees treated with CaCl₂ 2 % at two months after full bloom (62.7) while the lowest values appeared in the fruits of Urea 2 % at full bloom (23.4). The other treatments gained intermediate values. However, in the second one, all treatments gave higher values than control (22.0). As concerns of vitamin C, data presented in the same table reveal that in both seasons the highest values were obtained in the fruits harvested from trees sprayed with GA₃ 40 ppm at two months after full bloom (33.1 and 32.6 mg/100g), respectively. While, almost treatments trended to significantly decreased in values of vitamin C in both seasons in comparison with control.

Regarding total sugars, data in Table (4) reveal that the values of total sugar tended to increase as a result of spraying with all treatments more than control except treatments of GA_3 at one month after full bloom in both seasons and treatment of GA_3 20 ppm at two months after full bloom in the second season only.

Total phenols in the fruits throughout the two seasons had no clear trend. Anyhow, in the first season total phenols were higher in the fruits harvested from trees treated with NAA 60 ppm at one month after full bloom (0.114 %) while the lowest values appeared in the fruits of Urea 2 % at one month after full bloom (0.076 %). The other treatments gave intermediate values. However, in the second one, data showed significant response of total phenols to any treatment used in this study compared with control. In the same table, data reveal that in both seasons fruits carotenes content significantly increased in all treatments except treatments of GA_3 at two months

after full bloom which gave lowest values of carotenes (4.56 and 3.20 mg/100g) compared with control (5.42 mg/100g) in second seasons only.

Table	(1): Effect	of Spraying	Urea, NAA	, Ca Cl	2 and	GA ₃	on vegetative	growth	parameters	and leat	f macro
	eleme	nts content o	f mango cv.	'Succar	y Abia	ıd" in	2007 and 200	8 season	s.		

Treatment	Shoot (c	length m)	Num leaves	ber of /Shoot	Leaf (cr	area n) ²	Ν	%	Р	%	K	%	Ca	%
	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
Control	5.3 f	2.3 h	7.1 f	4.5 e	69.9 f	67.1 f	1.12 e	1.06 d	0.36 ab	0.36 b	0.52 d	0.52 d	1.23 cde	1.12 d
Urea 2% at F. B.	8.2 e	6.1 ef	8.3 def	6.7 cd	75.0 d	74.7 d	1.28 a	1.14 b	0.34 ab	0.67 a	0.52 d	0.58 c	1.28 c	1.24 b
Urea 2% at M. A. F. B.	15.6 b	16.0 a	11.5 b	6.9 cd	79.9 с	74.1 d	1.23b	1.15 ab	0.35 ab	0.46 b	0.59 c	0.69 a	1.20 e	1.20 bc
NAA 40 ppm at M. A. F. B.	8.3 e	6.7 e	9.4 cd	6.6 cd	79.3 c	71.5 e	1.21 c	1.14 b	0.38 a	0.48 b	0.53 d	0.63 c	1.20 e	1.21 b
NAA 60 ppm at M. A. F. B.	6.8 ef	5.7 f	7.6 ef	7.5 bcd	71.5 ef	77.6 bc	1.20 c	1.05 d	0.28 c	0.37 b	0.62 c	0.58 c	1.22 cde	1.13 d
Ca Cl ₂ 2% at M. A. F. B.	13.1 c	9.3 c	7.7 ef	10.2 a	73.8 de	82.2 a	1.23 b	1.19 a	0.35 ab	0.35 b	0.60 c	0.70 a	1.22 cde	1.02 e
Ca Cl ₂ 2% at 2 M. A. F. B.	7.3 e	11.5 b	8.4 de	6.3 d	73.3 de	77.3 c	1.23 b	1.16 ab	0.39 a	0.38 b	0.70 b	0.63 bc	1.44 a	1.31 a
GA ₃ 20 ppm at M. A. F. B.	22.1 a	4.7 g	16.6 a	8.3 b	79.9 с	82.2 a	1.15 d	1.09 cd	0.39 a	0.49 b	0.60 c	0.59 c	1.37 b	1.15 cd
GA ₃ 40 ppm at M. A. F. B.	7.6 e	6.7 e	10.4 bc	8.4 b	82.5 b	81.0 a	1.23 b	1.04 d	0.32 bc	0.38 b	0.61 c	0.61 c	1.27 cd	1.11 d
GA ₃ 20 ppm at 2 M. A. F. B.	6.8 ef	5.6 f	8.9 de	7.6 bc	85.0 a	81.1 a	1.12 e	1.12 bc	0.35 ab	0.35 b	0.76 a	0.69 ab	1.21 de	1.13 d
GA3 40 ppm at 2 M. A. F. B.	9.8 d	8.1 d	8.5 de	7.1 cd	82.8 ab	78.9 b	1.23 b	1.07 d	0.39 a	0.43 b	0.60 c	0.69 ab	1.22 cde	1.21 b

Values with the same small letter in each column are not significantly different at 5 % levelF. B. = Full BloomM. A. F. B. = One month after Full Bloom

2 M. A. F. B. = Two Months after Full Bloom

Table (2): Effect of Spraying Urea, NAA, Ca Cl2 and GA3 on tree yield of mango cv. "Succary Abiad" in 2007 and 2008 seasons.

	Emuit noto	(0/)	Yield/Tree					
Treatment	r fuit feter	Fruit retention (70)			Kg			
	2007	2008	2007	2008	2007	2008		
Control	12.91 d	11.57 e	130.0 g	118.0 g	42.7 f	38.8 g		
Urea 2% at F. B.	19.39 с	19.47 d	181.7 bc	165.0 bc	63.9 d	58.4 b		
Urea 2% at M. A. F. B.	25.68 a	25.42 a	213.7 a	194.3 a	73.0 a	80.4 a		
NAA 40 ppm at M. A. F. B.	19.91 с	19.03 d	143.0 ef	130.0 ef	50.2 e	53.7 с		
NAA 60 ppm at M. A. F. B.	21.09 bc	23.19 abc	177.7 cd	161.7 cd	67.3 c	55.2 c		
Ca Cl ₂ 2% at M. A. F. B.	21.29 bc	21.17 bcd	182.0 bc	165.3 bc	68.1 bc	53.1 c		
Ca Cl ₂ 2% at 2 M. A. F. B.	22.01 bc	21.58 bcd	186.3 b	169.3 b	70.7 ab	50.3 d		
GA ₃ 20 ppm at M. A. F. B.	24.15 ab	23.72 ab	171.0 d	155.3 d	64.3 d	58.2 b		
GA ₃ 40 ppm at M. A. F. B.	22.83 abc	22.40 abcd	149.0 e	135.7 e	52.5 e	46.0 e		
GA ₃ 20 ppm at 2 M. A. F. B.	21.09 bc	20.01 cd	138.3 f	125.7 f	51.4 e	44.1 ef		
GA3 40 ppm at 2 M. A. F. B.	19.86 c	20.52 bcd	136.7 fg	124.3 fg	44.8 f	41.8 f		

Values with the same small letter in each column are not significantly different at 5 % level F. B. = Full Bloom M. A. F. B. = One month after Full Bloom

2 M. A. F. B. = Two Months after Full Bloom

	Fruit	weight	Pulny	veight	Pulp v	veight/		Fruit di	mensions	6	Fruit	shape	Fr	uit
Treatment	(g)	1 up (g)	Fruit o	weight	Lengt	h (cm)	Widt	ı (cm)	(Ler Width	ngth/	firm (kg/	ness cm ²)
	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
	2007	2000	2007	2000	2007	2000	2007	2000	2007	2000	2007	2000	2007	2000
Control	328.7	328.6	285.7	265.5	86.9	80.8	10.7	10.7	8.0	7.7	1.33	1.38	1.02	1.00
	с	e	С	f	a	d	ef	cde	bc	bcd	С	bc	g	f
Urea 2% at F B	352.4	353.8	298.5	293.9	84.7	83.1	11.0	11.0	8.0	8.0	1.37	1.38	1.30	1.24
01ca 2 /0 at F. D.	b	с	а	d	b	b	cde	bc	bc	ab	abc	bc	de	d
Urea 2% at M. A. F.	341.5	413.8	272.4	339.1	79.8	82.0	11.2	11.8	07-	0.2 -	1.35	1.43	1.33	1.17
В.	b	а	d	b	d	с	bc	а	8.5 a	ð. 2 a	bc	а	de	е
NAA 40 ppm at M.	350.7	413.4	272.9	347.4	77.8	84.0	11.0	10.8	80.0	7.9	1.38	1.36	1.34	1.17
A. F. B.	b	а	d	а	e	ab	cd	cd	0.0 C	abcd	ab	bc	de	e
NAA 60 ppm at M.	378.3	341.4	296.1	270.5	78.3	79.3	11.1	10.9	0.2	7.9	1.34	1.38	1.23	1.13
A. F. B.	а	d	ab	ef	е	ef	bcd	cd	8.3 a	abcd	bc	bc	f	е
Ca Cl ₂ 2% at M. A.	374.8	321.5	284.6	255.7	75.9	79.6	11.0	10.6	8.1	7.7	1.37	1.37	1.43	1.24
F. B.	а	f	с	g	f	ef	cd	cde	bc	bcd	abc	bc	с	d
Ca Cl ₂ 2% at 2 M.	379.7	296.9	288.2	231.2	75.9	77.9	11.6	10.2	020	754	1.40	1.35	1.50	1.29
A. F. B.	а	g	bc	h	f	g	а	ef	0.5 a	7.5u	а	cd	b	bc
GA ₃ 20 ppm at M.	376.1	374.9	286.7	318.8	76.2	85.0	11.4	11.4	020	010	1.37	1.39	1.59	1.37
A. F. B.	а	b	с	с	f	а	ab	ab	0.5 a	0.2 a	abc	b	а	а
GA ₃ 40 ppm at M.	351.6	339.4	285.8	270.4	81.3	79.7	10.8	10.5	80.0	7.9	1.35	1.32	1.34	1.25
A. F. B.	b	d	с	ef	с	e	def	def	0.0 C	abc	bc	d	de	cd
GA ₃ 20 ppm at 2 M.	371.7	350.7	286.1	275.8	77.0	78.6	11.0	10.6	8.2	7.7	1.34	1.37	1.29	1.26
A. F. B.	а	с	с	e	ef	fg	cd	cde	ab	bcd	bc	bc	ef	cd
GA ₃ 40 ppm at 2 M.	327.6	336.5	254.4	273.5	77.6	81.3	10.6	10.0	701	7 (-)	1.35	1.32	1.37	1.33
A. F. B.	с	d	е	e	е	cd	f	f	7.ð d	7.0Cd	bc	d	cd	ab

 Table (3): Effect of Spraying Urea, NAA, Ca Cl₂ and GA₃ on physical characteristics of mango cv. "Succary Abiad" in 2007 and 2008 seasons.

Values with the same small letter in each column are not significantly different at 5 % level

F. B. = Full Bloom

2 M. A. F. B. = Two Months after Full Bloom

Table (4): Effect of Spraying Urea, NAA, Ca Cl₂ and GA₃ on chemical properties of mango cv. "Succary Abiad" in 2007 and 2008 seasons.

Treatment	SSC	C %	ТА	%	SSC ra	/ TA tio	Ascort (mg/	oic acid 100g)	Total	Sugars	Total I	Phenols	Caro (mg/	tenes 100g)
	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
Control	14.7	14.3	0.45	0.65	32.8	22.0	29.8	28.3	11.2	13.4	0.085	0.143	0.90	5.42
Control	f	f	с	а	e	h	b	b	de	e	с	а	i	h
Unon 20/ of E. D.	15.7	17.1	0.67	0.45	23.4	37.6	29.8	23.4	13.8	14.8	0.116	0.138	1.21	12.71
Urea 2 % at r. b.	cd	а	а	cd	f	bc	b	с	b	abc	а	b	g	b
Urea 2% at M. A.	15.7	16.4	0.66	0.56	23.9	29.1	22.9	20.3	14.7	15.2	0.076	0.084	5.09	16.45
F. B.	cd	b	а	b	f	fg	g	de	а	а	e	de	а	а
NAA 40 ppm at	15.8	16.7	0.47	0.53	33.5	31.4	26.2	22.1	13.0	15.0	0.078	0.075	5.13	7.14
M. A. F. B.	cd	ab	с	b	e	ef	d	cd	с	ab	de	f	а	f
NAA 60 ppm at	14.9	15.3	0.36	0.39	41.7	39.2	28.3	18.7	13.1	13.9	0.114	0.103	2.64	6.10
M. A. F. B.	ef	d	d	ef	d	ab	с	e	bc	de	a	с	f	g
Ca Cl ₂ 2% at M.	15.3	15.9	0.34	0.46	45.6	34.7	22.7	23.5	13.3	13.8	0.077	0.082	2.78	6.28
A. F. B.	de	с	de	с	с	cde	g	с	bc	de	e	e	e	g
Ca Cl ₂ 2% at 2	16.5	15.1	0.26	0.41	62.7	37.1	24.7	27.7	11.8	14.3	0.084	0.085	3.81	8.44
M. A. F. B.	b	de	f	de	a	bcd	e	b	d	bcd	cd	de	b	d
GA ₃ 20 ppm at	16.2	13.7	0.69	0.37	23.6	37.5	24.2	27.4	10.9	11.8	0.085	0.060	3.63	9.59
M. A. F. B.	bc	g	а	ef	f	bc	ef	b	e	f	с	g	с	с
GA ₃ 40 ppm at	17.1	15.1	0.55	0.56	31.0	26.9	29.9	24.0	11.3	12.0	0.103	0.103	3.34	7.68
M. A. F. B.	а	de	b	b	e	g	b	с	de	f	b	с	d	e
GA ₃ 20 ppm at 2	16.1	14.8	0.32	0.35	50.4	41.9	23.3	22.6	12.8	12.0	0.100	0.101	1.21	4.56
M. A. F. B.	bc	e	de	f	b	а	fg	с	с	f	b	с	g	i
GA ₃ 40 ppm at 2	15.1	15.3	0.29	0.45	51.4	34.0	33.1	32.6	14.7	14.1	0.088	0.087	1.07	3.20
M. A. F. B.	ef	d	ef	cd	b	de	а	а	а	cde	с	d	h	j

Values with the same small letter in each column are not significantly different at 5 % level

F. B. = Full Bloom

M. A. F. B. = One month after Full Bloom

2 M. A. F. B. = Two Months after Full Bloom

M. A. F. B. = One month after Full Bloom

4. Discussions

Concerning vegetative growth it could be attributed to the nitrogen effect on plants and easily incorporated into the plant metabolism. The obtained results are in agreement with the findings of Sergent *et al.* (2000). They found that spraying Urea on mango trees gave the largest significant effects on vegetative growth: shoot length, number of leaves per shoot and leaf area.

The present results regarding the influence of Urea, CaCl₂, NAA and GA₃ application on leaf macro element contents are in accordance with those found by El-Shewy (1999), Ghosh & Ghattopadhyay (1999), McKenzie (1995), Hermoso *et al.* (1997).

Regarding fruit retention and yield the obtained results are in harmony with these of Shinde *et al.* (2006), who reported that the foliar spray of Urea and NAA on mango trees produced significantly higher fruit yield per tree. Moreover, the results are in accordance with Rani and Brahmachari (2004) on application of $CaCl_2$ and GA_3 .

Concerning fruit physical characteristics the present results are accordance with those of Gupta and Brahmachari (2004) and Shinde *et al.* (2008) who found that the foliar application of Urea and NAA were effective in improving the fruit characters on mango. Regarding to the effect of GA₃ on fruit quality of mango, Sarkar and Ghosh (2005) mentioned that the spray application with GA₃ increased fruit weight, volume and length of fruit. The role of GA₃ was to multiply and to lengthen the meristem cells, which resulted in the increase of fruit volume and weight.

With regard to effect of Urea, CaCl₂, NAA and GA₃ application on fruit chemical properties the obtained results are in agreement with those of Jain (2006) who found that the application of Urea had shown significant increase in the soluble solids contents (SSC). Due to the application of Urea the functioning of number of enzymes might than been stimulated, affecting the physiological processes, which in turn hydrolyzed starch and helped in metabolic activity during the change available starch into sugar and SSC. Also, the obtained results are in harmony with those of Sharkawy (2006) who mentioned that CaCl₂ spraying increased SSC and fruit sugars. Moreover, the results of the tested treatments confirm those of Gupta and Brahmachari (2004), and Sarkar and Ghosh (2005) all of them mentioned that NAA spraving on mango trees increased SSC, SSC/acid ratio, sugars and decreased both acidity and vitamin C. finally the obtained results agree with Sarker and Ghosh (2005) who found that the spray application with GA₃ on mango trees increased SSC and total sugars.

5. Conclusion

Foliar spray mango trees with Urea, NAA, and GA₃ at all concentrations significantly increased shoot length, number of leaves per shoot and leaf area higher than control while urea showed the superiority effect. All treatments significantly increased the yield higher than control in the two seasons. Fruit quality in general improved by treatments. The fruit weight and volume were the highest within all treatments compared with control. Fruit firmness and SSC were increased within all treatments with significantly increments than control. Vitamin C was significantly increased in fruits harvested from trees sprayed with GA₃ 40 ppm at two months after full bloom. Total sugars in the fruits significantly increased higher than control within all treatments except GA₃ 20 ppm added at one month after full bloom.

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Economic effects of rural women's financial self-reliance

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Abstract: Since, village is suitable place for farming and additional activities, so it can be said that women's role at villages, has been toward this point and by developing agriculture sector and possibility to institutionalized appropriate infrastructure, we would have suitable attitude toward development process. Agriculture sector has critical responsibility, as one of the productive part of country for supplying needed food security, that it can assist this sector to access this main goal up to proper level, in accordance with workforce efficiency. To achieve this goal, women play main role, too. In spite of that, they couldn't represent their abilities in this field, because of limitations that they face.

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Keywords: rural women, financial self-reliance

Introduction:

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).

Most women, especially in developing countries are working three shifts in a day indeed, but, instead for their exhausting activities, they receive: less health care, less literacy and fewer wages. Compensation for them is vast sex discrimination that exists all over the worlds in various forms. For example in India, Pakistan and Bangladesh, about 1 million girls die, due to lack of proper health care.

World Health Organization estimated that women work 2times more than men averagely (Bahar, 2001). In United Nation researches, except Australia, Canada and US, women in all countries work more hours than men. But major problem here is that, work means everything that leading to financial income. So, in government statistics, women are considered as unemployed and few of female employees are counted as productive and employed forces.

In India, in one survey, and according to this perspective (Financial income) this result emerged that only 34% of women (compare to 63% of men) is counted as workforces of society. While if we also consider doing services and home productions and preparing family needs , as productive activities (without leading to Financial income), we would find different results and value of this deprived group of society , will be clear to us. By considering work and home productions in India, these results emerged: 75% of women compared with 64% of men are working (compared to 34% versus 64% of pervious statistics). (Balali, 2005)

Also in another survey in Nepal villages and according to financial income criterion, just 20% of women are working, while by considering home production criterion, women's share of workforce, reached to 53% (Bahar, 2001). By the way researches show that women have basic role in economics of family.

About rural women in agriculture productions, some researches is done by some scholars such (Shauver, Saches, Adams and Alston)

that all believe that women in activities such as cultivating (seeding) conservation (weeding, spading and sifting) and harvest (cut sugarcane branches) and they also participate in activities like doing pastoralist and caring animals, milking, nurturing poultry, gardening, fixing yard, snow removal, repairing building, handicrafts and etc

(Navab akbar 1997).

So, rural women are great part of workforce, needed for agriculture and rural societies. In 1966, according to F.A.O reports to food security congress, women doing 50% of productions in agriculture part averagely and this issue in developing country has very special importance. in African desert area about 70-80%, Asia 65%, in Latin America 45% and Caribbean and in Tunes 89% of workforces are women (Varzgar and azizi, 2001) and in Iran more than 50% of agriculture workforce are women (Banihashem, 1999).

Most rural women's service work, pertain to out of house. For example: rural women not only traditionally strive for environmental protection, they also take part in maintaining forest, plant cultivation and weeding in rural regions.

So rural women, doing major part of affairs in services and doing services inside and outside the house, isn't with any risk for them. They aren't secure while cleaning stall, milking, nurturing livestock and other activities. And they are at risk of common diseases between human and livestock. In fact they haven't security against any risk of work conditions (Emadi, 2001).

Summaries of woman service activities include housekeeping duties and also service activities out. So it's necessary to revise definition and classification service activities by women. Thus, according to rural women's basic role in productive activities and even rural developing, importance of rural women's role isn't considered properly. Maybe the reason of this inattention is that rural women's productions are used inside the family. This inattention caused that no changes happen for decreasing exhausting rural women's activities, in spite of development of technology and using various new tools that leads to remarkable decrease in using human resources. In so many developing countries yet, women use traditional cultivating tools that have little efficiency and demand more activities. Further, their agriculture activities accompanied by housekeeping duties that force them excessive efforts. One of the reasons is that rural women's role, remained unknown in economical productions. Because objective evidences at all over the world, especially in developing countries (particularly in our country) show that lost work or intangible activities is done by women that finally isn't considered as their efforts. Other reasons are: role of rural women and different productive activities remained unknown; and even lack of varying their position at different activities of village (Saadi and Arab Mazar, 2005).

Rural women are active at various fields of life as same as men and they accomplish their duties well. Thus undoubtedly, if we finance her as creative and active workforce and if credits sources can prepare her necessary credits, so she would be more active at different social, cultural, economic and even political areas and also would be more affective to flourish her family, village and then society. Until men and women can earn more as independently or collective, obviously they would have more lively and more healthy family; because more income leads to more purchasing and saving power and again more saving and investment cause economic, cultural and social prosperity (Fakhraee, 2002)

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 microcredit thought in village. (Najafi, 2007).

From 1970, the waves of thought about microcredits 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.

Women's access to farm land is very important in order to producing agriculture productions and realization of agriculture goals in order to increase women's efficiency. Rural economy of country is largely require to attention from point of increasing efficiency of rural economy activities, because waste growth, reduced efficiency of workforce and capital in close cycle, had led to loose economic activities attraction at these area and also special attention to this issue would work as rejection reasons for range of economic activities of village. Among this, according to very critical women's role at agriculture activities, focus and certain attention to this group and preparing them supporting, educational and extensional services, can help to remove obstacles and problems which they face, and consequently lead to increase and improve their efficiency about agriculture and finally lead to increase welfare of rural societies (Samadi Afshar, 2005).

Economic effects of rural women's financial self-reliance:

It is possible that rural women's financial selfreliance 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).

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).

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- 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).

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:

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.

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.

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.

But consider that aforementioned subjects, based on researches that has conducted at north of Iran that in these regions women have more active contribution from social, economic and cultural perspective.

Point that true about most of villages of Iran is that more active women economic contribution that leads to more income for family, cause that women be at higher rank for family decision making.

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.

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Evaluation of radio protective effects of wheat germ oil in male rats

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ABSTRACT: Wheat germ oil posses various biological properties as an anticancer and antioxidant agent. Present study was undertaken to evaluate the radio protective ability of wheat germ (WG) oil against whole body irradiation rat. Wheat germ oil was given to rats by oral injection in a concentration of 1 ml/kg and 3 ml/kg body weight/dose for 3 successive days, last dose administered 24 h pre-irradiation exposure with an acute single dose level of 2 Gry delivered at a dose rate of 0.564 Gry/ min at the time of experiment. With regard to cellular system, the results clearly indicated that pre-treatment with 3ml oil is more potent than 1ml and there are no significant differences between control group and groups that received oil only at either 1ml or 3ml in comparison to the control. Prior administration of WG oil to rats, significantly countered radiation induced biochemical disorder (liver enzymes and kidney function analysis, as well as, cholesterol level in the serum) and DNA damage (evaluated by DNA fragmentation assay and chromosomal aberration in bone marrow) in a dose dependent manner maximally at a concentration of 3 ml/kg. The results clearly indicated that wheat germ oil has significant potential to protect cellular system from radiation induced damage and ability to scavenge free radicals might be playing an important role in its radio protective manifestation without any toxicity

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Key words: wheat germ oil, DNA fragmentation, radioprotection, chromosome aberrations

1. INTRODUCTION

Radiotherapy is one of the most common therapies for treating human cancers. Several studies have indicated that irradiation induces reactive oxygen species (ROS), which play an important role in irradiation damage of the cell. Scientific and technological advancements have further increased the radiation burden in humans, because exposure to low levels of radiation has become common during medical diagnostic procedures, space or air travel, cosmic radiation and through the use of certain electronic gadgets. Other sources of radiation exposure include radon in houses, contamination from weapons testing sites, nuclear accidents and radiotherapy. The amounts of ionizing radiation that can be given to treat tumors are often limited due to the surrounding normal tissues and organs in the vicinity of the tumor that could be also exposed to the radiation causing damage, (Kunwar et al., 2010). Ionizing radiation may cause cancer, death, and loss of neural function in humans and animals. It also induces mutation, chromosomal aberrations and apoptosis in cells, Nair, et al (2001); Jagetia and Reddy (2005).

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Among the various physical/chemical agents, radiation is an important source in the generation of oxygen-derived free radicals and excited states. In actively metabolizing cells, there is considerable water apart from the target macromolecules of DNA, proteins, lipids and so on. The exposure of biological systems to radiation results in a radiolytic cleavage of water, giving rise to OH⁻ and H⁺. However, ionizing radiation can break chemical bonds and cause ionization of biologically important macromolecules such as nucleic acids, membrane lipids and proteins, (Lett 1992 and Daniniak and Tann 1995; Kamat et al., 2000). Due to the presence of polyunsaturated fatty acids, membranes are highly susceptible to oxidative damage induced by reactive oxygen species (ROS) generated during radiation, (Rice-Evans and Burdon (1993). Hence, compounds that are capable of protecting cellular membranes against ionizing radiation in particular, and free radicals in general, will have potential benefits as radio-protectors, antioxidants and anti-mutagens, Stavric (1994). Antioxidant systems have a fundamental role in defending organisms against irradiation-induced oxidative stress.

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Antioxidants are molecules that can prevent or reduce the extent of oxidative destruction of bio-molecules when present in small concentrations compared with the bio-molecules they are supposed to protect, Halliwell, (1990).

The essential oils and extracts of many plant species have become popular in recent years, and attempts to characterize their bioactive principles have gained momentum in many pharmaceutical and food processing applications, (Cowan, 1999). Plants (fruits, vegetables, medicinal herbs) contain a wide variety of free radical-scavenging molecules, such as phenolic compounds, nitrogen compounds, vitamins, terpenoids, and some other endogenous metabolites, that are rich in antioxidant activity, (Larson, 1988; Shahidi & Naczk, 1995; Cotelle *et al.*, 1996; Velioglu, et al., 1998; Zheng & Wang, 2001; Cai, *et al.*, 2003). Natural radio protectors are found in plant materials such as oil seed and wheat germ oil, (Singh *et al.*, 2009).

Wheat is an important source of vitamins, minerals, dietary fibre and phyto-chemicals. The oil is a rich source of toco-pherols and toco-trienols. The germ is the most nutritious portion of the wheat and it makes up about 2.5 % of the weight. During the milling process the germ is separated from the bran and starch. Wheat germ is a rich source of B complex vitamins, with wheat germ oil being the richest source of tocopherols. These nutrients and phytochemicals may have significant implications in chemoprevention, (Jensen et al., 2004; Lui, 2007). This oil is a source of easily assailable vitamin E which acts as inhibitor of oxidation processes in body tissues. It protects cells against the effects of free radicals, which are potentially damaging by products of the body's metabolism. Free radicals can cause cell damage that may contribute to the development of cancer, (Traber et al., 1999).

Recent studies have shown that a fibre rich diet reduces or causes a delay in fat digestion, impedes the absorption of cholesterol and fat in the intestine, reduces cholesterol synthesis by volatile fatty acids produced during fermentation and alters lipoprotein metabolism, (Cara *et al.*, 1992). A study conducted by Boateng *et al* (2007) concluded that dietary fat, depending on the source, quantity, fatty acid composition may have implications in the incidence of colon cancer. Wheat germ oil not only prevents autoxidation of unsaturated fatty acids but also generates DNA protective properties, (Gelmeza *et al.*,2009) .Hence it would be beneficial to determine the radio protective effects of wheat germ oil at 1 and 3

ml/kg which represents a normal and a high fat diet composition in the irradiated rats.

2. MATERIALS AND METHODS 2.1. MATERIALS

2.1.1. Chemicals:

Wheat germ oil was purchased from El-Captain Company (CAP PHARMA), 6th October City, Egypt. All other chemicals were of analytical grade.

2.1.2. Irradiation:

Source of irradiation used for Cobalt-60 gamma cell 3500. Whole-body Gamma-irradiation was performed at Middle Eastern Regional Radioisotopes Centre for The Arab Countries, Dokki, Cairo, Egypt. Animals were irradiated at an acute single dose level of 2 Gry delivered at a dose rate of 0.564 Gry/ min at the time of experiment.

2.1.3. Animal and Housing

Before commencing the work we obtained permission from the Institutional Animal Ethics Committee of NRC. Sixty Wister albino rats weighing 120 - 150 g (average age, 12 weeks) were used in this study. The animals were housed in stainless steel wire cages at 5 rates per cage under standard laboratory conditions. They were fed standard laboratory chow and water before the experiment for one week.

2.1.4. Wheat germ oil

In the present work wheat germ oil was given to rats by oral gavage in a concentration of 1 ml/kg and 3 ml/kg body weight/dose for 3 successive days as previously described based on the preliminary study, last dose administered 24 h pre-irradiation exposure.

2.2. Methods:

2.2.1. Experimental Design:

At the beginning of the experiment, rats were divided into six groups (10 rats / group).

<u>The 1^{st} one was the untreated control group.</u>

<u>The 2^{nd} group was exposed whole body to a single dose of γ -irradiation (2 Gry).</u>

<u>The 3^{rd} and 5^{th} groups were treated with wheat germ oil alone by oral administration at 1 and 3 ml/kg respectively for three consecutive days.</u>

<u>The 4th and 6th groups were treated with 1 and 3 ml/kg</u> respectively for three consecutive days pre-exposed to γ -irradiation.

All rats were left one hour after last dose of the treatment before exposure to γ -irradiation.

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2.2.2. Analytical procedures:

After 24 hours post-irradiation, rats were dissected under light anaesthesia and blood sample were collected by heart puncture using sterile syringes. Blood samples were incubated at 37°C then centrifuged to collect sera for biochemical analysis. Liver was dissected out and bone marrow was obtained for cytogenetic analysis (DNA fragmentation and chromosome aberrations).

2.2.3. Biochemical analysis:

For liver enzymes, serum ALT and AST were determined by kinetic method according to German Society for Clinical Chemistry (1970), Total serum cholesterol was determined by enzymatic colorimetric method according to Richmond (1973). To determine kidney function, serum creatinine was estimated by kinetic kits according to Henry (1974). While, blood urea determined by enzymatic and colorimetric method according to Patton and Crouch (1977).

2.2.4. DNA Fragmentation Assay:

Animals within different treatment groups were sacrificed 24h after last treatment and samples were collected. The method of DNA fragmentation assay was carried out according to Perandones *et al.* (1993). Rat liver was mechanically dissociated in hypotonic lysis buffer. The cell lysate was centrifuged at 11.000 rpm for 15 min. then the supernatant containing small DNA fragments was separated immediately, half the supernatant was used for gelelectrophoresis. The other half, as well as the pellet containing large pieces of DNA were used for the colorimetric determination Diphenylamine (DPA) assay.

2.2.5. Chromosomal aberrations:

In somatic cells, bone-marrow metaphases were prepared according to Yosida and Amano (1965). Briefly, bone marrow from the femur was aspirated, washed in saline, treated hypotonically (0.565% KCl), fixed in 3:1 methanol: acetic acid, spread on clean slides. Slides were stained with 7% Giemsa stain in phosphate buffer (pH 6.8). 100 well spread metaphases per animal were analyzed for chromosome aberrations. The types of aberrations in bone-marrow cells included breaks, deletions, fragments, centric fusions, centromeric attenuations, etc.

2.2.6. Statistical analysis:

Data were analyzed using One-way analysis of variance (ANOVA). The results obtained were expressed as means \pm standard error of the mean. Differences were considered significant at P<0.05.

3. RESULTS

The data of the present work showed the serum level of liver enzymes (AST and ALT), serum cholesterol and kidney function (serum creatinine and urea) of rats exposed to a single dose of γ -radiation (2 Gry) and rats pre-treated with 1 and 3 ml wheat germ oil. Serum level of liver function and kidney function were described in Table (1).

While serum cholesterol level illustrated in Fig 1. Serum AST revealed significant increases (P< 0.05) in rats exposed to γ -radiation than other treatments. WG oil decreased the elevation of serum AST activity particularly when rats orally received with 3 ml WG oil before exposure to γ -radiation. No significant differences among group III and V when compared with healthy control group. On other side, serum ALT level showed significant elevation in groups II, IV and VI as compared with control. Pretreatment with 3ml oil is more potent than 1ml to decrease the elevation level of ALT as a result of irradiation exposure and this means that 3 ml oil is more ameliorate the harmful effects of irradiation. No significant differences were observed between untreated control rats and rats either treated with 1ml or 3ml

There were decreases in the level of S. creatinine as a result of administration of oil before irradiation exposure in groups IV and VI but the decrease was pronounced at 3ml pre-treatment when compared with irradiated group.

However, a significant decrease in s. urea in groups IV and VI in comparison with group II, where as no significant change was obtained when compare each other .Serum cholesterol level declared significant elevation (P < 0.05) in the irradiated group and other groups which pre-treated with oil then exposed to irradiation II, IV and VI, when compared with control (I). However, the decrease was slightly more in the group pre-treated with 3ml oil but without significant difference with 1ml pre-treatment. There were no significant differences between control group and groups that received oil only at either 1ml or 3ml in compared to the control.

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Figure 1: Histogram showing the effects of wheat germ oil (WG) on the concentration of serum cholesterol in the different rat groups.

Parameters	Control	Irradiation	1ml WG	1ml WG &	3ml WG	3ml WG &IR
	Ι	(IR)	III	IR	V	VI
		II		IV		
S. AST U/L	271.38 ± 6.01 d	555.2 ± 3.89 a	251.18 ± 4.7 e	489.0 ± 1.09 b	248.32 ± 4.8 e	414.6 ± 5.87 c
S. ALT U/L	54.96 ± 2.1 d	189.39 ± 0.86 a	$56.2 \pm 1.02 \text{ d}$	$150.45 \pm 4.4 \text{ b}$	56.0 ± 1.79 d	124.33 ± 1.63 c
S. creatinine mg/dl	$0.6 \pm 0.005 d$	0.88 ± 0.014 a	0.59 ± 0.008 d	0.83 ± 0.009 b	0.59 ± 0.007 d	$0.71 \pm 0.007 \ c$
S. urea mg/dl	27.84 ± 0.42 c	36.38 ± 1.34 a	$27.22 \pm 0.46 \ \mathbf{c}$	33.1 ± 1.13 b	$25.9\pm0.48~c$	$32.0\pm0.57~b$

Table 1: Effects of wheat germ oil (WG) on liver enzymes and kidney function of different rat groups

Data are means of five replicates \pm standard error. Means in the same raw have the same letter are not significantly different at 0.05

DNA Fragmentation Results:

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The results presented in table (2) indicated that the percentage of DNA fragmentation in liver was increased significantly in irradiated animals compared to the control. Animals treated with WG alone at 1 and 3 ml/kg showed insignificant increase in the percentage of DNA fragmentation. On the other hand, animals treated with WG oil at both concentration and exposed to irradiation showed a significant decreased in the percentage of DNA fragmentation towards the control values although these treatments did not normalize it. Moreover, this improvement was pronounced in the liver of animals treated with WG at 3 ml/kg prior to irradiation. DNA fragmentation in response to irradiated animals was also detected by gel electrophoresis as DNA ladder representing a series of fragments that is multiples of 180–200 bp (Fig 2)

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Figure 2: Agarose gel electrophoresis showing the effects of wheat germ oil (WG) on percentage of DNA fragmentation on liver of irradiated rats Lane M: DNA molecular weight marker. Lane C: control group; Lane 1m: wheat germ oil at 1 ml, Lane 3 ml: wheat germ oil at 3 ml; Lane R+1m: Irradiated rats then received WG at Iml; Lane R+3ml: Irradiated rats then received WG at 3ml; Lane R+3ml; Irradiated rats.

Table 2:	Effe	cts of	wheat	germ	oil ((WG)	on
percentag	ge of	DNA	fragme	entatior	n on	liver	of
different	rat gr	oups					

Treatment	DNA Fragmentation %	The change %
Control	7.9	
(I) Irradiation (II)	32.0	+ 24.1
1 ml / kg WG (III)	9.6	+ 1.7
1ml/kg WG + Irradiation (IV)	17.4	+ 9.5
3 ml / kg WG (V)	10.0	+ 2.1
3ml/kg WG +	14.95	+ 7.05
Irradiation (VI)		

Chromosome aberrations:

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The frequencies of different types of structural (breaks, deletion, fragment and centromeric attenuation) and numerical chromosomal aberration (polyploidy and aneuploidy) for different treatment

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were presented in Table (3) and Fig (4). Total chromosomal aberrations showed a significant increase (P< 0.05) in γ -irradiated group compared to the control group. While animals treated with WG oil at 1 or 3 ml/kg body weight induced insignificant increased in the frequency of chromosome aberrations $(3.8 \pm 0.66 \text{ and } 4.6 \pm 0.68 \text{ respectively})$ when compared with that of the control groups $(3.0 \pm$ 0.32). A corresponding increase was found in all the individual aberrations in irradiated animals, however, the number of chromatid break and centromeric attenuation was found to be the most types of aberrations which significantly increased in all irradiated animals. Treatment with WG oil at either 1 ml or 3 ml before irradiation resulted in very significant decrease in the percent of aberrant cells (19.2 and 14.4 % respectively, (Fig 3A) and in the total number of aberrations (9.6 \pm 0.86 and 7.2 \pm 0.68 respectively) compared to the irradiated-group (19.3 \pm 1.56) Table (3). The pre-treatment with oil at both doses inhibited the frequency of chromosomal aberrations in bone marrow by 59.5 % at 1ml /kg while it reached to 74.2% at 3ml/kg, Fig (3B). The present data showed that administration of wheat germ oil ameliorate and improve the harmful effects of irradiation particularly when rats pre-treated with 3ml of the oil.

DISCUSSION

Ionizing radiation produces harmful effects on the organisms and due to wide spread use of radiation in diagnosis therapy, industry, so pharmacological intervention could be most potent strategy to protect human or ameliorates the deleterious effect of ionizing radiation, (Jagetia 2007). Ionizing radiations induce significant elevation in the physiological and metabolic processes, as well as, disorders in blood biochemical parameters, El-Masry and Saad (2005) and causing chain reaction of oxidation (Ammar, 2009). The degree of cellular damage is variable among different organs depending on the organ ratio sensitivity, so one approach to prevent such injury is by supplementation or administration with natural potent antioxidant as natural radio-protector (Zhou et al., 2001).

Radiation exerted significant (P< 0.05) elevation in the liver enzymes, cholesterol and kidney function. So current investigation in wheat germ oil was applied in view of minimizing the toxicity of ionizing radiation, Ammar (2009) and this agreed with the result of the present work that declared

significant elevation in liver enzymes, cholesterol, creatinine and blood urea as a result of γ -radiation exposure where as this elevation were alleviated when treated with wheat germ oil before γ -radiation exposure. In the present study, treatment with wheat germ oil without exposure to radiation revealed non significant changes in the investigated parameters indicating its safe in use.



(B)



Figure 3: Histogram showing the effects of wheat germ oil (WG) on the: A) percentage of chromosome aberrations and B) The inhibition percent in the irradiated rats

Sisodia *et al.*, (2007) and Kunwar *et al.*, (2010) and reported that ionizing radiation induce augmentation in the levels of serum AST and ALT that were significantly ameliorated by pre-treated with natural radio-protector which agreed with the present work.

Gamma irradiation caused significant increase in the levels of serum kidney and liver peroxidation but pre-treated with natural radio-protector were significantly decreases the level of serum kidney and liver function when compared to the irradiated group. Adaramoye et al., (2010) and these agrees with the results of the present work but on other side, Adaramove et al., (2010) reported that these were no significant difference (P>0.05) in the level of serum urea of irradiated and pre-treated animals when compared to the control. Increase in serum urea was due to increase in glutamate de-hydrogenase enzyme as a result of irradiation and this may increase carbamoyl phosphate synthetase activity leading to increase in urea concentration, Ramadan et al., (2001). Administration of WG oil before exposure to γ -irradiation reduced the level of serum urea when compared to the irradiated rats.

Natural radio-protector protected albino rats from the adverse effects of whole body irradiation, Adaramoye *et al.*, (2010). Gamma irradiation induced significant elevation in the level of serum cholesterol on the first day post-irradiation but administration with natural radio-protector before irradiation exposure restored the normal level, Ramadan *et al.*, (2002) and El-missiry *et al.*, (2007) and these were agreed with the present data. The administration of wheat germ oil before irradiation results in an increase in membrane permeability and fluidity causing decreased triglycerides and cholesterol, Yousri *et al.*, (1991).

Exposure of cells to ionizing radiation during the G0 or G1 phases of the cell cycle causes chromosomal aberrations (CAs) as breaks, dicentrics, acentrics, fragments, rings and translocations. These CAs are used as biomarkers of radio-sensitivity or radiation damage after medical, accidental and occupational exposure. Besides, some of these CAs may be strongly linked with different cancer types (Atanasova et al., 2004; Hande et al., 2005; Varella-Garcia et al., 2007). Additionally, radiation is a wellknown inducer of free radicals caused to chromosomal damages. In the present study exposure to γ -irradiation (2 Gry) induced chromosomal aberration in bone marrow and DNA fragmentation in liver by 38.6 % and 32.0 % respectively. The use of certain materials may help to decreasing of the genotoxicity created by radiation and may inhibit mutagenesis and carcinogenesis, De Flora et al., (2007).

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The results of our present study demonstrate the radio protective effect of wheat germ oil on radiation induced chromosomal aberrations and DNA fragmentation; wheat germ oil itself does not have any marked effect neither on the bone marrow chromosomes nor the DNA fragmentation percent. Wheat germ oil is a source of easily assimilable vitamin E which represented by 89.3 % and acts as inhibitor of oxidation processes in body tissues, Abd El-Azeim et al., (2005). Vitamin E has antioxidant and free radical scavenging activities, which suggests that this vitamin may modulate oxidative DNA damage in mammalian cells, (Odin, 1997, Paranich et al., 2000 and Jacobs et al., 2001). This activity could reduce the incidence of chromosomal aberrations caused by free radicals generation, Abd El-Azeim et al., (2005).

WG oil at both doses (1 ml and 3 ml) inhibited radiation-induced chromosomal aberrations by about 59.5 and 74.2 % respectively, indicating that WG oil has anti-mutagenic effect. In the same manner, Abd El-Azeim et al., (2005) reported that, both olive and wheat germ oils inhibited CP-induced chromosomal aberrations by about 76.8 % indicating that they have anti-clastogenic and anti-mutagenic effects. Similar trends were seen in studies by Field et al., (2008) where rats feed WG oil at 7 and 14 % significantly reduced the number of aberrant crypt foci which were good predictors of tumor outcome, Ishizuka et al., (2003). Wheat germ oil, a significant source of phyto-chemicals such as vitamin E may have played a beneficial role in reducing chromosomal aberration and DNA fragmentation induced by radiation exposure.

Table 3: Effects of wheat germ oil (WG) on the Frequencies of chromosome aberrations induced by exposit	ure
to 2.0 Gry of gamma rays in bone marrow of rats	

Groups	Total Aberrations	Structural aberrations					Numerical	aberrations
		Ch. break	deletion	Fragment	Centromeric attenuation	Dicentric	Polyploidy	Aneuploidy
Control I	3.0 ± 0.32	0 ^c	0.6 ± 0.25 ^c	0.6±0.25 ^b	0.8±0.37 °	0 ^b	0.6±0.24 ^b	0.4 ± 0.25 ^c
Irradiation (IR) II	19.3 ± 1.56	$2.8 \pm 0.37_{a}$	$3.2\pm0.2~^{\rm a}$	$2.4\pm0.24~^{\rm a}$	6.8 ± 0.37 ^a	$1.1 \pm 0.24_{a}$	$1.8\pm0.37~^{\rm a}$	$1.2\pm0.37~^{\rm a}$
1 ml/kg WG III	$3.8 \pm 0.66_{d}$	0 °	0.2 ± 0.2 ^c	$0.4\pm0.25~^{\rm b}$	$1.7\pm0.2\ensuremath{^{\rm c}}$ $^{\rm c}$	0 ^b	$0.8\pm0.37~^{\rm b}$	0.5 ± 0.24 ^c
1ml WG+IR IV	9.6 ± 0.86	$1.8\pm0.2~^{b}$	$1.2\pm0.37~^{\rm b}$	$1.0\pm0.20^{\rm b}$	3.0 ± 0.32^{b}	0.4 ± 0.2	$1.0\pm0.25^{\text{ b}}$	1.2 ± 0.25 ^a
3ml/kg WG V	$4.6 \pm 0.68_{d}$	0.4 ± 0.25	0.4 ± 0.25 ^c	$0.8\pm0.20^{\text{ b}}$	1.4 ± 0.25 ^c	0 ^b	$0.8\pm0.2^{\rm b}$	$0.8\pm0.37~^{\rm bc}$
3ml WG+IR VI	$7.2\pm0.68~^{\rm c}$	$1.4\pm0.4^{\rm b}$	1.4 ± 0.32 $^{\rm b}$	$1.2\pm0.32^{\text{ b}}$	$1.8\pm0.37~^{\rm c}$	0 ^b	$1.0\pm0.32^{\rm \ b}$	$0.4\pm0.37~^{\rm c}$

Ch break = Chromatid break, WG = wheat germ oil

Data are means of five replicates \pm standard error. Means in the same column have the same letter are not significantly different at 0.05

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Fig 4: Photo showing: (a) normal spread metaphase, (b) spread metaphase with chromatid break, fragment and deletion, (c) spread metaphase with polyploidy chromosomes

In conclusion, the treatment of rats with wheat germ oil either at 1ml or 3ml /kg body wt prior to whole body γ -irradiation seems to exert protective effects. The mechanism by which wheat germ oil mediated its effects may be due to inhibiting free radical liberating or scavenging these free radicals causing alleviate the damage induced in liver and kidney cells. Results from such studies should provide information of substantial value for making a better decision concerning the future use of wheat germ oil as a preventive measure to reduce injury from exposure to ionizing radiation.

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Replacement age of agricultural tractor (MF285) in Varamin region (case study)

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Abstract: One of the main aims in the management of farm equipment and tractors is deciding about their replacement, based on technical and economic conditions. The objective of this research was to determine the economic life time for common used tractor in Varamin region, Iran, namely Massey- Fergusen 285. First the annual depreciation and interest were calculated considering the initial purchase price of tractor, and then the economic life was calculated based on repair and maintenance costs. The results showed that the most suitable replacement age is nine years for Massey- Fergusen 285.

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Key words: Economic life; replacement age; MF285

1. Introduction

Agricultural tractors are the most common sources of power generating in the present mechanized agricultural operations. One of the aims in agricultural machinery management is decision making about the replacement of tractors considering the technical and economical conditions of the region which are used. Proper application and appropriate decisions about timely replacement of tractors and equipment causes to do agricultural operations in time with good quality and consequently results lower costs and higher revenue. In Iran, tractors are even used for some more years after their economic life by farmers and they don't have attention to the replacement issues, because the repair and maintenance costs are not calculated properly and the timeliness costs are not considered in the agricultural sections. It is clear that the breakdown cases will be increased after the economic life of the tractors while many of them may not be predicted, so the delay rate will be very high. On the other hand, the fuel consumption will be increased after economic life because of parts aging so the importance of the replacement is more clear due to the importance and

high value of the fossil fuels. Making decision about the replacement of aged machines is based on the economic life. Economic life, which is also known as useful life, is usually shorter than the machine's life and it depends on the repair costs of the machine. In general, the total costs of a machine are divided into fixed and variable costs. With increasing of the operating hours of the machine, the fixed costs including depreciation and interest have a declining trend per operation unit (area or time), while the variable costs including repair and maintenance will be increased. Economically the optimum time for replacing the aged machine is when the total cost is minimum per operation unit and after that will be increased again (ASAE Standards . 2000b).

The main aim of this research is to evaluate the operational conditions of more used tractor in this region namely Massey- Fergusen 285, in order to find the best replacement age for this tractors in Varamin region.

2. Materials and methods

The data and information for twenty MF285 (75hp) tractor in Varamin (40 km southeast of Tehran) was collected and examined over 10 years. The collected data includes of repair and maintenance costs, annual operating time, as well as purchase price. The price of MF 285 is taken as 12000 \$ based on the official documents of The Agricultural Machinery Development Agency, and the inflation rates posted by the central bank in 2008. Repair and maintenance costs were roughly calculated by referring to the owners and considering the costs of spare parts, used materials, and repair fee and then a questionnaire was completed for tractor. The annual operating hours were determined based on the tractor's working hour counter.

2.1 Depreciation

The declining balance method (reduction with fixed percent) was used for calculation of depreciation based on equations 1 and 2 (ASAE Standards, 2000a).

$\mathbf{V}_{n}=\mathbf{P}\left(1-\mathbf{x}/l\right)^{n}$	(1)
$\mathbf{D}_{n} = \mathbf{V} \cdot \mathbf{V}_{n+1}$	(2)

In which D_n is depreciation rate in the year of calculation, n is machine's age in the year of calculation, V_n is remaining value of the machine at the end of the nth year (\$), x is depreciation ratio (1<x<2), l is total machine life (year) and P is purchase price (\$).

In the computations, x is designated a mean value of 1.5 and L equals to 10 years (ASAE Standards, 2000b).

2.2 Interest

Equation 3 was used for calculation of interest (ASAE Standards, 2000b).



In which I_n is interest in the nth year (\$), i is interest rate in Iran based on central bank rate in 2008 (i=15%)

2.3. Replacement determination method

Following the calculation of repair and maintenance costs and operating hours over 10 years as well as depreciation and interest over the same period, the economic life of the tractor was calculated as follows. First, depreciation and interest costs, known as capital costs, were calculated. Total accumulated cost was calculated through adding accumulated repair and maintenance and accumulated capital costs. Total accumulated cost per operating unit was obtained by dividing the total accumulated cost by the accumulated operating hours. In this study, operation time is assumed as the operating unit. Economically, the optimum time for replacement of the machine is when the minimum total accumulated cost per operating unit occur (Cross, 1998).

3. Results

3.1. Depreciation and interest

The depreciation rate, interest rate, annual interest, and annual interest added depreciation as well as accumulated capital costs is shown in Table 1 for MF285 tractor in the first 10 years of the tractor's life. It is obvious that annual capital costs are reduce with time.

3.2. Repair and maintenance, capital and total accumulated costs

Tables 2 shows the rate and the percentage of costs of spare parts, repairs, oil and filter costs, total repair and maintenance costs in each year as well as the annual and total accumulated costs of repair and maintenance for MF285 tractor.

year	Annual depreciation costs(1000\$)	Annual interest of investment (1000\$)	Annual capital costs (1000\$)	Accumulated capital costs (1000\$)
1	18	15.3	33.3	33.3
2	15.3	13	28.3	61.6
3	13	11.05	24.05	85.65
4	11.06	9.39	20.45	106.1
5	9.4	7.98	17.38	123.48
6	7.99	6.78	14.77	138.25
7	6.78	5.77	12.55	150.8
8	5.77	4.9	10.67	161.47
9	4.9	4.17	9.07	170.54
10	4.18	3.54	7.72	178.26

Table1. Depreciation, interest, and capital costs for MF285 tractor

Table 2. Repair and maintenance costs for MF285 tractors over 10 years

Year	Spare parts		Repairs		Oil and filter		Total repair	Total
	Rate (1000\$)	Percent	Rate (1000\$)	Percent	Rate (1000\$)	Percent	maintenance costs in year	repair and maintenance costs
1	1.85	43.52	1.5	35.29	0.9	21.17	4.25	4.25
2	2.5	41.66	2.05	34.16	1.45	24.16	6	10.25
3	3.74	46.38	2.68	33.30	1.66	20.30	8.06	18.31
4	4.97	44.33	3.89	34.74	2.34	20.91	11.21	29.52
5	8.11	41.85	6.72	34.63	4.56	23.50	19.4	48.92
6	10.60	40.33	8.70	33.13	6.7	26.52	26.25	75.17
7	14.76	42.03	11.72	33.36	8.64	24.59	35.12	110.29

8	16.91	41.90	1	13.90	34.44	9.55	23.65	40.36	150.65
9	18.47	42.81	1	14.43	33.43	10.24	23.74	43.15	193.8
10	39.53	44.86	3	32.03	36.35	16.54	18.77	88.11	281.91
Mean		42.96			34.28		22.73		

Accumulated repair and maintenance, accumulated capital and total accumulated costs per year for tractor is shown in table 3. In this tables, the total accumulated costs in each year was obtained by sum of the total accumulated repair and maintenance costs and accumulated capital costs of that year.

Table 3. Repair and maintenance, capital, and total accumulated costs for MF285 tractors (1000\$)

Year	Accumulated repair and maintenance costs	Accumulated capital costs	Total of accumulated costs
1	4.25	33.3	37.55
2	10.27	61.6	71.87
3	18.31	85.65	103.96
4	29.52	106.1	135.62
5	48.92	123.48	172.4
6	75.17	138.25	213.42
7	110.29	150.8	261.09
8	150.65	161.47	312.12
9	193.8	170.54	364.34
10	281.91	178.26	460.11

4. Discussions

Based on the results shown in table 1, the annual capital costs including depreciation costs and annual investment interest decrease year by year. These costs have no direct relationship with the usage rate during machine life but somehow they are affected by them. Usage of machines causes less depreciation costs and investment interest, because a certain portion of annual costs will be divided to more operating hours (Ward *et al.*, 1985). Table 2 shows the repair and maintenance costs for MF285. These costs are depended on the operation time of the machine. Higher usage of the machine results higher repair and maintenance costs. The table also shows an increase in repair and maintenance, spare part and oil costs. The percentage of each item is different in different years and it may be higher or lower than the past or next year but in calculation the sum of repair and maintenance costs is important which increase in time. For example, considering table 2, the percent of spare parts costs in the eighth year is lower than those in the seventh and ninth years. Higher costs of spare parts may be caused by low quality of the parts, overuse or early replacement of the spare parts, improper usage of tractor caused by insufficiently trained

drivers, non-standard parts, improper repair and more importantly, excessive use after the economic life of the tractor .

Year	Total accumulated costs	annual operating costs	accumulated operating costs	Total accumulated costs per operating
1	37.55	1021	1021	36.77
2	71.87	1029	2050	35.05
3	103.96	1100	3150	33
4	135.62	1200	4350	31.17
5	172.4	1350	5700	30.24
6	213.42	1450	7150	29.84
7	261.09	1710	8860	29.46
8	312.12	1750	10610	29.41
9	364.34	1840	12450	29.26
10	460.17	1270	13720	33.54

Table 4. Accumulated, accumulated operating and total costs per operating hour for MF 285 tractors (1000\$)

Based on the data in table 4, the total accumulated costs per operating hour, calculated by dividing the total accumulated costs accumulated operating hours. According to table 4, the total accumulated costs per operating hour decrease first and then increase after the ninth year for MF285. Hence the best time for replacement is when the total accumulated costs per operating hour are minimum after which costs rise again (Hunt, 2001; Ward et al., 1985).It maybe concluded that the best age of replacement for MF285 is at the end of the ninth year. According to the available information in mechanization development center of Iran in 2002, the actual mean useful life for tractors in Iran is considered to be about 13 years while unofficial reports show higher levels. Obviously, there is a significant difference between economic life of tractors and their true and operational function. When the repair and maintenance costs of these tractors are not calculated properly, the true level of costs is not clear to use for replacement decisions and in most cases continued use of old tractors is not economic.

The time of agricultural proactive and timeliness costs are still overlooked in Iran especially in small farms. It is known that when the economic life of a tractor is exceeded, breakdown cases increase and down time delay will be high, whereas timely replacement can prevent these losses. The important aspects for increasing the economic life of tractors are timely servicing, repairs and maintenance, using good quality spare parts and materials and proper training of drivers. It is suggested to perform similar studies in different regions having different operational, climatic, economical and management conditions to determine the best time for replacement of tractor in that region. This process requires a system for collecting and recording data on operating hours, repair and maintenance costs, and calculating depreciation, interest and timeliness costs.

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A Weighted Usability Measure for E-learning Systems

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Abstract: Currently learning paradigms have been overcome, using information and communication technologies (ICT) to give rise to e-learning domain. Thus, classical classrooms based training has been substituted by online systems working on Internet. The aim of an e-learning system is to fulfill requirements of instructors as well as learners. However, institutions offering courses online have a lack of applying efficient evaluation methods to both teachers and students. Frequent preoccupation concerns with functionalities and interface that a system must satisfy for users needs. In our studied case, learners need to face up to functionality of e-learning infrastructure rather than to acquire knowledge. When users spend more time, resources (software, hardware) unnecessarily, consequently they spend more costs, instead quenching academic thirst. Thus, this research aims to evaluate the usability of e-learning systems, a pondered measure of usability evaluation is proposed as a result of the analysis of the inquiry applied to the system users. We study, evaluate, and compare the usability of two applications, to highlight recommendations for improvement.

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1. Introduction

An e-learning system manages software and hardware resources for engaging learners remotely [9]. This kind of system must be able to support the interaction among students and instructors, keeping track of training and authoring process. Users of this kind of groupware become experts of this activity when a system is well constructed. However, user's preferences may differ from each other. Thus, during the design, it is necessary to take into account not only basic requirements according with users profiles, but also the system should be adaptive according with the development acquired by users during the learning process, and with educational learning content. This point of view is related with the usability of systems.

International Standard Organization (ISO) defines usability as "The extent to which a product can be used by specified users to achieve specified goals with effectiveness, efficiency, and satisfaction in a specified context of use" [8]. Thus, in our context, an e-learning system is the product, while learners and instructors are the specific users. Based on educational background, cultural constraints, and level of computer literacy, learners can be characterized. Specific goal is to achieve certain level of knowledge delivery, enhancing learning quality and increasing user opportunities. The context of use concerns with learning environment, task at hand and user groups. Within the same learning environment,

each user can perform different tasks. But, changing the learning environment, and keeping other unchanged parameters, the context of use and hence the users' performance against a specific task, may suffer modifications. It means, system usability can be traduced as a measure of how much a product is effective, easy to employ, and enjoyable. Usability for an e-learning system has additional importance, because users may not be able to concentrate on learning content. Rather, they may spend time in understanding the system itself due to its complex interaction. Ensuring usability is a matter of great concern, so the most effective way of producing such a system is to evaluate usability of the system and if necessary to repeat its design until suitable solution [18].

Interaction among users is an important characteristic of a good parameter of usability. A set of usability attributes is identified in this work. We select some attributes and assigned a weight according with an impact and confirm each one by means of inquiries. Usability evaluation is based on the pondered measure. According to this value (below a certain level), the designer decides to revamp the system, concentrating on specific features which need improvement.

The paper organization is as follows: related works are discussed in Section 2. A usability criteria and an inquiry based on the usability criteria were proposed, that are described in Section 3. Our approach is applied on two e-learning systems as case studies, as we can see in Section 4. An overall usability measure is obtained taking into account users' information and the pondered usability criteria. Section 5 discusses obtained results. Section 6 recommends improvements based on obtained results. Finally, Section 7 presents conclusion and future work.

2. Related Work

Usability is considered a prime parameter for any system. No matter how well a system is compatible with the predefined set of guidelines to construct it, but these guidelines cannot be used as an alternate of assessment of usability [7]. Additionally, and due to an e-learning system is employed by two types of users: - learners who are characterized by cultural and educational background, abilities, technical expertise, and cognitive aptitudes; and – teachers who have different profile characterized by ability to transmit knowledge, to follows the learning process of students, thus, e-learning systems become a singular studied case.

One effective and popular usability evaluation technique is heuristics as guidelines to set up the design of a system. In e-learning, several heuristics have been adopted from the general context and applied to unveil specific usability problem, thus some research has come up different parameters as a checklist such as that of Nielsen [12]. In, a set of eight golden rules for designing user interface, a revision of Nielsen's ten heuristics is proposed in [19].

Efforts have been made to synthesize a systematic usability evaluation for e-learning systems [1] along with heuristics based evaluation method, like the concept of abstract task (AT) [1]. AT estimates the conformity of certain attributes of a particular application. AT methodology is more beneficial than those compared to user-testing and heuristic evaluation. Advantages showed by AT over techniques like inspection with user-testing and heuristic evaluation are more convincing. Three dimensions are chosen for comparison: effectiveness, efficiency, and satisfaction.

More advances features provided (Technology), Interaction (among different users groups), Content (learning material), and available Services (TICS) [9] constitute a new framework to guarantee the quality of the system, concerning the evaluation, this paper propose the eLSE methodology. Milano-Lugano Evaluation method, MiLE [21] based on scenario-driven inspection technique, MiLE incorporates profile, scenario, goal concepts, and usability attributes. MiLE fruitfulness is applied to a corporate e-learning platform, finding front-end (learner) and back-office (tutor) usability.

Several empirical studies are performed for usability evaluation that target a particular system and after using any usability evaluation approaches produce empirical results. For example, ISO standard model is applied to Blackboard learning management system [4]. "Relations" is an e-learning program or lesson, whose usability is tested using a specially designed questionnaire distributed among users [17]. The questionnaire is based on "Learning with Software" heuristics [20]. Heuristics combine usability and learning issues as an inspection by experts in human computer interaction domain. But, these heuristics are adopted and enhanced in order to develop questionnaires for lesson evaluation, rather than by experts.

SEMINOLE (SEaMless INtegrated Online Learning Environment) [10] is a customization of an open source learning management system called MOODLE, that is enhanced by web cast and multimedia recording and storage functionality provided by a system called ePresence. Students are able to attend classes remotely, access contents in the form of slides equipped with audio transcription, participate in forums, chat with a teacher and other student and check grades.

3 Usability Criteria

From the point of view of functionality of a system, activities can be divided into setup and execution phases. The definition of required elements to evaluate usability corresponds to the setup phase. These elements are taking into account during the execution phase to perform the evaluation process (see Figure 1). The setup phase starts with an exhaustive literature survey, giving rise to a set of comprehensive attributes, used as a criterion for evaluation. Each attribute has a weight which represents importance related with usability. Simultaneously, an inquiry is synthesized, reflecting the way in which the evaluation is performed.

During the execution phase, a user study is performed based on the prepared questionnaire, giving rise to a raw data for the system evaluation. The usability of the system is evaluated by a statistical process that combines collected data after user survey process with pondered attributes. The attributes are pondered according to its significance within the literature survey (see Table 1). The attributes are measured by means of a questionnaire. Topic questions/answers are listed in Table 2. TABLE 1: Usability Categories / Criteria Weight ages

Usability Criterion	Referred Material	Weight
Feedback & Interactivity	[1] [11][14] [23]	3
Learning Material	[1][3][4][13][14][23]	4
Assessment	[4][14] [17]	2
Visibility	[1][12][14][17][20][23]	4
Learner Facilitation & Support	[12][14][23]	2
Error Handling & Prevention	[1][12][17][20][23]	4
Collaboration Support	[3][4][5][13]	3



Fig 1. Activity Flow Diagram

Online Data Collection Module (DCM) is presented as a web page where questionnaire to evaluate a particular e-learning system is displayed. Questionnaire responses of participants are stored at the database for further processing (see Figure 2). DCM presents questions against five ordered scale (strongly agree to disagree). Questions, name and, email of participants are also stored.

TABLE 2.	Features	and	Questions
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Feature	Relevant answers
5	• Using this system I feel to be in a classroom
	interacting with instructors
Feedba	Performing some system functionalities I have
ck &	annropriate feedback
Interac	Fncouraging and multimedia feedback (sound
tivity	animation, icons) is properly provided, guiding
	me to complete knowledge process.
	Enough material and understandable is
Learni	provided, enhancing my learning.
ng	• Language used in the material is appropriate.
Materi	with examples.
al	• Learning content is updated frequently.
	• Assignments/quizzes are available for the
Assess	learned knowledge.
ment	• I have to use the learned knowledge to solve my
	assignments/quizzes.
	• Important lessons/topics/options are easily
	visible and accessible.
	• The interface shows only those options at a time
	that are needed.
Visibili	 Individual sections are clearly distinguishable
ty	from others.
	• Always I am able to know course/topic/action I
	am currently working with.
	 For each new topic, it is easy browsing the
	system content.
Learne	• I can choose multiple learning paths whichever
r	suitable to me.
Facilita	• Learning is enhancing by the well structuring
tion &	courses and planning.
Suppor	• Support is provided to complete all tasks.
t	
Error	• There is not technical error within the system.
Handli	• Error messages are clear presented that even a
ng &	layman can also understand.
Preven	• When errors occur (solving a quiz), undo/redo
tion	activity can be performed.
	 Support (email, chat, discussion forum) are
	available for learners and instructor
Colla	communication.
borati	 Secure communication is guaranteed, so
on	messages are always delivered.
Supp	• Communication with others is possible (to solve
ort	assignments).
	 System awareness provides me knowledge
	about actions taken by others.[15][16]


Fig. 2 Data Collection Module (DCM)

4 Case Studies

Virtual University (VU) (http://www.vu.edu. pk) is the first public-sector university based on information and communication technology for conducting e-learning education in Pakistan. Students are at remote location, with diverse backgrounds and level of computing skill. To cater with needs, provide better interaction of remote students and instructors, and delivery of content to student*s in a better way, VU the Virtual University Learning uses Management system (VULMS). VULMS is continuously in development to support, easy to use, it has a functionally rich interface for online learners. The interface of VULMS provides tools for students and instructors. But our focus is only on the student interface because an interactive and usable student interface plays a significant role in learning process.

After logging in, students are able to visit their registered courses and profile, submit assignments and quizzes, participate in discussions, communicate by mail, etc. Figure 3 shows important options for interaction, as well as courses currently registered by a student. A course is allocated to each registered student, containing information about content: description, outline, frequently asked questions, related links, downloads and grading scheme of the course. A distinctive feature of VULMS is the availability of Graded Discussion Board (GDB) and Moderated Discussion Board (MDB). Both intend to access the understanding of a student subject by reviewing his comments on a topic related to the course. Instructor can review student posted messages about specific topic. Depending on validity of student comments, instructor can grade them.



Fig. 3 Virtual University Learning Management system (VULMS)

The eFront (http://www.efrontlearning.net) e-learning system has been selected, because eFront provides facilities for both learners and instructors, and conduct courses at colleges, university, and business organizations. eFront is object-oriented and ajax-enabled multiple-language learning platform that is SCORM [2] Compliant and LDAP [22] Supportive.

Due to compliance with SCORM, eFront can interoperate with other SCORM compliant learning management system. In order to perform the respective transaction, individual interface is provided for administrator, instructors, and students. We are concerned with the student interface. Figure 4 shows a screenshot view of the eFront system when a user is logged in. This page shows options and material related to a course that a user registers. The left side of screen contains a pane which has options like theory, tests, and forums related to the course, main course page, change lesson, etc. Some other are: lessons, announcements, events, comments, and messages at forums related to the selected course.

5 The Usability Measure for eFront and VULMS

The overall weighted usability measure refers to a quantitative assessment of usability of an e-learning system taking into account several factors: responses from participants of survey, relative importance of each usability criterion, and number of questions in each usability category.

Ava Inte	ilable Options for racting with System	List of Available Course Lessons
Befront Refr Bo Edt Yow Refr Jone Refr Jone	Aczilla Firefox A	
exess écti 20 densra, hrío 20 denska 29 sparts 29 sparts 29 sparts 29 sparts 29 sparts 29 sparts 29 sparts 20 spart	kapand al / College al Moding Rutherlift in progress W Acceler Civilizations (C) Anayo difficultion (C) Anayo difficult	Sufer skarced programming too of for experienced programmer or sufer skarced programming too of for experienced programmer or any control of factor L. Eved
het El acout	Linguigi English	sifost (verba 363) - Canvelly Ettor - Consu

Fig. 4 The eFront E-learning System

A quantitative measure is necessary because qualitative usability evaluation only provides subjective information for analysis. A true measure of usability is not provided. An objective measure of usability evaluation can be extinguished by exploiting a quantitative measure. This quantitative measure is calculated based on the data collected during evaluation. The elements involved in the calculation of overall weighted usability measurement are:

Ni = Number of questions in usability category 'i' Wi = Weight assigned to category 'i' Mij = Median of responses to question 'j' in category 'i' Si = Summation of medians of category 'i' = Mij over j T = total Weight = (Si x Wi) R = Normalizing Factor = (Ni x Wi) Overall Weighted Usability Measure = U = T / R => U = (Si x Wi) / (Ni x Wi)

The total weight obtained by multiplying sum of medians and weight of a particular usability category makes that more importance is given to a usability category having more weight. The total weight serves as usability measure, although this factor is not within the range of 1 to 5. The normalizing factor is responsible for bringing the result within this range. Thus, 'U' provides an overall weighted usability assessment.

5.1 Pondered Usability Measure for eFront

At first glance eFront seems pretty good system. Thanks to the usability evaluation conducted, we see some problems in fulfilling certain parameters. Many participants' comments assert that "the system is not easy to use for beginners". Participants of survey are students from a university registered in Masters in CS program, and about 70 students out of them were registered in Human Computer Interface course. Although fewer number

of participants may have sufficed, but increasing this number is beneficial for more effective user testing [6]. Students filled the online questionnaire through Online Data Collection Module. These responses were used to measure "U. We save the official identification of participants, usability criterion, and questions measuring that usability criterion respectively. 'U' is calculated as follows:

Si = Summation of medians of category 'i' = Mij over j Si à S1 =14, S2 =19, S3 = 8, S4 =16, S5 = 19, S6 =10, S7 = 16 T = Weighted Total = (Si x Wi) T à (14*3, 19*4, 8*2, 16*4, 19*2, 10*4, 16*3) = 324 R was calculated as 89, hence Overall Weighted Usability Measure = U = T / R

 \tilde{O} U = 324 / 89 = 3.64

Note that the usability measure is graded out of 5.

5.2 Pondered Usability Measure for VULMS

After the usability evaluation of the VULMS system, it is found that overall participants are satisfied with the system in spite of facing problems in fulfilling certain usability criteria. This is also evident by several comments received by participants stating that "Overall VULMS is a good learning management system". Participants for the usability evaluation are those students under e-learning paradigm exclusively. They are the students of virtual university who are used to exploit VULMS for their ongoing education and any kind of interaction with their instructors or other students. The online questionnaire was presented to these 73 participants. We calculate 'U' as follows:

Si = Summation of medians of category 'i' = Mij over j

Si à S1 =15, S2 =20, S3 = 8, S4 =16, S5 = 20,

S6 =12, S7 = 18

T = Weighted Total = (Si x Wi)

T à (15*3, 20*4, 8*2, 16*4, 20*2, 12*4, 18*3) = 347

Note that R was calculated in section 4.3 as 89, hence Overall Weighted Usability Measure = U = T / R \tilde{O} U = 347 / 89 = 3.90

Note that the usability measure is graded out of 5.

5.3 eFront vs VULM – Comparing evaluations

The value of overall weighted usability measurement for the two systems revealed that VULMS enjoys a higher level of user satisfaction as compared to eFront. However, a relative comparison of two systems in individual usability category may expose useful information. A system can be more usable as compared to the other in a certain parameters, even if the overall measure is less that other system. To determine which parts of the system should be emphasized. The average of medians for each usability criterion is presented in Table 3.

Table 3 shows that eFront is slightly inferior to VULMS in terms of usability evaluation. However, the usability criteria of assessment and visibility were evaluated to be equally well-fulfilled by both systems. So, there is more need to concentrate on categories other than assessment and visibility. All other usability criteria need attention in case of eFront specially collaboration support.

6 Improvement of e-Leaning System Usability

A redesign of eFront system is highly recommended because lower usability measure is obtained. The redesign should focus on those features that have been shown to produce low usability measure. For instance, collaboration support when treated individually should be seriously considered.

Visibility, assessment, and learning material should also be taken into account. The learning material, for example, was reported as lacking enough examples to develop better understanding. This problem, however, can simply be eliminated by including more examples related to current learning

Usability Features	eFront	VULMS						
Feedback / Interactivity	14/4 = 3.5	15/4 = 3.75						
Learning Material	19/5 = 3.8	20/5 = 4						
Assessment	8/2 = 4	8/2 = 4						
Visibility	16/4 = 4	16/4 = 4						
Learner Facilitation & Support	19/5 = 3.8	20/5 = 4						
Error Handling and Prevention	10/3 = 3.33	12/3 = 4						
Collaboration Support	16/5 = 3.2	18/5 = 3.6						

TABLE 3: Comparison of Individual Usability	
Criterion Evaluation	

material. Same is the case with enough number of assignments/quizzes for assessment.

The collaboration support was bad evaluated because unavailability of online communication tools and the present/past awareness features. So, communication tools like a instant messenger should be included. Present/past awareness can be improved by making the status of current and previous activities of others, making it visible to concerned users, displaying the status of lessons in that how many users have read this lesson, displaying comments of others on a particular lesson, displaying poll responses (if any) by other students, most frequent activity on the system, etc.

Interactivity and feedback of the system should also be redesigned as stressed by users responses. This can be done by including multimedia and multimodal feedback in which different senses are simulated for feedback to be recognized and interpreted more easily. Specifically, along with visual and textual feedback, appropriate sound should be used in order to notify the occurrence of an event. Animation and graphical elements should be added in order to make the feedback more interactive. Finally, the technical errors handling problem can be eliminated by testing. For this purpose, testing techniques from software engineering should be used as a complement.

VULMS was proved to be more usable according with pondered usability measure. However, there is room for improvement in this system as well. as indicated by the low individual usability measure of collaboration support attribute. This feature was underrated because of the lack of present/past awareness elements. The recommendations in this regard are the same as those for eFront. However, students also complained that the system does not allow them to work in collaboration. To support such kind of activity, students should be allowed to discuss and share content online. They should be able to communicate online and modules should be developed that allow them to post comments, request for help, share files, and create new discussions on certain topics in which other students may also participate.

VULMS and eFront systems are just used as case studies for usability evaluation. The recommendations in this regard can equally be applied to any e-learning system.

7 Conclusion and Future Work

In this research, a pondered usability measure for e-learning system evaluation has been proposed. Evaluation is performed in user testing by means of a specially designed questionnaire. Each usability criterion is also applied individually based on responses to known what particular usability criterion needs further improvement.

To validate our approach, e-Learning system domain was selected, applying the methodology on two cases: eFront learning system and Virtual University Learning Management System (VULMS). Elements and statistical formulae involved in the calculation of overall usability measure under consideration were described, pondered usability measure was calculated for each system, as well as a comparison of obtained results. Based on this criterion, VULMS is better than eFront. Criteria and designed questionnaire can be used to evaluate usability, so designers of this system have the possibility to decide whether the system needs improvement.

Currently, we are evaluating the instructor profile, as well as we are formulating an inquiry ad hoc according with the obtained result from learners.

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Relationship between the Quality of Work Life and Employees' Aggression

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Abstract: People working in organizations have a lot of needs, all of which are regularly in competition to guide their behaviors; if these needs aren't met, they result in frustration, and failure in meeting the needs doesn't necessarily cause the quick death of organizations. One of the most common reactions against failure is aggression which is harmful and affects soul and spirit, working relations and performance of employees even though it doesn't cause physical damages. Thus, conditions of working environment which result in meeting the material and spiritual needs of people, represents the quality of work life; it is in such an environment that employees can feel possession, self-direction, responsibility and self-respect. The aim of the present research is to study the relationship between the quality of work life and employees' aggression. This research was carried out using correlation method in statistical universe of employees working in Kerman Bahonar Copper and Sarcheshmeh Copper Industries (approximately 5190 employees in 2007); the sample volume was 384 people. To gather and collect information, two closed-ended questionnaires of "quality of work life and aggression" were used. Data was analyzed using Kendal's Tau b Tests, Spearman Correlation Test and linear logarithm by means of SPSS Software. Results revealed that there is a relationship between quality of work life (and components of job security, justice and equality, received material salaries and allowances, skills improvement field and opportunity and employees' participation in decision making) and aggression. Gender, age, education level, marital status, working record, employment status and job title are of those intermediate variables that were studied in relation to the research main variables. Statistical results showed that two variables of quality of working life and aggression are independent concerning sex, marital status, age, education level, working record and employment status and are related regarding job title.

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Key words: Quality of Work Life, Aggression, Job Security, Justice and Equality, Material Salaries

Introduction

Organization and management aren't a new phenomenon, whose existence and nature have been discovered by man in the contemporary century. Rather, as man started his social life and worked on organizing and controlling affairs, he dealt with organization. Nowadays, he lives in an era where all his needs from birth to death have been met through organization. Therefore, organizations are present in all living levels of human beings and indeed people spend most of their lives in organizations or are affected by them. Existence philosophy of every organization is the need of every organization and the objective of these organizations is to meet the needs of people in society (Mashroteh, 2009).

People in every organization have some needs (Rezaeian, 2000, p. 32). According to Chris Argyris, the more the organization gets successful in meeting human needs, the more the tensions and conflicts existing in the organization decrease and therefore the more organization will get healthier (Fakhimi, 2002, p. 80). On the other hand, failures resulted from not meeting of needs can cause aggression and anger in

organizations; this aggression in organizations is mainly seen as an unfavorable behavior toward clients or inferiors (Mashroteh, 2009).

Aggression in organizations can be as a result of inconformity between one's needs and traditional official organization's wills: aggression can be reflected as ignoring clients or delaying in performing their work. Groups or people who have mostly been selected as shield and become the target of aggressive behaviors, are the weak ones who don't have power to defend themselves (economically, socially and psychologically). It makes aggression more successful (Korman, 2005, p. 14). Therefore, paying attention to employees' basic needs, preparing a suitable environment and creating innovation and growth fields in working environments have very useful and significant effects in organizations' performance (Hosseinizadeh & Saemiyan, 2002, p. 62). According to Dutch Landen, an environment in which people are considered organizational essential members, human souls are challenged, personal growth and development are inspired and works are performed, is an environment with high quality of work life (Belcher,

2001, p. 102). Thus, the quality of work life introduces an environment in which organizations give their employees some opportunities to meet their needs (M'adanipour, 2001).

Literature Review

The Quality of Working Life (QWL): concept and evaluation rates

Quality of work life is an appropriate human resource management strategy for developing countries (Pranee, 2010). The quality of working life could be defined as work place strategies, processes and combination, which environment stimulates employee's job satisfaction. It also depends on work conditions and organization's efficiency (Considine & Callus, 2002). Quality of work life (QWL) includes issues such as occupational hazards and safety, human resource development through welfare measures, professional training, working conditions and consultative work as well as participative mechanisms. Measures and strategies are focused on concern satisfying the minimal lower needs of employees, such as for example: security, safety, and welfare improving job contents, as well as participation and responsibilities in the decision making process (Pranee, 2010).

Moreover, QWL issues also address elements such as are involved high motivation, morale, healthy industrial relations and cooperation (Pranee, 2010). The QWL could be defined as work place strategies, processes and environment combination, which stimulates employee's job satisfaction. It also depends on work conditions and organization's efficiency(Akranavi i t & Ruževi ius, 2007: Considine & Callus, 2002). The OWL concept encompasses the following factors: job satisfaction, involvement in work performance, motivation, efficiency, productivity, health, safety and welfare at work, stress, work load, burn-out etc, these mentioned factors could be defined as physical and psychological results of the work which affect employee (Akranavi i t & Ruževi ius, 2007; Arts et al., 2001). Other authors suggest to involve in this concept more work factors: fair compensation, safe and hygienic working and psychological conditions, knowledge and opportunities to realize one's skills, social integration and relationship, life and work balance, work planning and organization (Akranavi i t & Ruževi ius, 2007; Looij & Benders, 1995).

The QWL domains and factors are:

1. Consideration of work (material and nonmaterial).

2. Emotional state (appreciation, esteem, stress, self motivation, job satisfaction, safety for job).

3. Learning and improvement (career opportunities, acquirement of new knowledge and skills).

4. Social relationship in the organization (relation with colleagues and supervisors, delegation, communication, command, division of work).

5. Self-realization (career opportunities, involvement in decisions making, self-sufficiency in one's workplace).

6. Physical state (stress, fatigue, burn-out, work load).

7. Safety and work environment (Akranavi i t & Ruževi ius, 2007; Arts et al., 2001; Gilgeous, 1998; Schoepke et al., 2003).

Effectively managed organizations are able to maximize both the quality of work life and their profitability for their workforces. Some of the critical factors that impact a workforces' quality of work life include for example:

- The physical aspects of QWL, such as working conditions the conditions of work, and managerial attitudes management attitudes towards pollution and safety, etc.
- The economic aspects of QWL, such as wages and salary administration and considerations for the standard of living that employees needs and enjoy.
- The psychological aspects of QWL such as the how and what of the assigned work, method to do work, and what kind of work (Cascio, 1998; Delamotte & Takezawa, 1984; Pranee, 2010; Stoddart, 1986).

What is aggression?

Aggression at work is usually defined as any form of aggressive behavior with the intention to harm the victim and the behavior used may be both physical and psychological in nature (Baron & Richardson, 1994; Geen, 1990; Høgh, 2005; J. Neuman & Baron, 1997).Traditionally aggression has been classified according to three aspects: physical-verbal, activepassive and direct-indirect aggression (Buss, 1963; Høgh, 2005). Verbal and psychological aggression seems to be more prevalent than physical aggression and violence (Barling, 1998; Baron & Neuman, 1996; Bulatao & Vanden Bos, 1998; Di Martino et al., 2003).

Although some aggressive incidents originate outside the workplace, our focus is on those aggressive acts that originate within the organization (Olson et al., 2006). Effects of workplace aggression are compelling. Individuals who work in aggressive environments experience detrimental psychological as well as physiological responses (Baron & Neuman, 1996; Leymann & Gustafsson, 1996; Magnuson & Ken, 2009; Pearson et al., 2001; Rayner et al, 2002). Workplace aggression also damages organizations (Johnson & Indvik, 2001; Keashly & Jagatic, 2003; Leymann & Gustafsson, 1996; Magnuson & Ken, 2009; Neuman & Baron, 1998; Tracy et al, 2006; Vickers, 2006) and negatively affects financial profit (Johnson & Indvik, 2001; Magnuson & Ken, 2009; Pearson et al., 2000). In an aggressive environment, targets' performance and contributions decrease, they miss work, and they leave their positions. Additionally, workers who witness workplace aggression leave the organizations (Rayner et al., 2002; Vickers, 2006; Magnuson & Norem, 2009).

Traditionally, negative effects of exposure to aggression may be divided into two subcategories: direct effects presented immediately after aggressive social interaction, and long-term effects, usually consequences of repeated exposure (Lanza, 1992). Direct effects of aggressive encounters reflect mostly in emotional sphere - victims frequently report feelings of irritations, anger, anxiety, helplessness, depression, discouragement, felling of guilt, and decreased self-esteem (Hoel et al., 2001; Mercez et al., 2009). Long-term exposure to workplace aggression leads to an impairment of social and professional life - relationships with coworkers are changed - the number of interpersonal conflicts increases, motivation and work involvement decreases (Barling, 1998; Merecz, et al., 2009).

There are some researches, known as Organizational Justice, which investigates how employees assess what is fair in an organization. The broad idea behind Organizational Justice is that employees are active observers in organizations - they see how rewards (and punishments) are allocated. Such allocations may be perceived as fair or unfair based on three things: whether someone deserves what they received (distributive justice), whether the allocation process was fair (procedural justice), and whether someone was treated with respect (interactional justice) (Colquitt et al., 2001; Everton et al., 2007). Distributive justice refers to whether outcomes are perceived as fair - do people get what they deserve? These outcomes are not just money, they can be decisions about who gets promoted, who gets fired, who gets special training, who gets to go on trips, which gets transferred, etc. How the fairness of outcomes is assessed can depend on any of three things:

Equality means that everyone gets an equal shot at receiving the outcome, for example, everyone may receive the same amount for a year-end bonus. Equity means that the outcome is distributed according to how much effort, skill, time, etc. recipients have put into the company. An example might be a year-end bonus based on the number of years one has worked for a company. Giving an award based on need would mean that those who need the resource the most receive the most. Needless to say, distributing most rewards in an organization based on need would cause many employees to perceive unfairness (Everton et al., 2007).

Relationship between QWL & Aggression

Existence philosophy of every organization is the need of every organization and the objective of these

organizations is to meet the needs of people in society (Mashroteh, 2009). According to Chris Argyris, the more the organization gets successful in meeting human needs, the more the tensions and conflicts existing in the organization decrease and therefore the more organization will get healthier (Fakhimi, 2002). On the other hand, failures resulted from not meeting of needs can cause aggression and anger in organizations; this aggression in organizations is mainly seen as an unfavorable behavior toward clients or inferiors (Mashroteh, 1999).

Aggression in organizations can be as a result of inconformity between one's needs and traditional official organization's wills; aggression can be reflected as ignoring clients or delaying in performing their work. Groups or people who have mostly been selected as shield and become the target of aggressive behaviors, are the weak ones who don't have power to defend themselves (economically, socially and psychologically). It makes aggression more successful (Korman, 2005). Therefore, paying attention to employees' basic needs, preparing a suitable environment and creating innovation and growth fields in working environments have very useful and significant effects in organizations' performance (Hosseinzadeh & Saemiyan2002, p. 62).

Elements of the work environment and a negative social climate are often associated with aggression at work (Høgh, 2005; Neuman & Baron2003). For instance, (authoritarian) leadership, role conflicts and interpersonal conflicts have been reported to correlate with aggression and bullying at work through tension, stress, and frustration in the work group (Chen & Spector, 1992; Einarsen, 2000; Høgh, 2005). Moreover, studies have suggested that conflicts between members of staff may increase the rate of e.g. violence at work (Beale et al., 1999; Bennett & Lehman, 1998; Høgh, 2005). Sometimes interpersonal conflicts at work escalate into harsh personified struggles and if one of the parties in such a conflict gets into a disadvantaged position he or she may become the victim of bullying (Einarsen, 2000). Interpersonal conflicts have also been associated with psychological strain, depression and frustration (Beehr1995; Beehr et al., 2000; Bergmann & Volkema, 1994).

It is clear that an organization's policies are tied to violence at work. Again, the perception of fairness and the idea that one's work life is closely monitored seems to increase aggressiveness. Such aggressiveness comes at great cost; employee victims suffer physical and emotional health problems and decreased job satisfaction and commitment to the organization. This may lead to increased absences and a host of other consequences.

Quality Work Life Domains



Organization Justice Domains





Research Variables and Dimensions



If an organization provides support to its employees, either informational or instrumental support, the impact of the violence should decrease dramatically (Everton et al., 2007).

Concerning the above mentioned items, the following basic questions will arise:

- How are the conditions of work life quality among statistical universe employees?
- Are statistical universe employees aggressive?
- Is there a relationship between level of work life quality and employees' aggression?

Answering all these questions requires a general research and scientific work.

Research Objectives

The present research aims to:

Realize and describe the relationship between level of quality work life and employees' aggression in statistical universe employees.

Research scope

Scope of the present research can be described and reviewed from three dimensions.

Ø **Research spatial scope**: It includes two Industries of Kerman Bahonar Copper and Sarcheshmeh National Copper Companies located at 10 and 160 Km away from center of Kerman.

Ø **Research temporal scope**: it is from the second half of year 2007 to the first half of year 2008.

Research topical scope: concerning research subject in relation to quality of work life, five dimensions of job security, justice & equality, material salary & allowance, Skills improvement field and opportunity and involvement in decision making from Thomas Tatel's viewpoint as well as aggression from Bass and Pery's viewpoint have been taken into consideration.

Words operational description

A) **Quality of work life**: in this research, quality of work life consists of job security, justice & equality, material salary & allowance, Skills improvement field and opportunity and involvement in decision making which are measured by questionnaire of quality of work life. A mark given to this questionnaire in Likert scale by tested, specifies the level of employees' quality of work life.

B) **Aggression**: A point obtained from employees' answer to the questions related to physical and verbal components as well as anger and hostility in Likert scale.

Types of variables:

- **Predictive variable**: in this research, quality of work life has been considered as the predictive variable.
- **Criterion variable**: in this research, aggression has been considered as the criterion variable

Intermediate variables: in this research, gender, age, marital status, working record, education, job title and employment status have been considered as the intermediate variable.

Research hypotheses

Main hypothesis:

There is a relationship between employees' quality of work life and their aggression

Specific hypotheses:

- 1- There is a relationship between level of employees' job security and their aggression
- 2- There is a relationship between level of observing justice and equality to employees and their aggression
- 3- There is a relationship between level of employees' received material salary and allowance and their aggression
- 4- There is a relationship between level of employees' skills improvement field and opportunity and their aggression
- 5- There is a relationship between level of employees' involvement in decision making and their aggression
- 6- There is a relationship between level of employees' quality of work life and their aggression, concerning intermediate variables (gender, marital status, age, education, working records, employment status and job title).

Methodology

Population and Sampling Procedure

Population in the present research includes all employees working in Kerman Bahonar Copper Company and Sarcheshmeh National Copper Industries in year 2007 (N=5190)

Sarcheshmeh National Copper Industries consists of five deputies which work under supervision of company's managing director; based on their activities, these five deputies are divided into five different groups namely: production deputy with 2845 employees; financial and administrative deputy with 752 employees; programming and development deputy with 118 employees; human resources deputy with 95 employees; and economical deputy with 70 employees. Bahonar Copper Company has also three deputies; they are called production deputy with 1027 employees; administrative and back-up deputy with 193 employees; and plan and program deputy with 90 employees.

To determine the required sample size, maximum sample size determination method was used N=n $_{max}=Z^2 P (1-p)/d^2=384$

In this research, sampling has been used by stratified sampling method proportional to stratified size. In this method,

the statistical universe (population) was divided into different stratifies and then every stratify of a random sample was chosen proportional to number of people of that universe (Ramezani, 2003). After determining the sample size, simple random method has been used to select sampling unit (respondent); that is, number of people working in every specified unit. Then based on sample size, number of people required for sampling was selected proportional to volume and finally the questionnaire was distributed among them.

Sampling from every level was calculated as follows:

n_i=Ni/N n

Where,

n =sample size; N =population; $n_i =$ sample size in M-i level; and Ni = population of M-i level.

	Tuble It bumple bille h	u terms of levels (uep	
	Deputies title	No. of employees	n=sample size from M-i level
Sarcheshmeh	Production	2845	n1=2845/5190×384=210
National Copper	Financial & administrative	752	n2=752/5190× 384=56
Industries	Planning & development	118	n3=118/5190× 384=9
	Human resources	95	n4=95/5190×384=5
	Economical	70	n5=70/5190×384=7
Kerman Bahonar	Production	1027	n6=1027/5190×384=76
Copper Industries	Back-up & administrative	193	n7=193/5190× 384=14
	Plan & program	90	n8=90/5190×384=7
	Total	5190	n= 384

Table 1: sample size in terms of levels (deputies)

Instruments and Data collection

In this research, date was collected using two close-ended questionnaires. The first one which measures the level of quality work life includes 24 questions. In this questionnaire, a five-degree Likert scale was used to measure the given factors and the answers were classified into five degrees of "very low, low, average, high and very high"; they were marked from one to five. Also, for reverse questions, they were marked from 5 to one. The resultant mark shows the level of employees' work life quality. To determine aggression, Bass and Pery's Questionnaire with 29 questions was used. In this questionnaire, a five-degree Likert Scale was used to measure the given factors and the questions were presented in the form of five choices of "never, seldom, sometimes, usually and always." They were marked from 1 to 5; for reverse sentences, marks 5 to 1 were used. The resultant mark showed the level of respondent's aggression.

Also, the socio demographic variables (gender, marital status, age, education, working record, employment status and job title) were asked in the beginning of questionnaire.

The researcher referred to Kerman Bahonar Copper and Sarcheshmeh National Copper Industries Companies directly and distributed 384 questionnaires among employees by the aid and cooperation of industrial relations. The questionnaires were distributed, answered and collected in one day and the respondents answered all questions.

Data analysis

In the present research, different tables and graphs were used to describe the collected data. To describe the personal specifications of people (including gender, marital status, age, education, working record, employment status and job title) as well as research hypotheses, frequency percent tables and graphs were used.

Job security variable was calculated from the mean of answers given to questions 1 to 4 of the questionnaire "quality of work life"; justice and equality variable was calculated from the mean of answers given to questions 5 to 9 of the questionnaire "quality of work life"; variable "received material salary and allowance" was calculated from the mean of answers given to questions 10 to 14 of the questionnaire "quality of work life"; skills improvement field and opportunity variable was calculated from the mean of answers given to questions 10 to 14 of the questionnaire "quality of work life"; skills improvement field and opportunity variable was calculated from the mean of answers given to questions 15 to 18 of the questionnaire "quality of work life"; variable "employees' involvement in decision making" was calculated from the mean of answers given to questions 19 to 24 of the questionnaire "quality of work life"; aggression variable was calculated from the mean of answers given to questions 1 to 29 of the questionnaire "aggression". In this case, SPSS Statistical Software was used. Then, using inferential statistics including Spearman and Kendal's Tau b Correlation Tests and independence test (q^2), to study the relationship between variables and to compare the variables distribute; moreover, linear logarithm analyses were studied and analyzed to study the relationship between main variables along with a intermediate variable.

Finding of the study Real-ground of Respond

Background of Respondents

Concerning data analysis, 87.5 percent of respondents were men and the remaining 12.5 percent were women. Among them, 7.6 percent were 25 years old or less; 23.8 percent were 26-30; 28.2 percent were between 31 and 35; 23.8 percent were between 36 and 40; 10.7 percent were between 41 and 45; 4.4 percent were between 45 and 50; and 1.3 percent were between 51 and 55. Concerning marital status, 80.2 of them were married and 19.8 were single. 14.4 percent of them didn't have high school diploma; 37.1 percent had high school diploma; 20.9 had associate's degree; 24.3 had bachelor's degree; and 3.4 percent had master's degree and higher. Regarding working record, 22.1 percent had a record of less than 5 years; 32.8 had a record of between 6 and 10 years; 20.3 percent had between 11 and 15; 16.7 percent had between 16 and 20; 5.7 percent had between 21 and 25 years; and 2.3 percent between 26 and 30 years. 73.3 percent were officially employed and 26.8 percent had contractual employment. And concerning job title, 38 percent were workers; 37.2 were employees; 13.3 percent were experts; 8.6 percent were supervisors; 2.6 percent were bosses; and 0.3 percent was managers.

<u>The research hypothesis is</u>: There is a significance relationship between level of employee's quality of work life and their aggression.

The null hypotheses stated that there is no relationship between level of employees' work life quality and aggression) H_0 : p=0

On the other hand, alternative hypotheses is, there is a relationship between level of employees' work life quality and aggression) H_1 : p 0

To proof the null hypotheses, Kendal & Spearman Correlation Coefficient was carried out. -0.330 and -0.3729 for the above-mentioned variables respectively and at significance level of = 0.05, the null hypotheses should be reject because *p* value (0.000) 0.001, therefore, the null hypothesis which points to the lack of a linear relationship between these two variables, is rejected.

Table 2: Kendal's & Pears	son's Correlation	Coefficients of I	Level of employees'	and Employees'	aggression

	Employees' aggress	ion				
Level of employees'	Kendal Correlation		Spearman Correlation			
quality work life	Correlation	Sig P	Frequency	Correlation	Sig P	Frequency
	coefficient			coefficient		
	-0.330	0.000	384	-0.372	0.000	384

Sub hypothesizes:

There is a relationship between level of employees' job security and their aggression.

(There is no relationship between level of employees' job security and aggression) H_0 : p=0 (There is a relationship between level of employees' job security and aggression) H_0 : p=0

(There is a relationship between level of employees' job security and aggression) H_1 : p 0

To proof the null hypotheses, Kendal & Spearman Correlation Coefficient was carried out. -0.158 and - 0.186 for the above-mentioned variables respectively and at significance level of = 0.05, the null hypotheses should be reject because *p* value (0.000) 0.001, therefore, the null hypothesis which points to the lack of a linear relationship between these two variables, is rejected.

 Table 3: Kendal's & Spearman's Correlation Coefficients of Employees' aggression & Level of employees' job security

	Level of Employees' job	Level of Employees' job security									
Employees'	Kendal Correlation			Spearman Correlation							
aggression	Correlation coefficient	Sig P	Frequency	Correlation coefficient	Sig P	Frequency					
	-0.158	0.000	384	-0.186	0.000	384					

There is a relationship between level of justice and equality toward employees and their aggression.

(There is no relationship between level of justice and equality toward employees and aggression) H₀: p=0

(There is a relationship between level of justice and equality toward employees and aggression) H_1 : p 0

To proof the null hypotheses, Kendal & Spearman Correlation Coefficient was carried out. -0.230 and -0.259 for the above-mentioned variables respectively and at significance level of = 0.05, the null hypotheses should be reject

because p value (0.000) 0.001, therefore, the null hypothesis which points to the lack of a linear relationship between these two variables, is rejected.

Table 4: Kendal's & Spearman's Correlation	Coefficients of Employees'	aggression & Level of	of justice and
equality toward employees			

	level of justice and equality toward employees								
Employees'	Kendal Correlation			Spearman Correlation					
aggression	Correlation coefficient	Sig P	Frequency	Correlation coefficient	Sig P	Frequency			
	-0.230	0.000	384	-0.259	0.000	384			

There is a relationship between level of employees' received material salary and allowance and their aggression. (There is no relationship between level of employees' received material salary and allowance and aggression) H_0 : p=0 (There is a relationship between level of employees' received material salary and allowance and aggression) H_1 : p 0. To proof the null hypotheses, Kendal & Spearman Correlation Coefficient was carried out. - 0.231 and -0.259 for the above-mentioned variables respectively and at significance level of = 0.05, the null hypotheses should be reject because *p* value (0.000) 0.001, therefore, the null hypothesis which points to the lack of a linear relationship between these two variables, is rejected.

Table 5: Kendal's & Spearman's Correlation Coefficients of Employees' aggression & Employees' received material salary and allowance

	Employees' received material salary and allowance							
Employees'	Kendal Correlation			Spearman Correlation				
aggression	Correlation coefficient	Sig P	Frequency	Correlation coefficient	Sig P	Frequency		
	-0.231	0.000	384	-0.259	0.000	384		

There is a relationship between level of employees' skills improvement opportunity and their aggression. (There is no relationship between level of employees' skills improvement opportunity and aggression) H_1 : p =0. (There is a relationship between level of employees' skills improvement opportunity and aggression) H_1 : p 0. To proof the null hypotheses, Kendal & Spearman Correlation Coefficient was carried out. -0.272 and -0.324 for the above-mentioned variables respectively and at significance level of = 0.05, the null hypotheses should be reject because *p* value (0.000) 0.001, therefore, the null hypothesis which points to the lack of a linear relationship between these two variables, is rejected.

Table 6: Kendal's & Spearman's Correlation Coefficients of Employees' aggression & Employees' skills improvement opportunity

	Employees' skills improvement opportunity									
Employees'	Kendal Correlation			Spearman Correlation						
aggression	Correlation coefficient Sig P		Frequency	Correlation coefficient	Sig p	Frequency				
	-0.272	0.000	384	-0.324	0.000	384				

There is a relationship between level of employees' involvement in decision making and their aggression. (There is no relationship between level of employees' involvement in decision making and aggression) H_1 : p =0. (There is a relationship between level of employees' involvement in decision making and aggression) H_1 : p 0. To proof the null hypotheses, Kendal & Spearman Correlation Coefficient was carried out. -0.254 and -0.304 for the above-mentioned variables respectively and at significance level of = 0.05, the null hypotheses should be reject because *p* value (0.000) 0.001, therefore, the null hypothesis which points to the lack of a linear relationship between these two variables, is rejected.

Table	7:	Kendal's	&	Spearman's	Correlation	Coefficients	of	Employees'	aggression	&	Employees'
involve	eme	nt in decisi	ion 1	making							

	Employees' involvement in decision making							
Employees'	Kendal Correlation			Spearman Correlation				
aggression	Correlation coefficient	Sig P	Frequency	Correlation coefficient	Sig P	Frequency		
	-0.254	0.000	384	-0.304	.000	384		

Next hypothesis: there is a relationship between level of employees' work life quality and their aggression and mediator variables (gender, marital status, age, education, working record, employment status and job title)

Linear logarithm analysis of employees' work life quality, aggression and gender:

To analyze a three dimensional agreed table which consists of three variables of gender (C), aggression (B) and quality of work life (A), we have used linear logarithm analysis. In Table 8, quality of work life of 2 or less than two was mixed and the new quality was called unfavorable quality of work life; quality of 2.5 and 3 was called average quality; and QWL more than three was called favorable quality of work life. Also, aggression of 2 or less than 2 was mixed and called low aggression; aggression of 2.5, 3 and 3.5 was called average aggression; and aggression of 4 or more was called high aggression. The last accepted hypothesis of the Table 9 is AC and BC. Test statistics level, df level and significance are also presented in this table.

Gender (C)	Aggression (B)	low	Average	High	Total
	QWL (A)				
Man	Unfavorable	79	33	34	146
	Average	149	23	1	173
	Favorable	17	0	0	17
	Total	245	56	35	336
Woman	Unfavorable	15	9	2	26
	Average	15	5	1	21
	Favorable	1	0	0	1
	Total	31	14	3	48
	$G^2 = 10.62962$	C	lf=8	P=0.	224

,	Table 8. (Calculating	the test	t statistics	ratio of	² accuracy	and its	significa	nce
	able o.	Calculating	the tes	i statistics	, i auv vi	. accuracy	anu no	Significa	nce

Therefore, hypothesis A, B C is accepted. Thus, quality of work life and aggression are independent of gender. Therefore, it can be said that A C and B C. However, partial analysis of quality of work life (A) and aggression (B) for men and women separately shows that A B C=2 while A B C=1. That is, women's idea that two variables of A and B are independent while men's idea that they aren't independent.

Table 9: Calculating the test statistics, ratio of accuracy and its s	significance
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	Accuracy ratio statistics (G ²)	df	Sig P value
Man (1)	71.860	4	0.000
Woman (2)	1.852	4	0.0763

Linear logarithm analysis of employees' quality work life, aggression and marital status:

To analyze a three dimensional agreed table which consists of three variables of marital status (D), aggression (B) and quality of work life (A), we have used linear logarithm analysis. The last accepted hypothesis of the table is AD and BD. Test statistics level, df level and significance are also presented in this table.

Table 10: Calculating	g the test statistics	, ratio of accuracy	y and its significance
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Marital Status (D)	Aggression (B) QWL (A)	low	Average	High	Total
Married	Unfavorable	78	30	23	131
	Average	134	25	2	161
	Favorable	15	0	0	15
	Total	227	55	25	307
Single	Unfavorable	15	12	13	40
	Average	30	3	0	33
	Favorable	3	0	0	3
	Total	48	15	13	76
	$G^2 = 10.76530$	Γ	Df=8	P=0.	215

Therefore, hypothesis A, B D is accepted. Thus, quality of work life and aggression are independent of marital status. Therefore, it can be said that A D and B D. However, partial analysis of quality of work life (A) and aggression (B) for married and single employees separately shows that A B D=2 while A B D=1. That is, concerning both single and married opinions, it can't be said that variables of A and B are independent.

Table 11: Calculating the test statistics, ratio of accuracy and its significance

	Accuracy ratio statistics (G^2)	df	Sig P value
Married (1)	42.381	4	0.000
Single (2)	31.058	4	0.000

Linear logarithm analysis of employees' quality of work life, aggression and age:

To analyze a three dimensional agreed table which consists of three variables of age (E), aggression (B) and quality of work life (A), we have used linear logarithm analysis. The last accepted hypothesis of the table is AE and AB. Test statistics level, df and level of significance are also presented in this table.

Age(E)	Aggression (B)	low	Average	High	Total
	QWL (A)				
30 or less	Unfavorable	31	13	14	58
	Average	44	9	2	55
	Favorable	7	0	0	7
	Total	82	22	16	120
30-40	Unfavorable	46	27	14	87
	Average	933	16	0	109
	Favorable	3	0	0	3
	Total	142	43	14	199
41 and more	Unfavorable	17	2	8	27
	Average	27	3	0	30
	Favorable	8	0	0	8
	Total	52	5	8	65
	$G^2 = 14.42161$	D	f=12	P=0.	275

Table 12: Calculating the test statistics, ratio of accuracy and its significance

Therefore, hypothesis B E A is accepted. Thus, it can be said that if the quality level of employees' work life is recognized, their age doesn't affect their aggression. However, partial analysis of quality of work life (A) and aggression (B) for different age groups separately shows that A B E=1 and A B E=2 and A B E=3. That is, both variables of A and B are related to all levels of E.

Age	Accuracy ratio statistics (G^2)	df	Sig P value
30 or less	18.570	4	0.001
30-40	38.032	4	0.000
41 and more	17.268	4	0.002

 Table 13: Calculating the test statistics, ratio of accuracy and its significance

Linear logarithm analysis of employees' quality of work life, aggression and education:

To analyze a three dimensional agreed table which consists of three variables of education (F), aggression (B) and quality of work life (A), we have used linear logarithm analysis. The last accepted hypothesis of the table is AF and AB. Test statistics level, df and level of significance are also presented in this table.

Therefore, hypothesis A F|B is accepted but A B|F. Thus, it can be said that if the level of employees' aggression is recognized, their education doesn't affect their quality of work life; while, if their education is recognized, it can't be said that aggression and quality of work life are independent. However, partial analysis of quality of work life (A) and aggression (B) for different education levels (F) separately shows that A B F=1 and A B F=2 and A B F=3. That is, both variables of A and B are related to all levels of F.

Education (F)	Aggression (B)	low	Average	High	Total
	QWL (A)				
High school	Unfavorable	53	21	23	97
diploma or less	Average	78	11	1	90
	Favorable	10	0	0	10
	Total	141	32	24	197
Associate's	Unfavorable	18	12	9	39
degree	Average	30	7	1	38
	Favorable	3	0	0	3
	Total	51	19	10	80
Bachelor's degree	Unfavorable	22	9	4	35
and more	Average	56	10	0	66
	Favorable	5	0	0	5
	Total	83	19	4	106
	$G^2 = 6.03088$	D	f=12	P=0.	915

Table 14: C	Calculating the	test statistics, ratio o	f accuracy and its	significance
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Table 15: Calculating the test statistics, ratio of accuracy and its significance

	Accuracy ratio statistics (G ²)	df	Sig P value
High school diploma or less	39.573	4	0.000
Associate's degree	14.479	4	0.006
Bachelor's degree and more	13.771	4	0.008

Linear logarithm analysis of employees' work life quality, aggression and working record:

To analyze a three dimensional agreed table which consists of three variables of working record (G), aggression (B) and quality of work life (A), we have used linear logarithm analysis. The last accepted hypothesis of the table is AG and AB. Test statistics level, df and level of significance are also presented in this table.

Working record	Aggression (B)	low	Average	High	Total
(G)	Life quality (A)				
Less than 15	Unfavorable	69	38	30	137
years	Average	116	23	2	141
	Favorable	11	0	0	11
	Total	196	61	32	289
More than 15	Unfavorable	25	4	6	35
years	Average	48	5	0	53
	Favorable	7	0	0	7
	Total	80	9	6	95
	$G^2 = 10.76530$	Γ	Df=8	P=0.2	215

 Table 16: Calculating the test statistics, ratio of accuracy and its significance

Therefore, hypothesis A G | B is accepted. Thus, it can be said that if the level of employees' aggression is recognized, their working record doesn't affect their quality of work life. However, partial analysis of quality of work life (A) and aggression (B) for different levels of working record separately shows that A B G=1 and A B G=2. That is, both variables of A and B are related to all levels of G

Table 17: Calculating the test statistics, ratio of accura	cy and its meaningfulness
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	Accuracy ratio statistics (G^2)	df	Sig P value
Less than 15	53.888	4	0.000
More than 15	14.601	4	0.006

Linear logarithm analysis of employees' quality work life, aggression and employment status:

To analyze a three dimensional agreed table which consists of three variables of employment status (H), aggression

(B) and quality of work life (A), we have used linear logarithm analysis. The last accepted hypothesis of the table is AH and AB. Test statistics level, df and level of significance are also presented in this table.

Employment	Aggression (B)	low	Average	High	Total
Status (H)	QWL (A)				
Official	Unfavorable	66	26	20	112
	Average	129	21	2	152
	Favorable	14	0	0	14
	Total	209	47	22	278
Contractual	Unfavorable	27	16	16	59
	Average	33	7	0	40
	Favorable	3	0	0	3
	Total	63	23	16	102
	$G^2 = 4.257$	Ι	Df=6	P=0.	624

Table 18: Calculating the test statistics, ratio of accuracy and its significant

Therefore, hypothesis B H A is accepted. Thus, it can be said that if the quality level of employees' work life is recognized, their employment status doesn't affect their aggression. But A B H is not correct. It can be shown through partial analysis. However, partial analysis of quality of work life (A) and aggression (B) for different levels of employment status separately shows that A B H=1 and A B H=2. That is, both variables of A and B are related to all levels of H

Table 19: Calculating the test statistics, ratio of accuracy and its sig	significance
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	Accuracy ratio statistics (G ²)	df	Sig P value
Official	40.497	4	0.000
Contractual	25.667	4	0.000

Linear logarithm analysis of employees' work life quality, aggression and job title:

To analyze a three dimensional agreed table which consists of three variables of job title (I), aggression (B) and quality of work life (A), we have used linear logarithm analysis. The last accepted hypothesis of the table is AI and AB. Test statistics level, df and level of significance are also presented in this table.

Job title(I)	Aggression (B)	low	Average	High	Total
	QWL (A)				
Worker	Unfavorable	37	18	26	81
(Labour)	Average	51	6	0	57
	Favorable	8	0	0	8
	Total	96	24	26	146
Employee	Unfavorable	38	21	4	63
	Average	60	12	2	74
	Favorable	6	0	0	6
	Total	104	33	6	143
Higher than	Unfavorable	19	3	6	28
employee	Average	53	10	0	63
	Favorable	4	0	0	4
	Total	76	13	6	95
	$G^2 = 11.799$	Ι	Df=8	P=0.	049

 Table 20: Calculating the test statistics, ratio of accuracy and its significance

Therefore, all three variables are related to each other and no independence can be found in the table because A B I. It can be shown through partial analysis. However, partial analysis of quality of work life (A) and aggression (B) for different job title separately shows that A B I=1 and A B I=2 and A B I=3. That is, both variables of A and B are related to all levels of I.

	Accuracy ratio statistics (G ²)	df	Sig. P Value
Worker (Labour)	47.312	4	0.000
Employee	11.82	4	0.025
Higher than employee	17.021	4	0.002

Table 21- calculating the test statistics, ratio of accuracy and its meaningfulness

Selected Descriptive Statistic of the Respondents

Level of quality work life: Respondent's ideas about quality of work life were ranked from 1 to 5; 1 is the least and 5 are the most.

able 22: Quality of work life among responder		
Variable specification	QWL	
Mean	2.4857	
Median	2.5000	
Mode	3.00	
Minimum	1.00	
Maximum	4.00	
Total	384	
Un-answered (missing)	0	

Table 22: Quality of work life among respondents:

Table 23: Ranks frequency:

Marks	Frequency
1 (very low)	26
1.50	6
2 (low)	140
2.50	24
3 (average)	170
3.50	5
4 (high)	13
Total	384



Level of aggression

Tastes' ideas about quality of work life were ranked from 1 to 5; 1 is the least and 5 is the most.

Variable specification	Level of aggression
Mean	2.0833
Median	2.000
Mode	2.00
Minimum	1.00
Maximum	5.00
Total	384
Un-answered (Missing)	0

Table 25- Ranks frequency

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Marks	Frequency
1 (very low)	116
1.50	2
2 (low)	158
2.50	1
3 (average)	68
3.50	1
4 (high)	35
5 (very high)	3
Total	384



Conclusions and Discussion

Research results and findings revealed that generally there is a relationship between level of employees' work life quality and their aggression. The existence of relationship between these two variables in the mentioned Companies was studied.

Statistical analyses showed that there is a negative significance relationship between aggression and quality of work life. In Mashroteh's research (2009) entitled "Study the relationship between traditional structure and aggression", this relationship has been confirmed; since traditional organizations are characterized by bureaucracy (hierarchy), since according to Koorman (mentioned in Mashroteh, 6, 2009), aggression in hierarchical organizations is more likely due to failure of not meeting employees' needs and since people in such environments face lower quality of work life as a result of dissatisfaction with material and mental needs, they face decreased success and increased aggression.

2- Research results revealed that there is a negative significance relationship between job security and aggression, and as job security increases, aggression decreases and vice versa.

In organizational life, job security is one of the issues all employees think about and a part of psychological and intellectual energy of the organization is spent on it. In a case employees achieve a favorable level concerning job security, they can allocate their power and intellectual energy to their jobs and then the organization experiences less human tensions (Soltani, 2000). According to Herci and Blanchard (1992) conscious need to security is completely apparent and is usual among almost all people. They believes that most organizations overemphasize the security by providing precise programs for job security and some benefits including heath, events, life insurance and retirement plans. Such emphasis causes people to become more predictable and obedient and thus behaviors like tension and aggression decrease on them

(Herci & Blanchard, 1992).

Statistical analysis revealed that there is a negative significant relationship between level of justice and equality toward employees and their aggression; and as justice increases, aggression decreases and vice versa.

In order for a person to feel that he is treated justly, his inputs like skill, knowledge, attempts, job performance and loyalty should be proportional to his outputs like fee, benefits, job satisfaction, fame and opportunities; that is, he should feel that he is treated fairly and his inputs and outputs are fair concerning other people's inputs and outputs. If employees believe that they are treated fairly and justly, they may likely to try more and undertake more load to keep their jobs. Results of a research showed that those employees who feel that organizational behavior has been fair or, in other words, think that organization and leaders have had a fair behavior toward them, they get along better with the organization and adapt themselves more to the organization's conditions, "that is, they are more flexible against unpredictable events and show more suitable reactions" (Desler, 1999, p. 237). In contrast, injustice toward members and existence of this feeling cause lack of success and consequently some behaviors like aggression appears; in this case, employees try to oppose leaders and groups aggressively. (Azarbayjani et al., 2006, p. 421). Similarly, Bin et al (1977) pointed out that physical violence is one of the reactions toward lack of organizational justice (Mahdad, 2006).

Results of the present research reveal that there is a negative meaningful relationship between employees' material salary and benefits and their aggression and as salary and benefits increase, aggression decreases and vice versa.

People want payment systems and promotion policy of organization to be fair, clear and according to their expectations. Concerning type of job and skill level of an employee (and the amount of salary paid at that society), if the salary and allowances are reasonable and fair, job satisfaction appears (Rabinz, 1999). Studies carried out by Locke showed that salary and fee is one of the most important factors satisfaction, especially determining job when employees feel their salaries are fair (Moghimi, 1998). Thus, compiling and applying a special salary system which can create a fair criterion for employees regarding their skills and abilities is of great importance (Abbaszadegan, 1992). Therefore, it should be taken into account that salary and fee is something more than meeting the living needs of human resources in an organization. Suitable salary and fee has a significant effect on behavior, life level, purchase power and mental-psychological health of people in one society and it eventually can increase production and efficiency in organizations; however, unfair salary

and fee result in economical crisis, decreased efficiency, bad behaviors and social corruptions. Thus, it should be taken into great concern (Abtahi, 1998). Otherwise, when a person becomes a victim of unfair social exchange, he tries to reform and correct the situation. Reform movements and measures include a range of different behaviors from a slight change in attitude or behavior to severe hostile behaviors for hurting unfair people (Rezaeian, 2006).

Statistical calculations revealed that there is a negative meaningful relationship between skills improvement field and opportunity and aggression; as this field increases, aggression decreases and vice versa.

Working environment will have high quality when people are considered as main members of the organization, have the opportunity to learn in different ways, can fulfill their job improvement paths, development opportunity is provided for them, their minds encounter intellectual and mental challenges and environment conditions result in the growth and improvement of their abilities; it is in this environment that works are done perfectly (Bahramzadeh & Khedmatgozar Baghan, 2004). Programs related to quality of work life can be positive incentives for employees to use their skills and abilities in their organizations and can provide them with a possibility to learn, grow, accept responsibility and create new skills to solve problems of organizations and manpower (Abtahi & Kazemi, 2004, p. 159) Most employees in various jobs use their manual and mental skills less. Lack of using skills and abilities can result in failure (Stat, 2003). Thus, failures can result in aggression (Rezaeian, 2006, p. 28). In contrast, people who improve their skills and knowledge aren't likely to show aggression because it is natural that when people are aware and well-informed in organizations, they are more patient and solve the problems more logically (Zakeri Afshar, 2006, p. 231).

Results of the present research reveal that there is a negative meaningful relationship between employees' involvement in decision making and their aggression; and as involvement increase, aggression decreases and vice versa.

Involvement in decision making is used as human aspects in one organization, generally as a suitable tool for motivating, increasing employees' performance and changing the status quo (Toolabi, 2002, p. 55). Charls Gibotz (mentioned in Shafiei Motahar, 39, 2004) regards asking help from others as one of the reasons of improvement in every organization. Letting people participate means accepting them through entrusting authority and responsibility in a social situation(Shafiee Motahar, 2004). Thus, participation culture resembles a cultural, social and ethical affair in managing society and organizations affairs. Concerning involvement necessity, Donald Micheal says: "Nowadays, it has become clear that when people can participate in decision making process in relation to their lives and jobs, they can trust themselves more, try more and present more ethical ideas (Shafiei Motahar, 49, 2002). Thus, increased involvement in an organization results in increased quality of work, improved quality of work life, improved organizational morale and loyalty, increased motivation and tendency to work, reduced conflict, hostility, unhealthy hostile competition, customers' dissatisfaction and tension in work (Toosi, 2001, p. 69).

Results of the present research revealed that the relationship between level of work life quality and employees' aggression concerning the mentioned intermediate variables is as follows:

Statistical calculations show the level of work life quality and employees' aggression concerning men and women; existence of this relationship in men is confirmed. Some research like Graham's research (1, 2002) entitled "Study two different worlds of aggression in men and women", which shows that aggression in men is more than women, can be the reason of confirming this relationship in men. That is, as quality of work life decreases in employed men, their aggression increases and vice versa (Graham, 2002).

Statistical studies show that quality of work life is related to aggression in both single and married employees concerning marital status, because both groups have the same working conditions and same needs in working environments. Therefore, if needs of employees are met, their quality of work life increases and aggression decreases and vice versa.

- Statistical studies show that, concerning age in three different groups, there is a relationship between quality of work life and aggression. Employees, in every age group, have some expectations from their working environments and level of these expectations may vary; but they exist in all groups. Employees expect environment and working conditions to meet these needs; if they are met, quality of work life increases and aggression decreases and vice versa.

Statistical calculations show that there is a relationship between quality of work life and aggression concerning education. In every three academic level, people show different reactions based on their knowledge of issues, needs and the way how they are met; if , in every level, the needs aren't met, quality of work life decreases and thus aggression increases and vice versa.

Statistical calculations show that there is a relationship between quality of work life and aggression concerning education. In every three academic level, people in every level show different reactions based on their knowledge of issues and needs

and the way how they are met; if , in every level, the needs aren't met, quality of work life decreases and thus aggression increases and vice versa.

Statistical studies show that there is a relationship between quality of work life and aggression concerning working record which is evaluated in two groups. As working record increases, stagnate appears in their futureless and unsuitable job; real abilities, professional incentives and objectives and lack of diversity in work make people mentally and physically tired (Sa'adat, 1996, p. 14). Thus, they consider themselves unsuccessful employees and consequently quality of their work lives decreases and aggression increases and vice versa.

Statistical studies show that there is a relationship between quality of work life and aggression concerning two official and contractual employment statuses. No matter what kind of employment they have, employees want their needs to be met and work environment provides this situation for them. If the environment of meeting these needs has an unfavorable condition, quality of work life decreases and thus aggression increases and vice versa.

Finally, statistical analyses showed that there is a relationship between quality of work life and aggression concerning job title. People, in every organizational position and every job title, need security, justice, enough salary and benefits, growth of their skills and abilities, respect and finally involvement in working environment. All of these items are related to an environment with high quality of work life; in a case every one of these meets isn't met, people become aggressive, angry and hostile with their colleagues and organization. Thus, as quality of work life decreases, aggression increases and vice versa.

Research findings regarding the comparison between the distribution of level of work life quality in Sarcheshmeh and Bahonar Copper Industries show that distribution of quality work life in employees working in these two companies aren't the same; quality of work life in employees of Sarcheshmeh Company is low or average while it is average in Bahonar Copper Company. Low to average quality in Sarcheshmeh Copper Company may depend on harder working condition exists in this complex. It should be mentioned that dispersion of level of quality work life is the same in both groups.

Also, distribution of level of aggression is not the same in these two groups; in spite of low median calculation in both groups, dispersion of level of aggression in Sarcheshmeh Company is higher than that of Bahonar Company. It can be said that higher number of samples in Sarcheshmeh Company can be the reason of higher dispersion of aggression among its employees.

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